Phase I trial of personalized peptide vaccination for cytokine-refractory metastatic renal cell carcinoma patients

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The aim of this clinical trial was to investigate the toxicity and immunological responses of personalized peptide vaccination for cytokine-refractory metastatic renal cell carcinoma patients. Patients were confirmed to be human leukocyte antigen (HLA)-A24 or HLA-A2 positive and had histologically confirmed renal cell carcinoma. Ten patients were enrolled in the present study. The peptides to be administered were determined based on the presence of peptide-specific cytotoxic T lymphocyte precursors in peripheral blood mononuclear cells (PBMC) and peptide-specific IgG in the plasma of cancer patients. Patients received subcutaneous injections of four different peptides (3 mg/peptide) every 2 weeks. Vaccinations were well tolerated without any major adverse events. A minimal increase in peptide-specific interferon-γ production in postvaccination PBMC was observed, regardless of higher levels of cytotoxic T lymphocyte activity in prevaccination PBMC. In contrast, an increase in peptide-specific IgG levels of postvaccination (sixth) plasma was observed in the majority of patients. After progression, five patients received interleukin-2 therapy and continuous vaccination, with survival of 31, 25, 23, 17, and 15 months, but interleukin-2 did not impede humoral responses boosted by the vaccination. These results encourage further clinical trials of personalized peptide vaccinations. (*Cancer Sci* 2007; 98: 1965–1968)

As metastatic renal cell carcinoma (RCC) is highly resistant to chemotherapy, interleukin (IL)-2 or interferon (IFN) is used as first-line therapy, but response rates with these cytokines are low (5–20%), and the median survival time is approximately 12 months.[1–4] Subsequently, to provide new therapeutic modalities, antiangiogenic agents such as kinase inhibitors (sunitinib and sorafenib) have been conducted with promising results.[5–8] The other approach could be peptide-based specific vaccine therapy using peptides capable of inducing cytotoxic T lymphocytes (CTL).[9,10] We recently developed a personalized peptide vaccination protocol in which we first prepared a dozen different peptide vaccine candidates and administered them to different cancer patients, measuring the reaction between T cells and antibodies in the blood before and after administering the test vaccines.[11–15] In the present study, we conducted a phase I trial of personalized peptide vaccination for cytokine-refractory metastatic RCC patients.

Materials and Methods

Patients and eligibility criteria. The study protocol was approved by the Institutional Ethical Review Boards of Hokkaido University, Tokushima University, and Kurume University. Written informed consent was obtained from all patients at the time of enrolment. According to the protocol, patients were confirmed to be human leukocyte antigen (HLA)-A24 or HLA-A2 positive and had histologically confirmed RCC. All patients had metastatic lesions with disease progression after standard cytokine therapy such as IL-2 or IFN-α (Table 1). The other eligibility criteria included aged 80 years or less, serum creatinine <1.4 mg/dL, bilirubin <1.5 mg/dL, platelet count ≥100 000/μL, hemoglobin ≥8.0 g/dL, and total white blood cell (WBC) ≥3000/μL. Hepatitis B surface antigen, hepatitis C antigen, and human immunodeficiency virus (HIV) were required to be negative. All patients had been untreated for at least 4 weeks before the study, and had an Eastern Cooperative Oncology Group performance status of 0–2.

Peptides and study design. Twenty-five or 23 peptides were provided for vaccination to HLA-A24$^+$ or HLA-A2$^+$ patients, respectively, as reported previously.[11–14] These peptides were prepared under the conditions of Good Manufacturing Practice by the Multiple Peptide System (San Diego, CA, USA), and peptide sequences are shown in a previous manuscript.[9–15] These peptides have the ability to induce HLA-A24- or HLA-A2-restricted and tumor-specific CTL activity in peripheral blood mononuclear cells (PBMC) of cancer patients and are expressed on various tumors, including RCC.[9–17]

A 2-mL volume of the peptide, which was supplied in vials containing 2 or 4 mg/mL sterile solution, was mixed with an equal volume of incomplete Freund's adjuvant (Montanide ISA-51VG; Seppic, Paris, France) and emulsified in a 5-mL sterilized syringe. Then, 1.5 mL of each peptide emulsion (maximum of four peptides per vaccination) was injected subcutaneously into the thigh or armpit area every 2 weeks. One cycle of treatment consisted of six vaccinations over a 12-week period. The cycle was repeated every 6 weeks as long as the patients agreed to continue and their condition was considered feasible for vaccination. Toxicity and immunological and clinical evaluations were conducted in those who received more than six vaccinations (i.e. one cycle).

Screening of peptide-specific CTL precursors and IgG. A 30-mL volume of peripheral blood was obtained before and after the sixth vaccination, and PBMC were isolated by means of Ficoll–Conray density gradient centrifugation. Peptide-specific CTL-precursors in PBMC were detected using a previously reported culture method.[11–15] After incubation of PBMC with the corresponding peptide for 14 days, these cells were harvested and tested for their ability to produce IFN-γ in response to

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Table 1. Patient characteristics

Patient no.	Age (years)	Sex	HLA-A allele	PS	Pathological stage	Cell type	Grade	Metastasis	Previous treatment	
									Duration of cytokine therapy (months)	Wash out (weeks)
1	39	M	A24	0	T3bN0M0	Clear > granular	G2 > G3	Pancreas, adrenal gland	IFN (11)	4
2	63	F	A24	1	T3bN0M1	Clear	G2	Lung	IFN (34), IL-2 (34)	11
3	69	M	A2/A24	0	T3aNXM1	Clear	G2	Lung mediastinum LN, bone	IFN (166), IL-2 (20), UFT (187)	5
4	47	M	A24	0	T2N0M0	Clear	G2	Adrenal gland, lung	IFN (22), IL-2 (17), UFT (7)	7
5	67	F	A24	0	T3aN0M0	Clear	G2	Lung, pelvic bone	IFN (25), IL-2 (14)	9
6	57	M	A2	0	T3bN0M0	Clear	G2 >> G3	Lung	IFN (69), IL-2 (8)	4
7	62	M	A24	0	T1bN0M0	Clear	G2	Lung	IFN (23), IL-2 (9), UFT (35)	4
8	71	M	A24	1	T2N1M1	Clear	G2 > G3	Lung	IFN (5), IL-2 (5)	10
9	72	M	A24/A2	0	T1aN0M1	Clear	G2	Mediastinum LN, adrenal gland	IFN (3), radiation (2)	78
10	53	M	A24	0	T4N1M1	Papillary	G2–G3	Spinal bone	IFN (53), IL-2 (11), radiation (3)	9

HLA, human leukocyte antigen; IFN, interferon; IL, interleukin; LN, lymphonodus; PS, performance status; UFT, Tegafur · Uracil.

CIR-A2402 or T2 cells that were preloaded with either a corresponding peptide or HIV peptides (RYLRQQLLGI for HLA-A24 and LLFGYPVYV for HLA-A2) as a negative control. The level of IFN-γ was determined by enzyme-linked immunosorbent assay (limit of sensitivity: 10 pg/mL). Two-tailed Student's *t*-test was used for statistical analyses. A well was considered positive when the level of IFN-γ production in response to a corresponding peptide was significantly higher ($P < 0.05$) than that in response to a HIV peptide, and when the mean amount of IFN-γ production in response to a corresponding peptide was >50 ng/mL, compared with that in response to a HIV peptide. The increment of CTL activity was judged as positive if postvaccination samples, but not prevaccination samples, showed CTL activity. It was also judged as positive if the level of IFN-γ produced by the postvaccination sample was two times higher than that by the prevaccination sample.

The levels of antipeptide IgG were measured using the Luminex system, as reported previously.[13] Postvaccination plasma values of >50 fluorescence intensity units compared with prevaccination plasma were considered to be elevated.

Combined cytokine therapy. Among the 10 patients enrolled, six received IL-2 and two received IFN-α in addition to the personalized peptide vaccination, according to their own wishes after disease progression with peptide vaccination alone had been tried. The doses were as follows: patient 2, 5 000 000 U IFN-α/week; patients 3 and 4, 1–2 000 000 U IL-2/day for 5 days/week; patient 6, 700 000 U IL-2/week; patient 7, 6 000 000 U IFN-α,/week; patient 8, 700 000–1 400 000 U IL-2/2 weeks; and patient 9, 350 000 U IL-2/2 weeks. Patient 1 also received IFN-α and IL-2 without vaccination after disease progression with peptide vaccination alone was noted.

Adverse events and clinical responses. Adverse events were monitored according to the National Cancer Institute Common Terminology Criteria for Adverse Events version 3.0. The clinical response was evaluated based on clinical observations and radiological findings. All known sites of disease were evaluated on a monthly basis by computed tomography scan or magnetic resonance imaging examination. Tumor size was estimated via direct measurement of the region of abnormal enhancement observed on computed tomography scan or magnetic resonance imaging examination. Patients were assigned a response category according to the Response Evaluation Criteria in Solid Tumors. Survival time was estimated from the date of the initial vaccination.

Results

Patient characteristics and toxicity. Between November 2002 and August 2005, 10 patients who were positive for HLA-A24 or HLA-A2 with cytokine-refractory metastatic RCC entered the clinical trial (Table 1). Their median age was 62.5 years, ranging from 39 to 72 years, and two patients were women. The pathological stages, cell types, and grades were described. All patients suffered from metastases and had received surgery and cytokine treatments prior to entry (Table 1).

All 10 patients were evaluated for common toxicities, and there was no severe toxicity, but dermatological reactions at the injection sites were seen in eight patients (five with grade 1 and three with grade 2). Nausea, diarrhea, fever, and nasal bleeding were seen in one patient (grade 1). Depression was seen in two patients (one grade 1 and one grade 2).

Personalized peptide vaccination and immune responses. We selected the top four candidate peptide vaccines among 48 peptides showing the strongest positive reactions for each individual patient and used them in our clinical research.[11–15] The results of the selection are listed in Table 2. All patients were vaccinated with four different kinds of peptide, and each set of four peptides was entirely different, with the most frequently used peptide being SART3109-118 (5/10).

We next examined whether personalized peptide vaccination could augment peptide-specific CTL or IgG in patients after the sixth vaccination (Table 2). The vaccination increased the peptide-specific CTL response to one of the four vaccinated peptides in patients 7 (CTL response to MRP3-509) and 9 (CTL response to Lck-488), and an increment in CTL activity after the sixth vaccination was observed with only 2 of the 40 vaccinated peptides (5%). Rather, CTL responses in prevaccination PBMC diminished in postvaccination PBMC in all 10 patients with 20 of the 40 peptides. For example, in patient 1, the CTL responses to SART2-93 or SART2-161 in prevaccination PBMC became undetectable in postvaccination PBMC, whereas the same levels of CTL response to SART3-109 were observed in both pre- and postvaccination samples.

The levels of peptide-specific IgG with at least one of the four peptides after the sixth vaccination increased compared with prevaccination plasma in 8 of 10 patients (80%) with 14 of the 40 vaccinated peptides (35%). IgG levels in prevaccination plasma were not diminished in postvaccination plasma in any of

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Table 2. Immune response during vaccination

Patient no.	Peptide	Peptide cellular response†		Anti-peptide IgG‡		Clinical response (after sixth)	TTP (weeks)	IL-2 and IFN combination therapy	Overall survival (months)
		Pre	After sixth	Pre	After sixth				
1	SART1-690	0	0	175	196	SD	43	None	40
	SART2-93	200	0	101	122				
	SART2-161	407	0	0	35				
	SART3-109	363	372	267	7010				
2	SART3-109	187	0	326	332	SD	25	IFN	14
	PSA-248	0	0	16	1560				
	HER2/neu-553	0	0	30	31				
	CEA-390	0	0	11	9				
3	Lck-208	52	0	1	2	SD	40	IL-2	25
	PSA-248	62	0	6	8855				
	EZH2-291	21	41	6	5349				
	PTHrP-110	122	0	0	29				
4	SART3-109	0	0	175	200	PD	12	IL-2	23
	PAP-213	78	0	14	35				
	PSA-248	31	0	12	44				
	HER2/neu-553	162	0	29	57				
5	Lck-486	211	0	77	75	SD	20	None	6
	HER2/neu-553	662	231	25	27				
	CEA-390	110	0	4	4				
	PTH-rP-110	226	0	3	3				
6	SART3-302	1009	0	101	94	SD	24	IL-2	17
	UBE-43	821	0	489	2481				
	UBE-208	151	0	11	8				
	EZH2-569	355	235	45	37				
7	SART2-93	291	360	17	13	PD	12	IFN	10
	MRP3-503	112	1579	18	10				
	PSA-248	366	0	10	*95*				
	EZH2-291	170	0	10	17				
8	Lck-208	63	0	805	1325	PD	10	IL2	31
	HER/2/new-553	245	267	841	5425				
	EZH2-735	0	0	1327	1327				
	PTH-rP-102	0	0	1727	2721				
9	SART3-109	0	0	432	2123	PD	7	IL-2	15
	Lck-488	0	283	81	122				
	MRP3-1293	142	105	144	361				
	HER2/neu-553	0	0	148	199				
10	SART3-109	0	0	238	233	SD	23	None	23+
	Lck-486	0	0	34	39				
	EZH2-291	275	0	27	16				
	PTH-rP-110	52	0	3	5				

†Values indicate interferon (IFN)-γ production of peptide-stimulated peripheral blood mononuclear cells reactive to the corresponding peptide. An increase in IFN-γ production after vaccination was judged positive when the level of IFN-γ production was more than 50 pg/mL and increased twice after the sixth vaccination. Positive responses are underlined. ‡Values indicate fluorescence intensity of IgG reactive to the corresponding peptide. An increase in fluorescence intensity after vaccination was judged positive when the level increased more than twice after the sixth vaccination. Positive responses are underlined. EZH, polycomb group protein enhancer of zeste homolog; IL, interleukin; MRP, multidrug resistance-associated protein; PAP, prostatic acid phosphatase; PD, progressive disease; PSA, prostatic specific antigen; PTH, parathyroid hormone; SART, squamous cell carcinoma antigen recognized by T cells; SD, stable disease; TTP, time to progression; UBE, ubiquitin-conjugated enzyme variant Kua.

the 10 patients or any of the 40 peptides vaccinated. These results indicate that humoral responses, but not cellular responses, were well boosted by the vaccination.

Vaccination combined with cytokine therapy. After the clinical responses of patients under vaccination alone resulted in progressive disease, five and two patients received IL-2 or IFN-α, respectively, at various doses in addition to the vaccination. Two patients continued with the vaccination alone, and the remaining patient (patient 1) received IFN-α and IL-2 without the vaccination. We then examined the levels of peptide-specific CTL or IgG in samples from precytokine therapy and those after the first of six vaccinations under the combined administration of IL-2 or IFN-α. As a result, combined therapy scarcely increased the peptide-specific CTL response (data not shown). However, at least one of the four peptides increased the levels of peptide-specific IgG in six of seven patients (data not shown). These results indicate that humoral responses, but not cellular responses, were also well boosted by combined therapy.

Clinical responses. This is a phase I trial to investigate toxicity and immune responses, and thus the evaluation of clinical responses was not a major objective of this study; however, description of the clinical responses could be important for the next stage of clinical trials. With the vaccination alone, the median time to progression was 23 weeks (range 7–43 weeks) with no major tumor regression (Table 2). The vaccination combined with cytokine therapies also showed no major tumor regression. Overall survival times after initiation of the vaccine in the five patients who received the vaccination alone followed

by the vaccination and IL-2 were 31, 25, 23, 17, and 15 months, those of the two patients who received both the vaccination and IFN-α were 14 and 10 months, and those of the two patients with the vaccination alone were 6 and >19 months. The remaining patient (patient 1) who received IFN-α and IL-2 without the vaccination when progressive disease was noted with the vaccination alone survived for 40 months. Collectively, the median survival time of 10 patients after starting the vaccination was 23 months.

Discussion

Among the 10 patients, no major adverse events were observed, although most of the patients developed grade 1 or 2 local dermatological reactions at the vaccine injection sites. Therefore, in terms of safety, the toxicity of the vaccination regimens reported here was tolerable and acceptable for cytokine-refractory RCC patients. With regard to peptide-induced immune reactions, a minimal increase in peptide-specific IFN-γ production in the postvaccination PBMC was observed, regardless of higher levels of CTL activity in prevaccination PBMC. In contrast, an increase in peptide-specific IgG in postvaccination (sixth) plasma was observed in the majority of patients. These results indicate that humoral responses, but not cellular responses, were well boosted by personalized peptide vaccination in cytokine-refractory metastatic RCC patients.

One of the reasons for this discrepancy might be the carry over of immune modulation caused by previous cytokine therapies. IL-2 or IFN-α therapy primarily activates cellular immunity, including T cells and natural killer cells, but does not largely affect humoral immunity. Cytokine-off intervals were set as at least 4 weeks in this study (Table 2), which may not be long enough for the recovery of CTL precursors in the circulation from *in vivo* cytokine effects to modulate immune responses to our own tumor cells. This issue needs to be further investigated.

We previously reported that most of the target antigens encoding the peptides used for vaccination were expressed in cell lines from RCC cells:[16,17] SART1, SART2, SART3, MRP3, EZH2, HER2/neu, and PTHrP. In those studies, however, prostatic specific antigen (PSA) and prostatic acid phosphatase (PAP) antigens were undetectable in the cell lines. Therefore, we further investigated the expression of PSA and PAP in the primary culture of both RCC cells and non-tumorous kidney cells by reverse transcription–polymerase chain reaction, and found PAP expression but not PSA expression in both types of cells (Komohara *et al*., unpublished results, 2002). Subsequently, we investigated PSA protein expression in metastatic RCC cells by means of immunohistochemical staining, and found that PSA antigens were expressed in RCC cells from two of four samples that were harvested surgically from lung metastases (Komohara *et al*., unpublished results, 2003). We also found that carcinoembryonic antigen (CEA), ubiquitin-conjugated enzyme variant Kua (UBE), and Lck antigens were expressed in both types of cells (Komohara *et al*., unpublished results, 2003). These results suggest that the peptides used for vaccination of patients under a personalized protocol are at least presentable on the HLA-A24 or HLA-A2 groove of RCC cells, although there is no direct evidence to prove this assumption at the present time.

Administration of IL-2 to vaccinated patients at the time of progression with vaccination alone neither increased the frequency of peptide-reactive CTL nor suppressed humoral responses to vaccinated peptides. These results suggest that IL-2 therapy did not largely impede peptide-induced immune responses, although this point should be confirmed in a large number of patients.

In conclusion, personalized peptide vaccination for cytokine-refractory metastatic RCC patients was well tolerated and the induction of peptide-specific humoral immunity was observed in most patients. These results encourage further clinical trials of personalized peptide vaccination. Vaccination combined with IL-2 therapy should be investigated because of the expected prolonged survival.

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References

1 Fisher RI, Rosenberg SA, Fyfe G. Long-term survival update for high-dose recombinant interleukin-2 in patients with renal cell carcinoma. *Cancer J Sci Am* 2000; **6** (Suppl 1): S55–7.
2 McDermott DF, Regan MM, Clark JI *et al*. Randomized phase III trial of high-dose interleukin-2 versus subcutaneous interleukin-2 and interferon in patients with metastatic renal cell carcinoma. (Published erratum appears in J Clin Oncol 2005; 23: 2877.) *J Clin Oncol* 2005; **23**: 133–41.
3 Motzer RJ, Murphy BA, Bacik J *et al*. Phase III trial of interferon α-2a with or without 13-cis-retinoic acid for patients with advanced renal cell carcinoma. *J Clin Oncol* 2000; **18**: 2972–80.
4 Negrier S, Escudier B, Lasset C *et al*. Recombinant human interleukin-2, recombinant human interferon α-2a, or both in metastatic renal-cell carcinoma. Groupe Francais d'Immunotherapie. *N Engl J Med* 1998; **338**: 1272–8.
5 Motzer RJ, Hutson TE, Tomczak P *et al*. Sunitinib versus interferon α in metastatic renal-cell carcinoma. *N Engl J Med* 2007; **356**: 115–24.
6 Escudier B, Eisen T, Stadler WM *et al*. Sorafenib in advanced clear-cell renal-cell carcinoma. *N Engl J Med* 2007; **356**: 125–34.
7 Brugarolas J. Renal-cell carcinoma – molecular pathways and therapies. *N Engl J Med* 2007; **356**: 185–7.
8 Hudes G, Carducci M, Tomczak P *et al*. A phase 3, randomized, 3-arm study of temsirolimus (TEMSR) or interferon-α (IFN) or the combination of TEMSR + IFN in the treatment of first-line, poor-risk patients with advanced renal cell carcinoma (adv RCC). *J Clin Oncol* 2006; **24** (**Suppl**): 930S.
9 Yang D, Nakao M, Shichijo S *et al*. Identification of a gene coding for a protein possessing shared tumor epitopes capable of inducing HLA-A24-restricted cytotoxic T lymphocytes in cancer patients. *Cancer Res* 1999; **59**: 4056–63.
10 Ito M, Shichijo S, Tsuda N *et al*. Molecular basis of T cell-mediated recognition of pancreatic cancer cells. *Cancer Res* 2001; **61**: 2038–46.
11 Tsuda N, Mochizuki K, Harada M *et al*. Vaccination with predesignated or evidence-based peptides for patients with recurrent gynecologic cancers. *J Immunother* 2004; **27**: 60–72.
12 Mine T, Sato Y, Noguchi M *et al*. 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**: 929–37.
13 Yajima N, Yamanaka R, Mine T *et al*. Immunologic evaluation of personalized peptide vaccination for patients with advanced malignant glioma. *Clin Cancer Res* 2005; **11**: 5900–11.
14 Noguchi M, Itoh K, Yao A *et al*. Immunological evaluation of individualized peptide vaccination with a low dose of estramustine for HLA-A24$^+$ HRPC patients. *Prostate* 2005; **63**: 1–12.
15 Itoh K, Yamada A. Personalized peptide vaccines: a new therapeutic modality for cancer. *Cancer Sci* 2006; **97**: 970–6.
16 Komohara Y, Harada M, Arima Y *et al*. Identification of target antigens in specific immunotherapy for renal cell carcinoma. *J Urol* 2007; **177**: 1157–62.
17 Arima Y, Matsueda S, Yano H, Harada M, Itoh K. Parathyroid hormone-related protein as a common target molecule in specific immunotherapy for a wide variety of tumor types. *Int J Oncol* 2005; **27**: 981–8.

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