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A phase I study of personalized peptide vaccination for advanced urothelial carcinoma patients who failed treatment with methotrexate, vinblastine, adriamycin and cisplatin

Kazumasa Matsumoto, Masanori Noguchi*, Takefumi Satoh, Ken-Ichi Tabata, Tetsuo Fujita, Masatsugu Iwamura, Akira Yamada†, Nobukazu Komatsu‡, Shiro Baba and Kyogo Itoh‡

*Department of Urology, Kitasato University School of Medicine, *Clinical Research Division and †Cancer Vaccine Division of the Research Centre for Innovative Cancer Therapy, Kurume University School of Medicine, and ‡Department of Immunology, Kurume University School of Medicine, Kurume, Japan*

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Study Type – Therapy (case series)
Level of Evidence 4

What's known on the subject? and What does the study add?
This phase I study showed the safety and boosted immune responses of personalized peptide vaccination for advanced urotherial carcinoma.

This study showed feasibility of personalized peptide vaccination as a new therapeutic modality for advanced urotherial carcinoma patients who failed cisplatin-based chemotherapy.

OBJECTIVE

- To investigate the safety and immune responses of 12 consecutive weeks of once-weekly personalized peptide vaccine (PPV) administration in patients with advanced urothelial carcinoma (UC) for whom therapy with methotrexate, vinblastine, adriamycin and cisplatin (MVAC) has failed.

PATIENTS AND METHODS

- A phase I trial was designed. Ten patients with MVAC-refractory advanced or metastatic UC were treated with weekly personalized peptide vaccine 12 times using positive peptides chosen from 14 and 16 peptides in patients with human leucocyte antigens A24 and A2, respectively.
- Peptide-specific cytotoxic T lymphocyte precursor analysis by interferon-γ production and peptide-reactive immunoglobulin G (IgG) using an enzyme-linked immunosorbent assay was monitored during the treatment.

RESULTS

- The peptide vaccination was safe and well tolerated with no major adverse effects. Increased cytotoxic T lymphocyte response and the anti-peptide IgG titre were revealed by the post-vaccination sera in eight patients.
- Clinical responses were as follows: one complete response, one partial response, two stable disease and six progressive disease.
- Median progression-free survival and overall survival were 3.0 and 8.9 months, respectively. In the four responders, median progression-free survival and overall survival were 21 and 24 months, respectively.

CONCLUSIONS

- This phase I study showed the safety of and boosted immune responses in response to PPV for advanced UC.
- The potential efficacy of 12 consecutive weekly vaccinations with PPV in patients with advanced UC merits further investigation based on these findings.

KEYWORDS

urothelial carcinoma, bladder cancer, peptide vaccine, personalized therapy, phase I clinical trial

INTRODUCTION

The currently available standard chemotherapy for advanced or metastatic urothelial carcinoma (UC) is a cisplatin-based treatment that includes methotrexate, vinblastine, adriamycin and cisplatin (MVAC) or gemcitabine and cisplatin [1–4]. However, there are no established therapeutic modalities for patients with UC who fail with these cisplatin-based therapies. Therefore, new approaches should be taken, and one of them could be specific immunotherapy. Recent advances in tumour immunology have resulted in the identification of a number of antigens and their peptides that are recognized by tumour-reactive and human leucocyte antigen (HLA) class I-restricted

cytotoxic T lymphocytes (CTL) [5]. Cancer vaccines have emerged as a promising therapeutic approach [6]. The efficacy of intravesical BCG in the treatment of superficial disease suggests a role for developing immune recognition strategies to enhance the treatment of UC. The presence of tumour-infiltrating CD8 T cells has been associated with survival in patients with UC [7]. CD8-expressing T cells can also recognize the NY-ESO-1 antigen [8], which occurs in approximately 30–40% of muscle-invasive bladder cancer. A recent clinical trial found that all six of six patients developed antigen-specific immune responses when treated with NY-ESO-1 vaccine [9]. Additional work evaluating the impact of immunomodulating therapy is ongoing, including the use of the anti-cytotoxic T-lymphocyte antigen-4 antibody to overcome inhibitory signals down-regulating T cells [10]. However, their clinical responses have been limited. To overcome this limitation, we devised a new regimen of peptide-based vaccination that consists of measuring pre-existing CTL precursors and IgG reactive to many kinds of vaccine candidates, followed by administration of the positively reactive peptides (personalized peptide vaccination: PPV) [11–14]. A recently conducted randomized clinical trial of PPV for advanced prostate cancer patients showed a favourable clinical response in the vaccinated group [15], whereas most of the other randomized cancer vaccine trials failed to obtain better clinical responses in the vaccine group [16–18]. In this phase I study, we addressed the feasibility of PPV for patients with advanced UC for whom MVAC therapy had failed.

PATIENTS AND METHODS

Eligible patients were included if they were ≥18 years of age with HLA-A24 and/or HLA-A2 status, as determined by commercially available serological tests (SRL, Tokyo, Japan), and were measurable or assessable and histologically proven to have locally advanced (≥T3, N1) or metastatic (M1) UC that included the urinary bladder and upper urinary tract. All patients received surgical treatment or biopsy and MVAC therapy had failed. Previous chemotherapy with radiation therapy for local treatment of the primary lesion was allowed if completed at least 4 weeks before enrolment. Patients were eligible if their disease had progressed at any time after therapy for advanced or metastatic disease or within 12 months of neoadjuvant or adjuvant treatment. Patients were required to have an Eastern Cooperative Oncology Group performance status of 0 to 1, adequate bone marrow reserve (white blood cell count ≥3000/μL, lymphocyte count ≥1200/μL, platelets ≥75 000/μL and haemoglobin ≥10 g/dL), hepatic function (serum bilirubin ≤1.5 mg/dL), and renal function (serum creatinine ≤1.5 mg/dL), and an estimated life expectancy of at least 12 weeks. Patients with non-malignant systematic disease that precluded them from receiving therapy, including active infection, autoimmune disease, any clinically significant cardiac arrhythmia, or congestive heart failure were not eligible. Patients also had to be negative for hepatitis B and C antigens. Patients with CNS metastases, second primary malignant lesions, or clinically significant pleural effusions or ascites or who had used any investigational agent 1 month before enrolment were not eligible. The study protocol was approved by the institutional ethical review boards of Kitasato University and Kurume University, and conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from all patients before entering this clinical trial.

The study design was for a non-randomized, open-label, phase I study in patients with advanced or metastatic UC previously treated with MVAC chemotherapy. The treatment was carried out at Kitasato University Hospital and Kurume University Hospital in the outpatients clinic. All immunological analyses were carried out at the Department of Immunology, Kurume University School of Medicine. The peptides used in the present study were prepared by Multiple Peptide Systems (San Diego, CA, USA) under the conditions of Good Manufacturing Practice. The peptide candidates consisted of $SART2_{93-101}$, $SART2_{161-169}$, $SART3_{109-118}$, $Lck_{208-216}$, $Lck_{486-494}$, $Lck_{488-497}$, $MRP3_{503-511}$, $MRP3_{1293-1302}$, $PAP_{213-221}$, $PSA_{248-257}$, $PSMA_{624-624}$, $EZH2_{735-743}$, $EGF\text{-}R_{800-809}$ and $PTH\text{-}rP_{102-111}$ for patients with HLA-A24, and $SART3_{302-310}$, $SART3_{309-317}$, $CypB_{129-138}$, $Lck_{246-254}$, $Lck_{422-430}$, $ppMAPkkk_{294-302}$, $ppMAPkkk_{432-440}$, $WHSC2_{103-111}$, $WHSC2_{141-149}$, $UBE2V_{43-51}$, $UBE2V_{85-93}$, $HNRPL_{140-148}$, $HNRPL_{501-510}$, $EZH2_{569-577}$, $PSCA_{21-30}$ and $EGFR_{479-488}$ for patients with HLA-A2 [8,9,13]. These peptides have the ability to induce HLA-A24-restricted or HLA-A2-restricted and tumour-specific CTL activity in peripheral blood mononuclear cells (PBMCs) of cancer patients, and are frequently expressed in various tumour cell lines [14,15,19]. The peptides were supplied in vials containing 3 mg/mL sterile solution for injection. Three milligrams of peptide with sterile saline was added in a 1:1 volume to the Monotide ISA-51 (Seppic, Paris, France), and then mixed in a Vortex mixer (Fisher, Alameda, CA, USA). The ISA51 is suitable for peptide vaccination because peptides solubilized in water phase are sequestered from peptidase-containing body fluid, and slow release of the peptides from the emulsion provides sustained antigenic stimulation [20]. The resulting emulsion (maximum of four peptides per vaccination) was injected subcutaneously into the femoral area, once a week for 12 weeks. This first cycle of treatment consisted of 12 consecutive weekly vaccinations. The cycle was repeated every 12 weeks for as long as the patients agreed to continue and their condition was considered appropriate for vaccination. Toxicity was evaluated in patients who received at least one vaccination, whereas both immunological and clinical evaluations were conducted in those who received more than six vaccinations. Blood samples for studies of immune responses were obtained on weeks 0, 6 and 12 during cycle 1. Supportive care could include blood transfusion and the administration of anti-emetics and analgesics, as appropriate. Further local therapy, including other chemotherapy regimens or radiation therapy, was allowed in patients with advanced disease after assessment of response to this regimen.

To measure peptide-specific CTL precursors, 30 mL peripheral blood was obtained before and after vaccination, and PBMCs were isolated by Ficoll–Conray density gradient centrifugation. Peptide-specific CTL precursors in PBMCs were detected using a previously reported culture method [21]. Briefly, PBMCs (1×10^5 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, Grand Island, NY, USA), 10% fetal calf serum, 100 U/mL interleukin-2 and 0.1 μM minimal essential medium non-essential amino acid solution (GIBCO BRL). Half of the medium was removed and replaced with a new medium containing a corresponding peptide (20 μM) every 3 days. After incubation for 14 days, these cells were harvested and tested for their ability to

Characteristics	No. of patients
Gender	
Male	8
Female	2
HLA typing	
A-2	4
A-24	5
A-2 and A-24	1
Primary organ	
Bladder	7
Upper urinary tract	2
Both	1
Surgical management	
TURBT	7
Nephroureterectomy	2
Radical cystectomy	1
Main target tumour	
Lymph node	5
Bladder	3
Bone	2
Previous treatment	
Chemotherapy	5
Chemotherapy and radiation therapy	5
Performance status*	
0	5
1	5

TABLE 1 Patient characteristics

*HLA, human leucocyte antigen; TURBT, Transurethral resection of bladder tumour. *Performance status by Eastern Cooperative Oncology Group score.*

produce interferon-γ (IFN-γ) in response to CIR-A2402 (kindly provided by Dr M. Takiguchi, Kumamoto University, Japan) or T2 cells that were pre-loaded 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 ELISA (limit of sensitivity: 10 pg/mL). All assays were performed in quadruplicate. A two-tailed Student's *t* test was employed for the 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 an 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 an HIV peptide. The increment of CTL activity was judged as positive if the post-vaccination sample, but not the pre-vaccination sample, showed CTL activity. It was also judged as positive if the level of IFN-γ produced by the post-vaccination (12th) sample was twice as high as that produced by the pre-vaccination sample. Our previous study showed that both increased IgG and a CTL response at least twice that of the vaccinated peptides correlated well with overall survival in patients with castration-resistant prostate cancer [22].

The levels of anti-peptide IgG were measured using the Luminex™ system, as previously reported [23]. In brief, plasma was incubated with 25 μL peptide-coupled colour-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 biotinylated goat anti-human IgG (chain-specific) for 1 h at room temperature. The plate was then washed, 100 μL of streptavidin-phycoerythrin was added to the wells, and the mixture was incubated for 30 min at room temperature on a plate shaker. The bound beads were washed three times followed by the addition of 100 μL Tween-PBS into each well. Fifty microlitres of sample was detected using the Luminex™ system. The sample was judged to be positive if the IgG level of the post-vaccination (12th) plasma was twice as high as that of the pre-vaccination plasma. This definition is the same as the CTL response according to our previous results [22].

Standard indirect immunoperoxidase procedures (ENVISION Kit; DakoCytomation California, Carpinteria, CA, USA) in combination with monoclonal antibodies were used for the detection of infiltrating lymphoid cells (CD45RA and CD45RA, 1:50; Dako, Glostrup, Denmark) [24]. Cells with known positive results were used as positive controls. The primary antibody was omitted for negative controls.

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 every 6 weeks by CT scan or MRI examination before and after each cycle. During treatment, blood counts and serum chemistries were performed weekly. Patients were assigned a response category according to the Response Evaluation Criteria in Solid Tumors (RECIST).

Student's *t* test was employed for evaluation of immunological assays. Progression-free survival time, overall survival time and response duration were calculated from the first day of peptide vaccination until the date of disease progression or death. The time-to-event endpoint was derived by the Kaplan–Meier method. All patients entering the trial were included in the survival determinations.

RESULTS

Between July 2007 and April 2009, 10 patients were treated with peptide vaccination at our institutions. Data were collected until December 2009. One patient did not meet the protocol entry criteria because cisplatin-based chemotherapy had not been received before the peptide vaccination. Median age was 71 years (range 44–77 years). Median follow-up time was 8.9 months (mean 12.0 months, range 2.5–29.3 months). Seven patients had bladder UC, two patients had upper urinary tract UC and one patient had bladder and upper urinary tract UC. Seven patients had metastatic disease, of whom five had lymph node metastasis and two had bone metastasis; three patients had locally advanced UC without distant metastasis after MVAC chemotherapy. The clinical characteristics of all entry patients are listed in Table 1.

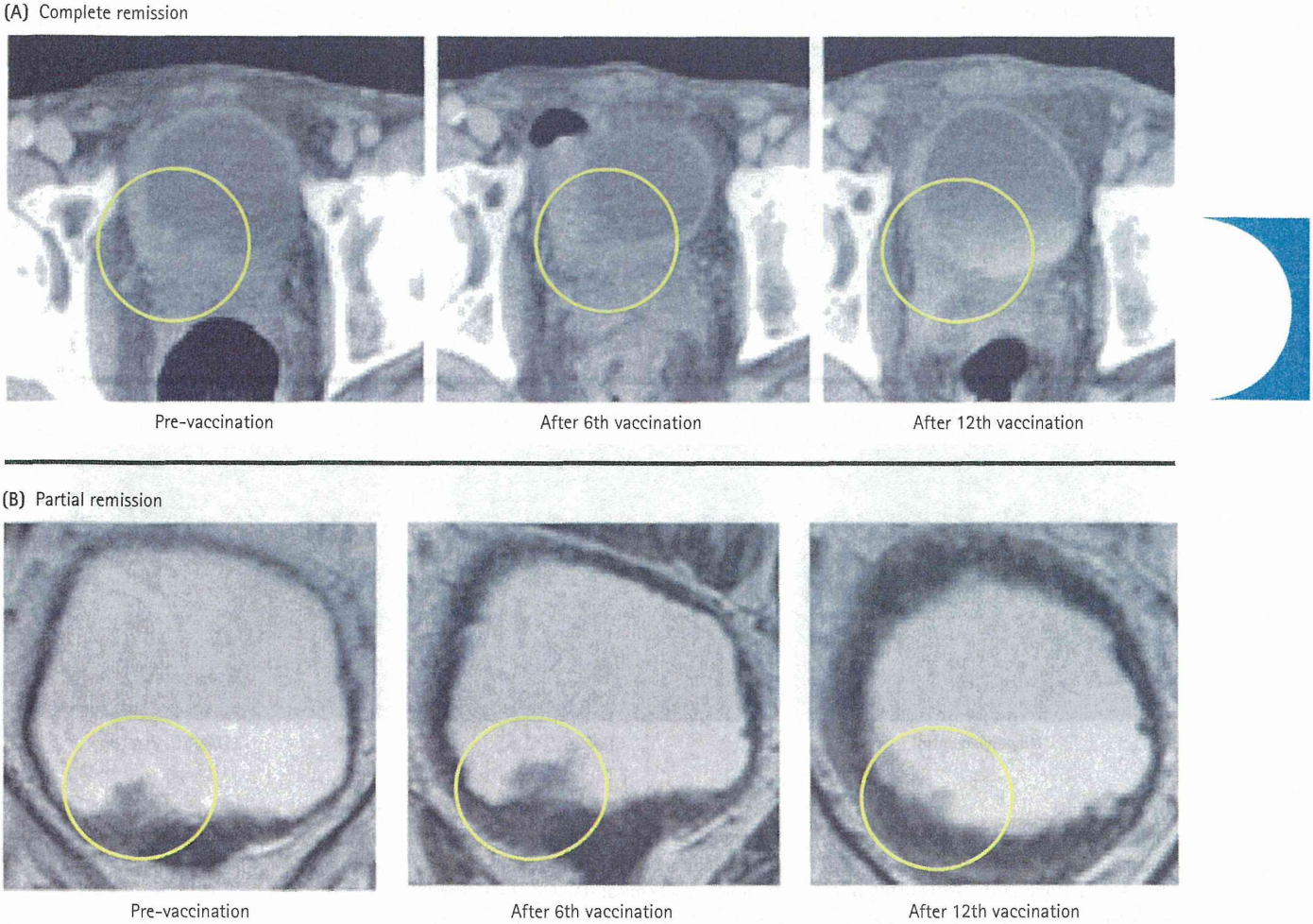
For the selection of peptides for the first to 12th vaccinations (the first cycle), pre-

TABLE 2 Immune responses and clinical outcomes

Patient no. Clinical stage	Peptide	No. of vaccinations	Cellular response*		Anti-peptide IgG†		Clinical response	PFS (months)	OS (months)	Prognosis
			Pre-	After 12th	Pre-	After 12th				
1 T4N0M1	PAP-213	10	–	NA	1753	NA	PD	1	3	Dead
	PSA-248		–	NA	110	NA				
	EZH2-735		–	NA	51	NA				
	PTHrP-102		–	NA	149	NA				
2 T3bN0M0	SART3-109	24	–	–	193	238	PR	22	28	Alive
	Lck-486		–	–	45	43				
	MRP3-1293		–	–	128	180				
	PAP-213		–	1923	167	23 959				
3 TisN2M1	SART3-109	12	–	–	48	13 261	PD	3	5	Dead
	Lck-486		155	–	53	156				
	MRP3-1293		–	–	228	2 144				
	PAP-213		–	–	353	25 892				
4 T3bN2M0	SART3-109	25	158	137	341	26 423	SD	22	29	Alive
	Lck-488		–	327	195	769				
	PAP-213		–	207	344	22 943				
	SART2-92		68	162	214	221				
5 T3bN1M0	MAP-432	12	–	113	37	128	CR	20	20	Dead
	Lck-422		–	216	32	25				
	WHSC2-103		57	2558	15	19				
	UBE2V-85		–	2684	20	26				
6 T3bN1M0	SART3-309	12	117	198	66	61	PD	3	4	Dead
	CypB-129		–	–	99	90				
	UBE2V-43		–	–	174	303				
	HNRPL-501		–	548	55	41				
7 T4aN2M1	SART3-109	12	–	–	62	25 796	PD	3	9	Dead
	Lck-486		–	–	31	42				
	Lck-488		–	–	89	131				
	UBE2V-43		–	6212	72	272				
8 T4N2M0	Lck-422	23	–	251	47	4 315	SD	3	9	Dead
	UBE2V-43		–	–	61	12 296				
	WHSC2-141		–	–	27	44				
	HNRPL-140		–	209	30	257				
9 T3N1M0	Lck-422	12	–	–	37	1 395	PD	4	5	Dead
	UBE2V-43		–	3252	129	11 845				
	HNRPL-140		–	–	33	2 231				
10 T4N0M0	Lck-208	16	–	193	216	232	PD	3	9	Alive
	MRP3-1293		–	1712	368	438				
	PAP-213		514	551	357	3 161				
	PSA-248		–	–	711	5 588				

*CR, complete response; NA, not available; OS, overall survival; PD, progressive disease; PFS, progression-free survival; PR, partial response; SD, stable disease. *Values indicate interferon-γ (IFN-γ) production of peripheral blood mononuclear cells reactive to the corresponding peptide (pg/mL). A two-tailed Student's t test was employed for the statistical analysis. 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 an HIV peptide, and also when the mean amount of IFN-γ production in response to a corresponding peptide was >50 ng/mL, compared with that to an HIV peptide. Increment of cytotoxic T lymphocyte activity was judged as positive if the post-vaccination samples, but not the pre-vaccination samples, showed the cytotoxic T lymphocyte activity. It was also judged as positive if the level of IFN-γ produced by the post-vaccination sample was more than twice as high as that produced by the pre-vaccination sample. The values showing the increment are underlined. †Plasma levels of peptide-specific IgG were measured using the Luminex™ system. Values indicated fluorescence intensity units of IgG antibodies reactive to the corresponding peptide. The sample was judged positive if the IgG level of the post-vaccination (12th) plasma was twice as high as that of the pre-vaccination plasma. The values showing positive response are underlined.*

FIG. 1. The kinetic CT images of the tumour lesion of a patient with complete remission (A) and a patient with partial remission (B). The yellow circle indicates the tumour region. Left: pre-vaccination; middle: after the sixth vaccination; right: after the 12th vaccination. Cystoscopy findings of the patient with complete remission after the 12th vaccination showed no visible tumours with negative urinary cytology and post-inflammatory lesions.



vaccination plasma was used to investigate the reactivity to each of the 14 or 16 peptides in the HLA-A24$^+$ ($n = 5$) or HLA-A2$^+$ patients ($n = 4$), respectively, followed by selection of the three or four peptides with higher levels of IgG reactivity to each of the peptides in order. For the one patient who was HLA-A24$^+$ and HLA-A2$^+$, all 30 peptides were used for the selection of peptides followed by selection of three peptides from the 14 peptides used for HLA-A24$^+$ patients and the remaining one peptide from the 16 peptides used for HLA-A2$^+$ patients; the peptides chosen had the higher levels of IgG reactivity. A summary of the administered peptides is shown in Table 2. For the second cycle (13th to 24th), the four peptides with highest reactivities were similarly chosen for administration on the basis of the results of screening both PBMCs and plasma. Eight patients received twelve vaccinations and two patients received twenty-four vaccinations without other chemotherapy treatment.

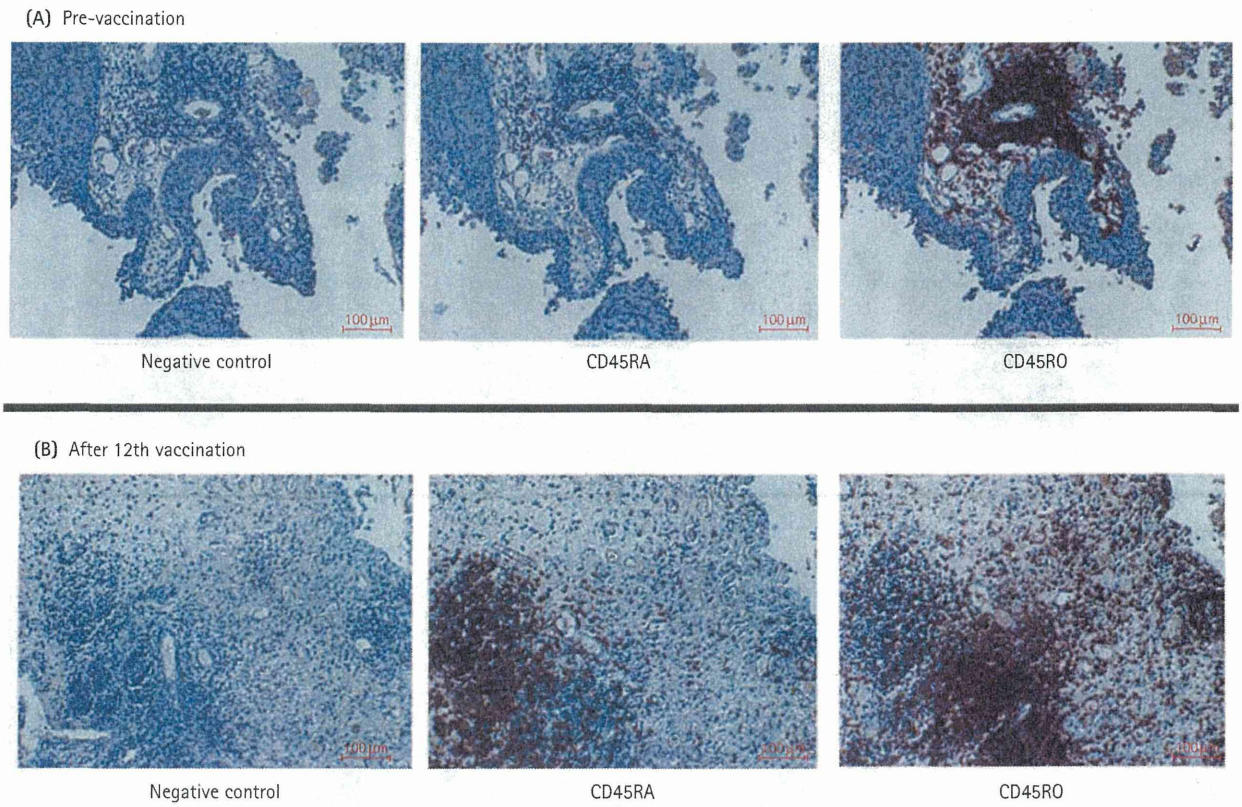
Representative non-haematological toxicity consisted of dermatological skin reactions including redness and heat at the vaccination site in all patients with grade 1 or 2 toxicity. There were no haematological toxicities or therapy-related deaths.

Peptide-specific cellular and humoral immune activities were measured at 12-week intervals for as long as the samples were available. The peptides used for vaccination and the corresponding immune responses are described in Table 2. One patient (#1) was not eligible because of rapid disease progression.

Among the nine patients tested, the augmentation of peptide-specific CTL responses in PBMCs taken after the 12th vaccination by IFN-γ production was observed in eight patients (#2, #4–10), and the augmentation of IgG responses in plasma taken after the 12th vaccination was also observed in eight patients (#2–5, #7–10). Both CTL and IgG responses were boosted in seven of nine the patients tested and CTL or IgG responses to more than two peptides were observed in four and six tested patients, respectively.

All clinical responses were confirmed by an independent review, and were as follows: one complete response, one partial response, two stable disease and six progressive disease (Table 2). A response was recorded on

*FIG. 2. Representative immunohistochemical stainings of both pre-vaccination tumour regions at the first visit before methotrexate, vinblastine, adriamycin and cisplatin therapy (**A**) and after the 12th vaccination (**B**); tumour regions with anti-CD45RO and -CD45RA monoclonal antibodies are shown. The magnification was ×100.*



radiological review in four patients. The remaining six patients had disease progressions. None of the six patients who had disease progression had any response to the peptide vaccinations. At the time of analyses, seven patients had died and all patients had progressed except for one patient who had a complete response but died from a cerebral infarction after complete peptide vaccination. The median progression-free survival was 3.0 months (range 0.5–14.1 months). The median overall survival was 8.9 months (range 2.5–29.3 months). Among the four responders, the median progression-free survival and overall survival were 21 (range 2.7–22.4 months) and 24 (range, 9.0–29.3 months), respectively.

It is of note that two patients (#2 and #5) with locally advanced bladder cancer showed obvious clinical responses on kinetic CT images (Fig. 1). To investigate host–tumour interaction, immunohistochemical staining of the biopsied samples taken at the first visit before MVAC therapy and after the 12th vaccination was performed. Immunohistochemical staining at the time of the first visit before MVAC therapy showed that there were a large numbers of tumour cells in the sample, whereas lymphocyte infiltration was limited in stromal lesions. $CD45RA^+$ naive lymphocytes were rare in the stromal lesions, whereas $CD45RO^+$ activated/memory lymphocytes were found around tumour vessels and stromal lesions, but not in tumour sites (Fig. 2A). Immunohistochemical staining after the 12th vaccination showed that there were very few tumour cells in the sample but many lymphoid cells with lymphoid follicles. $CD45RA^+$ naive lymphocytes were massively observed in lymphoid follicles, while $CD45RO^+$ activated/memory lymphocytes were massively observed not only in lymphoid follicles but also in the other lesions (Fig. 2B). These results suggest that PPV induced infiltration of both $CD45RA^+$ and $CD45RO^+$ cells into tumour sites, which in turn resulted in distraction of most of the tumour cells in this patient.

DISCUSSION

No severe adverse events were observed in any of the 10 patients enrolled, although all the patients developed grade 1 or 2 local dermatological reactions at the injection sites. Therefore, in terms of safety, the toxicity of the 12-week regimen of once-weekly PPVs was tolerable and acceptable for patients with MVAC-refractory UC.

With regard to peptide-induced immune reactions, an increase in peptide-specific IFN-γ production in response to at least one of the four vaccinated peptides was observed in most of the post-vaccination PBMCs (eight of nine cases), regardless of the absence ($n = 5$) or reduced levels ($n = 5$) of CTL activity in pre-vaccination PBMCs. Boosted CTL activities in response to all four peptides were seen in the

post-vaccination samples of the patient with complete remission (#5). Similarly, an increase of peptide-specific IgGs was observed in the post-vaccination plasma of most patients (eight of nine cases). There were more than 10-fold ($n = 7$) and 100-fold ($n = 6$) increases of the IgG levels in the post-vaccination samples, suggesting that clonal expansion of peptide-reactive B cells was induced by this regimen.

These results indicated that both the cellular and humoral responses were well boosted in most patients with UC under this regimen. The profile of positive peptides varied greatly from patient to patient, suggesting that the peptides suitable for use in each patient were different, which is consistent with the previously reported results in other types of cancers [11–15]. This would be because of the heterogeneous nature of the different tumours studied and the immunological diversity of the tumour-reactive CTLs in each patient.

Although cellular immunity is the predominant effector arm of antitumour responses, humoral immunity could also play an important role in host defence against cancer cells [25]. However, the mechanism of antibody production against the small vaccination peptides is unclear. One possible explanation is that pre-existing CD4 T helper type 1 cells specific to the vaccinated peptides recognize peptides loaded on HLA-class IA molecules and so facilitate both CTL induction and IgG production. Alternatively, some peptides may bind both class I and class II HLA and induce activation of CTL and T helper type 1 cells [26]. The biological roles of peptide-reactive IgGs will also need to be clarified in the near future.

This is a phase I trial designed to investigate toxicity and immune responses, but a description of the clinical responses could be important for the next stage of clinical trials. The overall response rate defined by radiological imaging is comparable to those seen in previously reported studies using chemotherapy combinations such as gemcitabine and paclitaxel [27,28]. The median survival time of our 10 patients was somewhat shorter than those reported for patients on chemotherapy regimens [27–29], but the four responders to peptide vaccination showed a median survival time of 24 months, suggesting that PPV has the potential to provide long-term survival in some patients with advanced UC.

In this study, we observed massive infiltration of both $CD45RA^+$ and $CD45RO^+$ cells into tumour sites of a PR patient after PPV, whereas they resided around vessels and connective tissues before the vaccination (at the first visit). We previously reported that PPV induced infiltration of $CD45RO^+$ lymphocytes, but neither $CD8^+$ T cells nor $CD20^+$ B cells, in tumour sites of patients with prostate cancer [24]. In considering CD45RO expression in activated or memory T cells and CD45RA expression in naive T cells [30], PPV induced infiltration of both $CD45RA^+$ and $CD45RO^+$ cells into tumour sites, which in turn resulted in destruction of most tumour cells in this patient. Further studies with other patients' samples will be needed to clarify this issue.

The potential efficacy of 12 consecutive weekly vaccinations with PPV in patients with advanced UC merits further investigation based on the safety and boosted immune responses shown herein.

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CONFLICT OF INTEREST

None declared.

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Correspondence: Masanori Noguchi, Clinical Research Division of Research Center for Innovative Cancer Therapy, Kurume University School of Medicine, 67 Asahi-machi, Kurume 830-0011, Japan.
e-mail: noguchi@med.kurume-u.ac.jp

Abbreviations: **UC**, urothelial carcinoma; **MVAC**, methotrexate, vinblastine, adriamycin and cisplatin; **HLA**, human leucocyte antigen; **CTL**, cytotoxic T lymphocyte; **PPV**, personalized peptide vaccination; **PBMC**, peripheral blood mononuclear cell; **IFN-γ**, interferon-γ.

総　説

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がんペプチドワクチンの課題と展望

伊東 恭悟，由谷 茂*

Current Status and Future Perspective of Cancer Vaccine Development

Kyogo ITOH and Shigeru YUTANI*

1. はじめに

がんワクチン療法は，1990年にがん関連抗原同定法が報告されてから多くの研究開発が実施され，我が国ではテーラーメイドペプチドワクチンが高度医療として承認されるまでに至った．企業による治験も進み，今後，3～5年間のうちには医薬品承認される可能性が高く，国民からは第4のがん治療法としての期待が高い．

一方，がんワクチンの安全性に関して不適切な免疫誘導が海外でのがん細胞を用いたワクチン治験で指摘され，また臨床効果を予測するバイオマーカーも未同定であるなど，課題も散見される．更にがん細胞は白血球抗原ロスなどの免疫逃避機構を有するために，ペプチドワクチン療法の限界も明らかになりつつある．

本稿では，今後到来が予想されるペプチドワクチン実用化時代を迎えるにあたって予想される諸課題を取り上げる．

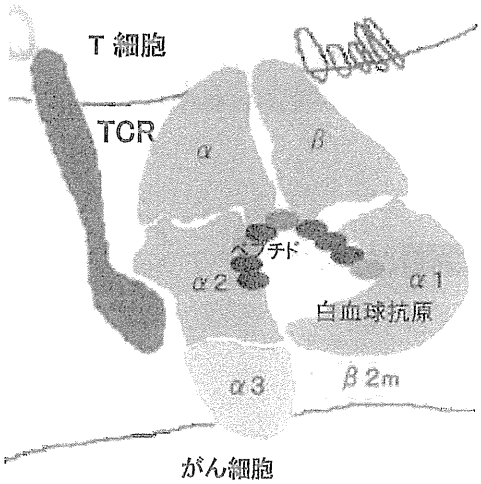
2. ペプチドワクチン療法の原理

免疫学の進歩に伴い，1980年代後半には「がん細胞上の白血球抗原上に結合する9～10個のがん抗原由来のアミノ酸（ペプチド）が患者の免疫系（T細胞）により，がん細胞として認識される」らしいことが予想されるにいたった（Fig. 1上）．しかし，T細胞はどのようにがん細胞のどこを認識するのか？ その抗原は何か？ などについては，まったくの謎であった．

1990年になりBoon博士らが，ヒトのがん関連抗原の同定法を報告し，がんワクチンの世界に大きな飛躍をもたらした[1]．その技術（cDNA expression cloning technique）を用いて次々とヒトがん関連抗原が同定され[2~6]，更に抗体が認識する抗原分子の同定法や，正常細胞とがん細胞のゲノム解析から同定する技術等も相次いで開発され，現在では，がん関連抗原として1000種類以上が同定されている．そのため，がん細胞上の白血球抗原上に結合する9～10個のがん関連抗原由来のアミノ酸（ペプチド）としては，数千にも及ぶことが明らかになっている．それらを大別すると，がん細胞に特異的（遺伝子変異などにより発現）に，もしくは，正常細胞に比べて過剰発現している抗原が大多数をしめる．また，胎児性抗原や精巣のみに発現されている抗原，ウイルス由来抗原などもあげられる（Fig. 1下）．

がん細胞の目印となるがん関連抗原は，がん細胞の中で産生と分解を繰り返している．そのがん関連抗原は分解されると短い蛋白質断片（ペプチド）となるが，その一部は主要組織適合抗原（ヒトではヒト白血球抗原，human leukocyte antigen，HLA）分子に結合して，HLA・ペプチド複合体を形成し，がん細胞の細胞膜表面に提示される（Fig. 1）．そして複合体が，100個以上の数としてまとまって提示された場合にのみ，宿主のT細胞にとって，がん細胞であることの目印になり，認識される．正常細胞では，そのようなHLA・がん関連抗原ペプチ

* 久留米大学　免疫・免疫治療学講座　福岡県久留米市旭町67（〒830-0011）
Department of Immunology and Immunotherapy, Kurume University School of Medicine 67 Asahimachi, Kurume-shi, Hukuoka 830-0011, Japan



がん拒絶抗原（1990年に発見）とその応用
癌細胞上の白血球抗原上に結合する9個のがん抗原由来のアミノ酸が患者の免疫系（T細胞や抗体）によりがんとして認識される．がん拒絶抗原：①腫瘍細胞に過剰発現している抗原，②胎児性抗原，③変異蛋白，④ウイルス抗原など

Fig. 1　T細胞のがん細胞認識

ド複合体の数は100個以下であるために，T細胞により認識されることはない．更に，がん細胞上のHLA-クラスI分子は，免疫逃避していない場合には，正常細胞と同様に10,000個以上あるために，少なくとも100個の異なるがん抗原ペプチドを提示できると想定されている．

哺乳類では，細胞内での非自己としての危険シグナルをこのような仕組みで宿主免疫系（T細胞）に伝えることができるために，免疫系による非自己細胞の排除が成立している．即ち，宿主（T細胞）は，がん細胞とそうでない正常細胞を，T細胞抗原受容体を介して識別している．T細胞が非自己として認識した場合には，速やかに増殖・分化（活性化，賦活化）する．活性化T細胞は，がん細胞を殺傷し（主にキラーT細胞），また抗体を産生させるシグナルをB細胞に指令する（主にヘルパーT細胞）と共に，各種サイトカインを産生し（キラーT及びヘルパーT細胞），マクロファージ，NK細胞をも活性化する．活性化したT細胞は，最短で8時間で2倍に増殖できるために，短期間の間に何十万倍にも増殖できる（クローン増殖）．

ペプチドワクチンは，上記の免疫の仕組みを活用したものといえる（Fig. 2）．まず，①がん細胞の目印になる分子（ペプチド）を化学合成して，添加剤と一緒にして，がん患者へ投与する．②免疫担当細胞のうち，抗原提示細胞（樹状細胞，dendritic cell，DC）が捕縛して，局所リンパ節に運び込み，そこで，HLA・ペプチド複合体をT細胞に提示する．③リンパ節に流入してきたT細胞はT細胞抗原受容体を介して，HLA・ペプチド複合体と結合し，特異的に結合可能な抗原エピトープ（数個のアミノ酸から構成）が100個以上提示されている場合に限って，活性化のシグナルを，CD3抗原を介して細胞内に伝達する．④ペプチド特異的に活性化されたT細胞は，リンパ流を経て全身をめぐり，同じHLA・ペプチド複合体を提示しているがん細胞に遭遇した場合には，速やかに攻撃して排除する．

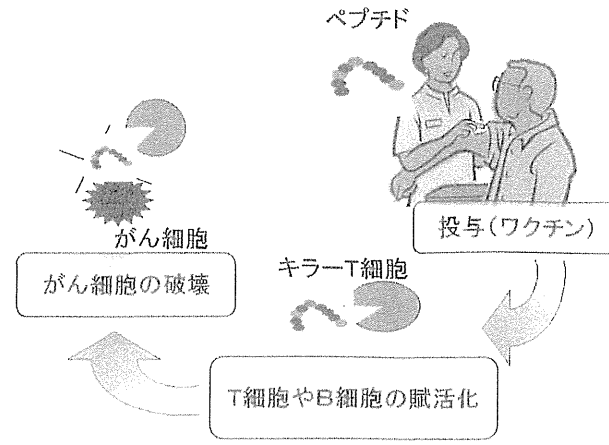


Fig. 2　ペプチドワクチンの原理

3.　ペプチドワクチン療法の特徴と限界

新しい治療法を説明する場合には，その特徴と限界を正しく理解してもらうことが肝要と思われる．特徴としては，ワクチン分子としての最小単位であるために抗原特異性が高いことや，T細胞活性化には最も効率がよいこと，正常細胞への悪影響などの副作用が少ないことがあげられる．実用化の観点からは，ペプチド合成の費用が比較的安価であることも利点としてあげられる．

一方，限界としては，まずHLAのタイプ毎に違うペプチドワクチンを準備しないといけないことや，単一の抗原エピトープであるために，複数のT細胞を活性化するには，複数のペプチドワクチンを必要とすること等があげられる．また，著者らが開発中の患者個々人の2次免疫反応を重視し患者毎に異なるペプチドを投与するテーラーメイドペプチドワクチン（ワクチン候補プールのなかから投与前の血液中に抗体陽性のペプチドのみを投与する）[7~9]以外は，同一のペプチドワクチンがすべての症例に投与されるために，1次免疫反応からがん免疫が誘導される症例と2次免疫反応から誘導される症例が混在する．そのため，免疫増強が遅延する場合（前者の症例）と早期からの免疫誘導が惹起される症例（後者の症例）が混在するために，開始後しばらくは，コントロール群に比して臨床効果の差異が認められないという短所も存在する．

最も大きな限界は，ペプチドワクチンに限らずがんワ