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# The present status and future prospects of peptide-based cancer vaccines

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**Abstract**

**Tumor cells commonly express several antigens, such as tumor-associated antigens (TAAs) or mutation-derived antigens (neoantigens), that can be regarded as foreign antigens and elicit anti-tumor immune responses in cancer patients. Various TAAs or neoantigens expressed in cancer cells have been identified and utilized as targets for cancer vaccines. One approach to elicit tumor-specific immune responses is termed peptide-based cancer vaccination; it involves administrating TAAs or neoantigen-derived peptide for treatment of cancers. There have been several forms of peptide-based cancer vaccines depending on which effector cells, such as CTLs or CD4$^+$ T-helper cells, are targeted to be activated. Many phase I and II clinical trials of peptide-based cancer vaccines using TAA-derived CTL epitopes, T-helper cell epitopes or dendritic cells loaded with TAA-derived peptides for various malignant tumors have been conducted and provide clinical benefits in a small fraction of patients. Nowadays, to improve the efficiency of peptide-based cancer vaccines, combination immunotherapy of peptide-based cancer vaccines with the immune-checkpoint blockade therapies using mAbs specific for CTLA-4, programmed cell death 1 (PD-1), or PD-1 ligand 1 (PD-L1) have been developed for clinical application. Furthermore, along with the recent technological progress in genetic and bioinformatic analysis, it has become easier to identify neoantigens from individual cancer patients. It is expected that peptide-based cancer vaccines targeting neoantigens as a personalized cancer immunotherapy will be developed.**

*Keywords:* neoantigen, peptide-based cancer vaccine, tumor-associated antigen, tumor-reactive T cell

## Introduction

Treatment of cancer through intervening in the immune system is termed cancer immunotherapy, and various approaches for activation or improvement of the immune system to fight against cancer in patients have been developed at present. The history of cancer immunotherapy is very old. In 1891, Dr William Coley, the pioneer of cancer immunotherapy, developed a mixture of heat-killed bacteria for intratumoral injection that occasionally showed durable regression of tumors (1). Since then, there have been significant advances in basic knowledge of tumor immunology. One example is the discovery of tumor-associated antigens (TAAs) that are expressed in tumor cells and recognized as if they are foreign antigens by the host immune system because of the dissimilarity of these substances with those expressed in normal cells. The identification of immunogenic TAAs makes it possible to develop tumor-specific cancer immunotherapies, such as administration of humanized mAbs specific to cell surface TAAs, adoptive immunotherapy with tumor-specific T cells or tumor-specific active immunization (cancer vaccines).

Peptide-based cancer vaccines are one form of cancer vaccines that induce tumor-specific T-cell responses by vaccination with TAA-derived tumor-specific peptides. Here, we introduce the background of peptide-based vaccination and its present status and provide an overview of current challenges and future directions of this immunotherapy.

## Background of peptide-based cancer vaccines

Nowadays, cancer immunotherapy has become an attractive therapeutic modality for cancers and is regarded as the fourth modality of cancer treatment after surgery, radiotherapy and chemotherapy (2). Cancer immunotherapies are generally classified into the two categories of ‘passive immunotherapies’ or ‘active immunotherapies’ according to their ability to stimulate the host immune system against cancer cells (3). Passive immunotherapies are defined as approaches in which immunologic reagents, such as tumor-targeting mAbs or adoptively transferred T cells that are thought to have

anti-tumor activities, are administered to cancer patients (4, 5). In contrast, active cancer immunotherapies are defined as activation of the host immune system by introduction of antigens that trigger immune responses against cancer cells.

Cancer vaccines are categorized as 'active cancer immunotherapy' and can provoke the immune system to destroy selectively cancer cells by targeting TAAs via vaccination with synthetic peptides derived from TAAs, recombinant TAA proteins, recombinant viral vectors encoding TAAs, TAA-loaded dendritic cells (DCs) or DNA/RNA-encoding TAAs (6). Peptide-based cancer vaccination is one of the therapeutic modalities of cancer vaccines that elicits endogenous tumor-specific T-cell responses after administrating TAA-derived peptides and is an attractive cancer treatment because it is easy to use and has shown low toxicity in many clinical trials (7, 8).

### Identification of TAAs as targets for cancer vaccines

To achieve effective and safe peptide-based cancer vaccines, it is important to identify appropriate TAAs as targets for peptide-based cancer immunotherapy. The ideal TAAs must have the three characteristics detailed below to be effective molecular targets of this immunotherapy (9).

First, tumor-specific expression patterns of TAAs. The expression of TAAs must be specifically in tumor tissues, but not (or highly restricted) in normal tissues. Cancer–testis antigens (CTAs) and oncofetal antigens (OFAs) that are over-expressed in tumor tissues, but not in normal tissues except for fetal organs and testis, are considered as ideal TAAs in terms of prevention of eliciting serious autoimmune responses.

Second, immunogenic characteristics of TAAs. The TAAs must have the capacity to be recognized in the same way as foreign antigens are and must elicit T-cell-mediated immune responses in cancer patients.

Third, oncogenic characteristics of TAAs. It is well-known that cancer cells can directly escape host T-cell recognition by down-regulating the expression of human HLA class I molecules or by down-regulating some TAAs. To prevent a loss of targeted antigens during cancer vaccination, TAAs must have biologically oncogenic characteristics because TAAs involved in oncogenesis are considered to be barely lost in the process of tumor progression.

Using genetic and immunological approaches, various TAAs have been identified. Melanoma antigen A1 (MAGE-A1), gp100 or melanoma antigen recognized by T cells (MART-1/Melan-A) were identified by screening of cDNA expression libraries established from melanoma cell lines using tumor-reactive T-cell clones generated from cancer patients as probes (10, 11). Other TAAs, such as New York esophageal squamous cell carcinoma 1 (NY-ESO-1), were discovered by serological analysis of recombinant cDNA expression libraries using autologous patients' antibodies as probes (SEREX technique) (12). Some of these TAAs have been in the process of clinical applications as targets for cancer immunotherapy (8, 13).

Nakamura *et al.* investigated the gene expression profiles of various tumor tissues (14–18) and normal tissues (19) using a genome-wide cDNA microarray analysis coupled with isolation of tumor tissues by laser microbeam microdissection. Various cancer-specific, immunogenic and oncogenic new TAAs have been identified as ideal targets for cancer immunotherapy using this technology. These TAAs have been shown to be frequently over-expressed in various cancer tissues including hepatocellular (14), pancreatic (15), lung (16, 17), esophageal (17, 18) and urinary bladder cancers; head and neck cancers (HNCs); and several other cancers and have characteristics of CTAs or OFAs, as shown in Fig. 1.

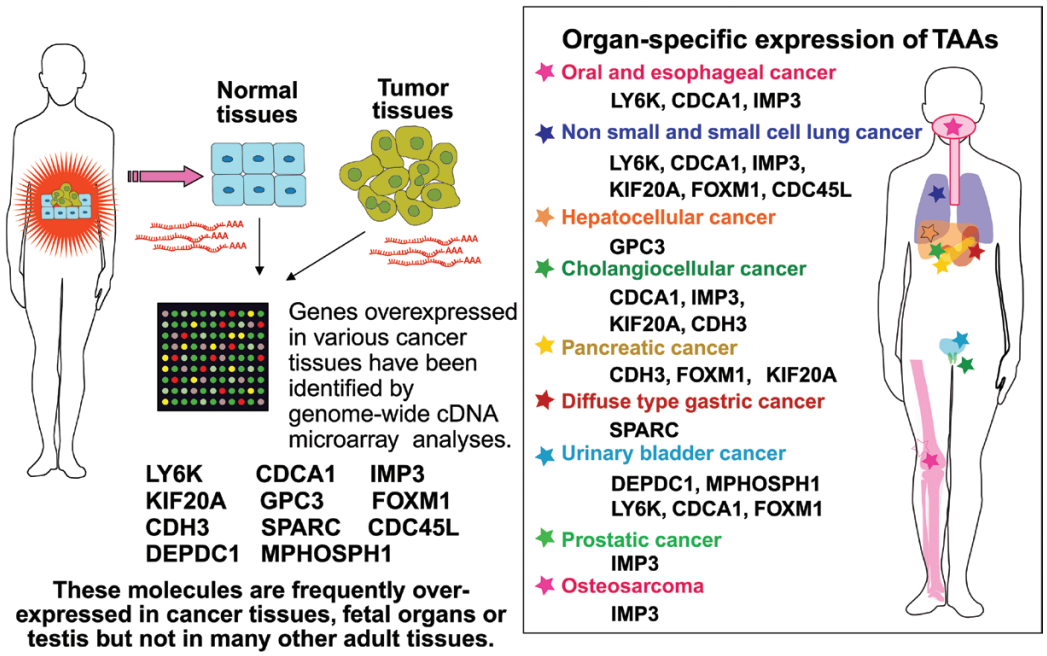


**Fig. 1.** Identification of ideal TAAs useful for peptide-based cancer vaccines by using genome-wide cDNA microarray analysis. The gene expression profiles of both various tumor tissues and normal tissues were investigated using a genome-wide cDNA microarray analysis. These data enable us to identify novel TAAs frequently over-expressed in various malignant tumors and have the characteristics of CTAs or OFAs as ideal targets for cancer immunotherapy. This figure is modified from Fig. 1 of our previous publication by Nishimura *et al.* (9).

### Peptide-based cancer vaccines using TAA-derived short peptides

Because CTLs have the ability to recognize TAA-derived CTL epitope peptides consisting of 8–10 amino acids [short peptides (SPs)] in the context of HLA class I molecules expressed on malignant cells and kill them, many cancer immunotherapies have focused primarily on how to activate the CTLs to attack malignant cells. To develop effective CTL-mediated cancer vaccines, many HLA class I-binding SPs derived from various TAAs have been identified for clinical application as cancer vaccines (20–22).

Using several well-established computer algorithms and HLA-A2- or A24-transgenic mice as shown in Fig. 2, we have also identified TAAs-derived SPs recognized by Japanese common HLA-A24 (*A*2402*)-restricted or HLA-A2 (*A*02:01*)-restricted CTLs, and these SPs are derived from several TAAs, such as cell division cycle associated 1 (CDCA1), lymphocyte antigen 6 complex, locus K (LY6K), glypican-3 (GPC3), or insulin-like growth factor-II mRNA-binding protein 3 (IMP-3), which were identified by genome-wide cDNA microarray analysis (23–26).

Alternatively, the other approach to identify novel CTL epitopes has been conducted using several proteomics technologies. After purification of peptide–HLA complexes expressed on the surface of cancer cells or DCs loaded with cancer cell lysates, the amino acid sequences of HLA-bound peptides were analyzed using proteomics technologies, such as mass spectrometry. Several TAAs and TAA-derived peptides have been identified by these methods (27, 28). The advantage of these methods is that we can select HLA-bound SPs expressed sufficiently on the surface of cancer cells, ensuring the potential targets for tumor-destructive CTLs. Recently, Walter *et al.* (29) identified TAA-derived and HLA-A2-restricted SPs by mass spectrometry and gene expression profiling from several renal cell cancer (RCC) tissues (Fig. 3), and clinical trials of peptide-based cancer vaccines with a mixture of these SPs combined with single-dose cyclophosphamide for RCC patients have been conducted (29). As a result of this trial, a survival advantage was observed with this cancer vaccine that was associated with an immune response of CTLs to the vaccinated SPs.

Many phase I and II clinical trials of SP-based cancer vaccines using identified TAA-derived SPs for various cancers, such as melanoma (30, 31), esophageal cancer (31, 32), lung cancer (33, 34), pancreatic cancer (35) and HNCs (36) have been conducted, and it has been reported that these cancer vaccines were well tolerated, induced measurable immune responses of CTLs and exhibited some anti-tumor clinical effects. Recently, we reported the result of the phase II clinical trial of SP-based cancer vaccines for metastatic/refractory HNC patients. In this trial, a mixture of HLA-A24-restricted multiple TAA-derived SPs, namely LY6K$_{177–186}$, CDCA1$_{64–72}$ and IMP-3$_{508–516}$, were injected subcutaneously with incomplete Freund's adjuvant to a total of 37 patients with advanced HNC. As a result, we observed that the overall

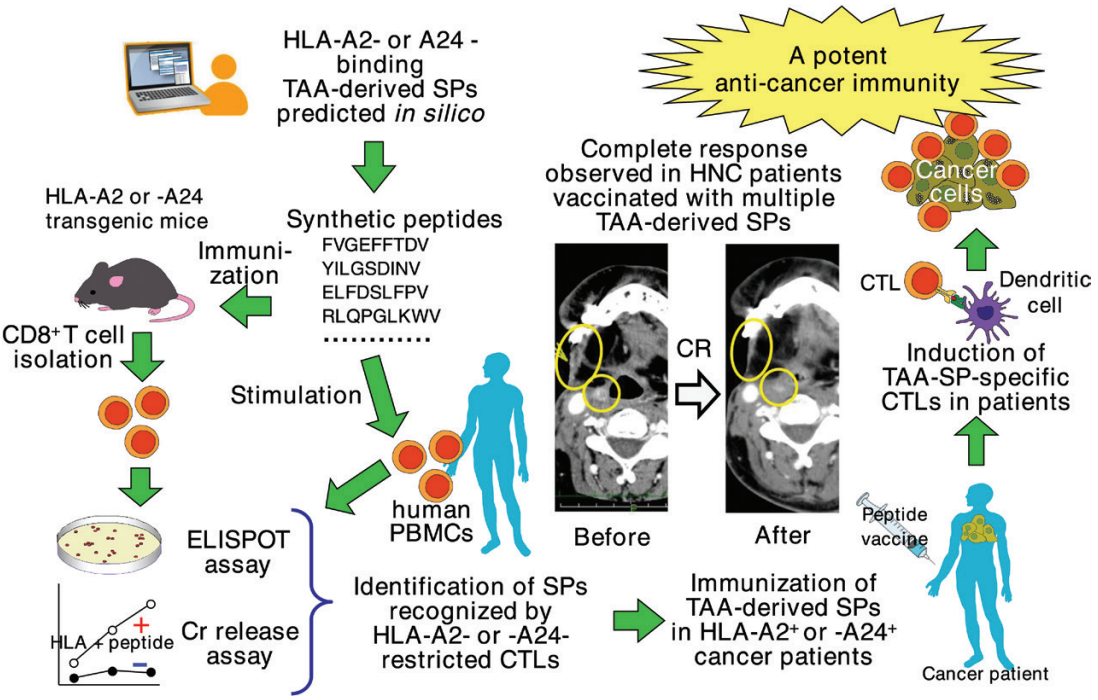


**Fig. 2.** Identification of TAA-derived SPs recognized by CTLs and the clinical application of these SPs for peptide-based cancer vaccines. At first, we used several well-established computer algorithms that predict SPs consisting of 9 or 10 amino acids possibly bound by HLA-A2 or HLA-A24 molecules that are most frequently observed in the Japanese population and synthesized these predicted SPs. Subsequently, the immunogenicity of these SPs was investigated using HLA-A2 transgenic mice or HLA-A24 transgenic mice *in vivo* or human PBMCs isolated from healthy donors and cancer patients *in vitro*. This strategy allowed us to identify many novel TAA-derived immunogenic CTL-epitope SPs. We have utilized some identified TAA-derived SPs as peptide-based cancer vaccines in clinical trials for patients with advanced HNC, and observed regression of tumors in some patients and prolonged OS of vaccinated patients. CR, complete response; ELISPOT, enzyme-linked immunospot assay. This figure is modified from Fig. 2 of our previous publication by Nishimura *et al.* (9).

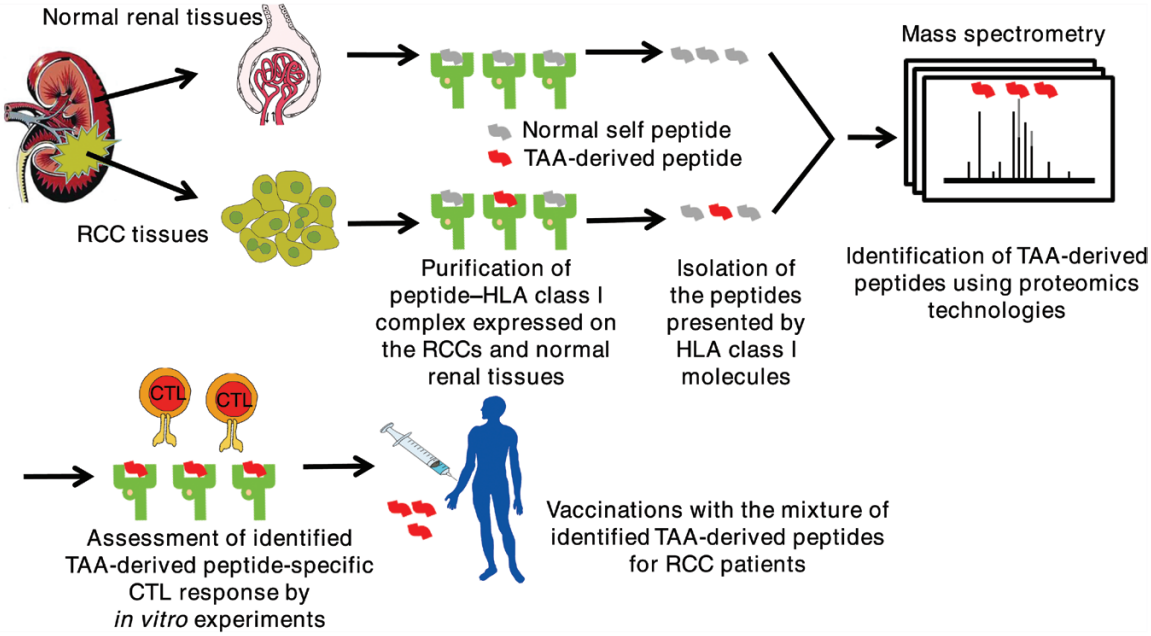


**Fig. 3.** Identification of T-cell epitope peptides expressed in the context of HLA-A2 on the surface of RCC tissues using proteomics technologies and the clinical application of these peptides as peptide-based cancer vaccines (29). After purifying peptide-HLA class I complexes expressed on the surface of RCCs and isolation of the peptides from HLA molecules, amino acid sequences of these peptide are analyzed using proteomics technologies, such as mass spectrometry. After immunogenicity of these peptides is confirmed by *in vitro* experiments, the identified peptides are utilized as peptide-based cancer vaccines for RCC.

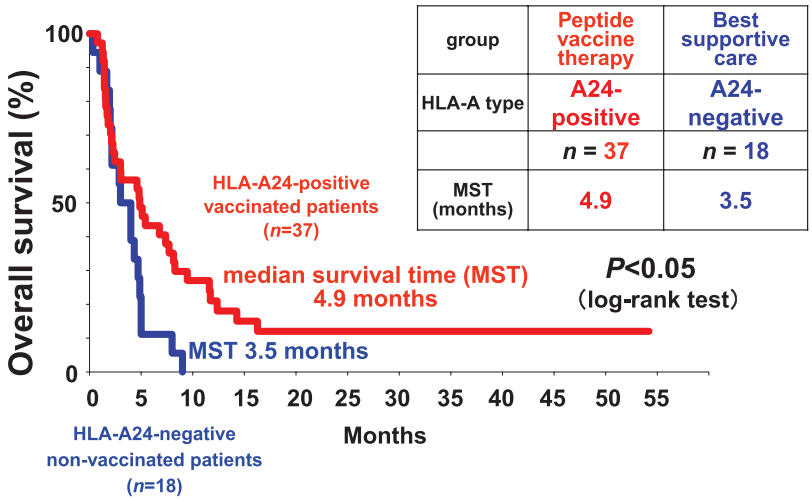


**Fig. 4.** Positive results of the phase II clinical trial of three TAA-derived SP-based cancer vaccines for patients with advanced HNC. A mixture of three SPs derived from three different TAAs, namely LY6K, CDCA, and IMP-3, was injected subcutaneously and weekly with incomplete Freund's adjuvant to patients with advanced HNC. The OS of HLA-A24-positive vaccinated patients was significantly longer than non-vaccinated patients who received the best supportive care. The median survival time was 4.9 months in vaccinated patients, whereas it was 3.5 months in non-vaccinated patients ($P < 0.05$). The OS was analyzed according to the Kaplan–Meier method, and statistical differences were assessed using the log–rank test. This figure is modified from Fig. 1A of our previous publication by Yoshitake *et al.* (36).

survival (OS) of vaccinated patients was significantly longer than non-vaccinated patients who received the best supportive care (Fig. 4). Furthermore, one of the advanced HNC patients showed a complete response after repeated vaccinations as shown in Fig. 2 (36).

Accumulating these clinical data of several SP-based cancer vaccines has demonstrated that SPs administered as cancer vaccines can indeed elicit tumor-targeting immune responses of CTLs in cancer patients. However, in spite of the detection of vaccine-induced T-cell responses, these immune responses were rarely associated with anti-tumor effects of cancer vaccines, and the effect of SP-based cancer vaccines have been limited to a small fraction (<10%) of cancer patients (37, 38).

### Peptide-based cancer vaccines using TAA-derived long peptides

It is well-known that $T_h1$ cells are essential to generate effective CTL-mediated anti-tumor responses as well as generation and maintenance of long-lasting memory CTLs (39–43). In addition to these CTL-supporting roles, T-helper cells have intrinsic effector functions. Tumor-specific T-helper cells can induce senescence of tumors by T-helper cell-derived IFN-γ and TNF-α (42). Moreover, $T_h1$ cells enable CTLs to infiltrate into the tumor microenvironment (43). Direct anti-tumor or antiangiogenic effects were also reported to be mediated by IFN-γ-secreting $T_h1$ cells (44–46).

As mentioned above, vaccination with HLA class I-restricted SPs alone does not always elicit a sufficient immune response to induce effective anti-tumor immunity (37). One of the causes for this ineffectiveness of SP-based cancer vaccines is considered to be the induction of immunological tolerance in $CD8^+$ T cells. SPs can induce tolerance or anergy of $CD8^+$ T cells when they are presented by HLA class I molecules expressed on nonprofessional antigen-presenting cells (APCs) because of the lack of signaling from co-stimulatory molecules (37, 47), whereas an extended long peptide (LP) encompassing several epitopes recognized by both CTLs and T-helper cells may overcome this problem because LPs cannot bind directly to HLA class I molecules expressed on nonprofessional APCs because of their long amino acid sequence. After injection of an LP, professional APCs, such as DCs, take up the LP, process it, then present CTL and T-helper cell epitopes in the context of HLA class I and HLA class II molecules, respectively (37).

The process by which DCs can present endogenous protein to HLA class I pathway is called cross-presentation, and this can prime naive CTLs to become effector cells, which is called cross-priming (48). Therefore, a TAA-derived LP that encompasses both CTL and T-helper cell epitopes may be an ideal candidate for peptide-based cancer immunotherapy, because LPs have the potential to elicit both $CD4^+$ and $CD8^+$ T-cell TAA-specific responses without inducing immunological tolerance. Indeed, the therapeutic activity of cancer vaccines with TAA-derived LPs including several epitopes recognized by both CTLs and T-helper cells is superior to that of TAA-derived SPs in mouse models (49, 50). Furthermore, Disis *et al*. reported that vaccination with a HER-2/*neu*-derived LP encompassing an HLA-A2-restricted CTL epitope elicited the embedded epitope-specific CTL responses in breast cancer patients (51), suggesting that vaccination with LPs has the potential to elicit both T-helper cell responses and CTL responses in cancer patients.

Several clinical trials of peptide-based cancer vaccines using TAA-derived LPs for various types of malignancies have been conducted (37, 51–56), and in some clinical trials, these LP-based cancer vaccines elicited LP-specific immune responses in vaccinated patients and showed some clinical benefits. Aamdal *et al.* reported the clinical trial of GV1001, a promiscuous telomerase-derived T-helper cell epitope vaccination for patients with lung cancer and melanoma (54, 55), and showed that this cancer vaccine prolonged the survival of cancer patients in combination with chemoradiotherapy. At this moment, there have been several clinical trials of LP-based cancer vaccines that are ongoing or recently initiated for treatment of various malignancies (Table 1).

Likewise, we have also succeeded in identification of several LPs derived from several TAAs, such as kinesin family member 20A (KIF20A), CDCA1, LY6K, GPC3 and IMP-3, using *in silico* analysis and immunological approaches (57–61) (Fig. 5). These LPs have the capacity to induce not only promiscuous HLA class II-restricted $T_h1$ cells but also tumor-specific CTLs through a cross-presentation mechanism. Furthermore, we showed that T-helper cells reactive to some of these LPs were detectable in PBMCs of HNC patients who were enrolled in a phase II clinical trial of SP-based cancer vaccines using a mixture of three TAA-derived SPs, and in the case of a GPC3-derived LP, the patient who exhibited GPC3-LP-specific T-helper cell responses showed significantly longer OS than those without any GPC3-specific T-helper cell responses (60). These results suggest that vaccination with these identified LPs has the potential to elicit both TAA-specific CTL responses and T-helper cell responses, and exhibit stronger anti-tumor immune responses.

On the other hand, it has been also reported that some cancer vaccinations with TAA-derived LPs induced LP-specific $T_h2$ cells or $T_{reg}$ cells in cancer patients that resulted in limited

**Table 1.** Clinical trials of TAA-derived LP-based cancer vaccines that are ongoing or recently initiated in the world

| Trial number | Phase | TAAs | Malignancy | Combination | Start date | Status |
|---|---|---|---|---|---|---|
| NCT01784913 | I/II | TERT | Prostate cancer | Combination with GM-CSF | Feb. 2013 | Ongoing |
| NCT01789099 | I/II | TERT | Non-small cell lung cancer | Combination with GM-CSF | Feb. 2013 | Ongoing |
| NCT02128126 | I/II | E6/E7[a] (HPV) | Cervical carcinoma | —[b] | Sep. 2013 | Recruiting |
| NCT01922921 | I/II | HER2/*neu* | Breast cancer | Combination with trastuzumab ± PSK | Feb. 2014 | Recruiting |
| NCT02427581 | I | Personalized LPs | Breast cancer | —[b] | Sep. 2015 | Recruiting |
| NCT02126579 | I/II | Multiple melanoma antigens | Melanoma | —[b] | Jan. 2016 | Recruiting |

[a]E6 and E7 proteins are virus oncoproteins derived from HPV that is associated with cervical and oropharyngeal cancers. [b] The dash '—' means that 'no other therapy is used'.
HPV, human papillomavirus; PSK, polysaccharide krestin; TERT, telomerase reverse transcriptase.
*Source:* www.clinicaltirals.gov.

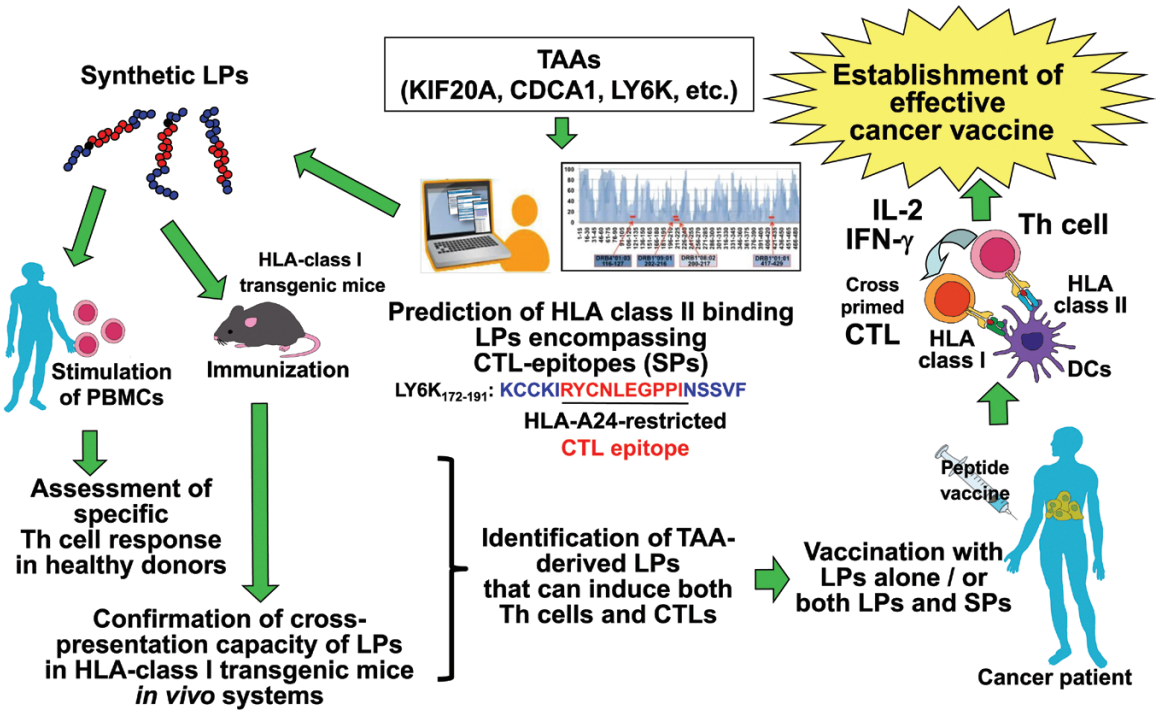


**Fig. 5.** The strategy of identification of TAA-derived LPs encompassing both T-helper cell and CTL epitopes and the clinical application of these LPs for peptide-based cancer vaccines. We attempted to identify TAA-derived LPs encompassing both LP-specific T-helper cells and CTL epitopes in order to further improve TAA-derived peptide-based cancer immunotherapy. At first, to select candidate LPs encompassing both T-helper cell and CTL epitopes (SPs), we combined *in silico* data obtained from a well-established computer algorithm predicting HLA class II-binding peptides and known HLA-A2-restricted or HLA-A24-restricted CTL epitope SPs. Their immunogenicity was determined by utilizing human T cells obtained from healthy donors, and several highly immunogenic TAA-derived LPs encompassing both T-helper cell and CTL epitopes were identified. Moreover, we confirmed the capacity of cross-presentation of these LPs using $CD8^+$ T cells *in vitro* in human and *in vivo* in HLA class I transgenic mice, suggesting that vaccination with these TAA-derived LPs can induce not only tumor-specific T-helper cells but also CTLs, leading to eliciting stronger anti-tumor responses in cancer patients. This figure is modified from Fig. 4 of our previous publication by Nishimura *et al.* (9).

clinical efficacy (62, 63). Therefore, one of the disadvantages of cancer vaccines using TAA-derived LPs is considered that not only $T_h1$ cells but also LP-specific $T_h2$ cells or $T_{reg}$ cells that generally do not provide help for TAA-specific CTLs can be induced after vaccinations, and this issue has to be resolved for future clinical applications of LP-based cancer vaccines.

### DC-based cancer vaccines using TAA-derived peptides

It has been widely known that DCs have an important role in eliciting antigen-specific adaptive immune responses to pathogens or tumor cells (64). DCs engulf the antigens, process them into small peptides and subsequently present them through HLA class II molecules to induce $CD4^+$ T-cell responses. Furthermore, DCs can activate $CD8^+$ T cells through the cross-presentation pathway. Therefore, the ability of DCs to elicit robust, therapeutically relevant anti-cancer T-cell responses is thought to be useful for cancer immunotherapies.

Several forms of DC-based cancer vaccines have been developed, and one of these vaccines utilizes DCs loaded with TAA-derived peptides. Generally, DCs for therapeutic use are generated by culture of monocytes isolated from peripheral blood of cancer patients and differentiation into immature DCs *ex vivo* in the presence of GM-CSF and IL-4. Then, DCs are loaded with TAA-derived peptides and subsequently reinfused into cancer patients to elicit TAA-specific anti-tumor T-cell responses (65). To date, many clinical trials of DC-based cancer vaccines using TAA-derived peptides have been widely conducted for various types of cancer patients (66), and accumulating data of these clinical trials have demonstrated that DC-based cancer vaccines are safe and can elicit anti-tumor immune responses, even though objective clinical responses of these vaccines have still remained low (67, 68).

### Neoantigen-derived peptide-based cancer vaccination

Cancer cells arise from accumulated multiple mutations that cause uncontrolled cell growth (69), and these mutations can result in the expression of mutant proteins. These mutation-derived epitopes can be presented by several HLA class I and class II molecules on the surface of cancer cells or APCs, and provide a form of 'foreign' antigen (neoantigen) detectable by the immune system. Recent development of genomics and bioinformatics approaches has enabled us to easily identify tumor-specific missense mutant proteins that function as neoantigens and the majority of them are products of passenger but not driver mutations (70, 71). In mouse melanoma models, the first evidence of the potential

of vaccination against neoantigens was provided, in which immune responses against mutant antigens was associated with the anti-tumor effect (72, 73). Likewise, in a clinical study of melanoma patients, accumulating evidence suggested that neoantigens could be recognized by intratumoral $CD8^+$ or $CD4^+$ T cells, and these immune responses have an important role in anti-tumor effects (74, 75).

The advantage of using neoantigens as targets for cancer vaccines over using TAAs is that neoantigen-reactive T cells are not subject to central immunological tolerance and therefore their TCRs often exhibit higher binding affinities for MHC-neoantigen peptide complexes in comparison with those of TAAs (76). Furthermore, it has been shown that the recent successful immunotherapeutic modalities, including adoptive T-cell therapy and immune-checkpoint blockade therapy, are suggested to be directed against neoantigens (74, 77). Therefore, neoantigens are considered to be potential targets of cancer vaccination, and several clinical trials of personalized cancer immunotherapies targeting neoantigens have been recently initiated in several institutions (Table 2).

Recently, using a combination of the whole-exome sequencing of cancer cells, *in silico* epitope prediction and immunological approaches (Fig. 6), Carreno *et al*. identified tumor-derived neoantigens and neoantigen-derived peptides (neoepitopes) recognized by HLA-A2-restricted CTLs in three melanoma patients' tumor samples individually and infused autologous DCs pulsed with these neoepitopes into each patient (78). They showed that these cancer vaccines elicited neoepitope-specific CTL responses that were undetectable prior to vaccination and enhanced the neoepitope-specific CTL responses preexisting before vaccinations. In this study, two of the patients showed stable disease and one patient showed no evidence of recurrence, without any evidence of serious adverse effects. Although the number of vaccinated patients was very limited in this trial, this report suggested that a neoantigen-derived peptide-based cancer vaccine is feasible and an attractive immunotherapeutic modality as a personalized cancer immunotherapy.

## Combination therapy incorporating peptide-based cancer vaccines

Immune-checkpoint blockade therapies have become a very promising cancer immunotherapy. They are a type of cancer immunotherapy using humanized mAbs specific to T-cell-inhibitory receptors (79), such as CTLA-4 (80), programmed cell death-1 (PD-1) (81) or its ligand, PD-1 ligand 1 (PD-L1) (82). The clinical responses to these immunotherapies are often durable, with some patients with different malignancies remaining free from disease progression for many years (81, 83).

In animal models, combination therapies with immune-checkpoint blockade therapies and various types of cancer vaccines have demonstrated enhanced activation of vaccine-induced tumor-specific T cells and synergistic anti-tumor effects (79, 84). Furthermore, recently, it has been reported that the effect of the immune-checkpoint blockade therapies was mediated by reactivating neoantigen-specific T cells in animal models (85). Likewise, some clinical studies of the immune-checkpoint blockade therapies for patients with melanoma, lung cancers and colon cancers with microsatellite instability have demonstrated that the clinical responses to the immune-checkpoint blockade therapies were associated with a high mutational load in the tumors (86–88), suggesting that the immune-checkpoint blockade therapies mediate an anti-tumor effect by reactivation of existing neoepitope-specific T cells. Therefore, combination therapies with peptide-based cancer vaccines including neoantigen-targeting cancer vaccines and the immune-checkpoint blockade therapies are expected to be a good candidate for more effective cancer immunotherapy (Fig. 6) (89).

**Table 2.** Clinical trials of neoantigen-derived peptide-based cancer vaccines that are ongoing or recently initiated in the world

| Trial number | Phase | Type | Malignancy | Combination | Start date | Status |
|---|---|---|---|---|---|---|
| NCT01970358 | I | Neonatigen-derived LP plus Poly-ICLC | Advanced melanoma | —[a] | Jan. 2014 | Recruiting |
| NCT02149225 | I | Neoantigen-derived peptide plus Poly-ICLC and GM-CSF | Glioblastoma | —[a] | Oct. 2014 | Recruiting |
| NCT02287428 | I | Neoantigen-derived LPs plus Poly-ICLC | Glioblastoma | Combination with radiotherapy | Nov. 2014 | Recruiting |
| NCT02427581 | I | Neoantigen-derived peptide plus Poly-ICLC and GM-CSF | Breast cancer | —[a] | Sep. 2015 | Recruiting |
| NCT02510950 | 0 | Neoantigen-derived LPs plus Poly-ICLC | Glioblastoma Astrocytoma | Combination with temozolomide | Nov. 2015 | Recruiting |
| NCT02419170 | 0 | Neoantigen-loaded DC vaccines | Non-small cell lung cancer | —[a] | Jan. 2016 | Not yet recruiting |
| NCT02600949 | 0 | Neoantigen-derived peptides | Pancreatic and Colorectal adenocarcinoma | —[a] | Mar. 2016 | Not yet recruiting |

[a]The dash '—' means that 'no other therapy is used'.
Poly-ICLC, polyinosinic acid polycytidylic acid polylysine in carboxymethylcellulose.
*Source:* www.clinicaltirals.gov.

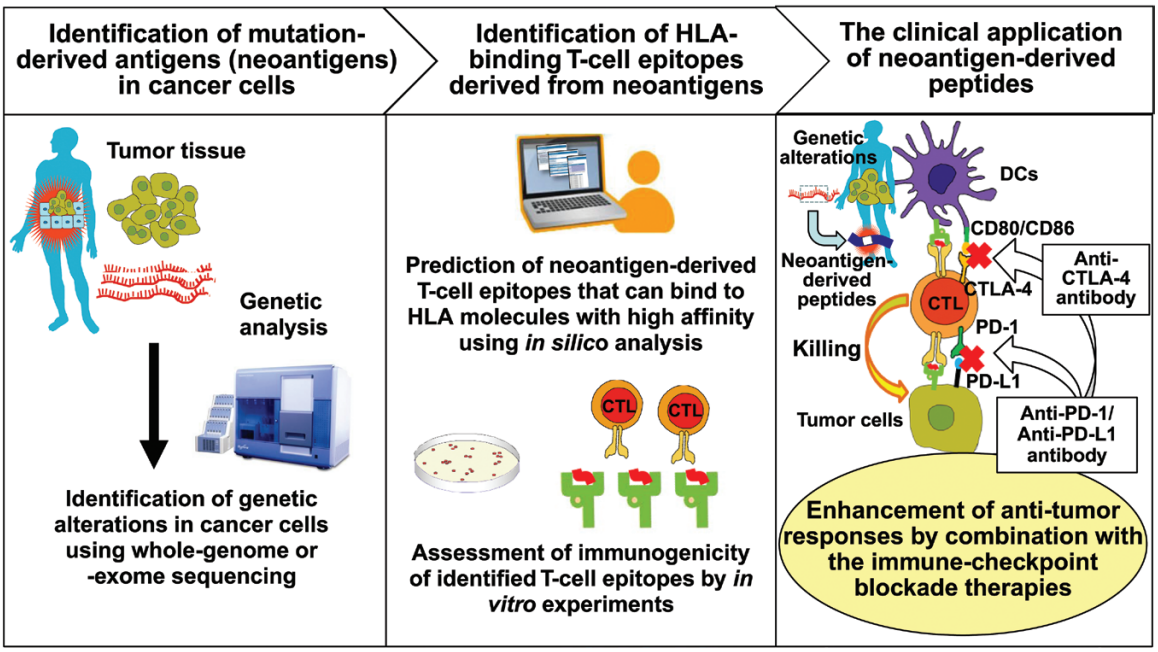


**Fig. 6.** Identification of neoantigen-derived peptides, the clinical application of these peptides for the personalized peptide-based cancer vaccines and combination immunotherapy with immune-checkpoint blockade therapies. First, using a whole-genome or whole-exome sequencing analysis, genetic alterations and neoantigens that resulted from these mutations in cancer cells can be identified and, subsequently, neoantigen-derived T-cell epitopes that can bind to HLA class I or II molecules with high affinity are predicted using *in silico* analysis. The immunogenicity of these epitope peptides is determined by utilizing human T cells *in vitro*, and immunogenic neoantigen-derived T-cell epitope peptides are identified. Using these neoantigen-derived peptides, personalized peptide-based cancer vaccines targeting neoantigens can be applied in a clinical setting. Furthermore, combination immunotherapy of peptide-based cancer vaccine targeting neoantigens with immune-checkpoint blockade therapies is expected to be a good candidate for more effective cancer immunotherapy.

## Conclusions and future prospects of peptide-based cancer vaccines

Although peptide-based cancer vaccines have sometimes shown survival advantages with few side adverse effects compared with the conventional therapy, this immunotherapy as a monotherapy is considered to be insufficient to elicit durable control of cancers and cures. Combination immunotherapy with peptide-based cancer vaccines and immune-checkpoint blockade therapies that are designed concurrently to activate tumor-specific immune responses and inactivate the immunosuppression in the tumor microenvironment may overcome this ineffectiveness, and lead to the induction of stronger anti-tumor responses.

Furthermore, the recent technical progress of genetic analysis enables us to easily evaluate the immunogenicity of tumor cells and the immune status of the tumor microenvironment in individual cancer patients. This information is expected to lead to the discovery of the predictive biomarkers to select patients for treatment with cancer immunotherapy and the development of the personalized peptide-based cancer vaccines that may improve the efficacy of this immunotherapy.

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