

# Personalized peptide vaccination

## A novel immunotherapeutic approach for advanced cancer

Tetsuro Sasada,<sup>1\*</sup> Masanori Noguchi,<sup>2</sup> Akira Yamada<sup>2</sup> and Kyogo Itoh<sup>1</sup>

<sup>1</sup>Department of Immunology and Immunotherapy; Kurume University School of Medicine; Kurume, Japan; <sup>2</sup>Research Center for Innovative Cancer Therapy; Kurume University; Kurume, Japan

Since both tumor cells and immune cell repertoires are diverse and heterogeneous, immune responses against tumor-associated antigens might be substantially different among individual patients. Personalized selection of right peptides for individuals could thus be an appropriate strategy for cancer vaccines. We have developed a novel immunotherapeutic approach, personalized peptide vaccination (PPV), in which HLA-matched peptides are selected and administered, based on the pre-existing host immunity before vaccination. Recent clinical trials of PPV have demonstrated a feasibility of this new therapeutic approach in various types of advanced cancers. For example, a randomized phase II trial for patients with castration resistant prostate cancer showed a possible clinical benefit in the PPV group. In the patients undergoing PPV, lymphocyte counts, increased IgG responses to the vaccine peptides, and inflammatory factors in pre-vaccination peripheral blood might be potential biomarkers for prognosis. Further randomized phase III trials would be recommended to prove clinical benefits of PPV.

### Introduction

The field of cancer immunotherapy has drastically moved forward during these two decades since Boon and his colleagues reported for the first time a tumor-associated antigen, MAGE-A1, recognized by cytotoxic T lymphocyte (CTL) in 1991.<sup>1</sup> In particular, there have recently been noteworthy advances in the clinical

application of cancer immunotherapy.<sup>2,3</sup> In 2010, sipuleucel-T (Provenge; Dendreon Corporation), an autologous cellular immunotherapy product designed to stimulate T cell immune responses against human prostatic acid phosphatase (PAP), was first approved for patients with castration-resistant prostate cancer (CRPC) by the US Food and Drug Administration (FDA).<sup>4</sup> In addition, another immunotherapeutic agent, ipilimumab, an anti-cytotoxic T lymphocyte antigen (CTLA)-4 monoclonal antibody, was also approved for melanoma patients by the FDA in 2011.<sup>5</sup> Despite these significant advances, however, most of other randomized clinical trials in cancer immunotherapy have so far failed to show beneficial therapeutic effects compared with existing treatments.<sup>6,7</sup> The failure of recent clinical trials has raised several issues to be addressed for development of cancer vaccines. Here, we have proposed a novel immunotherapeutic approach, "personalized peptide vaccination (PPV)" for advanced cancer patients.

### Rationale for Personalized Selection of Vaccine Antigens in Individual Cancer Patients

A large number of tumor-associated antigens have been identified by several different approaches, including cDNA expression cloning, serologic analysis of recombinant cDNA expression libraries (SEREX), and reverse immunological approach.<sup>8</sup> Although the number of cancer vaccine candidates is becoming almost limitless, antigens currently employed

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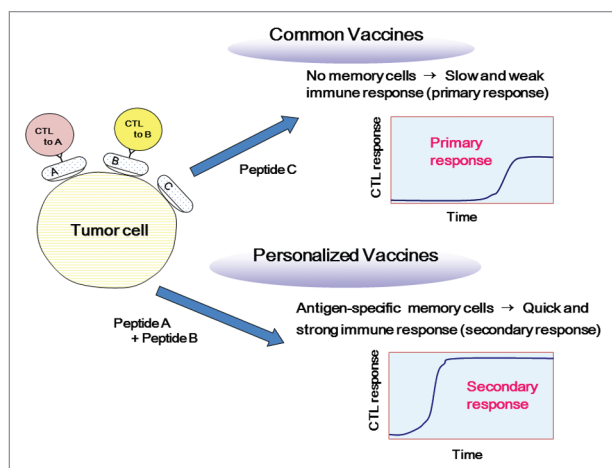
**Abbreviations:** PPV, personalized peptide vaccination; CTL, cytotoxic T lymphocytes; CRPC, castration-resistant prostate cancer; FDA, food and drug administration; MST, median survival time; HR, hazard ratio; CI, confidence interval

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\*Correspondence to: Tetsuro Sasada;  
Email: [tsasada@med.kurume-u.ac.jp](mailto:tsasada@med.kurume-u.ac.jp)



**Figure 1.** Personalized vaccines are more promising than common vaccines. Personalized antigens can induce quick and strong secondary immune responses, whereas common antigens without immunological memory induce slow and weak primary immune responses.

for vaccination against individual cancer patients might not always be appropriate. In general, anti-tumor immunity is known to be dependent on both immunological characters of tumor cells and immune cell repertoires. Since immune cell repertoires are quite diverse and heterogeneous, anti-tumor immunity might be substantially different among individuals. Therefore, it is likely that vaccine antigens that are selected and administered without considering the immune cell repertoires of the hosts could not efficiently induce beneficial anti-tumor immune responses. To increase the clinical benefits from cancer vaccines, particular attentions should be paid to immunological status of each patient by characterizing the pre-existing immune responses to vaccine antigens before vaccination.

Nevertheless, in most of current clinical trials of therapeutic cancer vaccines, common antigens are employed for vaccination independently of immunological status of patients. Patients, who have immunological memory to vaccine antigens, are expected to show quick and strong immune responses to them. In contrast, patients with no immunological memory against vaccine antigens would take more time for development of effective anti-tumor immune responses, because several rounds of repeated vaccinations might be required to prime antigen-specific naive T cells to functional effector cells (Fig. 1). In such situations, vaccinations could not

easily provide clinical benefits, especially in advanced cancer patients, who show a relatively quick disease progression. Moreover, immune responses induced by inadequate vaccines that are non-specific to tumor cells may not only be ineffective for tumor control, but also erode pre-existing immunity.<sup>9</sup> Based on the current paradigm that the size and composition of the adaptive immune system are limited and that individual immune cells are constantly competing each other in the limited space, inadequate vaccination may have negative consequences for the hosts by suppressing pre-existing beneficial memory cells specific to tumors and/or infections, which might result in acceleration of cancer progression or early death in vaccinated patients.<sup>10</sup> Considering these issues, it would be quite reasonable that vaccine antigens should be selected based on the pre-existing immunological status in each patient.

In addition, it should be noted that cancer cells possess or develop a variety of mechanisms to maintain their malignant behavior. For example, it has been well recognized that cancer cells escape from host immunological surveillance.<sup>11</sup> Through the interaction between host immune system and tumor cells at the equilibrium phase, immunological pressure often produces tumor cell variants that decrease or lose tumor-associated antigens. Therefore, to better control cancer cells, it would be recommended to

target multiple tumor-associated antigens to reduce the risk of outgrowth of antigen-loss variants.

### PPV as a Novel Immunotherapeutic Approach

In view of complexity and diversity of immunological characters of tumors and immune cell repertoires, we have developed a new concept of PPV.<sup>12</sup> In this “personalized” cancer vaccine formulation, appropriate peptide antigens for vaccination are screened and selected from a list of vaccine candidates in each patient, based on pre-existing host immunity. Currently, we employ 31 HLA class I-restricted peptide candidates, which were identified from a variety of tumor-associated antigens mainly through cDNA expression cloning method with tumor-infiltrating lymphocyte clones/lines; 12 peptides for HLA-A2, 14 peptides for HLA-A24, 9 peptides for HLA-A3 supertype (A3, A11, A31 or A33), and 4 peptides for HLA-A26. The safety and potential immunological effects of these vaccine candidates have been shown in previously conducted clinical studies.<sup>12-14</sup> A maximum of 4 peptides, which are selected based on the results of HLA typing and the pre-existing immune responses specific to each of the 31 different vaccine candidates, are subcutaneously administered in complex with incomplete Freund’s adjuvant weekly or bi-weekly.

Currently, we evaluate the pre-existing immune responses to vaccine candidates by B cell responses, but not by T cell responses, since the performance characteristics, such as sensitivity and reproducibility, of current T cell assays are unsatisfactory.<sup>3,15</sup> In contrast to these drawbacks inherent to T cell assays, B cell assays have more potential for screening and/or monitoring antigen-specific immune responses even to MHC class I-restricted peptides. Indeed, we have recently published several papers describing the clear correlations between clinical benefits and antigen-specific B cell responses measured by IgG antibody production in patient plasma after vaccination.<sup>16</sup> Notably, the multiplex bead-based LUMINEX technology that we have developed for monitoring B cell

**Table 1.** Clinical responses of advanced cancer patients treated with PPV

	Patient (n)	Evaluable patient (n)	Best clinical response (n)			Response rate (%)	Disease control rate (%)
			PR	SD	PD		
Total	500	436	43	144	249	9.9	42.9
Prostatic	174	155	29	36	90	18.7	41.9
Colorectal	74	68	1	23	44	1.5	35.3
Pancreatic	50	41	4	23	14	9.8	65.9
Gastric	42	35	0	8	27	0	22.9
Brain	33	30	5	11	14	16.7	53.3
Cervical	28	23	3	7	13	13.0	43.5
Non-small cell lung	22	21	0	11	10	0	52.4
Renal cell	13	12	0	9	3	0	75.0
Melanoma	12	11	0	5	6	0	45.5
Breast	11	10	0	1	9	0	10.0
Uroepithelial	10	7	1	2	4	14.3	42.9
Others	31	23	0	8	15	0	34.8

Best clinical responses were evaluated by RECIST criteria (or PSA values in prostatic cancer). PR, partial response; SD, stable disease; PD, progressive disease.

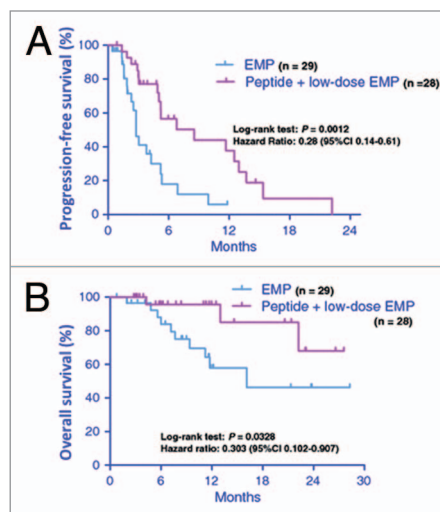
responses allows simple, quick and highly reproducible high-throughput screening of IgG responses specific to large numbers of peptide antigens with a tiny amount of plasma.<sup>17</sup>

In the clinical trials of PPV conducted during the past several years, we have shown promising results in various types of cancers.<sup>12,13,16,18,19</sup> Table 1 shows the clinical responses in 500 advanced cancer patients who received PPV from October 2000 to October 2008.<sup>16</sup> The best clinical response assessed in 436 evaluable patients were partial response (PR) in 43 patients (10%), stable disease (SD) in 144 patients (33%) and progressive disease (PD) in 249 patients (57%), with a median overall survival of 9.9 mo. Of note, as shown in Figure 2, a recently conducted phase II randomized clinical trial of PPV for 57 CRPC patients demonstrated that patients receiving PPV in combination with low-dose estramustine phosphate (EMP) showed a significantly longer progression-free [median survival time (MST), 8.5 vs. 2.8 mo; hazard ratio (HR), 0.28 (95% confidence interval (CI), 0.14–0.61);  $p = 0.0012$ ] and overall survival [MST, undefined vs. 16.1 mo; HR, 0.30 (95% CI, 0.10–0.91);  $p = 0.0328$ ] than those receiving standard-dose EMP alone.<sup>18</sup> In addition, PPV was also conducted in an early phase clinical trial of patients with

recurrent or progressive glioblastoma multiforme, one of the most aggressive brain tumors, with median overall survival of 10.6 mo.<sup>19</sup> Based on these promising results, randomized phase III trials are currently underway in CRPC and glioblastoma. To prove clinical benefits of PPV for accelerating cancer vaccine development, further randomized phase III trials would also be recommended in other different types of cancers.

### Lymphocyte Counts, Increased Humoral Responses to the Vaccine Antigens, and Inflammatory Factors as a Biomarker for PPV

Only a subset of patients show clinical benefits from cancer immunotherapy, including peptide-based cancer vaccines. In addition, even worse, some large clinical trials in the past several years



**Figure 2.** Progression-free and overall survival in patients with castration-resistant prostate cancer using personalized peptide vaccination. Kaplan-Meier curves of progression-free (A) and overall survival (B) in patients treated with personalized peptide vaccination plus low-dose estramustine phosphate (EMP) or standard-dose EMP. Adapted from Noguchi et al.<sup>18</sup>

have demonstrated that cancer vaccines might sometimes show worse clinical outcomes.<sup>6,7</sup> Therefore, it would be critical to identify biomarkers that accurately portray anti-tumor immune responses and predict prognosis in treated patients.<sup>3,6</sup> With regard to post-vaccination biomarkers, several factors, including CTL responses, Th1 responses, delayed-type hypersensitivity (DTH) and autoimmunity, have been reported to be associated with clinical responses in some clinical trials.<sup>20-23</sup> However, as they have not been always reproducible in other studies, there are currently no validated prognostic or predictive biomarkers in widespread use.

We also investigated immunological biomarkers in 500 advanced cancer patients who received PPV from October 2000 to October 2008.<sup>16</sup> By the statistical analysis in this patient population, both lymphocyte counts prior to the vaccination ( $p = 0.0095$ ) and increased IgG responses ( $p = 0.0116$ ) to the vaccine peptides, along with performance status ( $p < 0.0001$ ), were well correlated with overall survival.

To identify biomarkers useful for selecting appropriate patients before vaccination, we further addressed pre-vaccination prognostic markers in patients with several different types of advanced cancers who underwent PPV. In CRPC treated with PPV ( $n = 40$ ), a comprehensive study of soluble factors and gene expression profiles by microarray analysis demonstrated that higher IL-6 level and granulocytic myeloid-derived suppressor cells (MDSC) in the peripheral blood before vaccination were closely associated with poorer prognosis.<sup>24</sup> In patients with refractory non-small cell lung cancer ( $n = 41$ ), multivariate Cox regression analyses showed that higher C-reactive protein (CRP) level before vaccination was a significant predictor of unfavorable overall survival (HR = 10.115, 95% CI = 2.447–41.806,  $p = 0.001$ ).<sup>25</sup> In addition, in refractory biliary tract cancer patients ( $n = 25$ ), higher IL-6 and lower albumin levels before vaccination were significantly unfavorable factors for overall survival [HR = 1.123, 95% CI = 1.008–1.252,  $p = 0.035$ ; HR = 0.158, 95% CI = 0.029–0.860,  $p = 0.033$ ; respectively].<sup>26</sup> Collectively, these findings have demonstrated that less inflammation

may contribute to better responses to PPV, suggesting that evaluation of the inflammatory factors before vaccination could be useful for selecting appropriate cancer patients for PPV. Based on these findings, an early phase clinical trial is currently underway to show whether the blockage of IL-6-mediated inflammatory signaling with a humanized anti-IL-6 receptor monoclonal antibody, tocilizumab, would be beneficial for enhancing the immune and/or clinical responses of PPV.<sup>27</sup>

## Conclusions

The field of cancer immunotherapy has drastically moved forward during the past 20 years, but there have been several issues to be addressed for success of cancer vaccine development. In view of complexity and diversity of immunological characters of tumors and immune cell repertoires, we have developed a new concept of PPV. In the clinical trials conducted during the past several years, we have shown promising results of PPV as a new treatment modality for patients with various types of advanced cancers. Further randomized phase III clinical trials would be essential to prove clinical benefits of PPV. In addition, novel biomarkers for selecting patients who would most benefit from PPV remain to be identified.

## Disclosure of Potential Conflicts of Interest

The authors have no conflict of interest and financial relationships to disclose.

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