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REVIEW ARTICLE

# Cancer vaccine therapy based on peptides

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## ABSTRACT

Following numerous unequivocal clinical failures, immunotherapy has become an attractive therapeutic modality. Peptide vaccines are cost-effective compared to other vaccine approaches, and effective epitopes eliciting strong immune response can be selected experimentally *in silico* and *ex vivo*. However, the clinical benefits of cancer peptides vaccine have been disappointing in most studies; therefore, we need to prove the clinical beneficial effects for cancer treatment following induction of more powerful cytotoxic T lymphocytes (CTLs). First, the choice of ideal target antigen is essential. Epitopes derived from tumor-associated antigens (TAAs), oncoantigens, vascular endothelial cells and neoantigens are then developed. In particular, whole-exome sequencing enables us to identify the epitopes of neoantigens. The choice of therapeutic objectives is also important and peptide vaccines might be better to be developed as preventative vaccines. Dendritic cells (DCs) vaccine pulsed with peptides is an approach to induce powerful CTLs and might overcome several disadvantages of peptide vaccines as monotherapy. Targeting vaccine therapy against DC subsets *in vivo* is under development.

***Keywords**: immunotherapy; peptide vaccine; tumor-associated antigen; oncoantigen; VEGFR2; neoantigen; dendritic cells*

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## Introduction

It is well established that the immune system against cancer can be spontaneously activated. In melanoma, renal cancer, breast cancer and ovarian cancer, the tumor-infiltrating lymphocytes (TILs) in tumor micro-environment are positively related to long survival[1,2]. Moreover, adoptive transfer of TILs, as well as engineered tumor antigen-specific T cells, have induced the regression of tumors in melanoma patients[3].

Following numerous unequivocal clinical failures, immunotherapy has become an attractive therapeutic modality for cancer. One type of immunotherapy is immune checkpoint inhibitors using humanized monoclonal antibody (mAb) specific to cytotoxic T lymphocyte-associated protein 4 (CTLA4), programmed cell death protein 1 (PD-1) or its ligand PD-L1. These immune checkpoints are involved in the immune suppression of tumor-reactive T cells, and blockades of these molecules by antibody would enhance T-cell-mediated immune response to tumors[4,5]. Adoptive immunotherapy, such as chimeric antigen receptor T-cell (CAR-T) therapy, attracts rising attention for its remarkable clinical effects, especially for patients with hematological cancer[6–10].

Cancer vaccine therapy, offering potentially targeted therapy with fewer adverse effects compared with conventional therapy, is another immunotherapeutic approach based on the stimulation of anti-tumor immune system after immunization with synthesized tumor antigen. In cancer vaccine therapy, as well as immune checkpoint inhibitors and CAR-T, cytotoxic T lymphocytes (CTLs) play a leading role in attacking cancer cells. The markers recognized by CTLs to distinguish between cancer cells and normal cells are peptide human leukocyte antigen (HLA) complex expressed on the surface of these cells. Cancer vaccine therapy delivers

concentrated antigen to HLA class I and/or class II molecules of antigen-presenting cells (APCs) such as dendritic cells (DCs) and promotes the activation and proliferation of antigen-specific CTLs to attack cancer cells. The clinical benefits of cancer vaccines have not been presented clearly in most studies[11]. However, some recent clinical trials have reported encouraging results for this immunotherapy. For instance, Sipuleucel-T, which is an immune cell-based vaccine, resulted in increased overall survival in hormone-refractory prostate cancer patients. In 2010, it became the first therapeutic cancer vaccine to be approved by the United States Food and Drug Administration (FDA)[12].

## Therapeutic cancer peptide vaccine therapy

Several strategies for cancer vaccination are being investigated, including peptides, proteins, APCs, tumor lysate, tumor cells, DNA, mRNA and viral vectors. Peptide vaccines are cost-effective compared to other vaccine approaches. Moreover, peptide vaccines take advantage of computer algorithms to screen amino acid sequences for candidates with MHC class I-restricted peptide epitopes derived from cancer antigens. Those candidate epitopes are then tested for immunogenicity to induce and activate the specific CTLs experimentally. Therefore, an ideal epitope to elicit strong immune response against target antigens can be selected.

Cancer peptides are small fragments of tumor antigen protein forming a complex with HLA and are expressed on the surface of cancer cells. In cancer peptide vaccine therapy, patients are administrated a sufficient number of synthesized epitope peptides derived from tumor antigens (**Figure 1A**). These peptides then form a complex with HLA on the surface of APCs such as DCs. When the naive CTLs recognize the complex of peptide and HLA class I, these CTLs are activated and proliferated. The activated CTLs will then recognize the identical peptides presented on the surface of cancer cells and subsequently attack these cancer cells.

### Choice of target antigens

Improved results of cancer peptide vaccines can most likely be obtained by choice of antigens. Cancer-specific expression of antigens to avoid side effects, and oncogenic characteristics of antigens to avoid the escape from immune tolerance and high immunogenicity of antigens, are all required for ideal antigens to elicit effective and safe CTLs.

The most common approach to cancer vaccination is immunization with shared tumor antigens; for example, tumor-associated antigens (TAAs) such as differentiation antigens, overexpressed antigens, or cancer-testis antigens are expressed by many different patients' tumors (**Table 1**)[13]. Differentiation antigens (*e.g.* Tyrosinase, gp100, MART-1 and PSA) and overexpressed antigens (*e.g.* CEA, HER2 and h-TERT) are expressed at a much higher level in tumor cells than in normal cells. Cancer-testis antigens (*e.g.* NY-ESO-1 and MAGE-A3) are basically expressed only in tumor cells and in germ cells, which are unaffected by the immune system because of their physiological location[13].

**Table 1.** Target antigens of cancer vaccines

| | |
|---|---|
| **Differentiation antigen** | Tyrosinase, gp100, MART-1, PSA |
| **Overexpressed antigen** | CEA, HER2, h-TERT |
| **Cancer-testis antigen** | NY-ESO-1, MAGE-A3 |
| **Oncoantigen** | KIF20A, LY6K, DEPDC1, FOXM1, CDCA1, CDH3, IMP-3 |
| **Vascular endothelial cells** | VEGFR1, VEGFR2 |
| **Neoantigen** | Individual |

The National Cancer Institute prioritized cancer antigens based on pre-weighted objective criteria, including therapeutic function, immunogenicity, oncogenicity, specificity, expression level, stem cell expression, frequency of overexpression in tumors and in patients, and antigen cellular location[14]. WT1 emerged as the best antigen in this pilot study. WT1 is highly expressed in various malignancies and has been found to perform oncogenic function[15,16]. Both cellular and humoral immune responses against WT1 are naturally elicited in cancer patients, indicating strong immunogenicity of WT1[17,18]. Several clinical studies using WT1 peptide-based immunotherapies have been performed with encouraging results for patients, including children, with various kinds of malignancies[19,20]. Currently, a phase I/II trial for pediatric patients with relapsed or refractory high-grade gliomas and a pilot study combination with Nivolumab for recurrent ovarian cancer are ongoing.

### Oncoantigens

The development of genome-based technology has enabled us to obtain comprehensive gene expression profiles of malignant cells compared with normal cells[21]. By applying cDNA microarray

technology coupled with laser micro-dissection, novel oncoantigens are identified. An oncoantigen is a molecule with not only cancer-specific expression, but also oncogenic function that plays a critical role in tumor growth. The targeting oncogenic antigens can avoid the immune escape of cancer cells by lacking these proteins[22,23]. Epitope peptides of several oncoantigens such as kinesin family member 20A (KIF20A), lymphocyte antigen 6 complex locus K (LY6K), DEP domain-containing 1 (DEPDC1), forkhead box M1 (FOXM1), cell division cycle-associated 1 (CDCA1), cadherin 3/P-cadherin (CDH3), insulin-like growth factor-II mRNA-binding protein 3 (IMP-3) and others have been developed for clinical trials[24–33].

## Antigens targeting vascular endothelial cells

There are potential pitfalls limiting clinical efficacy of cancer vaccine therapy in targeting TAAs. One is the loss or downregulation of tumor antigens in cancer tissues, and another is HLA class I deficiency in cancer tissues[34,35]. On the other hand, neovascularization is associated with the expression of vascular endothelial growth factor receptor 1 (VEGFR1) and VEGFR2[36,37]. VEGFR1 and VEGFR2 are highly expressed in tumor vascular endothelial cells. Moreover, vascular endothelial cells play crucial roles in the growth and progression of tumors, and they stably express HLA molecules[38]. Therefore, epitope peptides derived from VEGFR1 (Pradimotide) and VEGFR2 (Elpamotide) have been developed[39–41].

Based on the promising results of phase I trial using Elpamotide, a randomized phase II/III trial was carried out for patients with advanced pancreatic cancer (PEGASUS-PC Study)[42,43]. Patients were randomly allocated to either Active group (Elpamotide + Gemcitabine) or Placebo group (Placebo + Gemcitabine) at 2:1 ratio. Definitively, the statistically significant differences between Active group and Placebo group in the prolongation of overall survival were not proven (Harrington–Fleming *P*-value = 0.918; log–rank *P*-value = 0.897; hazard ratio = 0.87; 95% confidence interval (CI), 0.486–1.557). Median survival time (MST) was 8.36 months (95% CI, 7.46–10.18) for the Active group and 8.54 months (95% CI, 7.33–10.84) for the Placebo group. However, subgroup analysis based on the degree of injection site reactions (ISR) showed that the survival time of patients group with severe ISR such as ulceration, observed in Active group only, was significantly prolonged (MST of 16 months) compared with that of other subgroups. This data strongly suggests that patients with a strong immunological response might have survival benefits from peptide vaccines therapy.

## Present status of cancer peptide vaccine development

Clinical benefits of cancer peptide vaccine therapy have been disappointing in most trials, including PEGASUS-PC Study, so far. Phase III trial of telomerase peptide vaccine (GV1001) for advanced pancreatic cancer (TeloVac) combined with chemotherapy did not improve overall survival[44]. Phase III trial of Tecemotide, a MUC1 antigen-specific vaccine, for stage III non-small cell lung cancer (START) also could not prove significant difference in overall survival[45,46]. Similarly, phase III trial of Rindopepimut, which is a EGFRVIII peptide, for glioblastoma patients failed to meet its pre-specified endpoint[47,48]. Proven clinical benefits of cancer peptide vaccines following the induction of more powerful CTLs must be decided based on these results' success.

One possibility to overcome this limit would be a cocktail of peptides to induce immunological response against multiple molecules. Multi-peptides cocktail vaccine is a strategy to overcome not only the low rate immunological responders of single vaccine, but also the heterogeneity of antigen expression on tumor cells, even though phase III trial of multi-peptides (IMA901) for advanced/metastatic RCC (IMPRINT) and phase III trial of multi-peptides (OCV-C01) for advanced pancreatic cancer failed to prove survival benefits[49–53].

## Neoantigens as novel targets of cancer peptide vaccines

Genetic alterations accumulated by cancer cells during the tumorigenesis process can result in mutant protein expression. Mutated proteins expressed exclusively in cancer cells and recognizable by the immune system are known as neoantigens. Each tumor has different mutations, and individual tumors express unique neoantigens. TAAs are hampered by the generated central T-cell tolerance, but neoantigens are not subject to the tolerance because they are generated after the accumulation of mutation in cancer cells. Therefore, neoantigens can be more immunogenic and tumor-specific than TAAs[54].

Whole-exome sequencing enables us to identify information on tumor-specific somatic missense mutations, fusion transcripts and frameshifts[55–58]. Then, a list of candidate epitopes derived from neoantigens is searched for by computer algorithms. These approaches are now being adapted to create

personalized therapeutic cancer vaccines. On the other hand, it might be difficult to select immunogenic epitopes eliciting active CTLs among candidate epitopes[59,60]. It takes time to assess the immunogenicity of candidate epitopes by *in vitro* experiments, though the vaccine should be delivered to patients quickly. However, the development of peptide immunogenicity prediction algorithms will overcome the problem. Early clinical trials have shown that personalized cancer vaccines based on each patient's mutation are immunogenic and can provide clinical benefits[61,62].

## Choice of therapeutic objectives

Another strategy to overcome the limited efficacy of cancer vaccines is the choice of therapeutic objectives. For example, preventative vaccines against human papillomavirus (HPV), which serves as the etiological factor and biological carcinogen for HPV-associated lesions and cancer, have been utilized to avert cervical cancer but they do not induce strong therapeutic effects against established HPV infections[63]. Therefore, cancer peptide vaccines targeting specific cancer antigens might also be better to be developed as preventative vaccines.

Post-operative cancer patients could elicit more powerful CTLs compared to far advanced tumor-burdened patients[64,65]. The phase III study of OCV-C01, a peptide cocktail vaccine of oncoantigen (KIF20A) and antigens targeting vascular endothelial cells (VEGFR-1 and VEGFR-2) for far advanced pancreatic cancer, was unable to prove survival benefit. However, phase II clinical trial using OCV-C01 as a post-operative adjuvant treatment for surgically resected pancreatic cancer patients showed encouraging results[66]. Disease-free survival (DFS), which is the primary endpoint of this study, was 15.8 months (95% CI, 11.1–20.6) in the OCV-C01 + Gemcitabine group. This is favorable when compared with 12 months in the Gemcitabine-alone group. Moreover, subgroup analysis suggested that the expression of KIF20A in surgical specimen is positively related to the induction of KIF20A-specific CTLs after the administration of vaccine. In addition, all four patients with positive KIF20A expression had no recurrence of pancreatic cancer. A randomized controlled trial is essential to demonstrate the clinical benefits of OCV-C01 for surgically resected pancreatic cancer patients.

Also, phase III trials of peptide cocktail vaccines derived from five oncoantigens for post-operative patients with resected esophageal cancer are currently ongoing.

## DC vaccine pulsed with peptides

In contrast to prophylactic vaccines, therapeutic cancer vaccines must break the tolerance acquired by the tumor cells so as to elicit powerful CTLs. DCs are known as the most effective APCs and play a pivotal role in coordinating innate and adaptive immune responses[67].

In cancer peptide vaccine therapy, administrated peptides are loaded directly on the MHC molecules of DCs *in vivo*. However, DCs *in vivo* are usually immature in their resting state. Mature DCs can migrate to lymphoid tissue, enhance expression of co-stimulatory molecules and produce cytokines to activate CTLs efficiently[68]. In contrast, immature DCs fail to induce antigen-specific responses or even induce the regulatory T cells[69–71]. Therefore, peptide vaccines are commonly administered with vaccine adjuvant to maturate the targeting DCs[32,72,73].

DCs vaccine pulsed with peptides is an approach to induce powerful CTLs. DCs can be generated and expanded from peripheral blood monocytes cultured with granulocyte macrophage-colony stimulating factor (GM-CSF) and IL-4. The DCs are then stimulated with adjuvant to maturate and be pulsed with peptides. Active CTLs can be induced more effectively by preparing optimized DCs *ex vivo* without the presence of tumor-derived negative factors, but with specific positive adjuvant to maturate DCs enough (**Figure 1B**).

It is also considered that peptides can induce anergy of CTLs when they are loaded on non-professional APCs because of the lack of signals from co-stimulatory molecules[74,75]. To overcome this problem, extended long peptides were developed. Long peptides do not bind to HLA directly and only professional APCs, such as DCs, can take it up[76]. However, it was reported that some long peptides might induce peptide-specific regulatory T cells (Tregs) and Th2 cells to limit the clinical efficacy of long peptides[77,78]. On the other hand, in DC vaccine therapy, peptides are pulsed on mature DCs *ex vivo* and they cannot be loaded on unprofessional APCs.

To date, many clinical studies of DC vaccine have been conducted, including short peptide, long peptide, protein, tumor lysate and mRNA targeting for TAAs, oncoantigens and neoantigens[48]. In 2010, the FDA approved Sipuleucel-T, which is a DC-based cancer vaccine for the treatment of hormone refractory prostate cancer. Sipuleucel-T consists of a mixture of DCs, B cells, monocytes, and NK cells that have been cultured *ex vivo* with

a recombinant fusion protein containing PAP and GM-CSF. The phase III IMPACT trial showed a 4.1-month improvement in median overall survival at 36 months and the survival rate was 31.7% in the treated patients versus 23.0% in the placebo patients[79].

In some prospective and retrospective studies of WT-1 peptide pulsed DC vaccine for patients with pancreatic cancer, prolonged survival and suggested positive delayed-type hypersensitivity (DTH) skin reaction are associated with good clinical outcome[80–82]. In another small-scale prospective study, WT1-specific HLA class I and class II peptides pulsed DCs were administered with Gemcitabine for patients with stage IV pancreatic ductal adenocarcinoma[83]. The survival of seven patients who received both class I and class II peptides pulsed DCs vaccine was significantly extended compared to that of three patients who received DC vaccine pulsed with class I or class II peptide alone ($P = 0.036$ in OS, $P = 0.010$ in progressive-free survival (PFS)). A phase III study using DCs pulsed with class I and class II peptides derived from WT-1 for patients with advanced pancreatic cancer is currently ongoing.

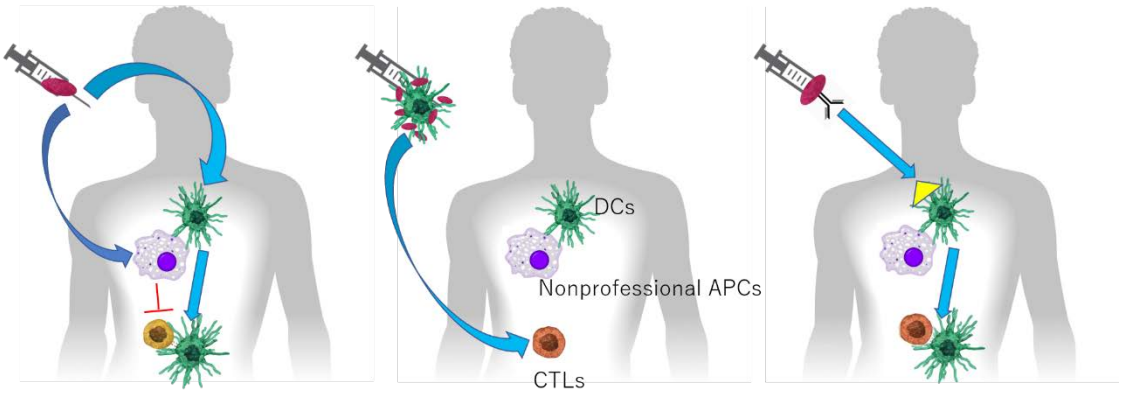
Recently, the novel concept of engineered vaccines that directly target antigens to *in vivo* DCs *via* ligation of C-type lectin receptor (CLR) or chemokine receptor has been developed (**Figure 1C**)[48]. DC subsets are known to express different CLRs and chemokine receptors. Therefore, DCs ligated with the ligand or antibody against CLRs or chemokine receptor will deliver to DC subsets *in vivo*. A human study using antigens targeted to DC DEC205, which is a kind of CLR expressed on DCs, demonstrated successful induction of tumor-specific T cell responses and several clinical trials of anti-DEC205-NY-ESO-1 are currently ongoing[84]. Another target is chemokine receptor XCR1, which is specifically expressed on CD141+ DCs. CD141+ DCs are believed to be the human equivalent of mouse CD8+ DCs, which can cross-present cell-associated antigens to CD8+ T cells. Vaccines ligated with the chemokine for XCR1 are being developed[85,86].

## Conclusion

The development of clinically effective peptide vaccine is a multi-component task and we need to explore the optimal combinations of antigens, adjuvant remedy, delivery tools and study design. On the other hand, active immunotherapy such as peptide vaccines must also address the immunosuppressive and tolerogenic mechanisms deployed by tumors, and combination with established immunotherapies such as immune checkpoint inhibitors are attractive strategies. However, the identification or arrangement of peptide vaccines, which can elicit powerful CTLs enough to eliminate tumor cells, as an ideal partner of immune checkpoint inhibitor is an essential task.

## Conflicts of interest

The authors declare no potential conflicts of interest with respect to the research, authorship, and/or publication of their article.




(A) Peptides are loaded on DCs and nonprofessional APCs *in vivo*

(B) Peptides *ex vivo* are loaded on DCs *ex vivo*

(C) Peptides are directly loaded on DCs *in vivo*

**Figure 1.** Comparison of peptide vaccine, DC vaccine and engineered peptide vaccine. **(A)** Administrated peptides are loaded both on DCs and non-professional APCs *in vivo*. **(B)** DC vaccine is pulsed with peptides *ex vivo* and activate CTLs directly following administration. **(C)** Administrated engineered peptides are specifically loaded on DC subset *in vivo*.

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