

Development of optimized cryptic peptides for immunotherapy

Jeanne Menez-Jamet & Kostas Kosmatopoulos*

Address

Vaxon Biotech, 3-5 impasse Reille,
75014, Paris, France
Email: kkosmatopoulos@vaxon-biotech.com

*To whom correspondence should be addressed

Specific immunotherapy is based on the use of tumor-specific antigens to induce an efficient antitumor immune response. Although tumors are known to be weakly immunogenic and therefore capable of escaping immune surveillance, the objective of tumor vaccination is to induce a frequent, strong and long-lasting antitumor immune response based mainly on the activation of cytotoxic T-lymphocytes. However, as widely expressed tumor antigens (universal tumor antigens) often correspond to normal proteins expressed not only by tumor cells but also by normal cells and tissues, these antigens are generally tolerated by the immune system. Thus, circumventing self tolerance to universal tumor antigens is a major goal of cancer vaccine research. Disappointing results obtained to date with most tumor vaccines has led to a shift in research toward determining ways of stimulating the immune response through the use of new adjuvants, immunostimulants and delivery vectors. However, although these aspects are clearly crucial to vaccine development, breakthroughs in the field may lie in the use of strong antigens as optimized cryptic peptides derived from universal tumor antigens, combined with a potent adjuvant. Targeting cryptic tumor peptides/antigens is an efficient way of overcoming tolerance. Indeed, the first vaccine based on an optimized cryptic peptide induced strong antitumor immunity and demonstrated promising clinical activity.

Keywords Cancer vaccine, immunotherapy, optimized cryptic peptide, universal tumor antigen, VX-001

Introduction

Antitumor immunotherapy is aimed at stimulating specific and/or nonspecific immunity, resulting in the activation of T-cells, B-cells, macrophages and NK cells to kill tumor cells. Nonspecific immunity, using cytokines such as IL-2, IL-12, GM-CSF and IFN γ tends to stimulate the global immune system to amplify a potential antitumor immune response. IL-2- or IFN γ -based therapies are commonly used to treat patients with melanoma or renal cell carcinoma, but success has been limited. However, toxicity related to nonspecific immune activation severely limits the dosing of the cytokine used.

Specific immunotherapy is based on the use of tumor-specific antigens to induce an efficient antitumor immune response. Tumors are known to be weakly immunogenic and are therefore capable of escaping immune surveillance. Nevertheless, the objective of tumor vaccination is to induce a frequent, strong and long-lasting antitumor immune response, based mainly on the activation of cytotoxic T-lymphocytes (CTLs). CTLs recognize peptides derived from endogenously processed tumor antigens and presented at the cell surface in association with HLA class I molecules (HLA-I). Tumor vaccines are captured by antigen-presenting cells (APCs) – mostly dendritic cells (DC) – that accumulate at the site of injection. The magnitude of an immune response to a vaccine depends on the immunogenicity of the targeted antigen/peptide and the potency of the associated adjuvant.

Substantial efforts have been directed toward developing adjuvants that can strengthen the weak immunogenicity of tumor antigens or can directly enhance antigen presentation. *In vivo*, adjuvants have been demonstrated to stimulate DCs through TLRs, CD40, or HLA-II to promote antigen presentation or to target DCs specifically. Optimization of antigen presentation may occur through adoptive vaccination with autologous DCs loaded with antigenic protein or fused to autologous tumoral cells, as well as through the use of vectors (eg, virosomes, liposomes, detoxified bacterial toxins and viral vectors).

Many clinical trials with cancer vaccines have been conducted, but these studies have often yielded disappointing results. Some of these vaccines had reached advanced stages of clinical development, including Canvaxin, an irradiated allogeneic melanoma cell line associated with BCG to treat melanoma; Melacine (discontinued in the US, but launched in Canada), an allogeneic tumor cell lysate associated with the adjuvant Detox B to treat melanoma; and PANVAC-VF, a recombinant attenuated vaccinia virus expressing CEA (carcinoembryonic antigen), MUC-1 and co-stimulatory molecules associated with a fowlpox vector to treat pancreatic cancer (development for the treatment of pancreatic cancer had been discontinued by Therion Biologics Corp at the phase III stage; however, in September 2008, the NCI was planning a phase I trial in this indication).

Nevertheless, at least two vaccines have obtained market authorization: OncoVax, an autologous tumor cell vaccine associated with BCG for the treatment of colorectal cancer; and vitespen (Oncophage), an autologous tumor-derived Hsp for the treatment of renal cell carcinoma. Other products that are currently in phase III clinical development have also produced encouraging results. Sipuleucel-T (Provenge; Dendreon Corp), an autologous preparation of prostatic acid phosphatase (PAP) tumor antigen associated with the adjuvant GM-CSF, has been demonstrated to prolong the survival of patients with prostate cancer, and is anticipated to attain market authorization in 2009. Moreover, two vaccines for patients with NSCLC, Stimuvax (Merck Serono SA) and MAGE-A3 ASCI (MAGE-A3 antigen-specific cancer immunotherapeutic; GlaxoSmithKline plc), have successfully completed phase IIb clinical trials.

Although vaccine-specific antitumor immune response depends on the adjuvant or on the antigen delivery efficacy, the choice of the tumor antigen is crucial. The use of optimized cryptic peptides derived from shared tumor antigens, has demonstrated compelling results in the field of cancer vaccines. Indeed, the first vaccine based on an optimized cryptic peptide induced strong antitumor immunity and demonstrated promising clinical activity. This paper discusses the development of optimized cryptic peptides for immunotherapy.

Tumor-associated antigen and derived peptide presentation

Tumor-associated antigens, apart from those derived from oncogenic viruses (eg, HPV and Epstein Barr virus), can be divided into two main groups:

- (i) Tumor-specific mutated self antigens (frequently patient-specific) result from somatic mutations in normal genes. Although the identification and isolation of mutated self antigens is technically feasible, these antigens are usually patient-specific and therefore of limited practical value.
- (ii) In contrast, shared non tumor-specific, non-mutated self antigens are common to most patients with a given type of cancer.

Thus, vaccination with defined and well-characterized shared antigens is preferred. However, because shared antigens correspond to normal proteins that are expressed not only by tumor cells but also by normal cells and tissues, these antigens are generally tolerated by the immune system, albeit to variable degrees. Tumor antigens corresponding to fetal gene products or products that are expressed at immunoprivileged sites, including CEA, 5T4 oncofetal antigen and MAGE-family antigens, trigger little or no tolerance. These antigens are therefore expected to be excellent tumor-rejection antigens. In contrast, tissue-specific products such as MART1 (melanoma antigen 1 recognized by T-cells), gp100 or widely expressed 'universal' tumor antigens such as HER2/neu are likely trigger some degree of tolerance and would be considered

weaker tumor-rejection antigens. The search for broadly expressed universal tumor antigens has been intensified with the identification of antigens with functions that are essential to the maintenance of the oncogenic phenotype, such as telomerase reverse transcriptase (TERT) and survivin.

Endogenous tumor antigens are recognized by antitumoral CTLs as short peptides (9 to 10 amino acids) associated with HLA-I at the surface of tumor cells. These peptides are produced during the intracellular processing of the relevant antigen, bind to HLA-I molecules, and are then exported to the cell surface. Peptides produced during intracellular antigen processing are not equally represented on the surface of tumor cells. Broadly, these peptides can be categorized into two groups: dominant peptides and cryptic peptides. This distinction is based on peptide density at the cell surface, depending mainly on HLA-I affinity. Dominant peptides exhibit strong HLA-I binding affinity, are abundantly expressed at the cell surface, and should be strongly immunogenic. In contrast, cryptic peptides exhibit weak HLA-I binding affinity, are weakly expressed at the tumor cell surface, and are essentially non immunogenic (Table 1.) Given that the tolerance to self-antigens depends on the elimination of CTLs that strongly recognize self-antigenic peptides, the distinction between dominant and cryptic peptides determines the level of tolerance to tumor antigens.

Circumventing self tolerance

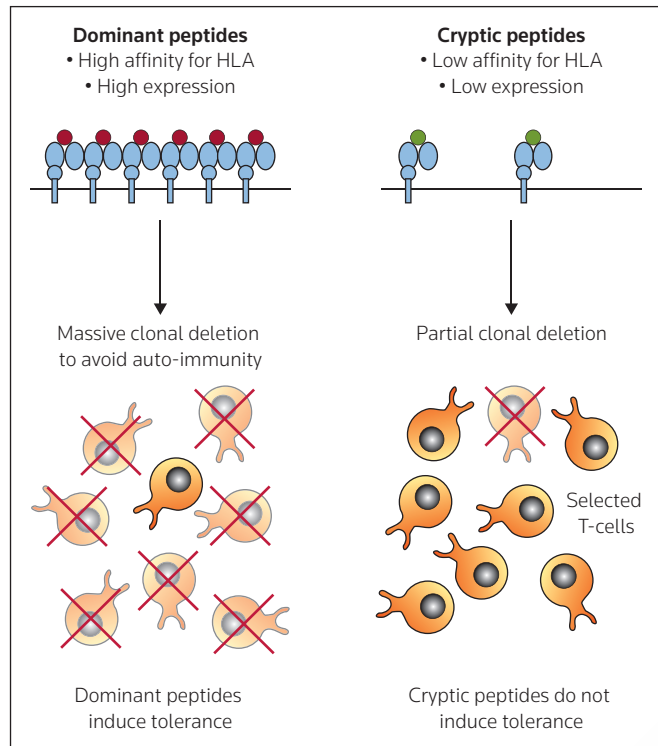
Self tolerance is a phenomenon by which autoreactive T-cells are inactivated or eliminated in order to prevent autoimmunity. Overcoming or circumventing self tolerance to shared tumor antigens is a major goal of cancer vaccine research. The deletion of autoreactive T-cells occurs mostly in the thymus, when immature T-cells encounter self antigens presented by thymic DCs or by medullary epithelial cells. The inactivation of autoreactive T-cells also occurs in the periphery, and is mediated by several mechanisms, such as the inappropriate or suboptimal presentation of antigens, immune suppression by regulatory T-cells and immunosuppressive cytokines.

However, the tolerance of self antigens, and thus the tolerance of shared tumor antigens, is not absolute. The residual antitumoral T-cell repertoire, which remains available for antitumor reactivity, depends on the efficiency of antigen presentation and on T-cell receptor affinity. As a result, the T-cell repertoire against poorly presented cryptic

Table 1 : Characteristics of dominant and cryptic peptides.

Characteristic	Dominant peptides	Cryptic peptides
HLA/peptide complexes at cell surfaces (frequency)	High	Low
HLA affinity	High	Low
Immunogenicity	High	Low

Figure 1. Tolerance of CTL repertoires specific for dominant and cryptic self peptides.



peptides escapes tolerance mechanisms and contains numerous T-cells with high affinity, whereas the T-cell repertoire against dominant peptides is composed of only a few T-cells, with low affinity (Figure 1). This observed pattern has been confirmed in experimental models. Likewise, in humans, a negative correlation has been demonstrated between HLA-I affinity and specific CTL affinity for several peptides derived from tumor antigens (eg, p53-derived peptides). The tolerance to dominant tumor peptides explains why, for example, the vaccination of mice with autologous tumor antigens such as gp100, tyrosinase, p53 and HER-2/neu failed to induce an immune response.

Several methods to overcome tolerance have been developed to mobilize a patient's own immune system to recognize and destroy tumor cells. As described previously, most of these mechanisms are based on the optimization of antigen delivery or through the use of new adjuvants. Another approach for increasing the immunogenicity of peptide-based vaccines involves epitope enhancement, consisting of modifying peptide sequences to increase HLA-I affinity and, consequently immunogenicity. Rosenberg *et al* (*J Immunother* (2000) **23**(4):487-498) first enhanced an HLA-A2-restricted peptide that is derived from gp100 to generate a strong T-cell immune response in almost 90% of patients with melanoma who were immunized. Similarly, Rivoltini *et al* (*Cancer Res* (2003) **63**(7):1560-1567) modified an HLA-A2-restricted MelanA/MART1 peptide that could elicit a robust peptide-specific CD8+ T-cell response. However, it is also important

to demonstrate that T-cell populations induced by the modified synthetic peptide are particularly able to recognize, and eventually lyse, target cells that express the wild-type epitope. Furthermore, the self tolerance limitation is not completely abolished, because the enhancement of the HLA binding capacity of a given antigenic peptide leads only to the stimulation of T-cells that escaped tolerance.

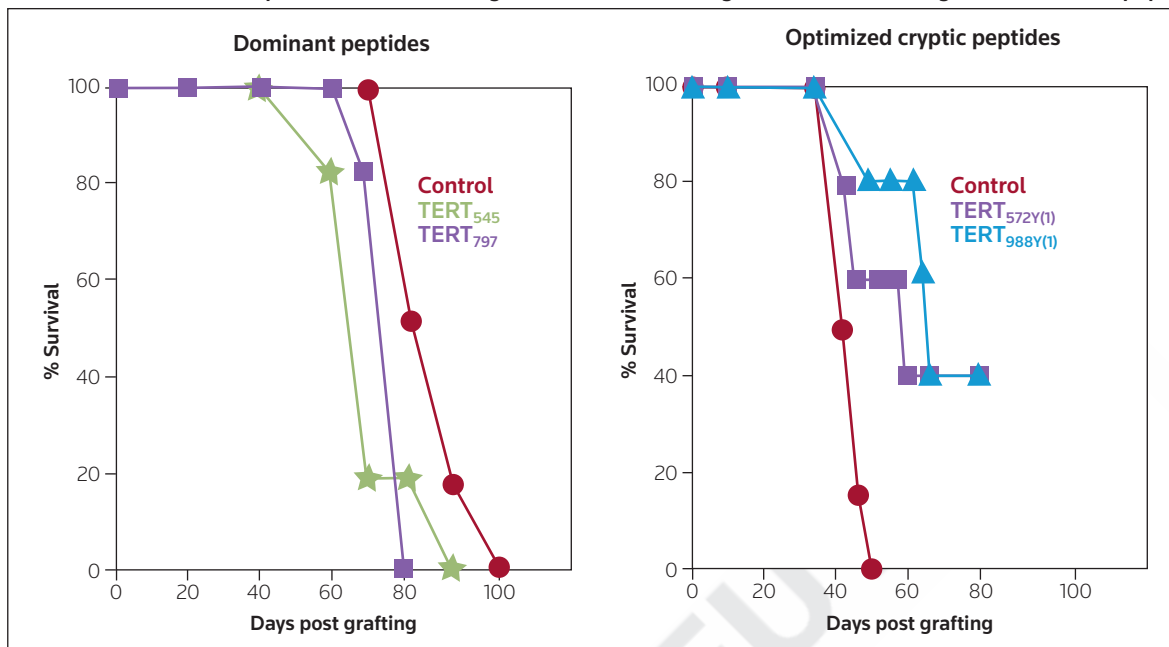
Vaccination with optimized cryptic peptides

Another way to circumvent self tolerance is vaccination with xenogeneic antigens. In this approach, xenogeneic antigenic peptides, which are homologous to poorly immunogenic human peptides that are not submitted to self tolerance, are able to stimulate specific immune responses. This method presumes the use of optimized cryptic peptides (*Biomed Pharmacother* (2007) **61**(2-3): 125-130).

Indeed, the most promising way of circumventing self tolerance has been through the use of cryptic tumor peptides, the specific T-cell repertoire of which remains relatively intact. This approach was first proposed by Moudgil and Sercarz 15 years ago (*Immunol Today* (1994) **15**(8):353-355). The main challenge is to elicit an immune response to peptides that, by definition, are not inherently immunogenic. One possibility is to enhance the HLA-I affinity of the target cryptic peptide. HLA-I affinity depends on the presence of primary and secondary anchor motifs within the peptide sequence that are specific for a given HLA-I allele. In theory, HLA-I affinity can be enhanced by identifying unfavorable primary and secondary anchor motifs and replacing these motifs with more favorable residues. However, replacing selected residues, especially in secondary motifs, may lead to a shift in the conformation (shape) of the peptide moiety that contacts the T-cell receptor, conferring antigenicity. A general approach that enhances the affinity of cryptic peptides presented by HLA-A*0201, the most frequent HLA-I allele (present in 40 to 45% of the population), has been described (*Eur J Immunol* (2000) **30**(12):3411-3421). The method consists of replacing the first residue, whatever its nature, by a tyrosine (Y1 modification). This modification increases the HLA affinity of the peptide, and has been shown to render immunogenic 90% of the cryptic peptides that have been studied. The antitumor vaccine efficacy of these optimized cryptic peptides has been demonstrated *in vivo* in HLA-A*0201-expressing humanized HHD mice. Indeed, vaccination with optimized cryptic peptides from the universal tumor antigen TERT (TERT_{572Y(1)} and TERT_{988Y(1)}) protected HHD transgenic mice from human TERT-expressing tumor growth, whereas vaccination with dominant peptides (TERT₅₄₅ and TERT₇₉₇) did not offer protection (Figure 2) (*J Clin Invest* (2004) **113**(3):425-433).

As described previously, TERT is a promising target for cancer immunotherapy because it is overexpressed in many human tumors and, therefore, is considered a universal tumor antigen. The TERT_{572Y} optimized cryptic peptide, which was shown to induce antitumor

Figure 2. *In vivo* antitumor immunity induced in HHD transgenic mice vaccinated against murine TERT high- and low-affinity epitopes.



immunity in HLA-A*0201 transgenic mice, was selected for testing in a phase I/II clinical trial in patients with advanced cancer.

VX-001 vaccination – A phase I/II clinical trial

An exploratory phase I/II trial was conducted with VX-001 (Vaxon Biotech SA), composed of an optimized TERT_{572Y} cryptic and the native TERT₅₇₂ peptide. A total of 116 patients with different cancers (mainly NSCLC, breast cancer, prostate cancer, renal cell carcinoma, colorectal cancer, melanoma and cholangiocarcinoma) were enrolled in the study. Patients received two injections of TERT_{572Y} and then four injections with the native TERT₅₇₂ peptide. This protocol was based on the rationale of selecting those T-cells recruited by TERT_{572Y} that had the highest specificity for the native TERT₅₇₂, which is naturally presented by tumor cells. VX-001 was safe and well tolerated in this trial; only grade I/II vaccine-related toxicity was observed in vaccinated patients. Immune responses were observed in the majority of patients and appeared as early as after the second vaccination, confirming that VX-001 was immunogenic. Moreover, the immune response was maintained with vaccination boosts with TERT₅₇₂ for more than 3 years. Encouraging results were also obtained in an exploratory analysis of efficacy. One patient (with breast cancer) experienced a complete response, three patients (two with NSCLC and one with hepatocellular carcinoma) experienced partial responses, and 34 patients demonstrated disease stabilization for more than 6 months.

Interestingly, an analysis of 33 vaccinated patients with NSCLC revealed a correlation between immune response to the vaccine and clinical response. Survival was significantly prolonged in patients who developed an

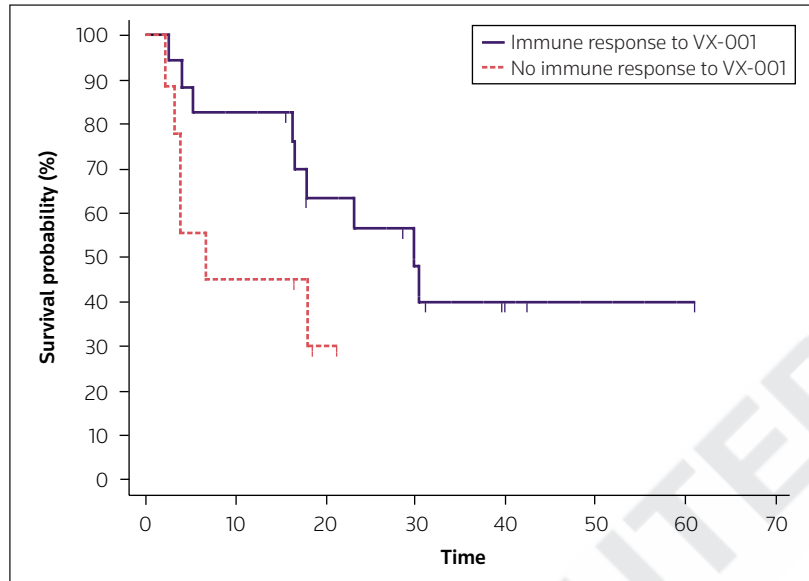
immune response to VX-001 compared with patients who failed to develop an immune response (Figure 3). This observation has been confirmed in patients with various cancers (other than NSCLC) who had progressive disease before vaccination. Given that immune responders had significantly longer survival than immune non-responders, clinical outcome appears to be related to the early development of immune response.

Conclusion

Despite the discouraging results of most cancer immunotherapy studies performed to date, widespread support continues to exist for the ultimate utility of using active cancer immunotherapy to engage a patient's own immune system to recognize and destroy tumor cells with minimal toxicity. Because few encouraging results have been obtained with most tumor vaccines, which are based on dominant peptides, research has undergone a shift toward determining ways of stimulating the immune response through the use of new adjuvants, immunostimulants and delivery vectors. Although these aspects are crucial to vaccine development, breakthroughs in the field may be based in the use of optimized cryptic peptides derived from shared tumor antigens, combined with a potent adjuvant.

The capacity of optimized cryptic peptides to induce antitumor immunity has now been demonstrated. These observations support the view that optimized cryptic peptides are more promising than dominant peptides as candidates for tumor immunotherapy, provided that the optimal vaccination protocol is determined. Encouraging preliminary clinical results of trials with VX-001 suggest that a new series of products that are effective against most cancers may be within reach.

Figure 3. Survival of patients with NSCLC who responded or failed to respond to treatment with VX-001.



Further reading

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