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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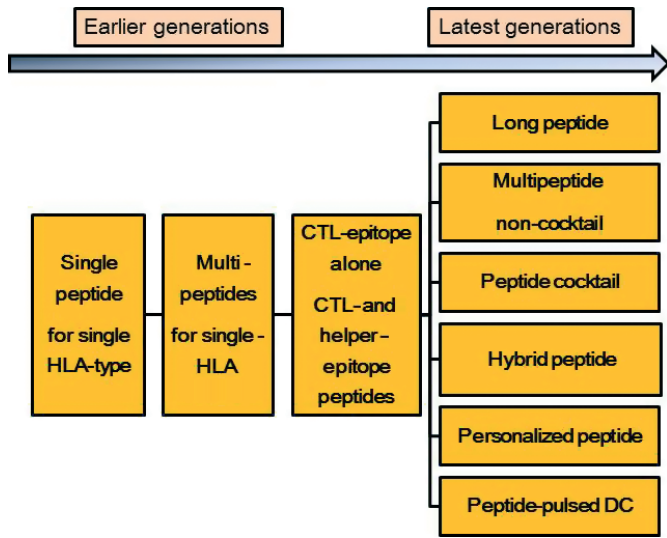E-mail: akiymd@med.kurume-u.ac.jp

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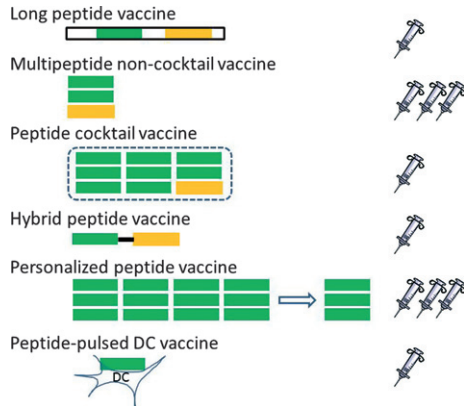

Fig. 2. Various types of latest generation peptide vaccines. The number of syringes indicates that of the final preparation for injection. Green, CTL-epitopes; orange, helper-epitopes. DC, dendritic cells.

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
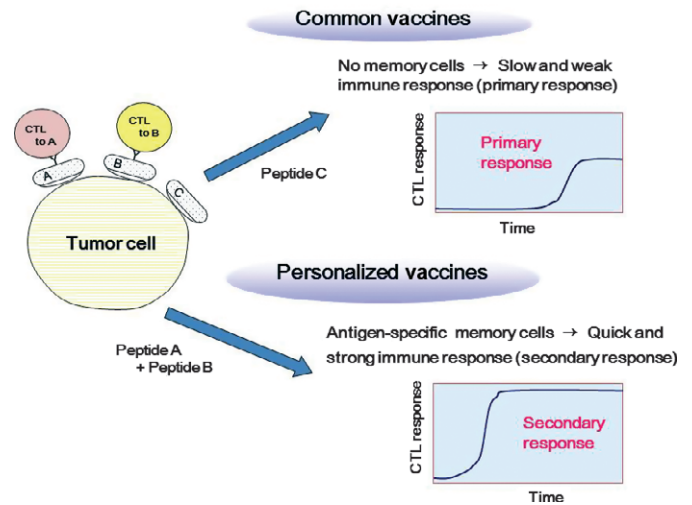

Fig. 3. Personalized peptide vaccine. In the classical type of vaccine, peptides derived from tumor-specific or overexpressed antigens are used as vaccine peptides and often mismatched to the pre-existing immunity of patients. In personalized peptide vaccines, appropriate peptides for vaccination are 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

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.
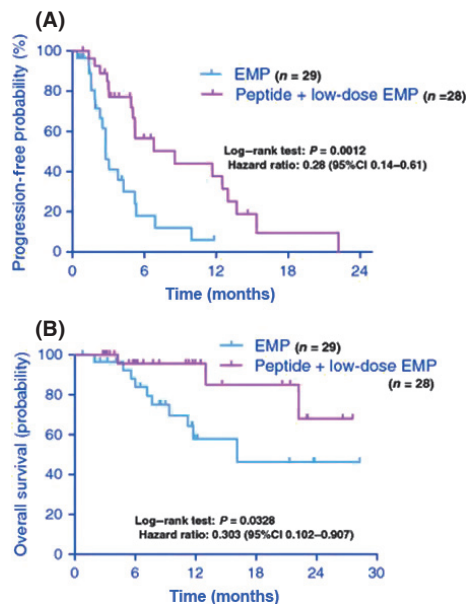
Fig. 4. Randomized phase II trial of personalized peptide vaccine (PPV) plus low-dose estramustine phosphate (EMP) versus standard-dose EMP in patients with castration-resistant prostate cancer. Patients were randomized into groups receiving either PPV plus low-dose EMP (280 mg/day) or standard-dose 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

Vaccine type	Pros						Cons					
	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	No	Yes	No	Yes	Yes	No	Yes	No	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.

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

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