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Dexamethasone Did Not Suppress Immune Boosting by Personalized Peptide Vaccination for Advanced Prostate Cancer Patients

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BACKGROUND. To evaluate the immunological responses of personalized peptide vaccination combined with low-dose glucocorticoids for advanced hormone refractory prostate cancer (HRPC) patients (pts).

METHODS. Eleven pts with advanced HRPC were treated with the vaccination and low-dose glucocorticoids; 6 pts with 10 mg/day of prednisolone (PDL) followed by 1 mg/day of dexamethasone at the time of progression, 1 pt with PDL, and 4 pts with dexamethasone. Peptide-specific cellular and humoral responses were employed to monitor pre- and post- (6th) vaccination samples.

RESULTS. The vaccination combined with glucocorticoids was well tolerated with no severe adverse effects. Increments of IgG responses were observed in 1 of 4 or 8 of 10 pts tested who received PDL or dexamethasone, respectively, increment of cytotoxic T lymphocyte activity was observed in 2 of 4 or 5 of 7 pts tested, respectively. Vaccination with PDL or dexamethasone resulted in a decline of PSA (at least 50%) in 1 of 7 or 6 of 10 pts with significantly longer median TTP in the dexamethasone group, respectively.

CONCLUSION. Vaccination combined with dexamethasone could be recommended for further clinical trials from both immunological and clinical points of view. *Prostate 68: 1753--1762, 2008.* © 2008 Wiley-Liss, Inc.

KEY WORDS: **prostate cancer; immunotherapy; peptide vaccine; glucocorticoids**

INTRODUCTION

Docetaxel-based therapy improved the overall survival of patients with hormone refractory prostate cancer (HRPC) to some extent [1,2]. There is no treatment modality for HRPC patients who have failed with docetaxel-based therapy, and the development of new therapeutic modalities is required, one of which could be specific immunotherapy since many antigens and their peptides recognized by host cytotoxic T lymphocytes (CTLs) were identified in the past decade

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[3–5]. We previously reported that a personalized peptide vaccination combined with a low dose (280 mg/day) of estramustine phosphate (EMP) could be a possible new treatment modality for patients with metastatic HRPC [6–8]. This approach, however, had a limitation, such as adverse events with EMP and disease progression, and thus we need another option instead of EMP. Glucocorticoids have anti-tumor activity in patients with HRPC, although they are potent activity to suppress immune responses [9,10]. Prednisone is now widely used in conjunction with docetaxel for the treatment of advanced prostate cancer when the large scale of randomized clinical trials showed the positive results [1,2], whereas a single use of a low dose of dexamethasone was also reported to be clinically effective for HRPC in a few manuscripts [11,12]. Subsequently, we investigated in this study whether a low dose of glucocorticoids, at first predonisolone followed by dexamethasone, suppressed immune boosting raised by personalized peptide vaccination. Clinical efficacy was also investigated.

PATIENTS AND METHODS

Patients

Patients with HLA-A24$^+$ or HLA-A2$^+$ and advanced HRPC who failed with combination therapy of peptide vaccine and EMP or peptide vaccine therapy were enrolled. Written informed consent was obtained from all patients at the time of enrolment. This study was approved by the Kurume University School of Medicine Ethics Committee.

Peptide Selection

All peptides have the ability to induce HLA-A24 or A2-restricted and tumor-specific CTL activity in peripheral blood mononuclear cells (PBMCs) of cancer patients [6–8,13–20]. These peptides were produced under Good Manufacturing Practice by the Multiple Peptide System (San Diego, CA). The peptide vaccination schedule was administrated according to a previous report [8]. Briefly, 30 ml of peripheral blood was obtained from patients receiving combination therapy, and then PBMCs and plasma were isolated by Ficoll–Conray density gradient centrifugation. Both anti-peptide IgGs in plasma and peptide-specific CTLs precursors in PBMCs were measured as described previously [21]. We reported that IgG specific to each of those peptides can be frequently detected in pre- and post-vaccination sera, and the level of anti-peptide IgGs is a laboratory marker to predict clinical responses to individualized peptide vaccination with a good relation to overall survival [21]. Therefore, peptides were chosen based on the evaluation of both anti-peptide IgGs levels in plasma and CTL precursors in PBMCs: for the first selection, a peptide with a high titer of anti-peptide IgGs and high level of responses in CTL precursors; for the second selection, a peptide with high titer of anti-peptide IgGs; for the third selection, a peptide with a high level of responses in CTL precursors; for no selection, a peptide with a low titer of anti-peptide IgGs and a low level (less than C rank) of responses in CTL precursors. Finally, four peptides were selected for each HLA type.

Vaccination and CombinationTherapy

A 2-ml volume of peptide, which was supplied in vials containing 2 or 4 mg/ml sterile solution, was mixed with an equal volume of incomplete Freunds adjuvant (Montanide ISA-51VG; Seppic, Paris, France) and emulsified in a 5-ml sterilized syringe, and 1.5 ml of each peptide emulsion (maximum of four peptides per vaccination) was injected subcutaneously into the thigh or armpit area every 2–4 weeks. The glucocorticoids were orally taken with 10 mg/day of predonisolone (PDL) or 1 mg/day of dexamethasone.

Immunological and Clinical Monitoring

For the evaluation of immune responses during the combination of peptide vaccine and glucocorticoids, peptide-specific CTLs precursors in PBMCs and plasma levels of peptide-specific IgGs were measured after every 6–7th vaccination.

Clinical monitoring was carried out according to previous reports [6–8]. In brief, patients were observed until disease death or intolerance, or consent was withdrawn. Clinical and laboratory assessments were performed at each visit, and patients were asked about adverse events, their severity and frequency. The severity of adverse events was scored according to the Common Terminology Criteria for Adverse Events (CTCAE, Version 3, 2003). Serum PSA was measured every 3–4 weeks during treatment. Bone scans and CT scans of the abdomen were performed every 6 months. The metastatic findings of bone scans were assessed by the extent of disease using the percentage of the positive area on the bone scan (% PABS) [22]. Clinical response was evaluated according to both changes in PSA levels and imaging studies in patients with measurable disease. PSA response was defined from two consecutive measurements at least 2 weeks apart that showed a 50% or greater decrease from baseline PSA levels (PR: partial response) or normalization of the PSA level (CR: complete response), and we defined that >25% increase of the PSA level form baseline was progressive disease

(PD). Time to PSA progression was registered at the time of the first of two consecutive PSA levels 25% above the baseline. Standard definitions were used for the response and progression of measurable and evaluable disease.

Evaluation of Peptide-Specific CTL Precursors

PBMCs (1×10^5 cells/well) were incubated with 10 μg/ml of each peptide in quadruplicate in a U-bottom-type 96-well microculture plate (Nunc, Roskilde, Denmark) containing 200 μl of culture medium. The culture medium consisted of 45% RPMI 1640, 45% AIM-medium (Gibco BRL, Gaithersburg, MD), 10% FCS, 100 U/ml of interleukin-2 (IL-2), and 0.1 mM MEM nonessential amino acid solution (Gibco BRL). Every 3 or 4 days, half of the culture medium was removed and replaced with new medium containing the corresponding peptide (20 μg/ml) and 100 U/ml IL-2. After 14 days, the cultured cells were separated into four wells. One set was used for culture with the corresponding peptide-pulsed T2 (HLA-A2) or the corresponding C1R-A24 (HLA-A24) cells, and the other set was used for the culture with HIV peptide-pulsed T2 (HLA-A2) or the corresponding C1R-A24 (HLA-A24) cells. After incubation for 18 hr, the supernatant was collected, and interferon (IFN)-γ production was determined using an enzyme-linked immunosorbent assay (ELISA). The successful induction of peptide-specific CTLs was judged to be positive when a value of $P < 0.05$ was obtained by a two-tailed Students *t*-test.

Evaluation of Anti-Peptide-Specific IgGs

The level of anti-peptide-specific IgGs from patients was measured using the Luminex™ method, as reported previously [23]. In brief, 100 μl of diluted plasma was incubated with 5 μl of color-coded beads (Luminex Corp., Austin, TX) conjugated with each kind of peptide in 96-well filter plates (MABVN1250; Millipore Corp., Bedford, MA) for 2 hr at room temperature on a plate shaker. The plates were then washed with Tween-PBS and incubated with 100 μl of biotin-conjugated goat anti-human IgG (BA-3080: Vector Laboratories, Burlingame, CA) for 1 hr at room temperature on a plate shaker. After the plates were washed, 100 μl of streptavidin-PE was added per well, and the samples were incubated for 30 min at room temperature on a plate shaker. The bound beads were washed four times, and 100 μl of Tween-PBS was added to each well, 50 μl of sample was examined using the Luminex™ system. Anti-peptide IgGs pre-dexamethasone administration were considered as the baseline and it was judged as suppression or increase if the level of anti-peptide IgGs after the first immunological monitoring from combination with glucocorticoids was <50% or >150% than that before the baseline level.

Cytotoxicity Assay

PBMCs were stimulated with peptide followed by testing their cytotoxicity against the prostate cancer cell line by a standard 6-hr ^{51}Cr-release assay as reported previously [6,7]. Prostate cancer cell lines used for the

TABLE I. Patient Characteristics

Patient no.	Age (years)	HLA type	Performance status[a]	Site of metastasis	Gleason score	Baseline PSA (ng/ml) Before PDL[b]	Baseline PSA (ng/ml) Before dexamethasone	Previous treatment[c]
1	72	A24/A26	0	Bone, nodal	4 + 4	1,577	2,619	LHRH, A, EMP, Vaccine + low EMP
2	71	A2/A24	0	Bone	4 + 4	1,054	1,529	LHRH, A, EMP, Vaccine + low EMP
3	79	A24/A24	0	Bone	4 + 4	327	—	LHRH, A, Vaccine + low EMP
4	71	A24/A33	0	Bone, nodal	4 + 3	6.8	19.3	LHRH, A, EMP, Vaccine + low EMP
5	79	A24/A24	0	Bone, nodal	4 + 3	235	260	LHRH, A, EMP, Vaccine + low EMP
6	69	A24/A3	1	Bone	4 + 3	—	436	LHRH, A, EMP + Docetaxel, Vaccine + low EMP
7	74	A24/A24	1	Bone, nodal	4 + 3	41	27	LHRH, A, EMP, Vaccine + low EMP
8	73	A2/A24	0	Bone	4 + 4	594	356	LHRH, A, EMP, Vaccine + low EMP
9	69	A24/A31	1	Bone, nodal	4 + 3	—	639	LHRH, A, EMP, Vaccine + low EMP
10	75	A24/A24	0	Bone	4 + 3	—	1,240	LHRH, A, EMP, Vaccine + low EMP
11	68	A24/A24	0	Bone	4 + 5	—	264	LHRH, A, EMP, Vaccine

[a]Performance status by EOCG score.
[b]PDL: predonisolone.
[c]LHRH: luteinizing hormone-releasing hormone therapy; A: anti-androgen; EMP: estramustine phosphate; Vaccine: personalized peptide vaccination.

study were PC93 (wild-type), PC93-A2 (HLA-A2$^+$), and PC93-A24 (HLA-A24$^+$) cells. Phytohemagglutinin (PHA)-activated T cells were used as a negative control of target cells. Two thousand ^{51}Cr-labeled cells per well were cultured with effecter cells in 96-round-well plates at three different effector to target cell ratios in a triplicate assay.

RESULTS

Patient Characteristics

From November 2005 to July 2007, 11 pts with advanced HRPC were treated with the vaccination and low-dose glucocorticoids; 6 pts with 10 mg/day of prednisolone (PDL) followed by 1 mg/day of dexamethasone at the time of progression, 1 pt with PDL, and 4 pts with dexamethasone. PDL was initially used for 7 pts primarily because it is now widely used in conjunction with docetaxel for HRPC patients [1,2]. We then used dexamethasone since elevated PSA levels were soon observed in most of them during the vaccination with PDL.

The median age of the patients was 72 (range, 68–79) years old and the median Eastern Cooperative Oncology Group performance status was 0 (range, 0–1). The median baseline PSA level of patients was 446.85 (range, 6.75–157) ng/ml. All patients had been treated by maximum androgen blockade therapy using LHRH analogue and anti-androgen, followed by the administration of a full dose of EMP before the peptide vaccination. One patient was administrated with chemotherapy using docetaxel. The patient characteristics are shown in Table I.

Adverse Events

All patients were evaluated for all common toxicities, and the overall toxicities are shown in Table II. All

TABLE II. Adverse Events

Toxicity	G1	G2	G3	G4	Total
Dermatologic	11				11/11
Bone pain	3	2			5/11
Edema					
Fatigue	3	1			4/11
Cushingoid					
Moon face		2			2/11
Centripetal obesity					
Cutaneous strias					
Buffalo hump					

Toxicities based on the National Cancer Institute common toxicity scale (version 3). Some patients had more than one toxic reaction.

TABLE III. Immune Response During Therapy

		Dexamethasone						Prednisolone			
		Anti-peptide IgG[a]			Cellular response to peptide[b]			Anti-peptide IgG[a]		Cellular response to peptide[b]	
Patient no.	Peptide	Pre	6th	12th	Pre	6th	12th	Peptide	Pre	6th	Pre	6th
1	Lck-208	11,306	8,139		2,181	1,662		Lck-208	21,611	16,676	1,973	800
	SART2-161	765	246		2,646	6,333		SART2-161	3,168	863	890	778
	SART3-109	13,688	13,896		790	32		SART3-109	24,527	25,725	0	0
	PSMA-624	16,084	10,395		1,533	1,409		PSMA-624	24,028	22,661	362	241
2	Lck-246	19,553	18,646		739	734		Lck-246	21,899	21,981	97	73
	SART3-309	20,748	18,662		0	383		SART3-309	8,904	12,417	1,079	54
	UBE-85	24,655	24,924		947	385		UBE-85	24,988	25,431	396	16
	EZH2-569	25,085	24,862		930	991		EZH2-569	1,014	2,950	219	347
3								SART3-109	29,196	27,733	0	**379**
								PAP-213	195,550	21,254	187	0
								PSA-248	15.35	22,001	57	0
								EZH2-291	NA	NA	505	993

4	PAP-213	19,825	19,571	19,234	1,206	67	110	SART3-109	28,455	27,166	0	**210**
	SART2-899	1	**1,208**	**3,762**	757	585	NA	PAP213	19,918	21,185	0	**325**
	lck486	12,094	17,186	15,824	0	0	NA	PSA-248	24,594	21,434	0	**123**
	EZH-2 291	6,090	**25,569**	**25,365**	0	**167**	NA	EZH2-291	607	**1,807**	81	0
5	SART3-109	29,268	26,154	28,457	153	0	NA					
	PAP-213	19,250	16,013	17,417	0	0	**83**					
	PSA-248	9,017	**14,421**	**19,775**	0	0	0					
	EZH-2 291	25,246	25,450	25,333	147	0	NA					
6	SART2-93	2,682	**23,970**	**21,486**	105	**313**	**7,386**					
	SART3-109	2,303	476	243	34	0	NA					
	MRP3-1293	432	298	205	0	**899**	NA					
	PSA-248	15,583	8,826	11,146	0	**74**	0					
7	SART3-109	514	**1,093**	**11,958**	1,032	254	1,630					
	PSA-248	16	**19,819**	**24,092**	0	0	0					
	Lck-488	3	**236**	**5,835**	0	0	0					
	lck486	1	**5,488**	**5,004**	0	0	**3,225**					
8	CypB-129	142	**253**	151	0	**175**	**11,298**					
	MAP-432	28	18	17	0	0	NA					
	UBE-43	25,448	24,128	23,021	891	0	748					
	HNR-501	312	51	34	48	0	**3,092**					
9	SART3-109	22,070	9,324									
	Lck-486	17,230	25,349									
	Lck-488	25	**154**									
	MRP3-1293	8,865	**25,450**									
10	SART3-109	6,323	**27,408**									
	Lck-488	2	55									
	PAP-213	270	**17,523**									
	PSA-248	9	**405**									
11	SART3-109	26,475	25,475									
	MRP3-1293	251	**1,673**									
	PAP-213	8,123	**20,923**									
	PSA-248	25,115	23,813									

NA, not available.

[a]The anti-peptide IgG response to peptide was performed by using cytometry assay. Above values indicate IgG titer pre- and duration of glucocorticoid administration.

[b]The CTL precursor response to peptide was performed by quadruplicate assay. Means of specific IFN-γ production (pg/ml) pre- and duration-vaccinated PBMCs were calculated by subtracting the response to the HIV-derived irrelent peptide, and compared by using Students *t*-test.Increased IgG titer (>150%) was shown in bold. Decreased of IgG titer (<50%) was shown as under lined.Increased IFN-γ (>200%) was shown in bold. Decreased of IFN-γ (<50%) was shown as underlined.

patients showed grade 1 dermatological reactions at the injection site during vaccination therapy. During the combination therapy of peptide vaccination with low-dose glucocorticoids, 5 pts developed grade 1 or 2 bone pain at the metastatic site, 4 pts complained of fatigue, and 2 pts demonstrated Grade 2 moon face, however, no serious adverse effects were observed during combination therapy.

Humoral Immune Responses

We first evaluated the humoral immune response of patients samples before and after the vaccination and PDL. Increment of peptide-specific IgG responses in the post (6th)-vaccination plasma (>150% increase of FTI as compared to that of pre-vaccination plasma at least in one peptide) was observed in 1 of the 4 pts who received the vaccination combined with 10 mg/day of PDL (Table III). Decrease of peptide-specific IgG responses in the post (6th)-vaccination plasma (>50% decrease of FTI as compared to that of pre-vaccination plasma at least in one peptide) was observed in 1 of the 4 pts tested. Post-vaccination samples were not available in the remaining 3 pts with PDL because of disease progression prior to the 6th vaccination. In contrast, the increment in post (6th)-vaccination plasma was observed in 8 of 10 pts who received the vaccination combined with 1 mg/day of dexamethasone (Table III). The increment was also observed post (12th)-vaccination from 4 of 5 pts tested. The decrease post (6th)-vaccination was observed in 4 of 10 pts tested, while that in the post (12th)-vaccination in 2 of 5 pts. The IgG level in post (6th)-vaccination/dexamethasone samples was significantly ($P = 0.046$) higher than that in pre-vaccination samples. These results suggested that peptide vaccination with dexamethasone had a positive immune response in the production of peptide-specific IgGs.

Kinetic studies of peptide-specific IgG and PSA levels in each of the 11 pts are shown in Figure 1. Inverse correlation, increase of peptide-specific IgGs and decrease of PSA levels, was observed in 3 pts (Pts. 7, 10, 11) among 6 pts who achieved >75% PSA decline during vaccination and dexamethasone administration. IgG levels were stable in the remaining 3 pts (Pts 4–6). Elevation of both IgG and PSA levels was observed in Pt 9, while IgG levels were stable in the remaining 2 pts (Pts 1 and 2) whose PSA levels were elevated during vaccination and dexamethasone administration.

Cellular Immune Responses

We next evaluated the cellular immune response of patients samples before and after the vaccination and glucocorticoids. IFN-γ production after glucocorticoid administration was compared to the baseline level of each patient (Table III). Increment of peptide-specific CTL activity post (6th)-PBMCs (>2-fold increase of IFN-γ production as compared to that of pre-vaccination PBMCs at least in one peptide) was observed in 2 of the 4 pts tested who received the vaccination combined with PDL. Decreased CTL activity in the post (6th)-PBMCs (>50% decrease of IFN-γ production as compared to pre-vaccination PBMCs at least in one peptide) was observed in all 4 pts tested. The increment in post (6th)-vaccination PBMCs was observed in 5 of 7 pts who received the vaccination combined with 1 mg/day of dexamethasone. The increment was also observed post (12th)-vaccination in 4 of 5 pts tested. The decrease post (6th)-vaccination was observed in 6 of 7 pts tested, while that post (12th)-vaccination was seen in 1 of 5 pts.

These results suggested that dexamethasone did not suppress the immune boosting raised by the vaccination, but PDL at least did not sustain it. Subsequently, CTL activity against prostate cancer cells by means of a 6-hr Cr release was conducted in the pre- and post (6th)-vaccination PBMCs from pts with the vaccination and dexamethasone administration (Fig. 2). Significant levels of CTL activity toward HLA-A matched prostate cancer cells in pre-vaccination PBMCs was observed in 4 of 6 pts tested, whereas those in post (6th)-vaccination was observed in all 6 pts. None of the PBMCs showed cytotoxicicity against normal cells (PHA blasts).

Clinical Response by CombinationTherapy

The clinical response, changes of PSA during the treatment, and TTP are summarized in Table IV. Peptide vaccination with PDL or dexamethasone resulted in a decline of PSA (at least 50%) in 1 of 7 or 6 of 10 pts, respectively. While bone scans in 6 of 7 pts during PDL showed new metastatic lesions, those in only 2 of 10 pts during dexamethasone showed new metastatic lesions. The remaining 7 pts with dexamethasone showed NC findings, and 1 pt with a 90% decline of PSA showed an improvement of pelvic bone metastasis on a bone scan with a decrease of % PABS (4.74–1.68%) after peptide vaccination with a low-dose of dexamethasone. However, there was no measurable response during the combination therapy with glucocorticoids. The median TTP in the vaccination combined with dexamethasone was significantly longer than that in the PDL (PDL vs. dexamethasone: 42 days vs. 115 days).

DISCUSSION

The most common toxicities in the peptide vaccine were dermatological reactions at the injection site as reported previously [6–8]. The similar results were

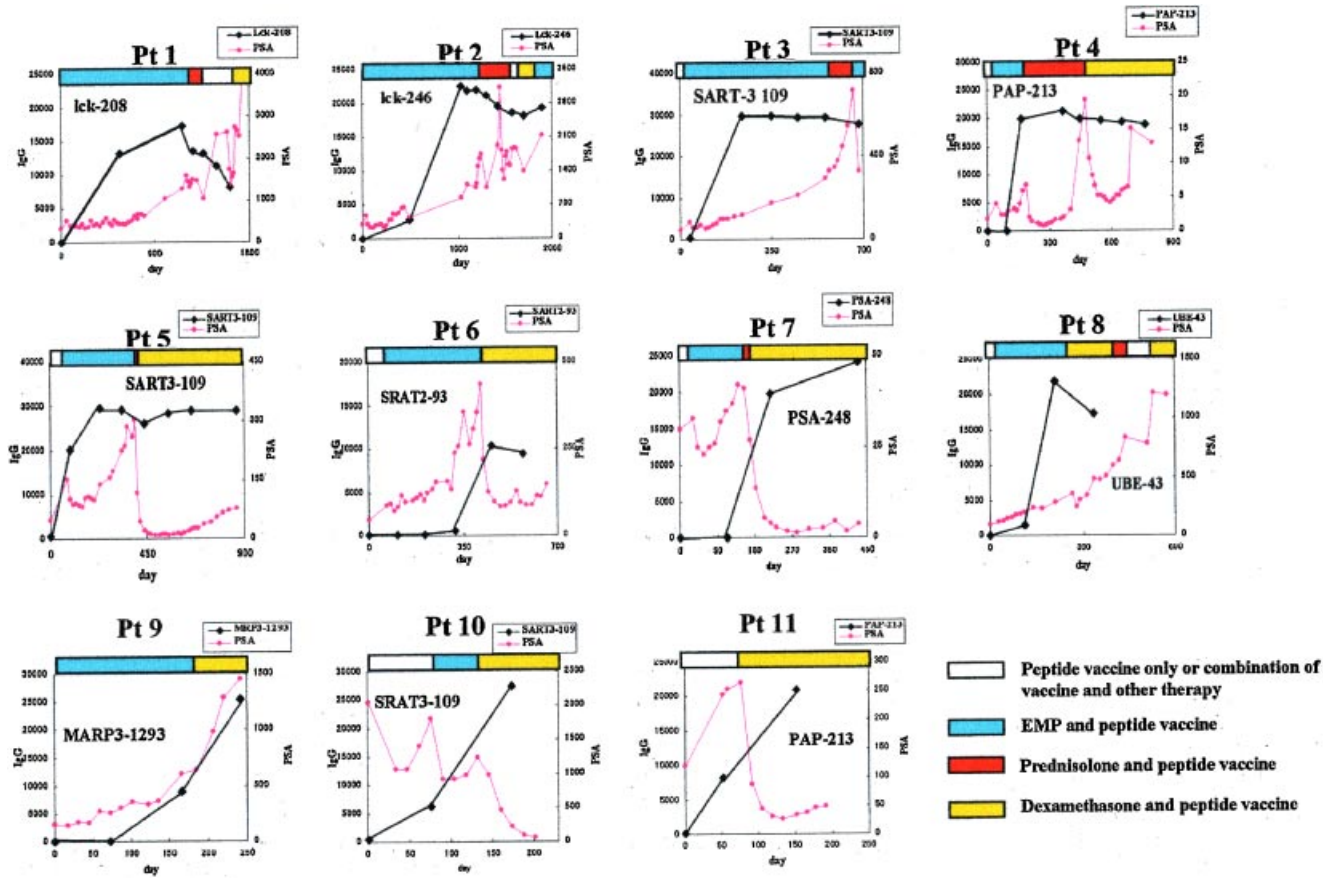

Fig. 1. IgG response and PSA levels. Kinetic studies of peptide-specific IgG and PSA levels during clinical trials with a vaccination combined with EMP, PDL or dexamethasone in each of the 11 pts are shown [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.].

observed in this trial, and neither PDL nor dexamethasone had inhibitory effects on the vaccination-induced dermatological reactions. Cushingoid effects were not observed in pts with PDL, but were observed in 2 pts with dexamethasone. There were no other glucocorticoid-associated toxicities (infection, peptic ulcer, hypertension, etc.). Collectively, this combination therapy could be administered safely.

In the humoral immune response, the IgG level could be maintained by vaccination in the PDL group, but immune boosting was scarcely observed. In contrast, IgG levels specific to the vaccinated peptides in the dexamethasone group increased as compared to those in the pre-combination stage. These results suggested that humoral immune-boosting by the peptide vaccination was not suppressed by a low dose dexamethasone. Similar results were observed with regard to cellular immunity. Increment of CTL activity was more often observed in the dexamethasone group (5 of 7) as compared to the PDL group (2 of 4). We could also detect HLA-A-restricted cytotoxicity against prostate cancer cells in the dexamethasone group. Collectively, these results suggest that dexamethasone did not suppress immune boosting by personalized peptide vaccination for advanced prostate cancer patients. In contrast, PDL might suppress it, although further studies with more patient samples are needed to confirm PDL-mediated immune suppression against personalized peptide vaccination.

It is known that PDL has the potential to induce a clinical response against HRPC [9,10]. A PSA decline of $\geq$50% was detected in 9–34% of patients in those reports of PDL. In contrast, only 1 pt showed a PSA decline of $\geq$50%, and the median of decreased PSA levels was $-$5% in our study. The reasons for this discrepancy are presently unclear, and further studies with more patient samples are needed to confirm our present results with regard to the clinical effects of PDL for HRPC pts under personalized peptide vaccination.

This study with a small number of pts has shown that a low dose of dexamethasone with personalized peptide vaccination might produce anti-tumor immune responses to prostate cancer. Glucocorticoids may exert an anti-tumor effect on HRPC by suppression of adrenal androgens. Low-dose glucocorticoids produce negative feedback on the pituitary gland, leading to a

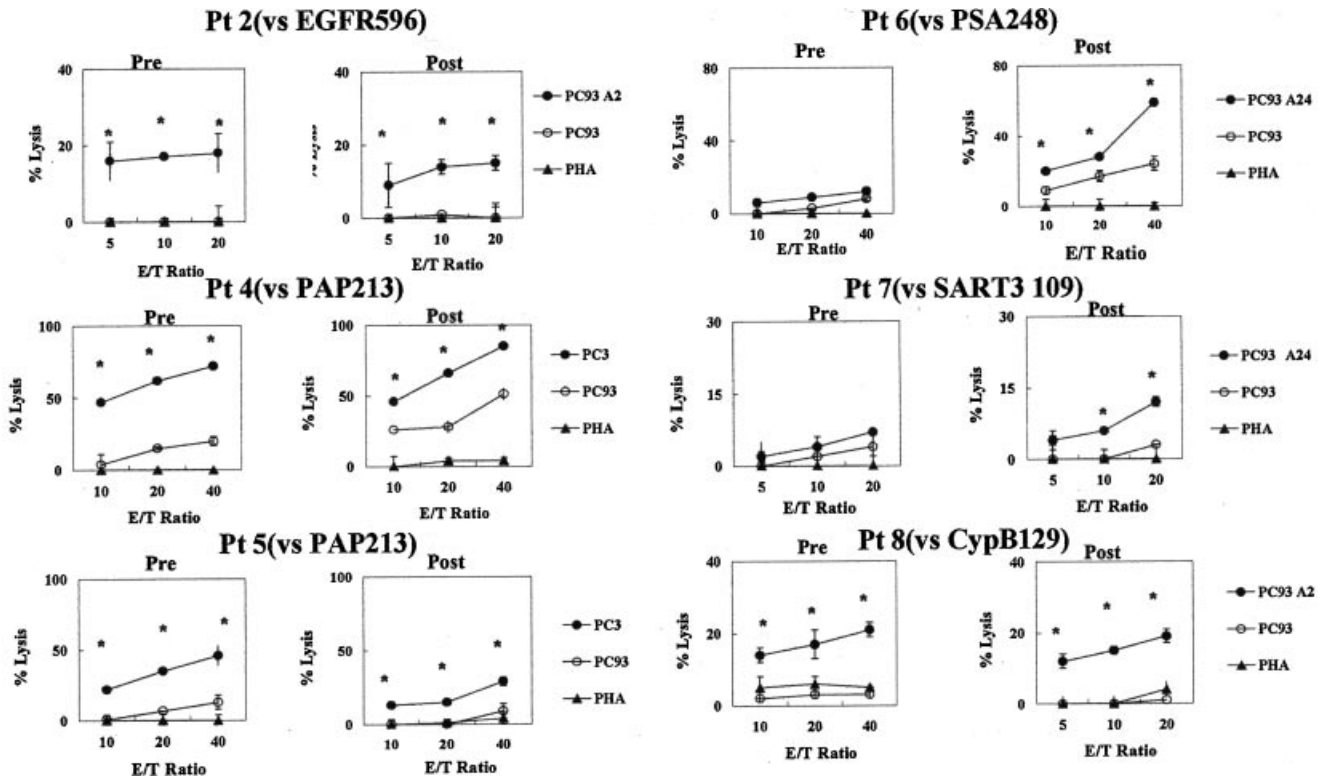

Fig. 2. Cytotoxicity. Pre- and post-vaccination PBMCs were incubated for 21–25 days with interleukin-2 (IL-2) and the corresponding peptide in culture followed by measurement of their cytotoxicity against PC93-A2 (HLA-A2), PC93-A24 (HLA-A24), PC3 (HLA-A24), PC93 (wild-type), and phytohemagglutinin (PHA)-blastoid T cells (−) (HLA-A24 + or HLA-A2 +) by a 6-hr ^{51}Cr-release assay at effector/target (E/T)ratios. The assay was carried out in triplicate, and the mean and standard deviations are shown. *A two-tailed Students *t*-test ($P < 0.05$) was used for statistical analysis.

TABLE IV. PSA Decline After Peptide Vaccination With Glucocorticoids

	Peptide vaccine with prednisolone				Peptide vaccine with dexamethasone			
Patients no.	Baseline PSA (ng/ml)	PSA at nadior or PD (ng/ml)	PSA decline (%)	TTP (day)	Baseline PSA (ng/ml)	PSA at nadior or PD (ng/ml)	PSA decline (%)	TTP (day)
1	1,577	1,309	17	98	2,619	1,374	48	140
2	1,054	1,497	−42	25	1,529	1,507	1	77
3	327	445	−36	49	—	—	—	—
4	6.8	0.7	89	259	19.35	4.11	79	207 +
5	235	286	−22	12	260	9	97	489 +
6	—	—	—	—	436	84.9	81	245 +
7	41	27	34	14	27	1.36	95	261 +
8	594	831	−40	42	356.4	246.3	31	70
9	—	—	—	—	639	983	−54	21
10	—	—	—	—	1,240	60	95	70 +
11	—	—	—	—	264.4	26	90	115 +
Median	327	445	−22	42	396.2	72.45	80	

PD, progressive disease; TTP, time to progression; +, continuation of combination therapy; —, not available.

decrease in both testicular and adrenal androgens [24]. Moreover, glucocorticoids inhibit prostate cancer cell growth by modulating cellular growth factors such as lipocortin, tumor growth factor beta-1, urokinase-type plasminogen activator and interleukin-6 [25].

A low dose of dexamethasone alone showed a marked clinical efficacy in the relatively small scale of clinical studies for relatively early stages of HRPC [11,12]. It was reported that 19 of 37 HRPC patients (51%) achieved a PSA declines of $\geq$75% along with increase of hemoglobin levels and improvement of bone pain [11]. The similar results were observed in the other study with 38 HRPC pts [12]. Our results are consistent with those studies from a clinical point of view. Namely, we showed that 6 of 10 pts (60%) achieved PSA decline of $\geq$75% with TTP for 4 months in the personalized peptide vaccination with dexamethasone for far advanced HRPC pts who failed all the standard therapy and also the vaccination with low dose of EMP. These results suggest that dexamethasone is an agent applicable for early as well as late stages of HRPC. It might also be applicable as monotherapy as well as combined therapy with vaccine therapy.

The present study showed that dexamethasone, but not PDL, had no suppression on immune boosting by personalized peptide vaccination. The clinical benefit was also associated with the vaccination with dexamethasone, but not with PDL. The molecular mechanisms involved in this discrepancy are presently unknown. One possible explanation is that apoptosis of prostate cancer cells was induced by dexamethasone, which in turn resulted in activation of antigen presenting cells for stimulation of the peptide-stimulated T cells at tumor sites. The other explanation is that PDL, but not dexamethasone, suppressed CD45RO positive activated/memory lymphocytes, a unique type of lymphocytes induced into tumor sites by personalized peptide vaccination as reported previously [26]. Further studies are needed to solve this issue.

Our analysis of a small number of patients encouraged the further evaluation of a combination of personalized peptide vaccination and low dose of dexamethasone for metastatic HRPC patients. Based on these preliminary findings, larger randomized study of this immune strategy could be warranted.

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REFERENCES

1. Tannock IF, de Wit R, Berry WR, Horti J, Pluzanska A, Chi KN, Oudard S, Theodore C, James ND, Turesson I, Rosenthal MA, Eisenberger MA. TAX 327 Investigators. Docetaxel plus prednisone or mitoxantrone plus prednisone for advanced prostate cancer. N Engl J Med 2004;351:1488–1490.
2. Petrylak DP, Tangen CM, Hussain MH, Lara PN Jr, Jones JA, Taplin ME, Burch PA, Berry D, Moinpour C, Kohli M, Benson MC, Small EJ, Raghavan D, Crawford ED. Docetaxel and estramustine compared with mitoxantrone and prednisone for advanced refractory prostate cancer. N Engl J Med 2004;351:1513–1520.
3. van der Bruggen P, Traversari C, Chomez P, Lurquin C, De Plaen E, Van den Eynde B, Knuth A, Boon T. A gene encoding an antigen recognized by cytolytic T lymphocytes on a human melanoma. Science 1991;254:1643–1647.
4. Kawakami Y, Eliyahu S, Sakaguchi K, Robbins PF, Rivoltini L, Yannelli JR, Appella E, Rosenberg SA. 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;180:347–352.
5. Rosenberg SA, Yang JC, Schwartzentruber DJ, Hwu P, Marincola FM, Topalian SL, Restifo NP, Dudley ME, Schwarz SL, Spiess PJ, Wunderich JR, Parkhust MA, Kawakami Y, Seipp CA, Einhorn JH, White DE. Immunologic and therapeutic evaluation of a synthetic peptide vaccine for the treatment of patients with metastatic melanoma. Nat Med 1998;4:321–327.
6. Noguchi M, Itoh K, Suekane S, Morinaga A, Sukehiro A, Suetsugu N, Katagiri K, Yamada A, Noda S. Immunological monitoring during combination of patient-oriented peptide vaccination and estramustine phosphate in patients with metastatic hormone refractory prostate cancer. Prostate 2004;60:32–45.
7. Noguchi M, Itoh K, Yao A, Mine T, Yamada A, Obata Y, Furuta M, Harada M. Immunological evaluation of individualized peptide vaccination with a low dose of estramustine for HLA-A24+ HRPC patients. Prostate 2005;63:1–12.
8. Noguchi M, Mine T, Yamada A, Obata Y, Yoshida K, Mizoguchi J, Harada M, Suekane S, Itoh K, Matsuoka K. Combination therapy of personalized peptide vaccination and low-dose estramustine phosphate for metastatic hormone refractory prostate cancer patients: An analysis of prognostic factors in the treatment. Oncol Res 2007;16:341–349.
9. Sartor O, Weinberger M, Moore A, Li A, Figg WD. Effect of prednisone on prostate-specific antigen in patients with hormone-refractory prostate cancer. Urology 1998;52:252–256.
10. Kelly WK, Curley T, Leibretz C, Dnistrian A, Schwartz M, Scher HI. Prospective evaluation of hydrocortisone and suramin in patients with androgen-independent prostate cancer. J Clin Oncol 1995;13(9):2208–2213.
11. Kazuo Nishimura K, Nonomura N, Yasunaga Y, Takaha T, Inoue H, Sugao H, Yamaguchi S, Ukimura O, Miki T, Okuyama A. Low doses of oral dexamethasone for hormone-refractory prostate carcinoma. Cancer 2000;89:2570–2576.
12. Storlie JA, Buckner JC, Wiseman GA, Burch PA, Hartmann LC, Richardson RL. Prostate specific antigen levels and clinical response to low dose dexamethasone for hormone-refractory metastatic prostate carcinoma. Cancer 1995;76:96–100.
13. Noguchi M, Kobayashi K, Suetugu N, Tomiyasu K, Suekane S, Yamada A, Itoh K, Noda S. Induction of cellular and humoral immune responses to tumor cells and peptides in HLA-A24 positive hormone-refractory prostate cancer patients by peptide vaccination. Prostate 2003;57:80–92.

14. Noguchi M, Itoh K, Suekane S, Yao A, Suetsugu N, Katagiri K, Yamada A, Yamana H, Noda S. Phase I trial of patient-oriented vaccination in HLA-A2 positive patients with metastatic hormone refractory prostate cancer. Cancer Sci 2004;95:77–84.
15. Harada M, Kobayashi K, Matsueda S, Nakagawa M, Noguchi M, Itoh K. Prostate-specific antigen-derived epitopes capable of inducing cellular and humoral responses in HLA-A24$^+$ prostate cancer patients. Prostate 2003;57:152–159.
16. Kobayashi K, Noguchi M, Itoh K, Harada M. Identification of a prostate-specific membrane antigen-derived peptide capable of eliciting both cellular and humoral immune responses in HLA-A24$^+$ prostate cancer patients. Cancer Sci 2003;94:622–627.
17. Matsueda S, Kobayashi K, Nonaka Y, Noguchi M, Itoh K, Harada M. Identification of new prostate stem cell antigen-derived peptides immunogenic in HLA-A2+ patients with hormone-refractory prostate cancer. Cancer Immunol Immun 2004;53:479–489.
18. Ogata R, Matsueda S, Yao A, Noguchi M, Itoh K. Identification of polycomb group protein enhancer of zeste homolog 2 (EZH2)-derived peptide immunogenic in HLA-A24+ prostate cancer patients. Prostate 2004;60:273–281.
19. Yao A, Harada M, Matsueda S, Ishihara Y, Shomura H, Takao Y, Noguchi M, Matsuoka K, Hara I, Kamidono S, Itoh K. New epitope peptides derived from parathyroid hormone-related protein which have the capacity to induce prostate cancer-reactive cytotoxic T lymphocytes in HLA-A2$^+$ prostate cancer patients. Prostate 2005;62:233–242.
20. Yao A, Harada M, Matsueda S, Ishihara Y, Shomura H, Noguchi M, Matsuoka K, Hara I, Kamidono S, Itoh K. Identification of parathyroid hormone-related protein-derived peptides immunogenic in human histocompatibility leukocyte antigen-A24$^+$ prostate cancer patients. Br J Cancer 2004;91:287–296.
21. Mine T, Sato Y, Noguchi M, Sasatomi T, Gouhara R, Tsuda N, Tanaka S, Shomura H, Katagiri K, Rikimaru T, Shichizo S, Kamura T, Hashimoto T, Shirouzu K, Yamada A, Todo S, Itoh K, Yamana H. Humoral responses to peptides correlate with overall survival in advanced cancer patients vaccinated with peptides based on pre-existing peptide-specific cellular responses. Clin Cancer Res 2004;10:929–937.
22. Noguchi M, Kikuchi H, Ishibashi M, Noda S. Percentage of the positive area of bone metastasis is an independent predictor of the disease death in advanced prostate cancer. Br J Cancer 2003;88:195–201.
23. Yajima N, Yamanaka R, Mine T, Tsuchiya N, Homma J, Sano M, Kuramoto T, Obata Y, Komatsu N, Arima Y, Yamada A, Shigemori M, Itoh K, Tanaka R. Immunologic evaluation of personalized peptide vaccination for patients with advanced malignant glioma. Clin Cancer Res 2005;11(16):5900–5911.
24. White RH, Chipps BE. Reduction of serum testosterone levels during chronic glucocorticoid therapy. Ann Intern Med 1986;104:648–651.
25. Fakih M, Johnson CS, Trump D. Glucocorticoids and treatment of prostate cancer: A preclinical and clinical review. Urology 2002;60:553–561.
26. Noguchi M, Yao A, Harada M, Nakashima O, Komohara Y, Yamada S, Itoh K, Matsuoka K. Immunological evaluation of neoadjuvant peptide vaccination before radical prostatectomy for patients with localized prostate cancer. Prostate 2007;67:933–942.