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.

Conflict of interest The authors indicated no potential conflict of interest except for Kyogo Itoh received a research grant from the Green Peptide Co., Ltd.; Kyogo Itoh own stock in the Green Peptide Co.

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Review Article

Next-generation peptide vaccines for advanced cancer

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Many clinical trials of peptide vaccines have been carried out since the first clinical trial of a melanoma antigen gene-1-derived peptide-based vaccine was reported in 1995. The earlier generations of peptide vaccines were composed of one to several human leukocyte antigen class I-restricted CTL-epitope peptides of a single human leukocyte antigen type. Currently, various types of next-generation peptide vaccines are under development. In this review, we focus on the clinical trials of the following categories of peptide vaccines mainly published from 2008 to 2012: (i) multivalent long peptide vaccines; (ii) multi-peptide vaccines consisting of CTL- and helper-epitopes; (iii) peptide cocktail vaccines; (iv) hybrid peptide vaccines; (v) personalized peptide vaccines; and (vi) peptide-pulsed dendritic cell vaccines. (*Cancer Sci* 2013; 104: 15–21)

A cDNA-expression cloning technique to identify genes and peptides of tumor-associated antigens was first reported by van der Bruggen *et al.* in 1991.[1] Subsequently, a technique using autologous antibodies was introduced for identification of genes and peptides recognized by the host immune system.[2] These advanced techniques have provided a large number of antigens and peptides applicable as cancer vaccines. Many clinical trials of peptide vaccines have been carried out since the first clinical trial of a melanoma antigen gene-1 (MAGE-1)-derived peptide-based vaccine was reported in 1996 by Hu *et al.*[3] The earlier generations of peptide vaccines were composed of one to several human leukocyte antigen (HLA)-class I-restricted peptides of a single HLA-type. The peptides were emulsified with Montanide ISA51, a clinical grade of Freund's incomplete adjuvant, or pulsed on antigen-presenting cells and used for vaccination. Various types of new generation peptide vaccines have since been developed (Figs 1,2). In this review, we discuss the recent clinical trials of the latest generation of peptide-based cancer vaccines mainly published from 2008 to 2012.

Multivalent long peptide vaccines

The classical types of peptide vaccines only contain one to several epitope peptides, which are recognized by CTLs or helper T cells. In contrast, the mother proteins of the peptide vaccines usually contain several HLA-type restricted epitopes recognized by both CTLs and helper T cells. Although the importance of helper T cells in the induction of CTLs has been established and protein vaccines are able to induce both CTLs and helper T cells, the protein vaccines have several demerits in terms of manufacturing and safety controls. To avoid these drawbacks, synthetic long peptide vaccines have been developed. Synthetic long peptide vaccines are predominantly taken up by antigen presenting cells (APCs), where they are processed for presentation by both MHC class I and II molecules.

Several clinical studies using mixes of synthetic long peptides have been reported, as mixes of synthetic long peptide are likely to contain multiple HLA class I and II T-cell epitopes, which allows the use of this type of peptide vaccine in all patients irrespective of the type of HLA of each patient. Kenter *et al.*[4] carried out a phase I study of high-risk type human papilloma virus (HPV) 16 E6 and E7 overlapping long peptides in end-stage cervical cancer patients. Cocktails of nine E6 peptides and/or four E7 peptides, each 25–35-mer, covering the entire sequences of E6 and E7 proteins, were given s.c. with Montanide ISA51 four times at 3-week intervals. Co-injection of E6 and E7 long peptides induced a strong and broad T-cell response dominated by immunity against E6. Subsequently, they carried out a phase II study of this vaccine in patients with HPV-positive grade 3 vulvar intraepithelial neoplasia.[5] Vulvar intraepithelial neoplasia is a chronic disorder caused by HPV 16. At 3 months after the last vaccination, 12 of 20 patients (60%) had clinical responses and reported relief of symptoms. Five women had complete regression of the lesions. At 12 months of follow-up, 15 of 19 patients (79%) had clinical responses with a complete response in 9 of 19 patients (47%).

A synthetic long peptide vaccine targeted for p53 was reported by Speetjens *et al.*[6] The p53 synthetic long peptide vaccine consisted of 10 synthetic 25–30-mer long overlapping peptides, spanning amino acids 70–248 of the wild type p53 protein. Ten patients with metastatic colorectal cancer were vaccinated with this vaccine. The p53-specific T cell responses were induced in 9 of 10 patients as measured by γ-interferon (IFN-γ). Subsequently, a phase II study of a p53 synthetic long overlapping peptide vaccine in patients with ovarian cancer was carried out by the same group.[7] Twenty patients with recurrent elevation of CA-125 were immunized with the vaccine. Stable disease, as determined by CA-125 levels and computed tomography scans, was observed in 2/20 (10%) patients as the best clinical response, but no relationship was found with vaccine-induced immunity. Interferon-γ-producing p53-specific T-cell responses were induced in all patients who received all four immunizations. Interestingly, the IFN-γ secreted cells were CD4 T-cells and no CD8 T-cell/CTL responses were detected. The absence of CD8 T-cell/CTL responses may be attributable to the dominant production of

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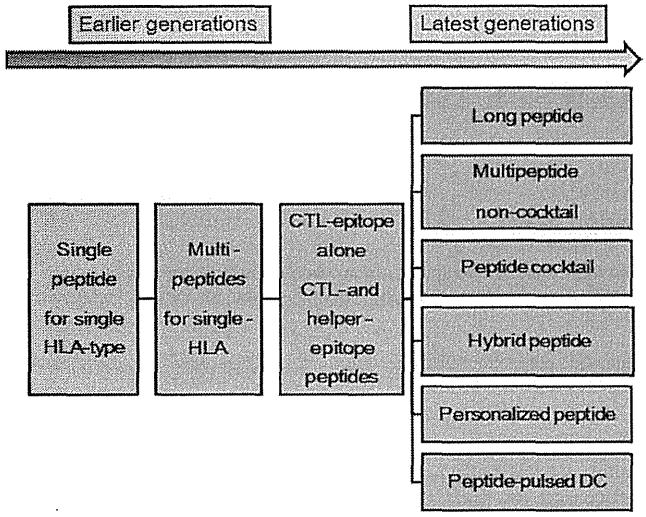

Fig. 1. Transition of peptide vaccine development for advanced cancer. DC, dendritic cells.

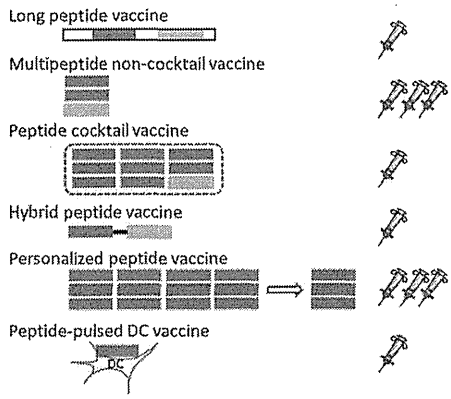

Fig. 2. Various types of latest generation peptide vaccines. The number of syringes indicates that of the final preparation for injection. Green, CTL-epitopes; orange, helper-epitopes. DC, dendritic cells.

Th2 cytokines, whose inhibitory effects on CTL induction are well known, although the vaccine immunization resulted in the expansion of p53-specific Th1 and Th2 CD4 T-cell responses.

Kakimi *et al.*[8] carried out a phase I trial of an NY-ESO-1 synthetic long peptide vaccine. A 20-mer NY-ESO-1f peptide, which includes multiple epitopes recognized by antibodies, and CD4 and CD8 cells, was given along with OK-432 and Montanide ISA51 to patients with advanced cancers. Both CD4 and CD8 T cell responses, as well as NY-ESO-1 antibody, were increased or induced in 9 of 10 patients.

Multipeptide vaccines consisting of CTL- and helper-epitopes

As mentioned above, helper T cells play crucial roles in the induction of CTLs. Some of the latest generation of peptide vaccines consist of HLA class-II restricted helper epitope peptides recognized by CD4 T cells in addition to class-I restricted CTL-epitope peptides to induce both CTLs and helper T cells. Numerous helper epitopes had been identified from the same target molecules of CTL-epitope vaccines and co-used as cancer vaccines.[9–17] A helper epitope peptide

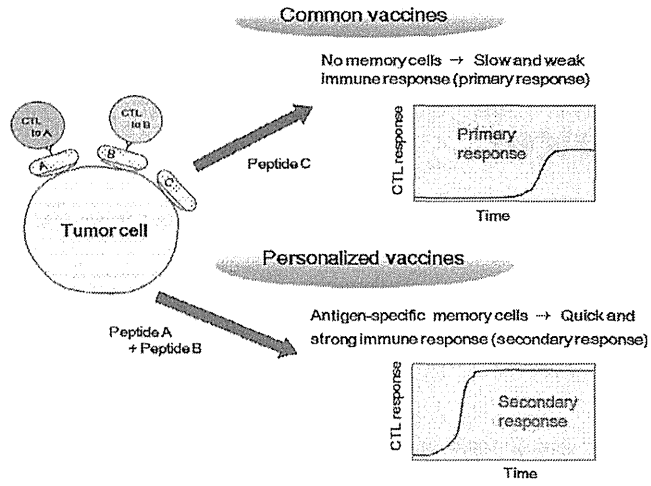

Fig. 3. Personalized peptide vaccine. In the classical type of vaccine, peptides derived from tumor-specific or overexpressed antigens are used as vaccine peptides and often mismatched to the pre-existing immunity of patients. In personalized peptide vaccines, appropriate peptides for vaccination are screened and selected from a panel of vaccine candidates in each patient, based on pre-existing host immunity and HLA types.

capable of binding pan HLA-DR (pan-DR epitope [PADRE]) has been reported,[18] and a clinical trial of a peptide vaccine using this helper epitope was reported. Kuball *et al.*[15] carried out a phase I study of CTL-epitope peptides of Wilms' tumor gene, proteinase 3, and mucin 1, and PADRE or mucin 1-helper epitope peptide with Montanide ISA51 and CpG oligonucleotide. Each peptide was formulated independently of the others and injected at a separate site. An increase in PADRE-specific CD4 T cells was observed after vaccination but these appeared unable to produce interleukin 2 (IL2), and the regulatory T cells were increased. This study indicates that helper epitope peptides have the potential to induce both helper T cells and regulatory T cells.

Peptide cocktail vaccines

Different peptides have different binding affinities to the corresponding HLA molecules. Therefore, if different CTL-epitope peptides with different binding affinities are loaded to APCs, there may be competition among the individual peptides to bind HLA molecules on the APCs. To prevent this, individual peptides of multipeptide vaccines were formulated independently of each other and injected at separate sites in most of the former clinical trials. In our case, a maximum of four peptides were individually mixed with Montanide ISA51 and injected s.c. at different sites on the same day. The maximum number of four peptides was similar to the maximum acceptable number of doses for patients on the same day, and no more than five peptides were used for vaccination. One of the strategies for overcoming the limitation of peptide number is the use of multipeptide cocktail vaccines. The multipeptide cocktail vaccines have no limitation of peptide number, as one preparation can contain more than 10 peptides. However, the issue of competition between the individual peptides of a cocktail vaccine for the binding of HLA molecules on the APCs still remains.

Different types of multipeptide cocktail vaccines have been developed, that is, vaccines consisting of CTL-epitope peptides alone,[19–21] or CTL-epitope and helper-epitope peptides.[9–13,16,17] The number of component peptides in the cocktail vaccines varies from around four to more than 10. Barve

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Table 1. Immunological and clinical responses to personalized peptide vaccines for advanced cancer

Disease status	Phase	HLA restriction	Total no. of patients	Humoral response (%)	Cellular response (%)	Clinical response (%)	MST (months)	Grade 3/4 toxicities	Ref. no.
Advanced CRPC	PI	A24	10	60	40	SD 50	Not ref.	0	31
Advanced CRPC	PI	A24	13	91	55	PR 63	24	G3, 5%	32
Advanced CRPC	PI	A2	10	70	40	SD 30	22	0	33
Advanced CRPC	PI/II	A24	16	50	71	PR 43	17	0	37
Advanced CRPC	PI/II	A2/A24	58	88	78	PR 24	17	G3, 7%	38
Localized PC	PII	A24	10	80	80	PR 20	Not ref.	0	39
Advanced CRPC	PI, extension	A24	15	47	67	PR 13	24	0	46
Advanced CRPC	PII, randomized	A2/A24	57	64	50	PFS 8.5 (vaccine) vs 2.8M (control)	22.4 (vaccine) vs 16.1M (control)	0	44
Advanced CRPC	PII	A2/A24/ A3sup/A26	42	44	34	PR 12	17.8	0	49
Advanced malignant glioma	PI	A2/A24	21	40–64	50–82	PR 24, SD 38	Not reached	0	36
Advanced glioblastoma multiforme	PI, extension	A24	12	17	75	PR 17, SD 42	10.6	0	47
Advanced corolectal cancer	PI	A24	10	70	50	PR 10	Not ref.	0	34
Advanced corolectal cancer	PI/II	A2/A24	7	71	57	SD 14	Not ref.	G3, 20%	40
Advanced pancreatic cancer	PI	A2/A24	13	69	69	PR 15, SD 54	7.6	0	41
Non-resectable pancreatic cancer	PII	A2/A24	21	72	78	PR 33, SD 43	9	0	45
Advanced gastric cancer	PI	A2/A24	13	80	50	SD 45	Not ref.	0	30
Advanced lung cancer	PI	A24	10	40	40	SD 80	15.2	0	29
Refractory SCLC	PII	A2/A24/ A3sup/A26	10	83	83	SD 20	6.2	G3, 4%	50
Refractory NSCLC	PII	A2/A24/ A3sup/A26	41	49	34	SD 56	10.1	G3, 7%	42
Metastatic RCC	PI	A2/A24	10	80	5	SD 60	23	0	43
Malignant melanoma	PI	A2/A24	7	57	86	SD 43	Not ref.	0	28
Recurrent gynecologic cancer	PI	A2/A24	14	86	85	SD 36	Not ref.	G3, 8%	35
Advanced urotherial cancer	PI	A2/A24	10	80	80	CR 10, PR 10	24	0	48

A3sup, A3 super type; CR, complete response; CRPC, castration-resistant prostate cancer; G3, grade 3; HLA, human leukocyte antigen; M, months; MST, median survival time; Not ref., not referred; NSCLC, non-small-cell lung cancer; PI, phase I clinical trial; PII, phase II clinical trial; PC, prostate cancer; PD, progressive disease; PFS, progression-free survival; PR, partial response; RCC, renal cell carcinoma; Ref., reference; SCLC, small-cell lung cancer; SD, stable disease.

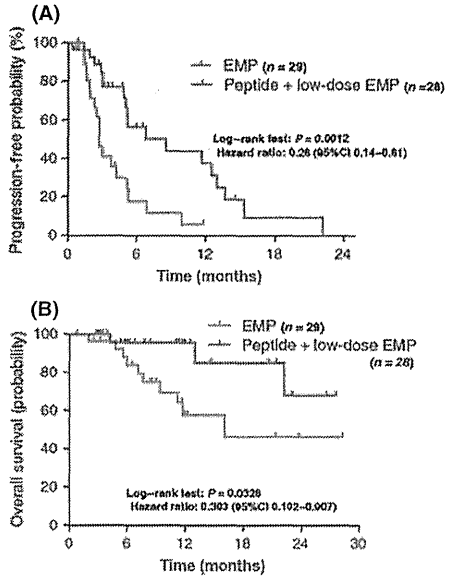

Fig. 4. Randomized phase II trial of personalized peptide vaccine (PPV) plus low-dose estramustine phosphate (EMP) versus standard-dose EMP in patients with castration-resistant prostate cancer. Patients were randomized into groups receiving either PPV plus low-dose EMP (280 mg/day) or standard-dose EMP (560 mg/day). (A) Duration of progression-free survival in the first treatment. (B) Overall survival of patients treated with PPV plus low-dose EMP and standard-dose EMP. CI, confidence interval.

et al.[9] carried out a phase I/II study of a cocktail vaccine IDM-2101 consisting of nine CTL-epitope peptides and the PADRE helper-epitope peptide with Montanide ISA51 in patients with metastatic non-small-cell lung cancer. No significant adverse events were noted except for low-grade erythema and pain at the injection site. One-year survival in the treated patients was 60%, and median overall survival was 17.3 months. One complete response case was observed in the total of 63 patients. Feyerabend and colleagues reported cocktail vaccines for patients with prostate cancer.[12] The cocktail vaccine consisted of 13 synthetic peptides, 11 HLA-A*0201 restricted CTL epitopes and two helper epitopes derived from prostate tumor antigens. A phase I/II trial of the vaccine was carried out in HLA-A2-positive patients with hormone-sensitive prostate cancer with biochemical recurrence after primary surgical treatment. The same group also developed another cocktail vaccine for renal cell cancer.[17] The vaccine, IMA901, consisted of nine HLA-A*0201 restricted CTL-epitopes and one helper epitope from renal cell cancer antigens with hepatitis B virus epitope as a marker peptide. A randomized phase II trial with a single dose of cyclophosphamide reduced the number of regulatory T cells and confirmed that immune responses to the vaccine component peptides were associated with longer overall survival.

Hybrid peptide vaccines

Peptide sequences of most of the single epitope vaccines as well as multi-epitope long peptide vaccines are native sequences with or without modification of anchor amino acids. Some of the latest generation of peptide vaccines are of hybrid-type, that is, a peptide fused with two epitopes. The Ii-Key/HER-2/neu hybrid peptide vaccine is a fusion peptide made up of the Ii-Key 4-mer peptide and human epidermal growth factor receptor-2 (HER-2)/neu (776–790) helper epitope peptide.[22,23] The Ii protein catalyzes direct charging

Table 2. Pros and cons of the latest generation of peptide vaccines

	Pros						Cons				
Vaccine type	Induction of CTL	Induction of Th	Applicable for multi-HLA type	Activation of memory T-cells	High efficiency of antigen presentation	Synthetic chemicals	No induction of Th	Possible induction of Treg	Not applicable for multi-HLA type	Multi formula	Induction of primary response	Biologics
Long peptide vaccine	Yes	Yes	Yes	No	No	Yes	No	Yes	No	No	Yes	No
Multipeptide non-cocktail vaccine	Yes	Yes	Yes	No	No	Yes	No	Yes	No	Yes	Yes	No
Peptide cocktail vaccine	Yes	Yes	Yes	No	No	Yes	No	Yes	No	No	Yes	No
Hybrid peptide vaccine	Yes	Yes		No	No	Yes	No	Yes	Yes	No	Yes	No
Personalized peptide vaccine	Yes	No	Yes	Yes	No	Yes	Yes	No	No	Yes	No	No
Peptide-pulsed DC vaccine	Yes	No	No	No	Yes	No	Yes/No	No	Yes	No	Yes	Yes

DC, dendritic cell; HLA, human leukocyte antigen; Th, helper T-cells; Treg, regulatory T-cells.

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of MHC class II epitopes to the peptide-binding groove, circumventing the need for intracellular epitope processing, and the shortest active sequence of the Ii protein is the Ii/Key peptide.[24] Holmes *et al.*[22] and Perez *et al.* [23] reported the results of phase I studies of the Ii-Key/HER -2/neu hybrid peptide vaccine in patients with prostate cancer. Significant decreases in circulating regulatory T cell frequencies, plasma HER-2/neu, and serum transforming growth factor-β levels were observed when compared with the native HER-2/neu (776–790) peptide vaccination.

Takahashi and colleagues developed a hybrid peptide of a helper-epitope and CTL-epitope of MAGE-A4.[25] The phase I study of the vaccine was carried out in patients with advanced cancers who were vaccinated with MAGE-A4-H/K-HELP combined with OK432 and Montanide ISA51. In a case report, there were no severe side-effects except for a skin reaction at the injection site. The vaccine induced MAGE-A4-specific Th1 and Tc1 immune responses and the production of MAGE-A4-specific complement-fixing IgG antibodies. Tumor growth and the carcinoembryonic antigen tumor marker were significantly decreased in the final diagnosis.

Personalized peptide vaccines

Virtually all prevaccination patients already have a weak immunity to cancer cells. However, the characteristics of cancer cells and of the immunological status against cancers differ widely among patients, even among those with the same histological types of cancer and identical HLA types. One of the reasons for the low clinical efficacies of the earlier generations of peptide vaccines might be a mismatch between the vaccine peptides and pre-existing immunity to the cancer cells. We therefore attempted to optimize the vaccine peptides so that they were appropriately matched to the pre-existing immunity of each patient (Fig. 3). There are two ways to detect pre-existing immunity, detection of CTL-precursors and detection of IgG in the peripheral blood. The PBMCs were cultured with vaccine peptide panels and the CTL responses to each peptide were measured. The second method is to detect IgG antibodies to the vaccine peptide panels. It is well known that the production of the IgG class of antibodies requires T-cell help. Therefore, the presence of a specific IgG indicates the presence of helper T cells. We carried out a series of clinical trials using personalized peptide vaccines (PPVs) for advanced cancer patients.[26–50] In this PPV formulation, appropriate peptide antigens for vaccination are screened and selected from a panel of vaccine candidates in each patient, based on pre-existing host immunity as mentioned above. Currently, we use 31 HLA class I-restricted peptide candidates, which were identified from a variety of tumor-associated antigens mainly through the cDNA expression cloning method with tumor-infiltrating T-lymphocyte 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 previous clinical studies.[26,27] A maximum of four peptides, which were selected based on the results of HLA typing and the pre-existing immune responses specific to each of the 31 different vaccine candidates, were injected s.c. with Montanide ISA51 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, as the performance characteristics, such as the sensitivity and reproducibility, of the current T cell assays are far from satisfactory. 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 HLA class I-restricted peptides. For example, 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. Notably, the multiplex bead-based Luminex technology that we have developed for monitoring B cell responses allow simple, quick, and highly reproducible high-throughput screening of IgG responses specific to large numbers of peptide antigens with a tiny amount of plasma.

In the clinical trials of PPV carried out during the past decade, we have shown promising results in various types of cancers.[26–50] Table 1 shows the summary of the immunological and clinical responses in 460 advanced cancer patients who received PPV. The best clinical responses assessed in the 436 evaluable patients were a partial response in 43 patients (10%), stable disease in 144 patients (33%), and progressive disease in 249 patients (57%), with a median overall survival of 9.9 months. Of note, a recent phase II randomized clinical trial of PPV for 57 castration-resistant prostate cancer patients showed that patients receiving PPV in combination with low-dose estramustine phosphate (EMP) showed a significantly longer progression-free (median survival time, 8.5 months *vs* 2.8 months; hazard ratio, 0.28 [95% confidence interval, 0.14–0.61]; $P = 0.0012$) and overall survival (median survival time, undefined *vs* 16.1 months; hazard ratio, 0.30 [95% confidence interval, 0.1–0.91]; $P = 0.0328$) than those receiving standard-dose EMP alone, suggesting the feasibility of this combination therapy (Fig. 4).[44] In addition, PPV was also used in an early phase clinical trial of patients with recurrent or progressive glioblastoma multiforme, one of the most aggressive brain tumors, with a median overall survival of 10.6 months.[47] Based on these promising results, randomized phase III trials are currently underway in glioblastoma. To prove the clinical benefits of PPV for accelerating cancer vaccine development, further randomized phase III trials would also be recommended in other types of cancers.

Peptide-pulsed dendritic cell vaccines

Many clinical trials of dendritic cell (DC)-based vaccinations using autologous DC and tumor-associated antigen peptides have been carried out to assess the ability of these vaccines to induce clinical responses in cancer patients.[51–54] Rahma *et al.*[54] carried out a comparative study of DC-based vaccine versus non-DC-based authentic peptide vaccine. Twenty-one advanced ovarian cancer patients were divided two groups: arm A received a p53 CTL-epitope peptide with Montanide with IL2; arm B received the same peptide-pulsed DCs with IL2. The median progression-free survival and overall survival were 4.2 (arm A) *i* 8.7 (arm B) months and 40.8 (arm A) versus 29.6 (arm B) months, respectively. This study suggests that the simple peptide vaccination and labor-consuming DC-based vaccination therapy are similarly effective.

Conclusion

Many investigators have attempted to develop more effective cancer vaccines, and in this review we discussed the resulting progress in the latest generation of peptide vaccines. The pros and cons of each type of vaccine are shown in Table 2. Each study used different adjuvants, cytokines, and/or other combination therapies with different doses. Moreover, the individual peptides themselves had different immunological and clinical potency as well as different amino acid sequences. Therefore, it is very hard to conclude that one type of vaccine was more efficient than another. The role of immune checkpoint molecules, such as CTLA-4 and programmed cell death-1, on antitumor immunity was clarified, and promising results have been reported in the clinical trials using combination therapies

with peptide vaccines and immune checkpoint blockades.[55–57] Further randomized phase III trials would be essential to prove the clinical benefits of these vaccine therapies, including immune checkpoint blockade combination therapies.

Disclosure Statement

The author Akira Yamada is an Executive Officer for Green Peptide Company, Ltd.

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COMMENTARY

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Current status of immunotherapy for the treatment of biliary tract cancer

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Biliary tract cancer (BTC) is one of the most aggressive malignancies. Although various promising regimens of chemotherapeutic and/or molecular targeted agents have been developed, further treatment modalities, including immunotherapies, still remain to be established for refractory patients who are unresponsive to or relapse after currently available therapeutic options for BTC. Recently, several clinical trials of immunotherapies, including peptide-based vaccines and dendritic cell (DC)-based vaccines, have been reported with promising results. Here we summarize the data from phase I or phase II clinical trials of immunotherapies for BTC. In particular, we introduce our novel immunotherapeutic approach called personalized peptide vaccine (PPV), in which HLA-matched peptides were selected and administered based on the pre-existing host immunity before vaccination, for the treatment of advanced BTC. Further clinical trials would be recommended to prove clinical benefits of these novel immunotherapeutic approaches. Recently concomitant treatments, such as chemotherapies and immune checkpoint blockade, have been reported to enhance the therapeutic effects of cancer immunotherapies through multiple coordinated immune mechanisms. Additional therapies in combination with immunotherapies could produce synergistic effects in the treatment of advanced BTC.

Keywords: biliary tract cancer, peptide vaccine, dendritic cell vaccine, personalized vaccine, immunotherapy, clinical trial, biomarker

Abbreviations: BTC, biliary tract cancer; DC, dendritic cell; PPV, personalized peptide vaccine; GEM, gemcitabine; TAA, tumor-associated antigen; WT1, Wilms tumor gene 1; MUC1, mucin 1; SD, stable disease; PD, progressive disease; OS, overall survival; PFS, progression-free survival; HR, hazard ratio; CI, confidence interval

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Introduction

Biliary tract cancer (BTC) is one of the most aggressive malignancies.[1,2] Only 10% of newly diagnosed patients present with early-stage disease and can be treated by a potentially radical excision of tumors. However, the remaining patients with unresectable, locally advanced and/or metastatic tumors show a poor prognosis, with a median survival of less than one year.[1,2] For advanced or recurrent BTC that are ineligible for surgery, various promising regimens of chemotherapeutic and/or molecular targeted agents have been studied.[1-4] For example, a combination of chemotherapeutic agents, gemcitabine (GEM) and cisplatin, has recently demonstrated a promising result in a randomized phase III trial in advanced BTC patients.[3] However, further treatment modalities still remain to be established for refractory patients who are unresponsive to or relapse after currently available therapeutic regimens for BTC.

Infiltration of different subsets of immune cells, including lymphocytes, macrophages, DCs and granulocytes, as well as immune-related microenvironments have been demonstrated to foster or inhibit tumor progression and/or metastatic potential in various types of cancers.[5,6] In BTC, higher frequencies of tumor-infiltrating $CD8^+$ cytotoxic T cells and/or $CD4^+$ T cells have been shown to be closely associated with favorable patient prognosis.[7,8] These findings have provided the rationale

Table 1. List of clinical trials of immunotherapies for biliary tract cancer

Type of vaccine	Disease condition	Phase of trial	Combined treatment	No. of patient	Clinical response	Median OS	Grade 3/4 toxicities (%)	Humoral response (%)	Cellular response (%)	Reference
MUC1 peptide	Advanced	I	(-)	3	PD 100%	NA	0	0	0	20
MUC1 peptide-loaded DCs	Adjuvant	I	(-)	2	No recurrence, 50%	NA	0	NA	NA	21
WT1 peptide	Advanced	I	GEM	16	SD 50%, PD 50%	288 d	0	NA	56	22
Tumor lysate-pulsed DCs plus activated T cell transfer	Adjuvant	I	(-)	36	PFS; 18.3M (vs 7.7M)	31.9M (vs 17.4M)	NA	NA	NA	24
Personalized peptide vaccine (PPV)	Advanced–(chemo-resistant)	II	chemotherapy	25	SD 80%, PD 20%	207 d	0	35	47	32

DCs, dendritic cells; GEM, Gemcitabine; OS, overall survival; PFS, progression-free survival; SD, stable disease; PD, progressive disease; M, months; NA, not available.

for further development of immunotherapies as a novel treatment modality against BTC. Here we summarize the current status of immunotherapies against BTC.

Recent Developments of Immunotherapeutic Approaches Against BTC

The field of cancer immunotherapy has drastically moved forward during these two decades since the first discovery of a tumor-associated antigen (TAA) recognized by cytotoxic T lymphocytes in 1991.[9-12] Advancement of molecular biological and immunological techniques has helped identify a large number of TAAs and peptide epitopes applicable as cancer immunotherapies.[13] For example, BTC has been reported to express a variety of TAAs, such as Wilms tumor gene 1 (WT1),[14] mucin 1 (MUC1)[15-17] and mutated K-RAS,[18,19] as potential targets for immunotherapies. Several clinical trials of immunotherapies targeting these molecules have recently been reported with promising results (Table 1).

Two groups employed a 100-mer peptide derived from MUC1 for the vaccination to BTC patients.[20,21] Yamamoto et al. reported a phase I clinical trial of vaccination with a 100-mer peptide consisting of the extracellular tandem repeat domain of MUC1 and incomplete Freund's adjuvant (Montanide ISA51) in patients with advanced pancreatic cancer (n = 6) or BTC (n = 3).[20] This study showed the safety of this vaccine formulation, but produced no substantial effects on antigen-specific immunological parameters or clinical outcomes in the vaccinated BTC patients. Lepisto et al. performed a Phase I/II clinical trial of vaccination with autologous DCs loaded with the 100-mer MUC1 peptide as an adjuvant therapy against pancreatic cancer (n = 10) or BTC (n = 2) patients following resection of their primary tumors.[21] The vaccine was well tolerated and no toxicity was observed. One of two patients with stage II intrahepatic cholangiocarcinoma had a long survival time without recurrence, although this patient showed no induction or boosting of MUC1 specific immune responses after vaccination.

Kaida et al. conducted an open-labeled, dose-escalation phase I trial of WT1 peptide vaccine combined with GEM to evaluate the safety and optimal immunological dose of this vaccine in HLA-A*0201, -A*0206, and/or -A*2402 positive patients with advanced pancreatic cancer (n = 9) or BTC (gallbladder carcinomas, n = 8; intrahepatic cholangiocarcinomas; n = 4; and extrahepatic cholangiocarcinomas, n = 4).[22] In this trial, 6 doses of GEM and 4 doses of WT1 peptide (1 or 3 mg) emulsified in incomplete Freund's adjuvant (Montanide ISA51) were administered. The adverse events were comparable to those with GEM alone, confirming the safety of this combination therapy. WT1-specific T cells in peptide-stimulated culture were detected by tetramer assay in 56% (9 of 16) of BTC patients. The clinical responses at 2 mo after vaccination showed 8 stable diseases (SD) and 8 progressive diseases (PD), and the median overall survival (OS) time for BTC was 288 d. Based on these promising data, the same group has started a phase I and randomized phase II study with WT1 peptide vaccine in combination with GEM and cisplatin for chemo-naive patients with unresectable or recurrent BTC.[23]

Shimizu et al. reported a phase I trial of autologous tumor lysate-pulsed DCs in combination with ex vivo CD3-activated T-cell transfer in an adjuvant setting for 36 postoperative patients with intrahepatic cholangiocarcinomas.[24] The median progression-free survival (PFS) and OS time of the patients receiving this adjuvant immunotherapy were 18.3 and 31.9 mo, respectively, which were significantly better than those of the control group receiving surgery alone [7.7 mo (p = 0.005) and 17.4 mo (0.022),

respectively]. In particular, patients with skin reactions (> 3 cm) at the vaccine site showed dramatically better prognosis. These results suggested a potential clinical benefit of this therapy for preventing recurrence and achieving long-term survival in intrahepatic cholangiocarcinoma patients, although a randomized trial will be needed for its confirmation.

Personalized Peptide Vaccine for BTC Patients

The anti-tumor immunity might differ widely among individual cancer patients, since the tumor cell characteristics and the host immune cell repertoires are reported to be quite diverse and heterogeneous among patients, even among those with identical HLA types and the same pathological types of cancer.[25-28] Considering the diversity of immune responses against heterogeneous tumor cells, tailored selections of vaccine antigens appropriate for individual patients could be a rational approach for developing effective cancer vaccines. We have developed a novel immunotherapeutic approach called personalized peptide vaccine (PPV), in which HLA-matched vaccine peptides are selected for vaccination based on the pre-existing host immunity from a list of vaccine candidates.[29,30] We have conducted a series of phase I and phase II clinical trials of PPV, which have shown better antigen-specific immune responses and promising clinical outcomes in patients with various types of advanced cancers.[31]

Recently, we conducted a phase II clinical trial of PPV for 25 chemo-resistant BTC patients (gallbladder carcinomas, n = 7; extrahepatic cholangiocarcinomas, n = 11; intrahepatic cholangiocarcinomas, n = 6; and periampullary carcinoma, n = 1) to evaluate the feasibility of this treatment and to identify potential biomarkers.[32] A maximum of 4 peptides were selected in consideration of the pre-existing host immunity before vaccination, as assessed by the titers of IgGs specific to each of the 31 different vaccine candidates [12 peptides for HLA-A2, 16 peptides for HLA-A24, 9 peptides for HLA-A3 supertypes (-A3, -A11, -A31, and -A33), and 4 peptides for HLA-A26], whose safety and immunological effects for other types of cancers were confirmed in previously conducted clinical studies. The selected peptides (3 mg/each peptide) were emulsified in incomplete Freund's adjuvant (Montanide ISA51) and subcutaneously administrated (weekly for 6 consecutive weeks and then bi-weekly thereafter) in combination with chemotherapeutic agents without severe adverse events. The median OS time was 207 d. In 10 patients who were radiologically evaluated before and after vaccination, the clinical response was classified as SD in 8 patients and PD in 2 patients. Humoral and T cell responses specific to the vaccine antigens were substantially induced in a subset of the vaccinated patients (35% and 47%, respectively). In the multivariate Cox regression analysis, lower IL-6 levels, higher albumin levels, and greater numbers of selected vaccine peptides were significantly favorable factors for OS [hazard ratio (HR) = 1.123, 95% confidence interval (CI) = 1.008 - 1.252, p = 0.035; HR = 0.158, 95% CI = 0.029 - 0.860, p = 0.033; HR = 0.258, 95% CI = 0.098 - 0.682, p = 0.006; respectively], suggesting that the evaluation of inflammation, nutritional status, and pre-existing antigen-specific immunity before vaccination could be useful for selecting appropriate BTC patients who would benefit from PPV. Based on this finding, we are planning an early phase clinical trial to reveal whether or not the blockade 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 after PPV in advanced BTC patients who show higher levels of plasma IL-6.[33,34]

Conclusions

Several clinical trials of immunotherapies for BTC have been reported with promising immunological responses and/or clinical outcomes. Further randomized trials would be essential to prove clinical benefits of these novel immunotherapies. Recently concomitant treatments, such as chemotherapies and immune checkpoint blockade, have been reported to enhance the therapeutic effects of cancer immunotherapies through multiple coordinated immune mechanisms, including activation of antigen-presenting cells or cytotoxic T cells and removal of suppressor cells.[35,36] Additional therapies in combination with immunotherapies could produce synergistic effects in the treatment of advanced BTC.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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Personalized Peptide Vaccine for Treatment of Advanced Cancer

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Abstract: The field of cancer immunotherapy has moved forward drastically in the past 20 years, since many tumor-associated antigens (TAA) have been identified. Although various approaches for therapeutic cancer immunotherapies, including peptide-based vaccines, have been developed and clinically examined, the complexity and diversity of tumor cell characteristics and host immune cell repertoires seem to limit the therapeutic efficacy of this treatment modality. Considering the diversity of immune responses against heterogeneous tumor cells, tailored selections of vaccine antigens appropriate for individual patients could be a rational approach for developing effective cancer vaccines. We have developed a novel immunotherapeutic approach called personalized peptide vaccine (PPV), in which a maximum of four human leukocyte antigen (HLA)-matched vaccine peptides were selected based on the pre-existing host immunity before vaccination. We conducted a series of phase I and phase II clinical trials of PPV, which have shown better antigen-specific immune responses and promising clinical outcomes in patients with various types of advanced cancers. Further randomized phase III trials would be recommended to prove the clinical benefits of PPV. In addition, novel biomarkers for selecting patients who would benefit most from PPV remain to be identified.

Keywords: Advanced cancer, biomarker, cancer immunotherapy, clinical trial, peptide epitope, personalized peptide vaccine.

1. INTRODUCTION

The field of cancer immunology and immunotherapy has moved forward drastically in the past 20 years, since many different tumor-associated antigens (TAA) have been identified [1-5]. Various approaches for therapeutic cancer immunotherapies have been developed and clinically examined, including cancer vaccines using tumor cells, proteins, peptides, viral vectors, DNA, or dendritic cells, and great advances have been made in the clinical efficacy of cancer immunotherapy [1-5]. Notably, two novel immunotherapeutic agents have recently been approved by the US Food and Drug Administration (FDA) for patients with advanced cancer [6, 7]. In April 2010, sipuleucel-T (Provenge; Dendreon Corporation, Seattle, WA), an autologous antigen-presenting cell (APC) product designed to stimulate antigen-specific immune responses against human prostatic acid phosphatase (PAP), was approved for the first time by the US FDA for the treatment of patients with castration-resistant prostate cancer (CRPC). The FDA granted this approval after treatment with sipuleucel-T improved overall survival by 4.1 months [mean survival time (MST), 25.8 months vs 21.7 months] in the largest phase 3 randomized controlled trial (the IMPACT study) [6]. In addition, in March 2011 the FDA approved ipilimumab (Yervoy; Bristol-Meyers Squibb, Princeton, NJ), an immunomodulating antibody that blocks cytotoxic T-lymphocyte antigen 4 (CTLA-4), one of the immune checkpoint molecules in T cells, to treat advanced melanoma patients. In the phase III randomized controlled trial, this agent resulted in a 3-month improvement in overall survival with a disease control rate of 28.5%, where 60% of the responding patients maintained disease control for more than 2 years [7].

Moreover, there have been promising results in immunotherapeutic approaches to the treatment of various types of advanced cancers, although they have not yet been officially approved. For example, blocking antibodies against a T-cell co-inhibitory receptor, programmed death 1 (PD-1), and one of its ligands, PD-ligand 1 (PD-L1), which have been reported to contribute to tumor cell escape from host immune surveillance, have shown feasible results against various types of cancers [8, 9]. Topalian *et al.* demonstrated that anti-PD-1 antibody revealed objective responses in approximately 20 to 25% of patients with non-small-cell lung cancer (NSCLC), melanoma, or renal-cell cancer [8]. Brahmer *et al.* reported that anti-PD-L1 antibody, which blocks the interaction between PD-1 and PD-L1, could induce durable tumor regression (objective response rates of 6% to 17%) and prolonged stabilization of disease (12% to 41% of patients at 24 weeks) in patients with advanced cancers, including NSCLC, melanoma, and renal-cell cancer [9]. Currently, these promising advancements are generating great optimism and heightened enthusiasm for the further development of cancer immunotherapies.

In addition to these significant advances, many other clinical trials of cancer immunotherapies have been under way to show beneficial therapeutic effects in patients compared to existing treatments [1-5]. In this review, we discuss the recent advances in peptide-based cancer vaccines. In par-

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ticular, we describe the details of our novel immunotherapeutic approach, called the personalized peptide vaccine (PPV), which has demonstrated promising results for advanced cancer patients in a series of clinical trials.

2. PERSONALIZED PEPTIDE VACCINE (PPV)

In 1991, Boon *et al.* for the first time reported a cDNA-expression cloning technique to identify TAA [10]. Subsequently, serologic analysis of recombinant cDNA expression libraries (SEREX), another technique for detecting TAA using autologous antibodies, was introduced for the identification of genes recognized by the host immune system [11]. Such advancement of molecular biological and immunological techniques has helped identify a large number of TAA and peptide epitopes applicable as cancer vaccines [12-14]. Since 1995, when Hu *et al.* reported the first clinical trial of the vaccination of a peptide derived from melanoma antigen gene-1 (MAGE-1) [15], many clinical trials of peptide vaccines have been reported [16, 17]. In earlier stages of clinical trials of peptide vaccines, one to several human leukocyte antigen (HLA) class I-restricted peptides emulsified with Montanide ISA51, a clinical grade of Freund's incomplete adjuvant, were employed. Although the early phase clinical trials demonstrated the feasibility and good toxicity profile of this approach, most of the late-phase randomized trials, other than few exceptions [18], failed to show beneficial therapeutic effects in patients compared to existing treatments [16, 17]. Therefore, a variety of new types of peptide-based vaccines have been developed [19, 20] (Fig. **1**). We first discuss our novel peptide-based approach, PPV, in which multiple vaccine antigens appropriate for each patient are selected from a panel of vaccine candidates based on pre-existing host immunity.

2.1. Rationale for Personalized Selections of Vaccine Peptides

Cancer patients possess anti-tumor immunity, which may depend strongly on both the tumor cell characteristics and the immunological status of the host [21-24]. The anti-tumor immunity might differ widely among individuals, since the tumor cell characteristics and the host immune cell repertoires are quite diverse and heterogeneous among patients, even among those with identical HLA types and the same pathological types of cancer. Nevertheless, before patients are enrolled in clinical trials of cancer vaccines, the expressions of vaccine antigens in tumor cells are sometimes confirmed, but the immunological statuses of the hosts are rarely evaluated. Considering the complexity and diversity of the host immune cell repertoires, it is likely that vaccine antigens that are selected and administered without considering the host immunological status might not efficiently induce beneficial anti-tumor immune responses [24]. Since, in most clinical trials of therapeutic cancer vaccines, common antigens are employed for vaccination independently of the immunological status of patients [16, 17], the low clinical efficacies might be explained at least in part by mismatches between the vaccine antigens and the host immune cell repertoires.

To evaluate the host immune cell repertoires, we examine patients' pre-existing immunity to a panel of vaccine candidates before vaccination and select appropriate vaccine antigens with immunological memory in each patient [25]. Vaccine antigens, to which patients already possess antigen-specific immunological memory, are expected to cause quick and strong secondary immune responses after vaccination (Fig. **2**). In contrast, vaccinations with inadequate antigens without immunological memory could not easily provide

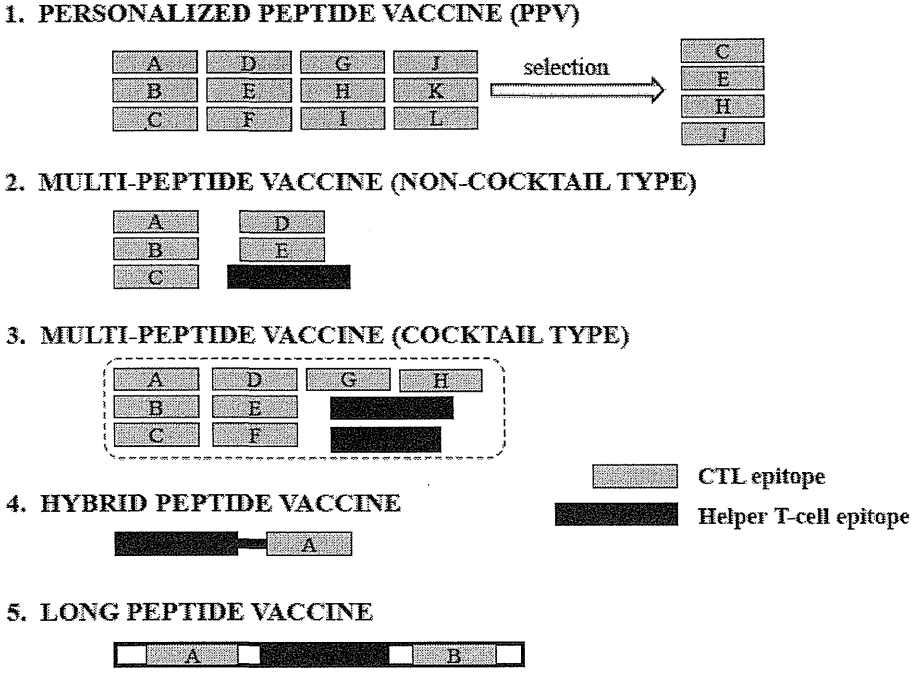

Fig. (1). Recent development of new types of peptide-based vaccines. Examples of new types of peptide-based vaccines are shown. Gray and black boxes indicate CTL and helper T-cell epitopes, respectively.

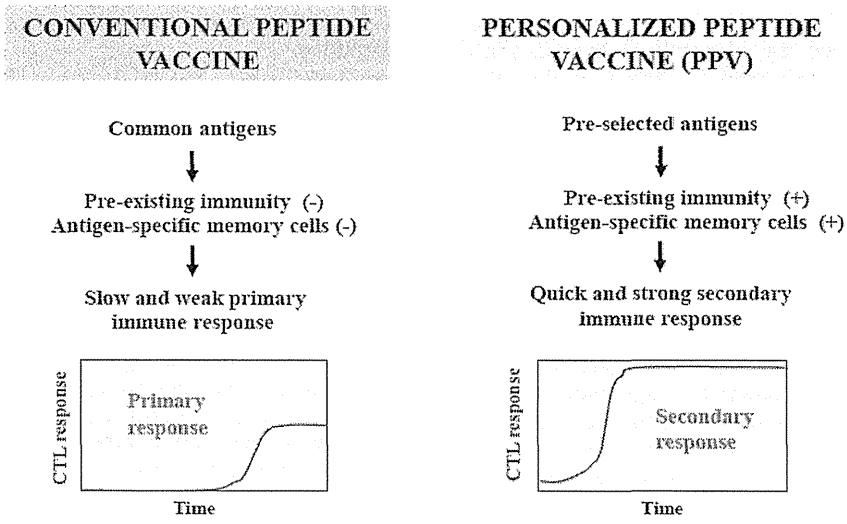

Fig. (2). Rationale of personalized peptide vaccine. In conventional peptide vaccines without pre-existing immunity, patients without immunological memory to vaccine antigens would take more time to develop effective anti-tumor immune responses because several rounds of repeated vaccinations might be required to prime antigen-specific naive T cells to functional effector cells. In personalized peptide vaccines with the pre-existing immunity, patients with antigen-specific immunological memory are expected to show quick and strong secondary immune responses to them.

clinical benefits, especially in advanced cancer patients who show rapid disease progression [26]. In light of this, it would be quite reasonable to select vaccine antigens on the basis of the pre-existing immune cell repertoires in each patient.

Cancer cells can develop various mechanisms to accelerate malignant behavior [21]. For example, it has been well recognized that cancer cells might escape the host's immunological surveillance. After the interaction/competition between tumor cells and host immune cells, tumor cell variants resistant to the immunological pressure often emerge through the selection of mutants with reduced antigenicity [21]. Therefore, the selection and administration of multiple vaccine antigens could reduce the risk of tumor escape through the existence and/or induction of antigen-negative variants escaping antigen-specific immune responses [22, 27], since it would be rare for tumor cells to simultaneously lose all of the multiple antigens selected for vaccination.

Collectively, our new concept of "personalized" cancer vaccine formulation, where multiple peptide antigens are selected for vaccination by the pre-existing host immunity from a list of vaccine candidates, may confer several advantages, including the possibility of bypassing both immunological diversity and tumor heterogeneity.

2.2. PPV Procedures

For PPV, a maximum of four peptides are selected based on the results of HLA typing and the pre-existing immune responses specific to each of the 31 HLA class I-restricted cytotoxic T lymphocyte (CTL) epitope peptides with minimal optimal lengths (9-mer or 10-mer): 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 (Table **1**). These peptides were identified mainly through the cDNA expression cloning method with tumor-infiltrating T-lymphocyte lines [25, 28-34]. The safety and potential immunological effects of these vaccine candidates have been demonstrated in clinical studies [25, 35, 36]. It should be noted that we currently employ these 31 CTL epitopes, which are also shown to induce antigen-specific B-cell immune responses, as vaccine antigen candidates for PPV, since it has been suggested that a CTL peptide with the ability to induce antigen-specific B-cell responses could provide more effective immune responses than a CTL peptide without it [37, 38].

Although short peptide epitopes with minimal optimal lengths have been reported to bear the potential to induce immune tolerance rather than activate antigen-specific immune responses [39-41], our PPV formulation with short epitopes has been demonstrated to efficiently induce antigen-specific IFN-γ-producing $CD8^+$ T cells, but not tolerance to them, possibly because only immunogenic epitopes are selected in each patient by screening before vaccination. Although long synthetic peptides have shown excellent immune responses and promising clinical results in some clinical trials [42, 43], we do not currently use long peptides for PPV, since they may contain undesirable T-cell epitopes that activate other immune cells, such as T helper 2 cells and/or regulatory T cells [44, 45], which could negatively affect beneficial antigen-specific immune responses.

Different peptides have their own different binding affinities to the corresponding HLA molecules. Therefore, if multiple CTL-epitope peptides with different HLA-binding affinities are loaded to APCs, the individual peptides may compete with each other to bind HLA molecules on the APCs [46]. For PPV, to prevent such competition among peptides at the vaccinated sites, a maximum of 4 immunogenic peptides selected from the 31 different vaccine candidates are individually mixed with incomplete Freund's adjuvant (Montanide ISA51; Seppic, Paris, France) and subcutaneously injected at different sites, but not at a single site as a mixture. Regarding the vaccination schedule,