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A phase II trial of personalized peptide vaccination in castration-resistant prostate cancer patients: prolongation of prostate-specific antigen doubling time

Masanori Noguchi[1,2*], Fukuko Moriya[2], Shigetaka Suekane[2], Rei Ohnishi[2], Satoko Matsueda[3], Tetsuro Sasada[3], Akira Yamada[4] and Kyogo Itoh[3]

Abstract

Background: Cancer vaccine is one of the attractive treatment modalities for patients with castration-resistant prostate cancer (CRPC). However, because of delayed immune responses, its clinical benefits, besides for overall survival (OS), are not well captured by the World Health Organization (WHO) and Response Evaluation Criteria in Solid Tumors (RECIST) criteria. Several surrogate markers for evaluation of cancer vaccine, including prostate-specific antigen doubling time (PSADT), are currently sought. The purpose of this study was to assess prospectively the PSA kinetics and immune responses, as well as the efficacy, safety, and biomarkers of personalized peptide vaccination (PPV) in progressive CRPC.

Methods: One hundred patients with progressive CRPC were treated with PPV using 2–4 positive peptides from 31 candidate peptides determined by both human leukocyte antigen (HLA) class IA types and the levels of immunoglobulin G (IgG) against each peptide. The association between immune responses and PSADT as well as overall survival (OS) was studied.

Results: PPV was safe and well tolerated in all patients with a median survival time of 18.8 months. Peptide-specific IgG and T-cell responses strongly correlated with PSADT ($p < 0.0001$ and $p = 0.0007$, respectively), which in turn showed correlation with OS ($p = 0.018$). Positive IgG responses and prolongation of PSADT during PPV were also significantly associated with OS ($p = 0.001$ and $p = 0.004$) by multivariate analysis.

Conclusions: PSADT could be an appropriate surrogate marker for evaluation of the clinical benefit of cancer vaccine. Further randomized trials are needed to confirm these results.

Trial registration: UMIN000001850

Keywords: Prostate-specific antigen doubling time, Personalized peptide vaccine, Prostate cancer, Surrogate marker, Overall survival

Background

Changes in serum prostate-specific antigen (PSA) can reflect the burden of disease and clinical benefit in patients with castration-resistant prostate cancer (CRPC) with cytotoxic chemotherapy or hormonal agents known to kill tumor cells; these changes can have practical utility by providing and updating prognostic information on an individual patient over time [1-4]. As observed in many clinical trials, however, immunotherapy can induce novel patterns of antitumor responses distinct from those of chemotherapy [5]. For example, an autologous dendritic-cell-based vaccine (sipuleucel-T) is known to improve survival without having an impact on early PSA decline [6], whereas docetaxel's improvement in overall survival (OS) correlates for the most part with a PSA decline within the first 3 months of therapy [7,8]. Thus, interpreting PSA decline in the context of novel immunotherapy must be

* Correspondence: noguchi@med.kurume-u.ac.jp
[1]Clinical Research Division of the Research Center for Innovative Cancer Therapy, Kurume University School of Medicine, 67 Asahi-machi, Kurume 830-0011, Japan
[2]Departments of Urology, Kurume University School of Medicine, Kurume, Japan
Full list of author information is available at the end of the article

carried out with caution on the basis of the mechanism of action, and may also depend on the time of sampling [9].

Personalized peptide vaccine (PPV) uses multiple peptides based on the pre-existing immunity. Under PPV treatment, each patient with human leukocyte antigen (HLA)-class IA types positive was tested for their immunological reactivity to 31 different peptides capable of inducing T-cell responses. The 31 peptides were derived from a number of tumor associated antigens: PSA, prostatic acid phosphatase (PAP), prostate-specific membrane antigen (PSMA), multidrug resistance protein and a variety of other epithelial tumor antigens. We previously demonstrated that PPV was safe and improved OS with immune responses in phase I, I/II, and II clinical trials in patients with CRPC [10-16]. However, it was not addressed whether PSADT could be an appropriate surrogate marker for evaluation of the clinical benefit of cancer vaccine. To address this, we evaluated data from a phase II clinical trial for CRPC using PPV.

Methods

Patient Eligibility

Eligibility required a histological diagnosis of prostate adenocarcinoma and progressive disease (PD) defined as at least two consecutive increases in PSA, new metastatic lesion on radionuclide bone scan, or progressive tumor lesions on cross-sectional imaging, despite adequate androgen ablative therapy. Patients showed positive IgG

Table 1 Peptide candidates for personalized peptide vaccination

Symbol for peptide	Origin protein	Position of peptide	Amino acid sequence	HLA type
CypB-129	Cyclophilin B	129-138	V	A2,A3sup[a]
Lck-246	p56 lck	246-254	KLVERLGAA	A2
Lck-422	p56 lck	422-430	DVWSFGILL	A2,A3sup
MAP-432	ppMAPkkk	432-440	DLLSHAFFA	A2,A26
WHSC2-103	WHSC2	103-111	ASLDSDPWV	A2,A3sup[a],A26
HNRPL-501	HNRPL	501-510	NVLHFFNAPL	A2,A26
UBE-43	UBE2V	43-51	RLQEWCSVI	A2
UBE-85	UBE2V	85-93	LIADFLSGL	A2
WHSC2-141	WHSC2	141-149	ILGELREKV	A2
HNRPL-140	HNRPL	140-148	ALVEFEDVL	A2
SART3-302	SART3	302-317	LLQAEAPRL	A2
SART3-309	SART3	309-317	RLAEYQAYI	A2
SART2-93	SART2	93-101	DYSARWNEI	A24
SART3-109	SART3	109-118	VYDYNCHVDL	A24,A3sup[a],A26
Lck-208	p56 lck	208-216	HYTNASDGL	A24
PAP-213	PAP	213-221	LYCESVHNF	A24
PSA-248	PSA	248-257	HYRKWIKDTI	A24
EGFR-800	EGF-R	800-809	DYVREHKDNI	A24
MRP3-503	MRP3	503-511	LYAWEPSFL	A24
MRP3-1293	MRP3	1293-1302	NYSVRYRPGL	A24
SART2-161	SART2	161-169	AYDFLYNYL	A24
Lck-486	p56 lck	486-494	TFDYLRSVL	A24
Lck-488	p56 lck	488-497	DYLRSVLEDF	A24
PSMA-624	PSMA	624-632	TYSVSFDSL	A24
EZH2-735	EZH2	735-743	KYVGIEREM	A24
PTHrP-102	PTHrP	102-111	RYLTQETNKV	A24
SART3-511	SART3	511-519	WLEYYNLER	A3sup[a]
SART3-734	SART3	734-742	QIRPIFSNR	A3sup[a]
Lck-90	p56 lck	90-99	ILEQSGEWWK	A3sup[a]
Lck-449	p56 lck	449-458	VIQNLERGYR	A3sup[a]
PAP-248	PAP	248-257	GIHKQKEKSR	A3sup[a]

[a]A3sup, HLA-A3 supertype (A3, A11, A31, and A33).

Table 2 Patient characteristics

Characteristics	Patients (N = 100) No.
Age, years	
Median	69
Range	51-92
ECOG performance status	
0	91
1	9
HLA typing	
A24	66
A2	21
A3 supertype	11
A26	2
Baseline PSA, ng/ml	
Median	29.8
Range	0.2-2481
PSADT, months	
Median	2
Range	0.3-36+
Lymphocyte, 1300/μL	
Low	41
High	59
CRP, 3 μg/mL	
Low	53
High	47
SAA, 8 μg/mL	
Low	27
High	76
IL6, 2.4 pg/mL	
Low	84
High	16
Gleason score	
≤7	34
≥8	57
Unknown	9
Site of metastasis	
no	14
Bone only	33
Bone and nodal/organ	40
Nodal/organ	13
Prior chemotherapy	
(-)	60
(+)	40

Abbreviations: PPV, personalized peptide vaccination; ECOG, Eastern Cooperative Oncology Group; HLA, human leucocyte antigen; PSA, prostate-specific antigen; PSADT, PSA doubling time; CRP, C-reactive protein; SAA, serum amyloid A; IL6, interleukin 6.

responses to at least two of the 31 different candidate peptides (Table 1). Any number of previous hormonal therapies was allowed. Patients were required to wait at least four weeks for entry into the study after the completion of prior radiation therapy, chemotherapy, or a change in hormonal therapy. Other inclusion criteria included age ≥ 20 years; Eastern Cooperative Oncology Group (ECOG) performance status 0 or 1; life expectancy of at least 12 weeks; positive status for HLA-A2, -A24, -A3 supertype (-A3, -A11, -A31, and -A33), or -A26; adequate hematologic, hepatic, and renal function; and negative status for hepatitis virus B and C. Exclusion criteria included an acute infection; a history of severe allergic reactions; pulmonary, cardiac, or other systemic diseases; and other inappropriate conditions for enrollment as judged by clinicians.

Study design and treatment

This was a single institution, single arm, open-label, phase II study. The endpoints of this study were primarily safety and feasibility of PPV in patients with CRPC. Secondary endpoints were to assess the PSA kinetics and immune responses. In addition, we identified potential factors for predicting OS and selecting suitable patients for this treatment. This study protocol was approved by Kurume University Ethical Committee. Written informed consent was obtained from all patients before any study procedures.

In this study, 31 peptides, whose safety and immunological effects had been confirmed in previously conducted clinical studies [10-18], were employed for vaccination [12 peptides for HLA-A2, 14 peptides for HLA-A24, 9 peptides for the HLA-A3 supertype (A3, A11, A31, or A33), and 4 peptides for HLA-A26] (Table 1). All peptides were prepared under conditions of Good Manufacturing Practice using a Multiple Peptide System (San Diego, CA). The selection of 2 to 4 peptides for vaccination to each patient was based on HLA typing and high titer level of peptide-specific IgG to candidate peptides. Each of the selected peptides was mixed with incomplete Freund's adjuvant (Montanide ISA-51VG; Seppic, Paris, France) and emulsified in the 5 ml plastic syringe, and a maximum of four peptides of 1.5 ml emulsion (3 mg/peptide) were injected subcutaneously into the lateral thigh area once a week for 6 weeks. The

peptides were re-selected according to peptide-specific IgG levels at every cycle of 6 vaccinations and administered at 2-, 3-, or 4-week intervals until withdrawal of consent or unacceptable toxicity.

Assessment of clinical activity

Patients were monitored at each visit by history and physical examinations. Serum PSA test and routine laboratory studies were performed every 6 vaccinations for any adverse effects. Toxicity was graded according to the National Cancer Institute Common Terminology Criteria for Adverse Events version 3.0 (NCI-CTCAE Ver3).

All patients underwent relevant radiologic studies and bone scans every 6 months or at the progression of symptoms. PD was defined as radiographic progression evaluated by Response Evaluation Criteria in Solid Tumors (RECIST) criteria [19] or clinical progression.

To assess the PSA response for each patient, percent PSA change from baseline was calculated for each phase of the study (pre- and during vaccination). In addition, PSA doubling time (PSADT) was calculated using all serum PSA values for a specified period, and using a minimum of three PSA values by the formula $\log_2/b$, where b denotes the least square estimate of the linear regression model of the log-transformed PSA values on time. For analytical purposes, negative PSADT estimates and high positive PSADT estimates (>36 months) were censored at 36 months.

To investigate biomarkers for OS that may allow patient selection and prediction of a response to PPV, serum amyloid A (SAA), C-reactive protein (CRP), and interleukin (IL)-6 in plasma at baseline were additionally examined by enzyme-linked immunosorbent assay (ELISA), respectively.

Measurement of humoral and T-cell responses specific to the vaccinated peptides

To study the humoral responses specific to the vaccinated peptides, peptide-specific IgG levels were measured by a Luminex system (Luminex, Austin, TX), as reported previously [20]. If the total titers of selected peptide-specific IgG in any cycles of post-vaccination plasma were more than 2-fold higher than those in the pre-vaccination plasma, the changes were considered to be a positive response.

Although T-cell subsets using flowcytometry was not analyzed in this study, T-cell responses specific to the vaccinated peptides were evaluated by IFN-γ ELISPOT assay using peripheral blood mononuclear cells (PBMCs), as reported previously [18]. Peptide-specific T-cell responses were evaluated by the differences between the numbers of spots per 10^5 x PBMCs in response to the vaccine peptides and those to the control peptide at pre- and 6th vaccination; at least 2-fold more spots at the 6th vaccination than at pre-vaccination was considered positive.

Statistical analysis

All patients who received more than 6 vaccinations were considered evaluable for tumor response, and all patients entered were included in the survival analysis. Data were

Table 3 Adverse events during peptide vaccination

	Grade 1	Grade 2	Grade 3	Total
Injection site reaction	73	24	13	43
Constitutional symptoms				
Bone pain	16	14	13	43
Appetite loss	29	5	1	35
Fatigue	23	11	0	34
Edema peripheral	10	3	0	10
Blood/bone marrow				
Lymphocytopenia	17	13	5	35
Anemia	7	7	16	30
White blood cell count decreased	6	6	5	17
Laboratory				
Hypoalbuminemia	27	13	0	40
ALP increased	20	8	6	34
AST increased	24	4	1	29
Hyponatremia	24	1	0	25
ALT increased	13	2	1	16
Blood triglycerides increased	10	2	0	12
Creatinine increased	6	1	2	8

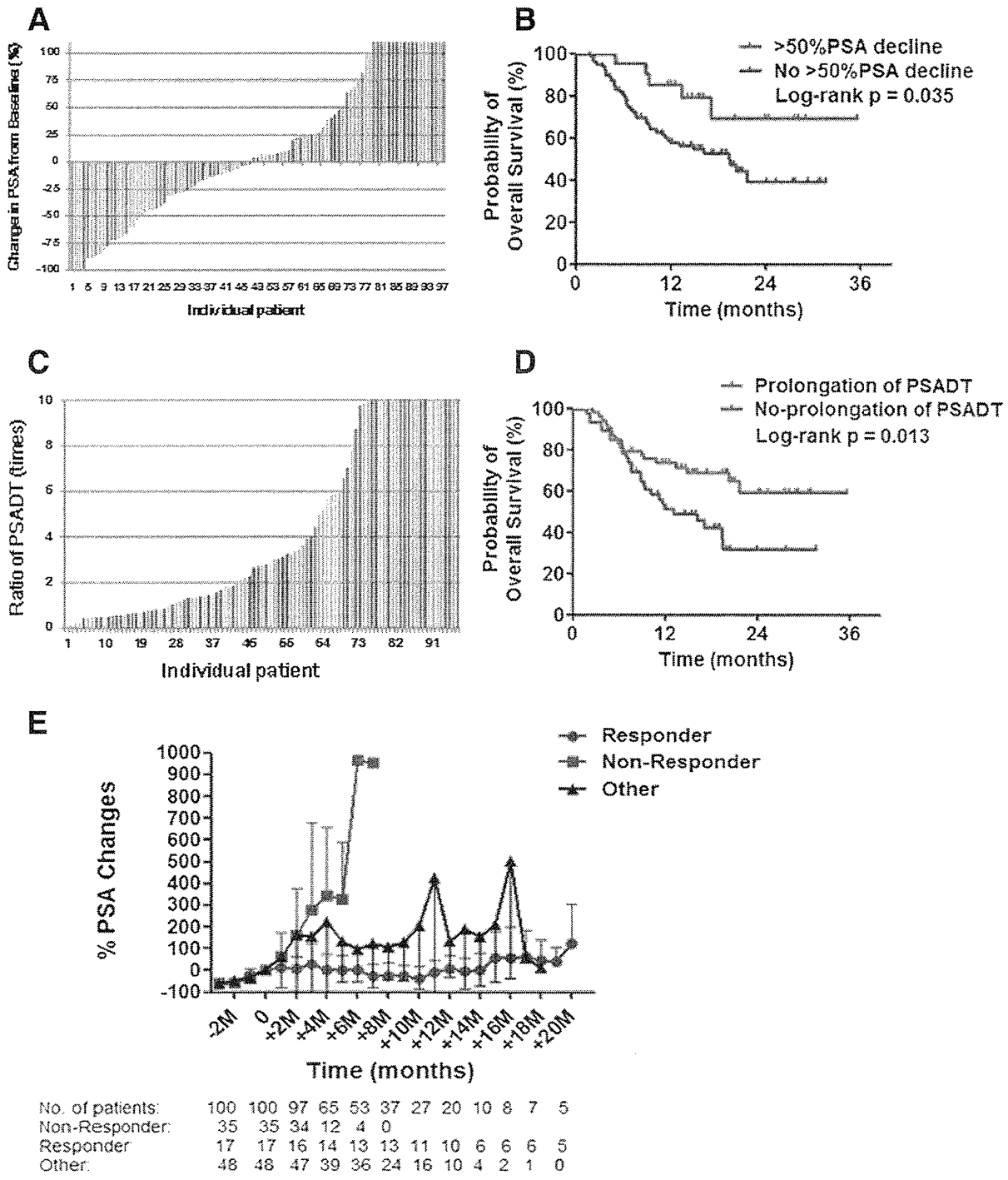

Figure 1 PSA kinetics and overall survival. **(A)** Waterfall plot showing the maximal PSA changes (%) from baseline during personalized peptide vaccination (PPV) at any time point. **(B)** Overall survival by >50% PSA decline. **(C)** The ratio of PSADT changes for each patient pre- and during PPV is plotted. The ratio of PSADT changes was calculated by dividing PSADT during treatment by pre-treatment PSADT. A ratio greater than 2 indicates prolongation of PSADT. **(D)** Overall survival by prolongation of PSDT. **(E)** Longitudinal average PSA changes (%) before and during PPV. Green histograms: Responder group (alive for more than 20 months). Red histograms: Non-responder group (death within 12 months). Gray histograms: Other group.

analyzed at the end of November, 2012 using commercially available computer software. The Student's t-test and the chi-square test were used to compare quantitative and categorical variables, respectively. Survival was calculated from the date of first treatment until the date of any cause of death. Patients lost to follow-up were censored at the last known date of survival. The Kaplan-Meier method was used to estimate actuarial survival curves, and groups were compared using a log-rank test. Cox proportional hazards regression model was used for univariate and multivariate analyses to identify factors that had a significant impact on survival. All baseline parameters in the survival and proportional hazards regression analysis were analyzed as dichotomous variables using median or cut-off values. A two-sided significance level of 5% was considered statistically significant.

Results

Characteristics of the patients

Between April 2009 and August 2011, 100 patients with CRPC were enrolled in this trial at Kurume University Hospital. All 100 patients received at least one vaccination with a median of 16 vaccinations (range, 1 to 40) and were included in the safety assessment and survival analysis. Three patients did not complete 6 vaccinations (1 cycle) and were excluded from the assessment of PSA response and immune responses. The reason for these failures to complete 6 vaccinations was withdrawal of consent. The

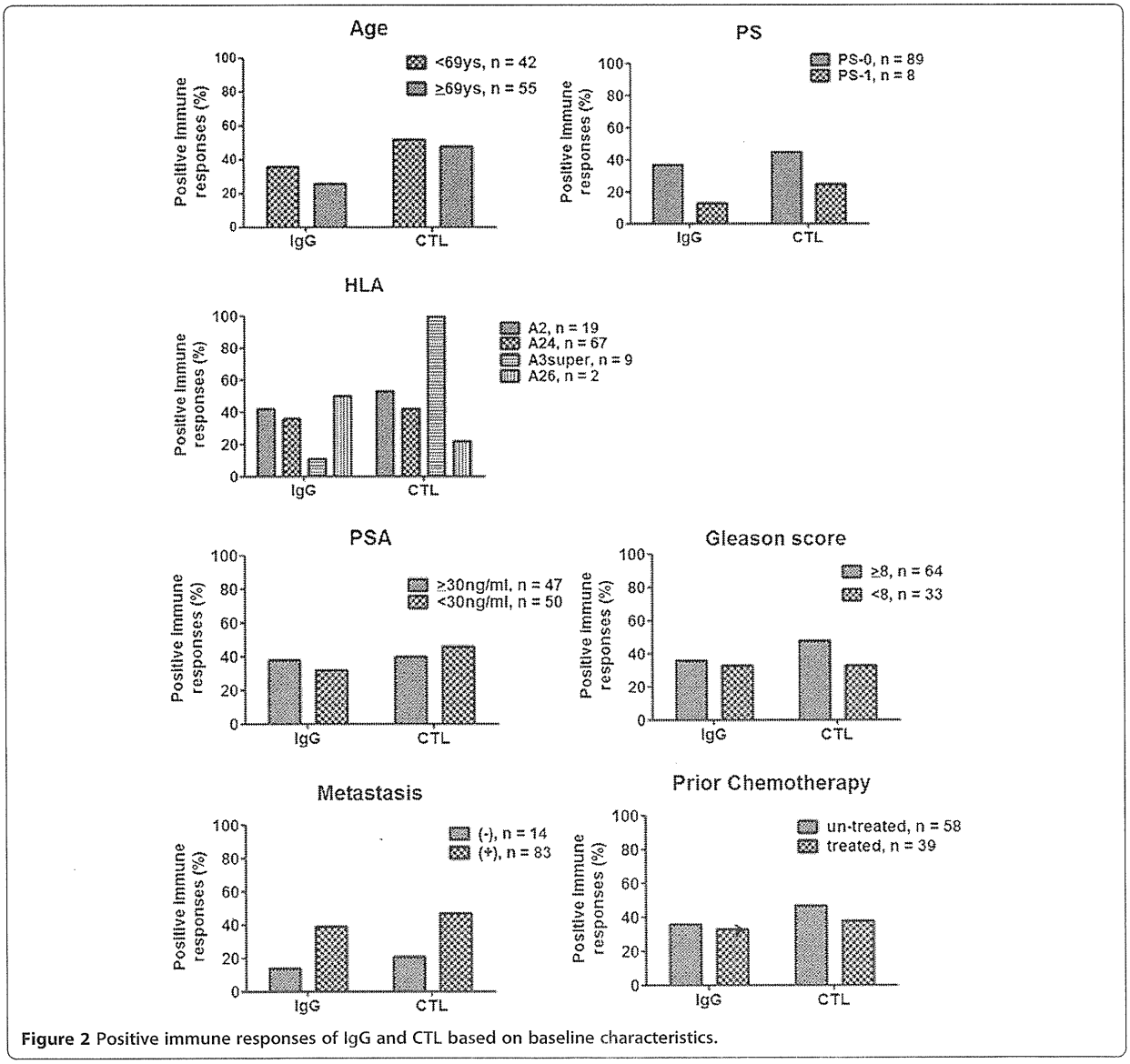

Figure 2 Positive immune responses of IgG and CTL based on baseline characteristics.

median age of participants was 69 years (range, 51 to 92 years), and the ECOG performance status was 0 in 91of the patients and 1 in the remaining 9. The median PSA and pre-vaccination PSADT at the entry to the study was 29.8 ng/ml (range, 0.2 to 2481 ng/ml) and 2 months (range, 0.3 to 36+ months), respectively. Fifty-seven patients had a Gleason score of ≥ 8 and 86 patients had metastasis. All patients had experienced progression after androgen deprivation therapy as an initial or secondary therapy. Forty patients had received docetaxel based chemotherapy with a median cycle of 6.5 as a third line treatment. Baseline patient characteristics are shown in Table 2.

Adverse events

The overall toxicities are shown in Table 3. The most frequent adverse events were local redness and swelling at injection sites, bone pain, hypoalbuminemia, lymphocytopenia, appetite loss, fatigue, increased ALP, and anemia, which were grade 1 or 2 in most cases. There were no grade 4 toxicities and no treatment-related deaths. A total of 51 grade 3 toxicities including anemia, bone pain, increased ALP, lymphocytopenia, decreased white blood cells, increased creatinine, injection site reaction, and increased AST and ALT were observed during the study. All of these severe adverse events were concluded to be not directly associated with the vaccinations, but with cancer progression or other causes by the independent safety evaluation committee in this trial.

Clinical outcome

Forty-eight (49%) patients exhibited some decrease in PSA from baseline, ranging from 1.9% to 99.6% (Figure 1A). Confirmed ≥50% PSA decline at any point during PPV was observed in 21 patients (22%), with a median time of 4 months to ≥50% PSA decline and a median duration of ≥50% PSA decline of 3 months. Delayed PSA response was observed. Patients with ≥50% PSA decline during PPV showed longer survival than remaining patients ($p = 0.035$) (Figure 1B). The median estimated PSADT pre- and during PPV were 2 and 3.89 months, respectively. Fifty-four (56%) patients displayed at least 2-fold increase over the pre-treatment PSADT (range, 2.1- to 75-fold), and these patients with a prolongation of PSADT showed longer survival than patients without a prolongation of PSADT ($p = 0.013$) (Figure 1C and D). To compare the difference in PSA responses with clinical outcomes, patients were divided into three groups: responder group with survival longer than 20 months after PPV, non-responder group with death within 12 months after PPV, and another group with the remaining patients. Average% PSA changes in the responder group were significantly lower than those in the non-responder group at 2 to 5 months ($p < 0.005$) and those in the other group at 5 to 10 months ($p < 0.005$) during the PPV. In addition, average% PSA changes in the responder group showed a trend of PSA plateau. Average% PSA changes from baseline among three groups before and during PPV are shown in Figure 1E.

There was no complete response or partial response in terms of measurable disease. The median time to disease progression, as defined by clinical and/or radiologic criteria, was 10.9 months (95% CI, 6 to 19 months). At the time of analysis with a median follow-up of 18 months (95% CI, 14.1 to 24 months), 64 deaths had occurred. Median survival time was 18.8 months (95% CI, 14.9 to 28.6 months) in all patients. Median survival time in chemotherapy naive patients and in patients after docetaxel chemotherapy were 21.6 months and 11.6 months, respectively.

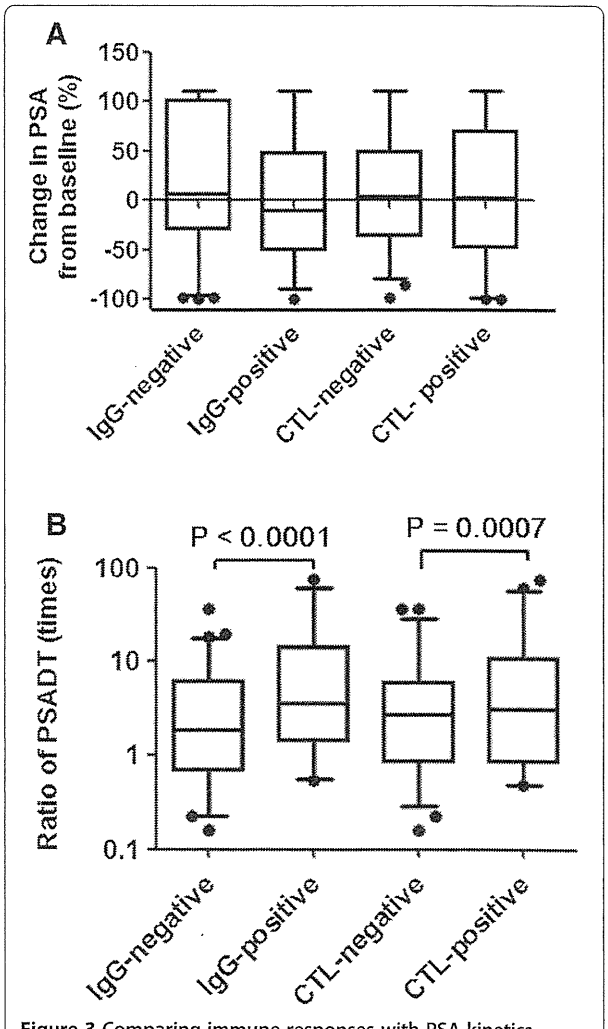

Figure 3 Comparing immune responses with PSA kinetics. **(A)** Change in PSA from baseline (%) based on immune responses. **(B)** Ratio of PSADT based on immune responses.

Immunological response

The number of selected peptides were 4 peptides in 62 patients, 3 peptides in 17 patients and 2 peptides in 21 patients at the first screening. Same peptide at the first screening were only selected in 29 of 97 (30%) patients at second screening and in 10 of 66 (15%) patients at the third screening, remaining patients received at least 1 different peptide during the study. The most frequently selected peptides were Lck486 (40 patients), CypB129 (31 patients), PAP213 (24 patients), SART2-93 (21 patients), PSA248 (20 patients), Lck488 (17 patients) and WHSC2-123 (16 patients) at the first screening. All 31 peptides were selected at any screening in the study.

Total IgG responses specific to the vaccinated peptide were augmented in 42 of 97 (43%) patients, 62 of 66 (94%) patients, 36 of 36 (100%) patients, 16 of 16 (100%) patients, and 7 of 7 (100%) patients at the 6th, 12th, 18th, 24th, and 30th vaccinations, respectively. Finally, positive IgG responses during PPV were observed in 76/97 (79%) patients. PBMCs from 97 patients were available for IFN-γ Elispot assay at the pre- and 6th vaccination. Peptide-specific T-cell responses were detectable in 42 patients (43%) at the 6th vaccination. There was no obvious correlation between IgG and CTL responses. Positive immune responses of both IgG and CTL based on baseline characteristics including age, PS, HLA typing, PSA, Gleason score, presence of metastasis and prior chemotherapy are shown in Figure 2. There was no difference in positive immune responses among baseline characteristics. In comparing immune responses with PSA kinetics, although average PSA changes did not correlate with immune responses, average ratio of PSADT was significantly higher in patients with positive IgG (8 vs. 4, $p < 0.0001$) and CTL (8.8 vs. 6.1, $p = 0.0007$) responses (Figure 3).

Survival analysis

Cox proportional hazards regression analysis was performed to determine factors that would predict disease death (Table 4). Univariate Cox analysis showed that good performance status ($p < 0.0001$), positive IgG response ($p < 0.0001$), low CRP ($p = 0.012$), prolongation of PSADT ($p = 0.018$), low PSA ($p = 0.004$), prior chemotherapy status ($p = 0.037$), positive T-cell response ($p = 0.039$), and presentation of ≥50% PSA decline ($p = 0.046$) were significantly associated with survival.

The factors showing p less than 0.05 in the univariate analysis were included in multivariate analysis of the model. Finally, positive IgG response ($p = 0.001$) and prolongation of PSADT ($p = 0.004$) during PPV, as well as baseline good performance status ($p = 0.004$), low CRP levels ($p = 0.006$), and low PSA levels ($p = 0.008$), were significantly favorable factors for OS (Table 4).

Discussion

As observed in several clinical trials, immunotherapy can induce novel patterns of antitumor responses distinct from those of chemotherapy, which are consequently not captured by the WHO or RECIST criteria [5]. On the other hand, there is debate regarding the utility of PSA changes, especially with immunotherapy, and the PSA Working Group 2 has advocated using radiographic progression-free survival as a preferred endpoint for phase

Table 4 Cox proportional hazards regression analysis of association between potential factors and death after PPV in the 100 CRPC patients

Factors	Cut-offs[a]	Univariate			Multivariate		
		p value	Hazard ratio	95% CI	p value	Hazard ratio	95% CI
IgG response	Positive vs. negative	<0.0001	0.19	0.101-0.355	0.001	0.272	0.125-0.592
ECOG performance status	0 vs. 1	<0.0001	0.073	0.031-0.174	0.004	0.179	0.056-0.569
CRP	Low (<3000 ng/mL) vs. high	0.012	0.461	0.252-0.842	0.006	0.389	0.199-0.759
PSADT	Increase (2 times) vs. no	0.018	0.477	0.258-0.881	0.004	0.357	0.176-0.725
PSA	Low (<30 ng/mL) vs. high	0.004	0.407	0.221-0.749	0.008	0.361	0.171-0.762
Prior chemotherapy	Untreated vs. treated	0.037	0.536	0.298-0.962	0.329	0.695	0.335-1.445
T-cell response	Positive vs. negative	0.039	0.51	0.269-0.967	0.273	0.679	0.340-1.357
>50% PSA decline	Positive vs. negative	0.046	0.387	0.152-0.984	0.553	0.733	0.263-2.042
Number of lymphocytes	High (>1300/μL) vs. low	0.054	0.562	0.313-1.009	-	-	-
IL6	Low (<2.4 pg/mL) vs. high	0.057	0.491	0.236-1.021	-	-	-
Pts. age	Low (<69 years) vs. high	0.186	0.666	0.364-1.218	-	-	-
Gleason score	Low (<8) vs. high	0.623	1.162	0.637-2.128	-	-	-
SAA	Low (<8 μg/mL) vs. high	0.709	0.875	0.433-1.767	-	-	-

Of the 100 men, 64 died.
[a]Lymphocyte, PSA, and patient age are based on median values.
Abbreviations: PPV, personalized peptide vaccination; CRPC, castration-resistant prostate cancer; CI, confidence intervals; ECOG, Eastern Cooperative Oncology Group; PSA, prostate-specific antigen; PSADT, PSA doubling time; CRP, C-reactive protein; SAA, serum amyloid A; IL6, interleukin 6.

II trials [21]. Others have argued that changes in PSADT may be a marker of drug effect, understanding that shorter PSADT corresponds to worse prognosis and, thus, a favorable change in PSADT suggests drug activity [22,23]. However, clinical trials of recently developed drugs, such as sipuleucel-T [6], cabazitaxel [24], and abiraterone acetate [25], for the treatment of progressive CRPC patients did not analyze the usefulness of PSADT as a surrogate marker of response in CRPC patients. In the current study, we attempted careful and stringent collection of multiple PSA values in order to calculate PSADT changes before and during PPV accurately. While delayed PSA responses were observed, we did see a statistically significant increase in PSADT. Importantly, patients with prolongation of PSADT showed statistically longer survival ($p = 0.018$). These results suggest that the development of late immune responses is associated with changes in PSADT.

The evaluation of T-cell immune responses to target self antigens after vaccine clinical trials presents several challenges. Antigen-specific T-cells can be evaluated by their peptide target specificity, proliferative capacity, cytokine secretion, cytolytic activity, and membrane markers of activation. At present, the best measure of antigen-specific T-cells is unknown, as is the optimal time to evaluate immune responses. In our current analysis, we evaluated both humoral responses determined by peptide-specific IgG levels using a Luminex system and antigen-specific CD8+ T-cell responses by using IFN-γ ELISPOT assays, to provide a more direct quantitative assessment after immunization. Delayed 50% PSA decline and prolongation of PSADT were observed in patients with positive IgG and T-cell respkonses, and these immune responses were associated with OS. These results suggest that further immunological analysis at multiple time points might be needed to determine whether T-cell response or the development of late immune responses is associated with clinical responses.

Cancer vaccinations do not always extract good immune and/or clinical responses in vaccinated patients. This study showed that IgG responses and prolongation of PSADT during PPV, along with baseline performance status, CRP, and PSA levels, were well correlated with OS in patients with CRPC treated by PPV. These results suggest that risk stratification based on these factors could be helpful for estimating the OS in patients with CRPC treated by immunotherapy.

Despite these encouraging observations, the current study must be interpreted as hypothesis-generating due to several limitations. This single-arm phase II study without a concurrent control arm did not allow estimation of the potential clinical or immune effects of this treatment. Another potential limitation of this study regarding OS is the lack of treatment data after the treatment phase of the trial. Imbalances due to chance may have occurred in treatments after progression. However, only docetaxel has been shown to affect survival in this population of patients, and only by a few months. The median survival of 18.8 months (95% CI, 14.1 to 24 months) observed in this study surpassed the survival that was observed from docetaxel-based clinical trials in a similar population by TAX-327 (median survival, 19.2 months) and South West Oncology Group 9906 (median survival, 17.5 months) [7,8]. Thus, we think it unlikely that a potential imbalance in post-study treatments could explain the survival results.

Conclusions

This study showed that PPV in patients with CRPC was active and well tolerated, improving survival with immune responses, delayed PSA responses, and prolongation of PSADT. Further randomized trials are needed to confirm these preliminary results.

Abbreviations

CR: Complete response; CT: Computed tomography; CRPC: Castration-resistant prostate cancer; CTL: Cytotoxic T lymphocytes; EOCG: Eastern cooperative oncology group; HLA: Human leukocyte antigen; IFN- γ: Interferon-γ; IgG: Immunoglobulin G; OS: Overall survival; PBMC: Peripheral blood mononuclear cells; PPV: Personalized peptide vaccination; PSA: Prostate specific antigen; PSADT: Prostate specific antigen doubling time.

Competing interests

K. Itoh is a consultant/advisory board member in Green Peptide Co. A. Yamada is a part-time executive of Green Peptide Co. No potential conflicts of interest were disclosed by other authors.

Authors' contributions

NM conceived of the study, and participated in its design and coordination and drafted the manuscript. KI and AY participated in its design and helped to draft the manuscipt. FM, SS, RO performed the clinical trial and collected the data. SM and TS carried out the immunoassays. All authors read and approved the final manuscript.

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Author details

[1]Clinical Research Division of the Research Center for Innovative Cancer Therapy, Kurume University School of Medicine, 67 Asahi-machi, Kurume 830-0011, Japan. [2]Departments of Urology, Kurume University School of Medicine, Kurume, Japan. [3]Immunology and Immunotherapy, Kurume University School of Medicine, Kurume, Japan. [4]Cancer Vaccine of the Research Center for Innovative Cancer Therapy, Kurume University School of Medicine, Kurume, Japan.

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FOCUSSED RESEARCH REVIEW

Personalized peptide vaccination: a new approach for advanced cancer as therapeutic cancer vaccine

Masanori Noguchi · Tetsuro Sasada · Kyogo Itoh

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Abstract Since both tumor cells and host immune cell repertoires are diverse and heterogeneous, immune responses against tumor-associated antigens should differ substantially among individual cancer patients. Selection of suitable peptide vaccines for individual patients based on the preexisting host immunity before vaccination could induce potent anti-tumor responses that provide clinical benefit to cancer patients. We have developed a novel immunotherapeutic approach of personalized peptide vaccination (PPV) in which a maximum of four human leukocyte antigen (HLA) class IA-matched peptides are selected for vaccination among pooled peptides on the basis of both HLA class IA type and the preexisting host immunity before vaccination. In this review, we discuss our recent results of preclinical and clinical studies of PPV for various types of advanced cancer.

Keywords Immunotherapy · Personalized peptide vaccine · Cancer vaccine · Advanced cancer

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M. Noguchi
Department of Urology, Kurume University School of Medicine, Kurume, Japan

M. 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

T. Sasada · K. Itoh
Department of Immunology and Immunotherapy, Kurume University School of Medicine, Kurume, Japan

Abbreviations

APC	Advanced pancreatic cancer
CR	Complete response
CRPC	Castration-resistant prostate cancer
CTL	Cytotoxic T lymphocytes
DBC	Docetaxel-based chemotherapy
EMP	Estramustine phosphate
GBM	Glioblastoma multiforme
GEM	Gemcitabine
HLA	Human leukocyte antigen
IgG	Immunoglobulin G
MHC	Major histocompatibility complex
MRP3	Multidrug resistance-associated protein 3
MST	Median survival time
NSLC	Non-small cell lung cancer
PBMC	Peripheral blood mononuclear cells
PAP	Prostatic acid phosphatase
PD	Progressive disease
PFS	Progression-free survival
PPV	Personalized peptide vaccine
PR	Partial response
PSA	Prostate-specific antigen
PSMA	Prostate-specific membrane antigen
SART	Squamous cell carcinoma antigen recognized by T cells
SCLC	Small cell lung cancer
TAA	Tumor-associated antigen

Introduction

Since the identification of tumor-associated antigens (TAA) in different tumor histological types, many cancer vaccination strategies have been investigated, including peptide-based vaccines, recombinant DNA- or protein-based

vaccines, and cell-based vaccines. Results from early trials, although demonstrating the feasibility and the good toxicity profile of this approach, provided evidence of clinical activity in only a minority of patients [1]. However, there have recently been noteworthy advances in the clinical application of immunotherapy. In 2010, sipuleucel-T (Provenge; Dendreon Corporation, Seattle, WA, USA), 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 U.S. Food and Drug Administration (FDA) [2]. 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 [3]. Despite these significant advances, however, most other randomized clinical trials of immunotherapies, including peptide vaccines, recombinant DNA- or protein-based vaccines, and cell-based vaccines, have so far failed to show beneficial therapeutic effects in patients compared to existing treatments [4]. The failure of recent clinical trials has raised several issues that need to be addressed for the successful development of cancer vaccines. We describe here a novel immunotherapeutic approach, "personalized peptide vaccination (PPV)," in which a maximum of four human leukocyte antigen (HLA) class IA-matched peptides are selected for vaccination from a pool of peptides on the basis of both HLA class IA type and the preexisting host immunity before vaccination. This strategy may confer several advantages, such as the possibility of bypassing both immunological diversity and tumor heterogeneity. For example, "personalized" antigens with preexisting immunity, which are designed to stimulate antigen-specific memory T cells, could be expected to induce rapid and strong secondary immune responses. For example, we previously reported that PPV quickly induced infiltration of $CD45RO^+$ memory T cells, rather than naïve T cells or B cells, into cancer tissues [5]. In addition, selection of multiple epitopes for PPV could reduce the risk of tumor escape through existence and/or induction of antigen-negative clones escaping peptide-specific immune responses. Indeed, it would be relatively rare that tumor cells escape from peptide-specific immune responses by simultaneously losing all of multiple antigens selected for vaccination.

Characteristics of candidate peptides for PPV

A large number of tumor-associated antigens (TAA) have been identified by several different approaches, including complementary DNA (cDNA) expression cloning [6], serologic analysis of recombinant cDNA expression libraries (SEREX) [7], and a reverse immunological approach. We have identified a number of TAA genes and their peptides, some of which have been used as vaccine antigens for PPV, by cDNA expression cloning techniques. For example, a series of the squamous cell carcinoma antigens recognized by T cells (SART), including SART1, SART2, and SART3, were identified from a cDNA library of a squamous cell carcinoma cell line for the first time as TAA derived from epithelial cancers except for melanoma, by using a CTL line established from a patient with esophageal cancer [8–11]. Similarly, other TAAs with interesting characteristics, such as $p56^{lck}$ and multidrug resistance-associated protein 3 (MRP3), have also been identified as vaccine antigen candidates for PPV. $p56^{lck}$, the *src* family tyrosine kinase essential for T-cell development and function is reported to be aberrantly expressed in colon, small cell lung carcinoma, and prostatic cancer cells with a trend toward preferential expression in metastatic cancer cells [12, 13]. This molecule encodes epitopes, which can frequently induce cytotoxic T lymphocytes (CTLs) in the peripheral blood lymphocytes of $HLA\text{-}A2^+$, $HLA\text{-}A24^+$, or HLA-A3 $supertype^+$ cancer patients with distant metastases [14–16]. Peptides derived from MRP3, which are recognized by CTLs in an HLA-A2402-restricted manner [17], often show positive immune responses in advanced cancer patients in whom standard chemotherapy had failed.

One of the notable characteristics of PPV is to screen CTL epitope candidates for therapeutic cancer vaccines on the basis of their ability to induce CTL and/or humoral responses in pre-vaccination samples, since all of the CTL epitopes currently employed for PPV have B-cell epitopes as well. This is based on the hypothesis that a CTL peptide possessing a B-cell epitope could provide more effective clinical benefits than a CTL peptide without it. Although this hypothesis has not been confirmed by randomized clinical trials yet, it has been well recognized that both cellular and humoral immune responses are crucial to induce potent anti-tumor immunity in animal models [18, 19]. As a result of basic and clinical studies, we have focused on 31 HLA class I-restricted peptide epitopes with minimal optimal length for PPV, [2 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] (Table 1). These peptides were identified from 15 different TAAs, including SART2, SART3, $p56^{lck}$, MRP3, prostate-specific antigen (PSA), prostatic acid phosphatase (PAP), prostate-specific membrane antigen (PSMA), and a variety of other epithelial tumor antigens [8–11, 14–17, 20–28]. The safety and potential immunological effects of these vaccine candidates have been shown in previously conducted clinical studies.

Although short peptide epitopes with minimal optimal length have been reported to bear the potential to induce tolerance rather than effective immune responses [29], PPV

Table 1 Information of peptide candidates used for personalized peptide vaccination

Peptide name	Original protein	Position of peptide	Amino acid sequence	HLA type	References
CypB-129	Cyclophilin B	129–138	KLKHYGPGWV	A2, A3sup[a]	[20]
Lck-246	p56 lck	246–254	KLVERLGAA	A2	[15]
Lck-422	p56 lck	422–430	DVWSFGILL	A2, A3sup	[15]
MAP-432	ppMAPkkk	432–440	DLLSHAFFA	A2, A26	[21]
WHSC2-103	WHSC2	103–111	ASLDSDPWV	A2, A3sup[a], A26	[21]
HNRPL-501	HNRPL	501–510	NVLHFFNAPL	A2, A26	[21]
UBE-43	UBE2 V	43–51	RLQEWCSVI	A2	[21]
UBE-85	UBE2 V	85–93	LIADFLSGL	A2	[21]
WHSC2-141	WHSC2	141–149	ILGELREKV	A2	[21]
HNRPL-140	HNRPL	140–148	ALVEFEDVL	A2	[21]
SART3-302	SART3	302–310	LLQAEAPRL	A2	[8]
SART3-309	SART3	309–317	RLAEYQAYI	A2	[8]
SART2-93	SART2	93–101	DYSARWNEI	A24	[9]
SART3-109	SART3	109–118	VYDYNCHVDL	A24, A3sup[a], A26	[10]
Lck-208	p56 lck	208–216	HYTNASDGL	A24	[14]
PAP-213	PAP	213–221	LYCESVHNF	A24	[22]
PSA-248	PSA	248–257	HYRKWIKDTI	A24	[23]
EGFR-800	EGF-R	800–809	DYVREHKDNI	A24	[24]
MRP3-503	MRP3	503–511	LYAWEPSFL	A24	[17]
MRP3-1293	MRP3	1293–1302	NYSVRYRPGL	A24	[17]
SART2-161	SART2	161–169	AYDFLYNYL	A24	[9]
Lck-486	p56 lck	486–494	TFDYLRSVL	A24	[14]
Lck-488	p56 lck	488–497	DYLRSVLEDF	A24	[14]
PSMA-624	PSMA	624–632	TYSVSFDSL	A24	[25]
EZH2-735	EZH2	735–743	KYVGIEREM	A24	[26]
PTHrP-102	PTHrP	102–111	RYLTQETNKV	A24	[27]
SART3-511	SART3	511–519	WLEYYNLER	A3sup[a]	[11]
SART3-734	SART3	734–742	QIRPIFSNR	A3sup[a]	[11]
Lck-90	p56 lck	90–99	ILEQSGEWWK	A3sup[a]	[16]
Lck-449	p56 lck	449–458	VIQNLERGYR	A3sup[a]	[16]
PAP-248	PAP	248–257	GIHKQKEKSR	A3sup[a]	[28]

[a] A3sup, HLA-A3 supertype (A3, A11, A31, and A33)

using short epitopes has been reported to efficiently induce antigen-specific IFN-γ-producing $CD8^+$ T cells with cytotoxic activity, but not tolerance to them, possibly because only immunogenic epitopes are selected in each patient by pre-vaccination screening. Although long peptides have shown excellent immune and clinical responses in some of clinical trials [30], we do not currently employ long peptides for PPV, since it may be possible that they contain undesirable T-cell epitopes that stimulate immune suppressive cells, such as regulatory T cells or T helper-2 cells [31], which may negatively regulate beneficial immune and clinical responses.

For PPV, a maximum of 4 peptides, selected on the basis of the results of HLA typing and the preexisting immune responses specific to each of the 31 different vaccine candidates, are subcutaneously administered in complex with incomplete Freund's adjuvant (Montanide ISA51; Seppic, Paris, France) weekly or biweekly. To prevent interaction/competition among peptides at the vaccinated sites, each of vaccine peptides is injected separately at different sites, but not in a mixture at a single site. Since more than five peptides per vaccination seemed intolerable due to adverse skin reactions, which sometimes may cause unpleasant symptoms, such as itching and pain, in our previous feasibility studies (unpublished data), four peptides per vaccination have currently been employed. Regarding the vaccination schedule, selected peptides are administered at the weekly schedule at least for the first cycle of six vaccinations, since a clear trend toward better immune responses was observed among the patients who underwent the weekly administration protocol, compared to the biweekly protocol in previous clinical trials [32].

Rationale for PPV

Although the number of cancer vaccine candidates is becoming almost limitless, antigen peptides employed for vaccination against individual patients might not always be appropriate. In general, anti-tumor immunity is known to be dependent on both the immunological characteristics of tumor cells and the host immune cell repertoires. Since immune cell repertoires of the hosts are quite diverse and heterogeneous, anti-tumor immunity might differ substantially among individuals. Therefore, it is likely that vaccine antigens that are selected and administered without considering the host immune cell repertoires would not efficiently induce beneficial anti-tumor immune responses. To increase the clinical benefits from cancer vaccines, particular attention should be paid to the immunological status of each patient by characterizing the preexisting immune responses to vaccine antigens before vaccination. However, in most of the current clinical trials of therapeutic cancer vaccines, common antigens are employed for vaccination independently of the immunological status of patients.

Patients who have an 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 to develop 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 may erode preexisting immunity. On the basis of the current paradigm that the size and composition of the adaptive immune system are limited and that individual immune cells are constantly competing with each other in a limited space, inadequate vaccination may have negative consequences for the host by suppressing preexisting beneficial memory cells specific to tumors and/or infections, which might result in acceleration of cancer progression or early death in vaccinated patients. In addition, the approach, which is designed to stimulate antigen-specific memory T cells, but not to prime naïve T cells, might not need additional immune boosting, such as the blocker of checkpoint molecule, CTLA-4, since it has been known that memory T cells are less dependent on costimulatory molecules for recall responses [33, 34]. Considering these issues, it would be quite reasonable for vaccine antigens to be selected on the basis of the preexisting 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 [35]. Through the interaction between the 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, for better control of cancer cells, it would be recommended to administer multiple tumor-associated antigens to reduce the risk of outgrowth of antigen-loss variants.

In view of the complexity and diversity of the immune cell repertoires of hosts and the immunological characteristics of tumors, we have developed the new concept of

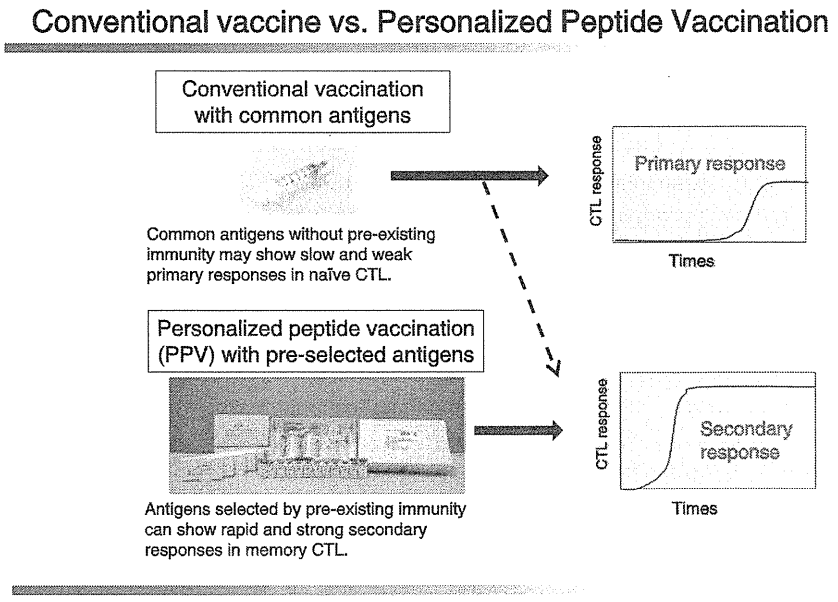

Fig. 1 Concept of personalized peptide vaccination. Patients who have an immunological memory to vaccine antigens are expected to show quick and strong immune responses to them. In contrast, patients with no immunological memory against vaccine antigens would take more time to develop effective anti-tumor immune responses because several rounds of repeated vaccinations might be required to prime antigen-specific naïve T cells to functional effector cells

PPV. In this "personalized" cancer vaccine formulation, multiple peptide antigens appropriate for vaccination are screened and selected from a list of pooled vaccine candidates in each patient, based on preexisting host immunity. In the early-phase translational study of PPV, the preexisting immunity was defined by the frequencies of CTL precursors in pre-vaccination PBMCs by using the peptide-specific IFN-γ production assay with the cutoff level of around 1 of 10,000 cells as reported, since we found that the magnitude of CTL activation could be in part dependent on the frequencies of peptide-specific CTL precursors in circulation, which were determined by this assay [36]. Indeed, when CTL precursors were measured in pre-vaccination PBMC followed by administration of peptides with higher CTL precursor frequencies, rapid and strong activation of CTL with potential clinical benefits was induced in certain patients of a series of clinical trials for advanced cancers [32, 37, 38].

Nevertheless, we are currently evaluating the preexisting immunity to vaccine candidates by peptide-specific immunoglobulin G (IgG) responses in pre-vaccination plasma, which are determined by the multiplex bead-based LUMINEX assay with the cutoff level of 10 FIU [39], rather than by CTL responses, since we have found that the IgG-based selection is useful for predicting CTL boosting after vaccination in our clinical trials, which showed the safety, high immunogenicity, and possible clinical benefits of PPV. For example, in the phase I trial of PPV for recurrent or progressive glioblastoma patients, CTL responses were boosted in 23 of 48 vaccinated peptides (48 %), which were chosen solely by humoral responses [40]. In addition, CTL responses were induced in 16 of 28 vaccinated peptides (57 %), which were chosen solely by humoral responses in another phase I trial of PPV for castration-resistant prostate cancer (CRPC) patients [41]. Based on these results, the prediction power of evaluating the preexisting immunity solely by the humoral responses for the existence of CTL responses could be estimated around 50 % when four peptides were chosen for the vaccination. Figure 2 shows one example of original data for determining the preexisting immune responses before vaccination by the humoral responses to vaccine candidates. As shown in this figure, immune responses to multiple peptides can be detected before vaccination in most of the patients treated with PPV. In such situation, the peptides showing higher IgG responses are selected for vaccination from the list of peptides that match the patient's HLA types.

There are some reasons for assessing IgG responses, instead of CTL responses, to define the preexisting immune responses. The most critical reason is a technical issue that standard methods to measure the CTL activity have not been well established yet. The performance characteristics, such as sensitivity and reproducibility, of currently available CTL assays require more modification/sophistication to detect low frequencies of antigen-specific CTL, and it seems difficult to validate the quality of the assays in clinical trials [42, 43]. In contrast, the multiplex bead-based

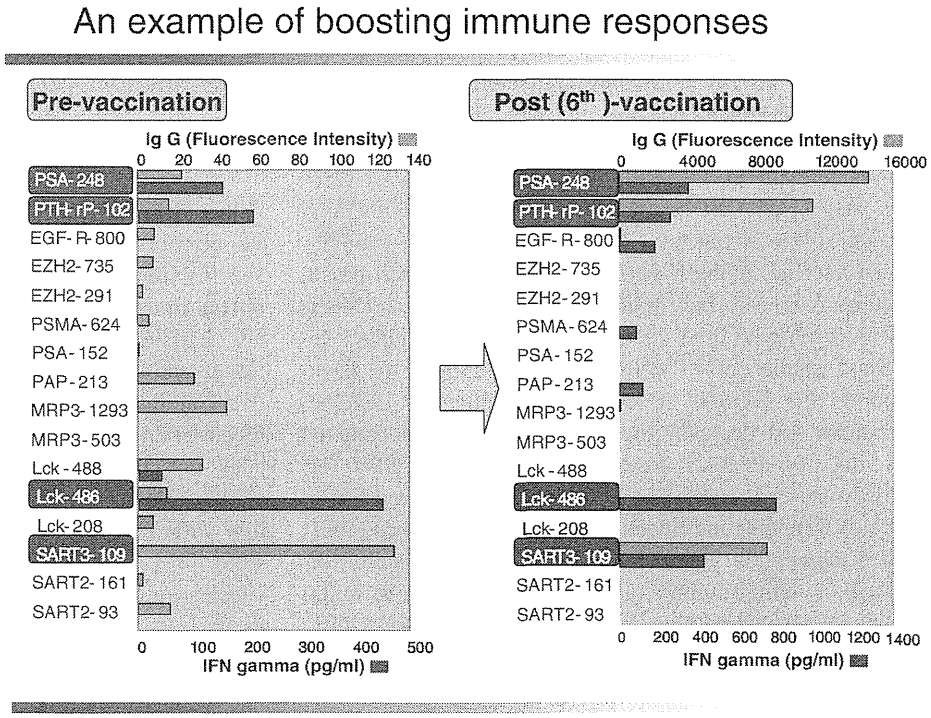

Fig. 2 An example of boosting immune responses. IgG responses to PSA-248 peptide increased from 20 to 14,000 FIU in the post (6th)-vaccination samples. The similar boosting was observed in the other two peptides. CTL response was also increased in all four vaccinated peptides. Clinical response of this case was PR

LUMINEX technology that we have developed for monitoring IgG responses allows simple, quick, and highly reproducible high-throughput screening of IgG levels specific to large numbers of peptide antigens with a tiny amount of plasma [39]. 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 [44, 45]. Of course, we believe that cellular immune responses might represent the most important marker if appropriate CTL assay conditions are defined and become available. More sophisticated CTL assays remain to be developed for the further evolution of cancer vaccination.

Clinical trials of PPV for advanced cancer

To date, a series of phase I, I/II, and II clinical trials using PPV have been conducted [5, 32, 37, 38, 40, 41, 46–62]. We have summarized the observed immune and clinical responses in advanced cancer patients induced by the PPV (Table 2). In the following sections, we provide a more detailed account of these studies, categorized by the different cancer types.

Castration-resistant prostate cancer (CRPC)

Most prostate cancer-related deaths occur in patients with advanced CRPC. Chemotherapy plays only a palliative role in the treatment for prostate cancer, although two docetaxel-based randomized clinical trials demonstrated a survival benefit of only 2.4 months compared with those with mitoxantrone and prednisone in CRPC patients. A large number of agents and treatment strategies including immunotherapy are currently under investigation for various stages of CRPC. Indeed, several immunotherapy strategies for advanced CRPC, such as single-peptide-based vaccine, multiple-peptide-based vaccine, cell-based vaccine, viral vaccine, antibody-based therapy, and their combination with other therapies, have been evaluated. In phase I studies of PPV for advanced CRPC, we have reported the increase in cellular and humoral immune responses and decrease in PSA levels in some patients [41, 46, 48]. Phase I dose-escalation study of PPV for CRPC with 1, 3, and 5 mg/peptide injection showed that a dose of 3 mg/peptide injection was better than those of 1 and 5 mg/peptide injections in terms of the induction of cellular immune responses to peptides, although the maximum tolerated dose (MTD) was not estimated [41]. In a phase I/II study, 58 patients with HLA-$A2^+$ or HLA-$A24^+$ with CRPC were treated with a combination of PPV and low-dose estramustine phosphate (EMP) [50]. As a result, the majority (76 %) of patients showed a decreased serum PSA level, along with a median survival time (MST) of 17 months (95 % CI, 12–25 months). In addition, this study showed that a small number of lymphocytes, a negative immunological response after PPV, and poor performance status were independent predictors of disease-related death. In this study, long MST with the combination therapy supports the hypothesis that this combination with a low-dose cytotoxic drug produces additional antitumor effects with minimum immunosuppression. Sequentially, we conducted a randomized, cross-over, phase II trial of PPV plus low-dose EMP comparing standard dose EMP in HLA-$A2^+$ or HLA-$A24^+$ patients with CRPC [51]. Median progression-free survival (PFS) was 8.5 months in the PPV group and 2.8 months in the EMP group with a hazard ratio (HR) of 0.28 (95 % CI, 0.14–0.61; log-rank $P = 0.0012$), and the MST for the PPV plus low-dose EMP group was 22.4 months, while the MST for the standard dose EMP group was 16.1 months (95 % CI, 8.0–13.4 months) ($P = 0.0328$). The HR for overall survival was 0.3 in favor of the PPV plus low-dose EMP group. These results suggest that PPV is well tolerated and active in CRPC patients. In another phase II study, we compared the MST in docetaxel-based chemotherapy (DBC)-resistant CRPC patients treated by PPV ($n = 20$) with a historical control ($n = 17$) [52]. MST from the first day of progressive disease (PD) were 17.8 and 10.5 months in DBC-resistant CRPC patients receiving PPV and those with no PPV, respectively. These encouraging preliminary study results suggested that PPV warrants further study as a novel therapy for CRPC patients with PD after DBC. Now, we are conducting a phase III randomized clinical trial of PPV in DBC-resistant CRPC patients.

Glioblastoma multiforme (GBM)

Although immunotherapy is theoretically attractive due to the discovery of TAAs and peptides capable of inducing specific immunity in patients with GBM, previously conducted immunotherapy trials failed to provide evidence of any definite clinical benefit in patients with GBM. One of the potential hurdles hindering the development of effective immunotherapy for the treatment of GBMs is the blood–brain barrier, but recent studies have shown that it does not always function in cases involving recurrent GBMs. We previously showed the feasibility of vaccination with PPV for advanced GBM patients in a phase I study [32]. Twenty-one patients received more than six vaccinations, and clinical responses were five cases of partial response (PR), eight of stable disease (SD), and eight of PD with MST of 20.7 months in this study. More importantly, significant levels of peptide-specific IgG were detected in the post-vaccination tumor cavity or spinal fluid

Table 2 Immunological and clinical responses of personalized peptide vaccination for advanced cancer

Tumor site	Disease status	Phase	HLA restriction	Total No.of Pts	Combined chemotherapy	Humoral response (%)	Cellular response (%)	Clinical response	MST (months)	Grade 3/4 toxicities	References
Prostate	Advanced CRPC	PI	A-24	10		60	40	SD 50 %	Not ref.	0	[46]
	Advanced CRPC	PI	A-24	13		91	55	PR 63 %	24	G3, 5 %	[47]
	Advanced CRPC	PI	A-2	10		70	40	SD 30 %	22	0	[48]
	Advanced CRPC	PI/II	A-24	16		50	71	PR 43 %	17	0	[49]
	Advanced CRPC	PI/II	A-2/A-24	58	Low-dose EMP	88	78	PR 24 %	17	G3, 7 %	[50]
	Localized PC	PII	A-24	10		80	80	PR 20 %	Not ref.	0	[5]
	Advanced CRPC	PI, Extension	A-24	15	Low-dose EMP	47	67	PR 13 %	24	0	[41]
	Advanced CRPC	PII, Randomized	A-2/A-24	57	Low-dose EMP	64	50	PFS		0	[51]
								8.5 versus 2.8 M	22.4 versus 16.1 M		
	Advanced CRPC	PII	A-2/A-24/ A-3sup/ A-26	42		44	34	PR 12 %	17.8	0	[52]
Brain	Advanced malignant glioma	PI	A-2/A24	21		40–64	50–82	PR 24 %, SD 38 %	Not reached	0	[32]
	Advanced glioblastoma multiforme	PI, Extension	A-24	12		17	75	PR 17 %, SD 42 %	10.6	0	[40]
Colorectal	Advanced colorectal cancer	PI	A-24	10		70	50	PR 10 %	Not ref.	0	[53]
	Advanced colorectal cancer	PI/II	A-2/A-24	7	TS-1	71	57	SD 14 %	Not ref.	G3, 20 %	[54]
Pancreas	Advanced pancreatic cancer	PI	A-2/A-24	13	GEM	69	69	PR 15 %, SD 54 %	7.6	0	[55]
	Non-resectable pancreatic cancer	PII	A-2/A-24	21	GEM	72	78	PR 33 %, SD 43 %	9	0	[56]
Stomach	Advanced gastric cancer	PI	A-2/A-24	13		80	50	SD 45 %	Not ref.	0	[57]
Lung	Advanced lung cancer	PI	A-24	10		40	40	SD 80 %	15.2	0	[37]
	Refractory SCLC	PII	A-2/A-24 A-3sup/ A-26	10	Chemotherapy	83	83	SD 20 %	6.2	G3, 4 %	[58]
	Refractory NSCLC	PII	A-2/A-24 A-3sup/ A-26	41	Chemotherapy	49	34	SD 56 %	10.1	G3, 7 %	[59]
Kidney	Metastatic RCC	PI	A-2/A-24	10		80	5	SD 60 %	23	0	[60]
Skin	Malignant melanoma	PI	A-2/A-24	7		57	86	SD 43 %	Not ref.	0	[61]

Table 2 continued

Tumor site	Disease status	Phase	HLA restriction	Total No.of Pts	Combined chemotherapy	Humoral response (%)	Cellular response (%)	Clinical response	MST (months)	Grade 3/4 toxicities	References
Uterine	Recurrent gynecologic cancer	PI	A-2/A-24	14		86	85	SD 36 %	Not ref.	G3, 8 %	[38]
Bladder	Advanced urothelial cancer	PI	A-2/A-24	10		80	80	CR 10 %, PR 10 %	24	0	[62]

A-3 sup A-3 supertype, *CR* complete response, *CRPC* castration-resistant prostate cancer, *EMP* estramustine phosphate, *GEM* gemcitabine, *G3* grade 3, *HLA* human leukocyte antigen, *MST* median survival time, *NSCLC* non-small cell lung cancer, *PI* phase I clinical trial, *PC* prostate cancer, *PD* progressive disease, *PFS* progression-free survival, *PR* partial response, *RCC* renal cell carcinoma, *SCLC* small cell lung cancer, *SD* stable disease, *TS-1* 5-fluorouracil derivative; *Total No. of Pts* total number of patients

of all of the tested patients who showed favorable clinical responses. Another clinical study showed the safety and increased immune boosting with potential clinical benefits in cases of recurrent or progressive GBM, even in temozolomide refractory settings [40]. On the basis of these promising results, double-blind randomized phase III trials are currently underway in GBM patients.

Colorectal and gastric cancer

We reported previously that SART3 is expressed in the majority of colorectal cancers and that two to three SART3-derived peptides are present in the majority of cancer patients with HLA-A24$^+$ and HLA-A2$^+$ [8–10, 14]. In a phase I clinical trial of PPV on 10 patients with advanced colorectal cancer, we observed one PR and one SD continuing for more than 6 months [53]. These PR and SD cases were vaccinated with three kinds of SART3- and p56^{lck}-derived peptides, suggesting that the combination of these peptides might constitute a promising vaccine strategy for advanced colorectal carcinomas. In addition, a phase I/II clinical trial of PPV in combination with oral administration of a 5-fluorouracil derivative (TS-1) in advanced gastric or colorectal cancer patients indicated that administration of the standard dose of TS-1 in combination with PPV does not necessarily impede immunological responses in these cancer patients, and actually maintains or augments them [54]. Another phase I clinical trial of PPV in 13 patients with advanced gastric cancer demonstrated prolonged survival and cellular and humoral immune responses to the vaccinated peptides in the post-vaccination samples, including those of all four patients with the scirrhous type [57]. Even though only a small number of selected patients were treated, the encouraging clinical response warrants further studies of PPV in colorectal and gastric cancers.

Pancreatic cancer

For patients with advanced pancreatic cancer (APC), the treatment options are limited, although gemcitabine (GEM) is currently used as the standard therapy. We have conducted a phase I trial of PPV in 13 HLA-A24- or HLA-A2-positive patients with APC, in which patients were treated by PPV at three different dose settings of 1, 2, and 3 mg/peptide with GEM [55]. This combination therapy was well tolerated, and 11 of 13 patients (85 %) showed clinical responses, such as reduction in tumor size and/or the level of tumor markers. Augmentation of peptide-specific CTL activity against pancreatic cancer cells was observed at each dose level, and the increment of peptide-specific IgG antibodies was dependent on peptide dose. These results suggested that GEM did not inhibit the immune responses induced by PPV.

Subsequently, we have evaluated the safety, clinical efficacy, and immune response to PPV with GEM as the first therapy in 21 patients with APC [56]. This phase II study showed a longer survival (MST of 9 months with a 1-year survival rate of 38 %) than in previously reported results of GEM alone (MST of 5.7 months with a 1-year survival rate of 18 %). Importantly, MST was 15 months in the patients who showed immunological responses to vaccinated peptides in the early stages of vaccination. In view of these findings, the survival benefit in comparison with GEM alone needs to be confirmed in future clinical studies.

Lung cancer

The prognosis of advanced lung cancer patients remains very poor with a median survival time of around 6–10 months. Phase I and II studies of PPV in a small number of patients with refractory non-small cell lung cancer (NSLC) showed longer survival (MST of 10.1–15.2 months) [37, 59] than in previous reports. A clinical study of advanced small cell lung cancer (SCLC) showed the feasibility of PPV since there were higher rates of peptide-specific immunological boosting after PPV [58]. In order to identify potential biomarkers for predicting overall survival in advanced lung cancer patients, we retrospectively analyzed pre-vaccination clinical findings and laboratory data. In patients with refractory NSLC, a higher C-reactive protein (CRP) level before vaccination and a low frequency of $CD3^+CD26^+$ cells after vaccination were significant predictors of unfavorable overall survival [59]. In patients with refractory SCLC, the number of previous chemotherapy treatments and the frequency of $CD3^+CD26^+$ cells in PBMCs before vaccination were potential prognostic predictors in patients who received PPV [58]. These findings demonstrate that less inflammation may contribute to better responses to the PPV, suggesting that evaluation of the inflammatory factors before vaccination could be useful for selecting appropriate cancer patients for PPV.

Other cancers

We have also conducted phase I clinical trials for other advanced cancers including metastatic renal cell carcinoma (RCC), malignant melanoma, gynecologic cancers, and bladder cancer [38, 60–62]. All of these studies demonstrated that PPV was safe and well tolerated with no major adverse effects and that more immune responses were observed in the majority of patients after PPV than with the pre-designated peptide vaccination. Some patients treated by PPV showed objective clinical responses evaluated by the response evaluation criteria in solid tumors criteria with boosted immune responses: CR in one patient with chemotherapy-resistant advanced bladder tumor and PR in two patients with cervical cancer [38, 62]. These results indicate that PPV can be applied in further clinical trials aimed at the treatment for these cancers.

Conclusions

The field of immunotherapy has advanced dramatically during the past 20 years, but there have remained several issues to be addressed in order to achieve successful cancer vaccine development. In view of the complexity and diversity of the immunological characteristics of tumors and the immune cell repertoires of hosts, selection of suitable peptide vaccines for individual patients based on the preexisting host immunity before vaccination could induce potent anti-tumor responses that provide clinical benefit to cancer patients. We have shown promising results of PPV in this review article as a new treatment modality for patients with various types of advanced cancer. Further randomized phase III clinical trials are essential to prove the clinical benefits of PPV. In addition, novel biomarkers for selecting patients who would benefit most from PPV remain to be identified.

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