SPANDIDOS PUBLICATIONS

EXPERIMENTAL AND THERAPEUTIC MEDICINE 2: 109-117, 2011

Phase I clinical study of a personalized peptide vaccination available for six different human leukocyte antigen (HLA-A2, -A3, -A11, -A24, -A31 and -A33)-positive patients with advanced cancer

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Received October 1, 2010; Accepted December 2, 2010

DOI: 10.3892/etm.2010.177

Abstract. The majority of peptide-based cancer vaccines under development are for human leukocyte antigen (HLA)-A2- or -A24-positive patients. To overcome this limitation, we conducted a phase I clinical study of peptide vaccines designed for cancer patients with six different HLA-A types. Eligible patients were required to have failed prior standard cancer therapies and to be positive for the HLA-A2, -A24 or -A3 (A3, A11, A31 and A33) supertype. Three sets of 8 candidate peptides (24 peptides in total) were provided for vaccination to HLA-A2$^+$, HLA-A24$^+$ and HLA-A3$^+$ patients, respectively. Personalization of the vaccination peptides from the candidate pool was made by considering the patients' HLA types and pre-existing levels of IgGs to the candidate peptides. Seventeen patients were enrolled in this study. The peptide vaccinations were well tolerated in all patients with no vaccine-related severe adverse events. Augmentation of cytotoxic T lymphocyte (CTL) or IgG responses specific to the vaccinated peptides was observed in 11 or 10 out of 13 cases tested, respectively. This new type of vaccine is recommended for phase II clinical trial because of its tolerability and the immune responses to the vaccinated peptides.

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Abbreviations: CTL, cytotoxic T lymphocyte; HLA, human leukocyte antigen; PBMCs, peripheral blood mononuclear cells

Key words: personalized, cancer vaccine, peptide, clinical trial, phase I

Introduction

Immunotherapy is one of the most promising modalities for cancer treatment. Identification of T-cell epitopes of cancer antigens enables the development of peptide-based cancer vaccines. More than 200 T-cell epitopes of cancer antigens have been identified, and clinical trials using these peptides are in progress at various institutions with promising results (1,2).

It is well known that recognition of cancer antigens by T-cell receptors is restricted by class I-human leukocyte antigen (HLA) molecules, and epitope peptides recognized by T-cells differ according to the HLA type of the patients (1-5). This fact, together with the presence of many different types of class I-HLAs, hampers the development of peptide-based cancer vaccines at the industrial level (1,2).

Currently, peptide-based cancer vaccines are mainly developed for HLA-A2$^+$ or HLA-A24$^+$ patients and rarely developed for the other types of class I-HLAs (1,2). The approximate frequencies of the HLA-A2 and -A24 populations are as follows: 40 and 60% in Japanese, 50 and 20% in Caucasians, and 30 and 12% in African blacks, respectively (6). Therefore, the currently developed HLA-A2 and -A24 vaccines only cover 76% [40 + 60% x (100 - 40)/100%] of Japanese, 60% of Caucasians and 38% of African blacks, and development of new vaccines for the other types of class I-HLAs is required. After HLA-A2 or -A24, the most frequent class-I HLA types in Japanese are HLA-A26 and -A11 (occurring in approximately 20% of the population), followed by -A31 and -A33 (~15%) (6). HLA-A11, -A31 and -A33, as well as -A3, make up the A3 supertype, and common binding motifs of antigenic peptides for these HLA types have been identified (7).

In the present study, to overcome HLA-based limitations in the development of peptide vaccines, three sets of 8 candidate peptides (24 peptides in total) were provided for vaccination to HLA-A2$^+$, HLA-A24$^+$ and HLA-A3$^+$ patients, respectively. Personalization of the vaccination peptides from the candidate

peptide pool was conducted by considering the HLA types of the patient and the pre-existing levels of IgGs to the candidate peptides (1,2,8-10), and the safety and immunological effects of the vaccination were analyzed.

Patients and methods

Patients. Between April 2007 and September 2008, 17 patients with different types of HLA-A alleles and different types of cancers who visited the outpatient clinic of Kurume University Hospital were enrolled in this study. The study was approved by the Kurume University Ethics Committee and was registered in UMIN-CTR (UMIN000000619). Eligibility criteria of the patients were as follows. i) Patients were pathologically diagnosed with cancer which was refractory to standard cancer therapies; ii) patients possessed target lesions for evaluation of response; iii) patients were positive for HLA-A2, -A3, -A11, -A24, -A31 or -A33; iv) patients had significant levels of plasma IgGs reactive to at least one of the vaccine candidate peptides; and v) any patients who previously received pre-therapies, including chemotherapy, immunotherapy or radiation therapy, started vaccine therapy more than 4 weeks after the last treatment. In the case of 5-FU and 5-FU-related compounds, the vaccine therapy started after a washout period of more than 2 weeks, and patients did not exhibit any remaining anti-tumor effects or adverse effects of pre-therapy at the time of vaccine therapy; vi) patients had an Eastern Cooperative Oncology Group (ECOG) performance status score of 0-2; vii) patients were expected to survive more than 3 months; viii) patients satisfied the following: WBC $\geq$3,000/mm^3, lymphocytes $\geq$1,000/mm^3, Hb $\geq$9.0 g/dl, platelets $\geq$100,000/mm^3, serum creatinine $\leq$1.4 mg/dl and total bilirubin $\leq$1.5 mg/dl; ix) patients were negative for hepatitis virus B/C; x) patients were more than 20 years and less than 80 years of age; and xi) all of the patients provided written informed consent prior to study entry.

The following patients were excluded from the study: i) patients with severe symptoms (active or severe infectious, circulatory, respiratory or kidney disease, immunodeficiency and disturbance of coagulation); ii) patients with a past history of severe allergic reactions; iii) patients who were pregnant or nursing, or who were currently attempting to become pregnant. (This included patients who had failed to use effective contraception during or for at least 70 days after study participation.) and iv) patients who were judged inappropriate for the clinical trial by doctors.

Clinical protocol. This was an open-label phase I study. The primary endpoint of this study was adverse events (evaluation of safety) and the secondary endpoint was immunological effects of group 3 peptides (see Peptides and vaccine preparation). Plasma levels of IgGs specific to the 24 different vaccine candidate peptides were measured for screening. Based on the results of the HLA typing and the IgG screening, peptides showing higher immune responses (a maximum of 4 peptides) were selected from an appropriate group(s) of candidate peptides for injection as reported previously (8-10). If two groups of candidate peptides were appropriate for a patient, a maximum of 2 peptides were selected from each group. The peptides (3 mg/peptide) were subcutaneously injected with incomplete Freund's adjuvant (IFA) once a week for 6 weeks as reported previously (8-10). We investigated immunological responses to the vaccinated peptides after every six vaccinations. After the 6th vaccination, the vaccinated peptides were re-selected according to the results of immunological tests. Physical examination was performed weekly throughout the entire treatment period. Complete blood counts and serum chemistry tests were performed once every 2 weeks. All the vaccinated patients (n=17) were assessed for toxicity. Thirteen patients whose samples were available both pre- and post- (after the 6th) vaccination were assessed for immunological responses. Toxicity was assessed according to the common toxicity criteria for adverse events (CTCAE) version 3.0.

Peptides and vaccine preparation. Three sets of 8 candidate peptides (24 peptides in total) were provided for vaccination to HLA-A2$^+$, HLA-A24$^+$ and HLA-A3$^+$ supertype patients, respectively. The peptide name, 'mother' protein name, amino acid (a.a.) positions in the protein and a.a. sequences are provided in Table I.

The peptides were prepared under the conditions of Good Manufacturing Practice by the PolyPeptide Laboratories (San Diego, CA, USA) and the American Peptide Company (Vista, CA, USA). The safety and immunological effects of the HLA-A2 and -A24 peptides were confirmed in previously conducted clinical studies (8-10). By contrast, the 8 peptides included in the pool for the HLA-A3 supertype were not previously used in clinical trials, although these peptides were previously shown to induce cytotoxic T lymphocyte (CTL) activity *in vitro* in HLA-A3$^+$ supertype cancer patients (11-13).

The peptides were dissolved in the appropriate solvents and stored at -80˚C. The stock solutions were diluted with saline, sterilized by filtration and mixed with an equal volume of IFA to make the emulsion preparation. The IFA formulation designated as 'NH2' consisted of sorbitan monooleate (NOFABLE SO-991; NOF Corporation, Tokyo, Japan) 11.4 w/w% and mineral oil (Hicall M-72; Kaneda, Tokyo, Japan) 88.6 w/w%. NOFABLE SO-991 and Hicall M-72 are pharmaceutical-grade products, and their regulatory status meets the requirements of the Japanese Pharmaceutical Excipients and the Japanese Pharmacopeia, respectively.

Peptide-specific CTL response. Screening of peptide-specific CTL precursors was conducted using 30 ml of peripheral blood obtained from each patient. Peripheral blood mononuclear cells (PBMCs) were separated by means of Ficoll-Conray density gradient centrifugation. Peptide-specific CTL responses in PBMCs were detected using a previously reported culture method (14). Briefly, PBMCs (1x10^5 cells/well) were incubated with 10 μM of a peptide in 200 μl of culture medium in U-bottom-type 96-well microculture plates (Nunc, Roskilde, Denmark). The culture medium consisted of 45% RPMI-1640 medium, 45% AIM-V medium (Gibco BRL, Walkersville, MA, USA), 10% fetal calf serum, 100 IU/ml of interleukin (IL)-2 and 0.1 μM MEM non-essential amino acid solution (Gibco BRL). Half of the medium was removed and replaced with new medium containing a corresponding peptide (20 μM) every 3 days.

Table I. Vaccine candidate peptides.

HLA	Peptide name	Mother protein	a.a. Position	a.a. Sequence
HLA-A2				
	SART3-302	SART3	302-310	LLQAEAPRL
	CypB-129	Cyclophilin B	129-138	KLKHYGPGWV
	Lck-246	p56 lck	246-254	KLVERLGAA
	Lck-422	p56 lck	422-430	DVWSFGILL
	ppMAPkkk-432	ppMAPkkk	432-440	DLLSHAFFA
	WHSC2-103	WHSC2	103-111	ASLDSDPWV
	UBE2V-43	UBE2V	43-51	RLQEWCSVI
	HNRPL-501	HNRPL	501-510	NVLHFFNAPL
HLA-A24				
	SART2-93	SART2	93-101	DYSARWNEI
	SART3-109	SART3	109-118	VYDYNCHVDL
	Lck-208	p56 lck	208-216	HYTNASDGL
	Lck-488	p56 lck	488-497	DYLRSVLEDF
	MRP3-1293	MRP3	1293-1302	RYLTQETNKV
	PAP-213	PAP	213-221	LYCESVHNF
	PSA-248	PSA	248-257	HYRKWIKDTI
	EGF-R-800	EGF-R	800-809	DYVREHKDNI
HLA-A3 supertype				
	SART3-511	SART3	511-519	WLEYYNLER
	SART3-734	SART3	734-742	QIRPIFSNR
	Lck-90	p56 lck	90-99	ILEQSGEWWK
	Lck-449	p56 lck	449-458	VIQNLERGYR
	PAP-248	PAP	248-257	GIHKQKEKSR
	PSA-16	PSA	16-24	GAAPLILSR
	IEX1-47	IEX-1	47-56	APAGRPSASR
	β-tubulin5-154	β-tubulin5	154-162	KIREEYPDR

After incubation for 14 days, these cells were harvested and tested for their ability to produce interferon (IFN)-γ in response to T2, CIR-A11, CIR-A24, CIR-A31 or CIR-A33 cells (stable transformants of CIR cells with HLA-A1101, -A2402, -A31012 and -A3303, respectively) that were pre-loaded with either a corresponding peptide or a negative control peptide from human immunodeficiency virus (HIV) as reported previously (8-13). The level of IFN-γ was determined by enzyme-linked immunosorbent assay (limit of sensitivity, 10 pg/ml). All assays were carried out in quadruplicate and were analyzed by the Student's t-test. The peptide-specific IFN-γ production (net value) was estimated as the difference between the IFN-γ production in response to target cells with a corresponding peptide and the IFN-γ production in response to target cells with an HIV peptide; differences of $P \leq 0.05$ were considered statistically significant. The IFN-γ production of individual wells in the quadruplicate cultures was considered positive when the net value was >50 pg/ml.

Measurement of peptide-specific IgGs. The peptide-specific IgG levels were measured using a Luminex system (Luminex, Austin, TX, USA) as reported previously (8,10). In brief, plasma was incubated with 25 μl of peptide-coupled color-coded beads for 2 h at room temperature on a plate shaker. After incubation, the mixture was washed with a vacuum manifold apparatus and incubated with 100 μl of biotinylated goat anti-human IgG (γ-chain specific) for 1 h at room temperature. The plate was then washed, followed by the addition of 100 μl of streptavidin-phycoerythrin per well and incubation for an additional 30 min at room temperature on a plate shaker. The bound beads were washed three times, followed by the addition of 100 μl of Tween-20 phosphate-buffered saline into each well. Each sample (50 μl) was then analyzed using the Luminex system.

Results

Patient characteristics. Between April 2007 and September 2008, 17 patients with different types of advanced cancer were enrolled in this study (Table II). There were 15 male and 2 female subjects, with a median age of 70 years (range 53-76). All patients had advanced-stage cancer and were previously treated with and failed to respond to the standard therapy for the particular cancer type, including surgery, chemotherapy, hormone therapy, radiation therapy or a combination of two or more of the above. There were 2 HLA-A2, 8 HLA-A24 and 12 HLA-A3 supertype-positive patients.

Table II. Patient characteristics.

Patient ID	HLA type	Disease	Disease stage	Age/Gender[a]	Previous treatment
D1	A2/A24	Vulvar carcinoma	IVb	76/F	Surgery, chemo
001	A24	Prostate cancer	D2	68/M	Hormone
002	A24/A11	Bladder cancer	IV	63/M	Surgery, chemo
003	A24	Prostate cancer	D1	58/M	Hormone, chemo
004	A24/A33	Penile SCC	III	74/M	Surgery, chemo-radiation
005	A26/A31	Prostate cancer	D1	71/M	Hormone
008	A24	Prostate cancer	D2	75/M	Hormone, chemo
009	A24/A31	Prostate cancer	D2	69/M	Hormone
010	A24/A26	Prostate cancer	C	72/M	Hormone
011	A26/A31	Gallbladder cancer	IV	68/M	Surgery, chemo, tumor-cell vaccine
101	A2/A33	Renal cell carcinoma	IV	54/M	Surgery
301	A11/A33	Prostate cancer	D1	56/M	Surgery, hormone, chemo, radiation
302	A11/A33	Prostate cancer	C	70/M	Hormone, chemo, radiation
303	A11	Prostate cancer	D2	70/M	Hormone
304	A11/A31	Skin SCC	III	74/M	Radiation, chemo
305	A11/A26	Prostate cancer	D2	65/M	Surgery, hormone, chemo, radiation
306	A11/A33	Adorenocortical cancer	IV	53/F	Surgery, chemo

[a]Median age of the patients was 70 years (range 53-76). SCC, squamous cell carcinoma.

Toxicities. The overall toxicities are shown in Table III. The most frequent adverse events were injection site reactions (n=9), tumor site pain (n=7) and limb edema (n=4). All of the adverse events were grade 1 or 2, with the exception of one case of tumor site pain (grade 3). Severe adverse events (grade ≥3) were as follows: increase in serum ALT (n=1, grade 3), increase in serum creatinine (n=2, grade 3), rectal fistula (n=1, grade 3), decrease of hemoglobin (n=2, grade 4; n=2, grade 3), duodenal hemorrhage (n=1, grade 3), hypotension (n=1, grade 4), penile infection (n=1, grade 4), lymphopenia (n=1, grade 3), stricture of the ureter (n=1, grade 4, n=1, grade 3), urticaria (n=1, grade 3) and death (n=1, grade 5). All of the severe adverse events (grade ≥3) observed in this study were evaluated by an independent safety evaluation committee, who concluded that the events were associated with cancer progression.

Peptides and immunological responses. The HLA-A types of the patients (n=17) were HLA-A2 (n=2), -A11 (n=4), -A24 (n=8), -A26 (n=3), -A31 (n=5) and -A33 (n=2). The numbers of peptides vaccinated to the patients were 4 peptides to 8 patients, 3 to 7 patients, and 2 to 2 patients. Post 6th vaccination, blood samples were obtained from 13 of the patients. Since 14 patients received at least six vaccinations, 13 patients were eligible for immunological analysis (Table IV). The median number of vaccinations was 12, with a range of 6-37. Both CTL and IgG responses to the vaccinated peptides were analyzed in the pre- and post- (6th and 12th) vaccination samples (Table IV). The remaining 3 patients withdrew from the trial earlier due to disease progression, terminating treatment after only one, one and four vaccinations, respectively.

When the IgG level of the post-vaccination plasma was more than 1.5-fold higher than that of the pre-vaccination plasma, the increment was considered to be significant. When a significant increment was observed for at least one vaccinated peptide, the specific IgG response was considered to be augmented. Under these criteria, the peptide-specific IgG response was augmented in 8 out of 13 patients after the 6th vaccination and in all 6 patients after the 12th vaccination.

We considered the IFN-γ production of individual wells of the quadruplicate culture to be positive when the net value was >50 pg/ml. If the number of positive wells of the post-vaccination samples increased – or in cases in which the number of positive wells was unchanged between the pre- (before the 1st vaccination) and post-vaccination samples, if the net values of positive wells increased more than 2-fold – then the IFN-γ response was considered to be augmented. Under these criteria, augmentation of CTL activity in response to at least one of the vaccinated peptides after the 6th and 12th vaccination was observed in 11 out of 12 and 2 out of 3 patients tested, respectively. Patient 301 had two different types of HLA-A3 supertypes (HLA-A11 and -A31), and each type-restricted CTL response to the vaccinated peptides was investigated. An augmented CTL response to the Lck-449 peptide was observed in both HLA types, whereas augmentation to the IEX-47 peptide was observed only in an HLA-A33-restricted manner (Table IV). Similarly, patient 306 had two different types of HLA-A3 supertypes (HLA-A11 and -A33), and an augmented CTL response to the SART3-511 and Lck-449 peptides was observed only in an HLA-A33-restricted manner (Table IV). The SART3-109 peptide, which has the ability to induce CTL activity in PBMCs of both HLA-A24 and -A3 supertypes, but not in HLA-A2$^+$

Table III. Adverse events.

Adverse events	No. of patients presenting with each event					Total	Frequency (%)
	G1	G2	G3	G4	G5		
						0	0
Injection site reaction	3	6				9	52.9
ALT, SGPT	1		1			2	11.8
AST, SGOT	2					2	11.8
Creatinine			2			2	11.8
Death not associated with CTCAE – disease progression NOS					1	1	5.9
Dermatology/skin-other, herpes zoster		1				1	5.9
Diarrhea	1					1	5.9
Edema: limb		4				4	23.5
Fatigue	2	1				3	17.6
Fever	1	2				3	17.6
Fistula, GI-rectum			1			1	5.9
Hemoglobin			2	2		4	23.5
Hemorrhage, GI-duodenum, stomach			1			1	5.9
Hypotension				1		1	5.9
Infection-other, penis				1		1	5.9
Lymphopenia			1			1	5.9
Pain-tumor pain	1	5	1			7	41.2
Potassium, serum-high		1				1	5.9
Somnolence		1				1	5.9
Stricture/stenosis, GU-ureter			1	1		2	11.8
Urticaria			1			1	5.9
Vomiting	2	2				4	23.5

patients, was vaccinated to patient 009 (HLA-A24 and -A31). Therefore, CTL activity to the SART3-109 peptide in both an HLA-A24- and -A31-restricted manner was investigated. As a result, an augmented CTL response was observed in both the HLA types, with the HLA-A24-restricted induction being much stronger (Table IV).

Discussion

HLA restriction is the main feature of T-cell-mediated recognition of antigenic peptides on the binding groove of HLA molecules (15). Antigen epitope peptides recognized by T-cells are different in each HLA type (4,5). Therefore, it is difficult to develop peptide-based cancer vaccines applicable for patients with rare types of HLAs at the industrial level.

To overcome the hurdle of HLA restriction, protein vaccines are sometime developed. Protein vaccines usually contain multi-epitope peptides, recognized by both CTLs and helper T-cells, which may cover several different HLA types. However, whole protein vaccines may also contain allergic epitopes, since some of the target molecules for cancer vaccines have been identified as allergens for atopic dermatitis (16). In addition, the protein vaccines have the following disadvantages when compared to peptide vaccines. i) Protein vaccines are biologics rather than chemicals and therefore the industrial cost of chemistry, manufacturing and control is much higher for protein vaccines than for peptide vaccines, and it is difficult to prepare many different types of proteins as candidates for personalized vaccines. ii) The relative molar contents of the epitope peptides in the protein vaccine preparations are generally lower by a factor of several tens compared to that of peptide vaccines. For these reasons, we sought to develop a peptide-based personalized vaccine applicable for virtually all patients with different types of HLAs. Our recent study demonstrated that several vaccine candidate peptides which were originally identified in HLA-A24$^+$ patients, were recognized by CTLs of different types in an HLA-restricted manner (18). Furthermore, HLA-A11, -A31 and -A33, as well as -A3, make up the A3 supertype, and common binding motifs of antigenic peptides for these HLA types have been identified (7). Approximately 95% of Japanese possess at least one of the HLA-A2, -A24 and -A3 supertypes. Our cancer vaccine candidate peptides were derived from commonly expressed antigens in different tissue types and origins of cancer cells (1,2). Therefore, the sets of 8 peptides used for each of the HLA-A2, -A24 and -A3 supertypes in this study could be applicable for the majority of Japanese cancer patients irrespective of their HLA type. These vaccine candidate

Table IV. Peptide-specific IgG responses induced by the peptide vaccination.

Patient ID (HLA type)	Total no. of vaccinations	Vaccinated peptides	Peptide-specific IgG (FIU)		
			Before 1st	Post 6th	Post 12th
001 (A24/A24)	28	SART3-109	5,043	**19,505**	**19,164**
		MRP3-1293	116	**193**	**2,822**
		PAP-213	46	**5,154**	**10,168**
		PSA-248	8,288	**21,420**	**24,132**
002 (A11/A24)	16	SART3-511	71	**333**	**417**
		SART3-734	11	<10	<10
		SART2-93	10	**20**	**154**
		SART3-109	531	450	359
003 (A24/A24)	9	SART2-93	22	22	ND
		SART3-109	945	**7,675**	ND
		lck-488	18	17	ND
		MRP3-1293	16	16	ND
008 (A24/A24)	29	SART3-109	79	**2,826**	**38,887**
		PAP-213	179	**428**	**2,322**
009 (A24/A31)	25	SART3-109	544	527	**18,131**
		SART3-734	242	241	**1,636**
		PAP-248	34	34	38
010 (A24/A26)	8	SART3-109	44	45	ND
		lck-488	98	96	ND
		MRP3-1293	82	84	ND
		PAP-213	69	68	ND
011 (A26/A31)	11	SART3-109	262	242	ND
		SART3-734	373	**627**	ND
		lck-449	204	194	ND
101 (A2/A33)	18	CypB-129	220	105	**22,217**
		SART3-511	802	**1,244**	**2,467**
		SART3-734	54	25	30
301 (A11/A31)	6	SART3-511	260	319	ND
		lck-449	185	239	ND
		IEX1-47	21	<10	ND
302 (A11/A31)	6	SART3-511	10	ND	ND
		Lck-449	290	ND	ND
		IEX1-47	15	ND	ND
303 (A11/A11)	6	SART3-511	347	**1,219**	ND
		lck-449	201	**587**	ND
		β-tubulin5-154	93	86	ND
304 (A11/A31)	6	SART3-734	346	267	ND
		lck-449	312	251	ND
		PAP-248	27	25	ND
305 (A11/A26)	37	SART3-511	231	186	**744**
		SART3-734	68	58	**243**
		lck-90	73	53	**21,307**
		lck-449	143	124	**32,987**
306 (A11/A33)	12	SART3-511	107	52	ND
		SART3-734	38	<10	ND
		lck-90	48	40	ND
		lck-449	85	**136**	ND

Values in bold print indicate significant augmentation (>1.5-fold higher than that of the pre-vaccination plasma) of IgG response. FIU, fluoresence intensity unit; ND, not determined.

Table V. Peptide-specific CTL responses induced by the peptide vaccination.

Patient ID (HLA)	Vaccinated peptides (for HLA-A type)	HLA-restriction	Peptide-specific IFNγ production (ng/ml)[a]		
			Before 1st	Post 6th	Post 12th
001 (A24/A24)	SART3-109 (A24)	A24	- (0)	182, 273 (2)	ND
	MRP3-1293 (A24)	A24	- (0)	96,267,643,60 (4)	ND
	PAP-213 (A24)	A24	- (0)	- (0)	ND
	PSA-248 (A24)	A24	- (0)	1401 (1)	ND
002 (A11/A24)	SART3-511 (A11)	A11	- (0)	- (0)	ND
	SART3-734 (A11)	A11	- (0)	- (0)	ND
	SART2-93 (A11)	A11	- (0)	365 (1)	ND
	SART3-109 (A11/A24)	A11	- (0)	- (0)	ND
	SART3-109 (A11/A24)	A24	ND	ND	ND
003 (A24/A24)	SART2-93 (A24)	A24	- (0)	75, 58 (2)	ND
	SART3-109 (A24)	A24	- (0)	- (0)	ND
	lck-488 (A24)	A24	- (0)	- (0)	ND
	MRP3-1293 (A24)	A24	- (0)	- (0)	ND
008 (A24/A24)	SART3-109 (A24)	A24	- (0)	281,62 (2)	ND
	PAP-213 (A24)	A24	- (0)	- (0)	ND
009 (A24/A31)	SART3-109 (A24/A31)	A24	- (0)	1564, 889, 171 (3)	1261, 1511, 141 (3)
	SART3-109 (A24/A31)	A31	- (0)	84 (1)	46 (1)
	SART3-734 (A31)	A31	- (0)	- (0)	75 (1)
	PAP-248 (A31)	A31	- (0)	52 (1)	108 (1)
010 (A24/A26)	SART3-109 (A24)	A24	- (0)	- (0)	ND
	lck-488 (A24)	A24	- (0)	- (0)	ND
	MRP3-1293 (A24)	A24	- (0)	- (0)	ND
	PAP-213 (A24)	A24	- (0)	1346 (1)	ND
011 (A26/A31)	SART3-109 (A31)	A31	- (0)	- (0)	ND
	SART3-734 (A31)	A31	- (0)	177 (1)	ND
	lck-449 (A31)	A31	- (0)	- (0)	ND
101 (A2/A33)	CypB-129 (A2)	A2	- (0)	- (0)	- (0)
	SART3-511 (A33)	A33	- (0)	101, 41 (2)	107, 99 (2)
	SART3-734 (A33)	A33	- (0)	- (0)	- (0)
301 (A11/A31)	SART3-511 (A11/A31)	A11	- (0)	- (0)	ND
	SART3-511 (A11/A31)	A31	- (0)	- (0)	ND
	lck-449 (A1/A31)	A11	- (0)	136, 157 (2)	ND
	lck-449 (A1/A31)	A31	- (0)	213 (1)	ND
	IEX1-47 (A11/A31)	A11	- (0)	- (0)	ND
	IEX1-47 (A11/A31)	A31	- (0)	60 (1)	ND
302 (A11/A31)	SART3-511 (A11/A31)	A11	ND	ND	ND
	SART3-511 (A11/A31)	A31	ND	ND	ND
	Lck-449 (A11/A31)	A11	ND	ND	ND
	Lck-449 (A11/A31)	A31	ND	ND	ND
	IEX1-47 (A11/A31)	A11	ND	ND	ND
	IEX1-47 (A11/A31)	A31	ND	ND	ND
303 (A11/A11)	SART3-511 (A11)	A11	- (0)	- (0)	ND
	lck-449 (A11)	A11	- (0)	1165, 1557, 719 (3)	ND
	β-tubulin5-154 (A11)	A11	- (0)	- (0)	ND
304 (A11/A31)	SART3-734 (A11/A31)	A11	- (0)	- (0)	ND
	SART3-734 (A11/A31)	A31	ND	ND	ND
	lck-449 (A11/A31)	A11	- (0)	- (0)	ND
	lck-449 (A11/A31)	A31	ND	ND	ND
	PAP-248 (A11/A31)	A11	- (0)	- (0)	ND
	PAP-248 (A11/A31)	A31	ND	ND	ND

Table V. Continued.

Patient ID (HLA)	Vaccinated peptides (for HLA-A type)	HLA restriction	Peptide-specific IFNγ production (ng/ml)[a]		
			Before 1st	Post 6th	Post 12th
305 (A11/A26)	SART3-511 (A11)	A11	- (0)	ND	- (0)
	SART3-734 (A11)	A11	- (0)	ND	- (0)
	lck-90 (A11)	A11	- (0)	ND	- (0)
	lck-449 (A11)	A11	- (0)	ND	- (0)
306 (A11/A33)	SART3-511 (A11/A33)	A11	- (0)	- (0)	ND
	SART3-511 (A11/A33)	A31	- (0)	- (0)	ND
	SART3-734 (A11/A33)	A11	- (0)	- (0)	ND
	SART3-734 (A11/A33)	A31	- (0)	- (0)	ND
	lck-90 (A11/A33)	A11	- (0)	- (0)	ND
	lck-90 (A11/A33)	A31	- (0)	- (0)	ND
	lck-449 (A11/A33)	A11	- (0)	- (0)	ND
	lck-449 (A11/A33)	A31	- (0)	517 (1)	ND

[a]Values of IFNγ production (pg/ml) in the positive wells are indicated. The number of positive wells in the quadruplicate cultures is also shown in parenthesis. ND, not determined.

peptides could be applicable for 95-99% of Asians, 80-83% of Caucasians, 80-84% of Spanish, 81-88% of Indians and 52-66% of Blacks. Therefore, these sets of peptide vaccines may be applicable for large numbers of cancer patients with different HLA-A types worldwide.

Augmentation of CTL activity to at least one of the vaccinated peptides was observed in 11 out of 12 patients after the 6th vaccination and in 2 out of 3 cases after the 12th vaccination. The frequency of CTL augmentation was higher than in any of our previously conducted clinical trials of personalized peptide vaccines for advanced cancer patients (8-10). This may have been at least partly due to the IFA used in the trials. In this trial we used a new formulation of IFA which we designated 'NH2', whereas previously conducted trials used the commercially available ISA51 made by Seppic (Paris, France). Indeed, we found that 'NH2' was more effective than ISA51 with regard to CTL induction specific to peptides in murine models (Iseki *et al*, unpublished data).

We previously reported that the SART3-109 peptide induces *in vitro* CTL activity restricted, not only to HLA-A24, but also to the HLA-A3 supertype, but not CTL activity restricted to HLA-A2 molecules (18). We also reported that all 8 peptides used for the HLA-A3 supertype induce *in vitro* CTL activity restricted to HLA-A11, -A31 and -A33 molecules (11-13). Subsequently, we investigated whether these results could be replicated *in vivo* in 3 patients (patients 009, 301 and 306) whose PBMCs were available for study. Indeed, the results showed that the SART3-109 peptide induced CTL activity restricted to both HLA-A24 and -A31 molecules, while the Lck-449 peptide induced CTL activity restricted to both HLA-A11 and -A31 molecules, in agreement with the results from the *in vitro* assays. To our knowledge, this is the first study to show CTL induction restricted to two different HLA molecules in clinical trials. Similarly, this could be the first report of peptide vaccination to cancer patients with the HLA-A3 supertype.

All of the subjects enrolled in this study had failed to respond to various types of standard therapies, and the majority of the patients could not continue the 2nd cycle of vaccination after completion of the 1st cycle. In addition, this was a phase I study designed to investigate the safety of and immune responses to a new type of personalized peptide vaccination applicable for six different HLA-A types. Therefore, the clinical effects of the vaccine were not evaluated in this study.

In conclusion, this phase I clinical study of a personalized peptide vaccine for HLA-A2, -A24 and -A3 supertype (A3, A11, A31 and A33)-positive cancer patients confirmed the safety and immunological effects of the vaccination. These results suggest that this new type of vaccine is applicable for the majority of cancer patients in Japan and other countries.

Acknowledgements

This study was supported in part by a grant from the 'High-Tech Research Center' Project for Private Universities, by matching fund subsidies from the Ministry of Education, Culture, Sports, Science and Technology (MEXT) of Japan, and by Grants-in-Aid from MEXT.

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