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The Present and Future of Peptide Vaccines for Cancer: Single or Multiple, Long or Short, Alone or in Combination?

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Abstract

Peptide vaccines incorporate one or more short or long amino acid sequences as tumor antigens, combined with a vaccine adjuvant. Thus, they fall broadly into the category of defined-antigen vaccines, along with vaccines using protein, protein subunits, DNA, or RNA. They remain one of the most immunogenic approaches, based on measures of T cell response in the blood or in draining lymph nodes. However, existing peptide vaccines have had limited success at inducing clinical tumor regressions, despite reliable induction of T cell responses. Several new developments offer promise for improving peptide vaccines, including use of long peptides, optimization of adjuvants including toll-like receptor agonists, and combination with systemic therapies that may reduce tumor-associated immune dysfunction, such as blockade of PD-1/PD-L1 interactions. To apply these new approaches optimally, it will be critical to study their effects in the context of defined antigens, for which peptide vaccines are optimal.

Keywords

Peptide; Neoplasm; Melanoma; Immune therapy; TLR agonists; Vaccine; cancer vaccine

Peptide vaccines for cancer offer the promise of inducing T cells reactive to well-characterized tumor antigens and also enabling assessment of vaccination effect, by monitoring antigen-specific T cell responses. Cancer cells express peptide antigens recognized by CD8⁺ cytotoxic T lymphocytes (CTL)¹, which are typically 8-10 amino acids long and are presented in association with Class I MHC molecules. The peptides recognized by helper (CD4⁺) T-cells are presented in association with Class II MHC molecules and are usually longer (13-18 amino acids in length), although peptide elution studies have indicated no apparent restriction on peptide length. For melanoma, the melanocytic differentiation proteins (MDPs) and the cancer-testis antigens (CTAs) are the most common source proteins for these defined shared peptide antigens. Now, a large number of peptide epitopes recognized by melanoma-reactive human CTL and helper T-cells are known^{2,3}, making it possible to design vaccines using these antigens. We and others have found that selected peptides can induce circulating T cell responses in a majority of patients,^{4,5} and that vaccination with a mixture of peptides is immunogenic in up to 100% of patients.⁴ The magnitude of T cell responses sometimes is substantial, with 1-5% of circulating CD8 T cells reactive to single antigens⁶⁻⁹, which rivals the magnitude of T cell responses to individual CMV antigens in humans¹⁰; however, responses in most patients are 1-2 orders

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of magnitude lower, which may or may not be adequate for clinical benefit. T cell responses to vaccines may be durable for months or years, but are at least as likely to be transient, sometimes declining even while still receiving vaccines.¹¹ However, T cells induced by vaccination can recognize and lyse melanoma cells expressing the relevant protein and MHC^{12,13}. Thus, peptide vaccines induce promising immunogenicity. However, the transience and low magnitude of responses in many patients presents a need for improving the immunogenicity and for ensuring that memory responses are induced.

Clinically, there have been durable clinical responses in some patients receiving melanoma vaccines, suggesting the potential for clinical activity.¹⁴ However, overall clinical response rates are only about 3-5%¹⁵. Thus, vaccines are not optimized: the antigens and the adjuvants may both be improved. Also, circulating immune responses have not consistently correlated with clinical outcome^{4,16}. Arguably, that is not surprising, especially in the setting of advanced tumor burden. Both antigenic heterogeneity and tumor-associated immune dysfunction are characteristic of the tumor microenvironment (TME). Adoptive therapy with T cell clones specific for a single antigen has led to eradication of melanoma cells expressing that antigen, but the tumors have not regressed, because of the persistence of antigen-loss variants¹⁷. Furthermore, T cells infiltrating tumor deposits are commonly found to be anergic or poorly responsive to antigenic stimulation, leading to the perception that the tumor microenvironment is hostile to the T cell response¹⁸. Effective immune therapy may require induction of T cell responses to multiple numbers of antigens simultaneously, and maintenance of T cell activation in the tumor deposits. Combination with approaches to block immunoregulatory mechanisms may well also be needed for immune therapy to be most successful.

ADVANTAGES OF PEPTIDE VACCINES

There are several advantages of peptide vaccines over other cancer vaccine approaches (Table 1). Aside from the ease of synthesizing them, and their safety demonstrated in many trials, they have been effective at inducing T cell responses. Short peptides (typically nine amino acid residues) bind to Class I MHC molecules and induce CD8 T cells that can lyse melanoma cells expressing the cognate MHC and peptide^{12,13}. Immune response rates vary, depending on the peptides and adjuvants used, and depending on the assay method. However, immune response rates approaching 100% can be achieved^{4,8,19,20}, and the proportion of CD8+ cells responding to individual peptide antigens can exceed 1%^{7-9,20}. Though MHC-restriction of individual peptides limits their use to a subset of patients, we have found that mixtures of a dozen peptides restricted by HLA-A1, A2, A3, or A11 can be prepared as a stable mixture²¹ and can induce immune responses in the 85% of patients with melanoma who express one or more of those MHC molecules^{4,8,20}, without negative effects from competition among the peptides⁴. Other experience supports the ability to induce T cell responses to multiple peptides when vaccinating with peptide mixtures²².

LIMITATIONS OF PEPTIDE VACCINES

Short peptides restricted by Class I MHC molecules can bind directly into the peptide-binding groove on the exposed surface of the appropriate Class I MHC molecule. In vivo, when a peptide vaccine is administered into the subcutaneous tissue (or other sites), the peptides may be able to bind to numerous types of cells, only a few of which are professional APC. When they bind to non-professional APC (e.g. fibroblasts), they are presented without optimal co-stimulation; presentation of antigen in this way can even be toleragenic. Thus, there is concern that the effectiveness of vaccination with short peptides may be limited by this phenomenon. Also, these short peptides have little or no tertiary structure and thus are subject to rapid degradation by tissue and serum peptidases. We have

estimated the half-life of a MelanA/MART-1 peptide in fresh human plasma to be approximately 22 seconds, and found that both exopeptidases and endopeptidases were involved in the degradation. It is possible that the low magnitude and transience of T cell responses observed in many patients vaccinated with short peptides may be explained in part by rapid degradation of these peptides *in vivo*, before they can be presented by professional APC, and also by suboptimal antigen presentation when it occurs.

Another factor is that when a vaccine is administered in the skin, it is generally believed that antigen presentation depends on dendritic cells bearing the antigen to migrate to the regional draining nodes and to present the antigen there to naïve circulating lymphocytes. When an antigen is presented by dendritic cells (DC) that have taken up a whole protein or DNA, the peptide of interest is going to be presented on an ongoing basis because it is generated inside the cell over time and presented on MHC. However, when a short peptide is used, and its ability to be presented to T cells in the draining node depends on its ability to remain bound to the MHC; so short peptides with low affinity for the MHC may be less immunogenic than they would be if they were more continuously being presented, for which one example may be gp100₂₈₀₋₂₈₈^{23,24}.

SINGLE VS MULTIPLE PEPTIDES

Melanomas often lose expression of one or all melanocytic differentiation antigens as they progress²⁵, and cancer-testis antigens are expressed only in a subset of patients. Thus, no single antigen will be adequate for all melanomas. Also, antigenic heterogeneity, and antigen loss phenotypes are common; so even if a melanoma expresses a specific antigen, some of the cells in that tumor may not express it. Thus, immunologic control of melanoma likely requires a broad immune response against multiple antigens. It is hypothesized that an effective immune response against a single antigen can induce epitope-spreading – induction of immune responses against other antigens. However, it is also possible for vaccines to target multiple antigens directly.

In developing multi-peptide vaccines, there has been some concern that if multiple peptides binding the same MHC molecule are co-administered, binding of lower-affinity peptides to the MHC may be competitively inhibited by higher-affinity peptides. Because of this concern, multi-peptide vaccines at some centers have been administered so that each peptide is administered at a different body site²⁶. This approach will become increasingly unwieldy if the number of peptides exceeds three or four. In preclinical studies from our group, we have found that competition among peptides for MHC binding does not significantly inhibit T-cell induction or T-cell effector function^{27,28}. On the other hand, a clinical study raised concern about the effect of mixing the peptides YMDGTMSQV (tyrosinase₃₆₉₋₃₇₇) and IMDQVPFSV (gp100_{209-2M}²⁹). That report was based on two sequential clinical trials, so that there may have been other variables at play. In a prospective randomized trial, we tested the immunogenicity of a 12-peptide vaccine compared to a vaccine containing just 4 of those 12 peptides. We found that immunogenicity of the index peptides was maintained in those patients receiving the 12-peptide vaccine, and there was also a marked and significant increase in cumulative T-cell reactivity toward the 12-peptide vaccine. Thus, addition of two to three peptides binding the same class I MHC allele does not inhibit immunogenicity of an index peptide when administered at equimolar concentrations, and use of multiple peptides in a cancer vaccine is supported.

ADDITION OF CD4 EPITOPES

The role of CD4⁺ helper T Lymphocytes in anti-tumor immune responses

Most peptide vaccines have been designed to activate the CD8⁺ cytotoxic T cell arm of the host immune system, which plays a critical role in tumor eradication³⁰⁻³⁴. However, some recent approaches target CD4⁺ T_h cells. This is based in part on results from earlier studies which demonstrated depletion of CD4⁺ T-cells abrogates all or part of protective immune response to vaccines³⁵. Furthermore, adoptive therapy with CD4⁺ T-cells has been shown to induce tumor protection in some model systems³⁶, and there has been anecdotal clinical benefit in a patient after adoptive therapy with melanoma-reactive CD4⁺ T-cells³⁷. Thus, protective immunity induced by tumor cell vaccines and by adoptive T cell therapy may be mediated both by CD4⁺ T-cells.

Natural immune responses to pathogens consist of an integrated response including T_h responses to epitopes presented by class II MHC molecules and CTL responses to epitopes presented by class I MHC molecules³⁸. T_h cells can activate dendritic cells (DC) for heightened antigen presentation, causing the DC to secrete IL-2 and other cytokines that may help to direct the immune response. Furthermore, strong T_h1 help produces the proper cytokine milieu (IFN γ , TNF α , IL-2) which is critical to the induction of immune-mediated tumor destruction^{39,40}. In addition, T_h responses are believed to be involved in the establishment of memory responses⁴¹. Thus, there is rationale for induction of CD4⁺ T cells with cancer vaccines, either on their own, or in combination with stimulation of CD8⁺ T cells.

Induction of non-specific T cell help

One approach to induction of CD4⁺ T cell help is to use molecules that stimulate CD4⁺ T cell responses that are not specific for cancer antigens but may stimulate recall responses or other nonspecific help. Commonly used approaches have been to add keyhole limpet hemocyanin (KLH)⁴²⁻⁴⁴, PADRE peptide⁴⁵, or a tetanus toxoid helper peptide tetanus^{46,47}. In one study, CD8⁺ T cell responses to Class I MHC-associated tyrosinase peptides appeared to be increased by co-administration of KLH⁴⁴. Although, KLH may have broader adjuvant properties than simply the effects on helper T cells, these data suggest a benefit of non-specific helper peptide stimulation. We have used a peptide epitope for T-helper cells derived from tetanus toxoid. (tetanus helper peptide) modified from the reported sequence by adding an alanine residue to the N-terminus, to avoid spontaneous conversion of glutamine at the N-terminus to pyroglutamate, and to increase stability (AQYIKANSKFIGITEL). Responses to that peptide are induced in over 90% of patients when vaccinating with IFA^{8,20}, and these responses appear to be predominantly T_h1 responses⁴⁸.

Induction of melanoma-specific T cell help

HLA-DR restricted peptides have been identified from melanoma-associated proteins,⁴⁹⁻⁵⁵ but there is limited *in vivo* human experience with them. Epitopes for CD4⁺ helper T cells are typically longer than those for CD8⁺ T cells, and they are promiscuous in binding to many different Class II MHC molecules. Most prior studies with these “helper” peptides used only one or two peptides, and they limited enrollment to a single MHC Class II allele⁵⁶⁻⁵⁸. We have tested a multi-peptide helper peptide vaccine to stimulate melanoma-reactive CD4⁺ T cells, using 6 melanoma helper peptides (6MHP) from melanocytic differentiation antigens and cancer testis antigens, restricted by HLA-DR molecules (Figure 1)¹⁴. The findings demonstrate safety and immunogenicity. Immune responses to the 6 MHP pool were detected in over 80% of patients, across a wide range of HLA-DR molecules, suggesting these peptides may be broadly relevant to the immune response to

melanoma.¹⁴ Findings also suggest promiscuity of these helper peptides across a wider range of HLA-DR molecules than originally reported. Some immune responses were detectable through week 39, more than 6 months after the last vaccine, suggesting induction of memory in those patients. However, the immune responses were transient in some other cases, suggesting immune regulatory processes that should be identified and targeted for combination immunotherapy in the future.

In addition to *in vitro* evidence of immunogenicity of this 6-helper peptide vaccine, we also observed *in vivo* evidence of immune reactivity, based on DTH responses in 7 of 24 evaluable patients (29%). There were also autoimmune reactivities in 21% of patients, including vitiligo in 10%, without associated symptoms. Durable objective clinical responses were observed in 2 of 17 patients with measurable disease, and durable disease stabilization occurred in 2 additional patients.¹⁴ Immune responses were identified to 6MHP for all patients with DTH responses, all patients with autoimmune toxicities, and all four patients with partial clinical responses or stable disease. Together, these data suggest both biological activity and evidence of clinical activity. This phase I/II trial provided data supporting larger studies of this 6 helper peptide mixture with or without immunogens to stimulate CD8+ T cells.

Combining class I MHC restricted peptides & class II MHC restricted peptides in vaccines

Because CD4+ cells have a fundamental and comprehensive role in initiation and maintenance of cytotoxic T cell responses⁴¹, vaccination with cytotoxic T-cell epitopes may be more successful when the vaccine includes helper epitopes from the same protein(s) rather than with non-specific helper epitopes. In HIV patients, there are data suggesting that induction of CD4+ responses to HIV antigens with an HIV-specific peptide augments HIV-specific CD8+ T cell reactivity⁵⁹. Considering the strong rationale for the induction of helper T cell responses to melanoma antigens, and human data supporting the approach in the HIV setting, it is rational to test whether vaccination with melanoma-associated helper peptides that can induce antigen-reactive CD4+ T cell responses can increase CD8+ responses, compared to vaccination with a nonspecific helper peptide. Several trials have tested this concept in the setting of a single Class I restricted peptide and a single Class II restricted peptide. In a study with the gp100₄₄₋₅₉ helper peptide, helper T cell responses were not induced, but CTL responses were paradoxically reduced compared to a prior study⁶⁰. That report raises a question of whether the addition of helper peptides may be harmful, rather than helpful, to the anti-tumor response. However, the conclusions of that report were confounded by several critical weaknesses: (1) comparison of outcomes between two non-randomized pilot studies only, (2) marked differences in the proportion of patients with prior chemotherapy between the study groups (23% vs 58%), (3) very low immunogenicity of the MART-1 peptide in both arms, raising questions about vaccine immunogenicity, (4) complete absence of immunogenicity of the DR-restricted gp100 peptide, and (5) use of high-dose IL-2 in some patients, that may alter measured responses.¹¹

In another study with class II peptides only, that same gp100 peptide was not immunogenic, but another helper peptide, from MART-1/MelanA, was immunogenic⁶¹. That study did not assess the impact of the helper peptide responses on CTL responses to Class I peptides, but there were some cytotoxic CD4+ responses generated⁶¹. In another study, in epithelial cancers, with Her-2/neu peptides comprising overlapping epitopes for CD4+ and CD8+ T cells, CD8+ T cell responses were observed, but CD4+ responses were not reported⁶².

To address formally the question of whether addition of melanoma helper peptides would increase CD8 T cell responses in a multi-peptide vaccine, we have performed a multicenter randomized trial, Mel44, which enrolled 167 eligible patients with resected stage IIB-IV

melanoma, who were randomized to 4 vaccination groups. Patients were vaccinated with 12 MHC Class I-restricted melanoma peptides (12MP) to stimulate CD8⁺ T cells, and randomized to receive a tetanus helper peptide or a mixture of 6 melanoma helper peptides (6MHP) to stimulate CD4⁺ T cells. Prior to vaccination, patients were also randomly assigned to receive CY pretreatment or not. T cell responses were assessed by ex vivo IFN- γ ELISpot assay. Vaccination with 12MP plus tetanus induced CD8⁺ T cell responses in 78% of patients and CD4⁺ T cell responses to tetanus peptide in 93%. Vaccination with 12MP plus 6MHP induced CD8⁺ responses in 19% and CD4⁺ responses to 6MHP in 48%. CY had no significant effect on T cell responses. Thus, in this adjuvant setting, melanoma-associated helper peptides paradoxically decreased CD8⁺ T cell responses to a melanoma vaccine ($p < 0.001$), and CY pretreatment had no immunologic or clinical effect²⁰. Similar negative effects of combining helper peptides with class I peptides have been observed in an Eastern Cooperative Oncology trial 1602⁶³. Possible explanations for negative effects on CD8 responses include modulation of homing receptor expression or induction of antigen-specific regulatory T cells, and new data also raise the possibility that these negative results may be explained in part by effects of the vaccine adjuvants, locally at the site of vaccination.

PHOSPHO-PEPTIDES

As immune therapy becomes an effective and accepted approach for durable clinical regressions of melanoma and other cancers, the other most exciting area of new drug development for cancer therapy is in targeted therapies that inhibit molecular activation that is critical to the transformed phenotype. Most of the targeted therapies, including those of the MEK/BRAF pathway, are focused on blocking the activity of bioactive phosphoproteins. As effective as these therapies can be, rapid disease progression can occur after initial responses. A promising approach would be to combine such targeted therapies with immune therapy that is active against the phosphoproteins that maintain the malignant transformed phenotype. A new class of peptides being brought to the clinic in the near future are peptides that contain phosphoserine or phosphotyrosine residues, and which thus represent biologically active phosphoproteins that may be critical to the transformed phenotype Engelhard⁶⁴⁻⁶⁶. They offer promise for a new and important class of antigen to target in future cancer vaccines.

ADJUVANTS FOR PEPTIDE VACCINES

IFA as immunologic adjuvant

This paper is focused on peptide vaccines, but the peptides cannot be isolated from their adjuvants. Surprisingly, effects of immunologic adjuvants in vivo in humans are not well-understood. The most common adjuvant for peptide vaccines for melanoma has been an incomplete Freund's adjuvant (IFA), commonly Montanide ISA-51. There have been questions about whether the commonly used incomplete Freund's adjuvant (IFA) Montanide ISA-51 (Seppic, Inc.; Paris, France) is a reliable immunological adjuvant^{67,68}. We have found it useful even in its newer formulation.⁶⁷ However, it is increasingly evident that effective immunotherapy depends on the quality of the adjuvant, whose optimization will require an understanding of its function in vivo in humans at the vaccine site microenvironment (VSME). In a study of vaccination with peptides in IFA, we found that 1 week after 1 vaccine, Th2 cells (GATA-3⁺), but not Th1 cells (T-bet⁺), were increased in number, suggesting that peptide vaccination in IFA may induce a Th2-dominant VSME; this was supported also by induction of a large number of eosinophils at the vaccine site. Only after 3 vaccines was there conversion to a more balanced Th1/Th2 microenvironment⁶⁹. Also, the frequency of FoxP3⁺ cells (putative regulatory T cells) increased with repeated vaccination⁶⁹. The impact of FoxP3⁺ cells here can be debated, but this adjuvant does not

appear to induce an optimal immunologic milieu at the VSME. These findings may contribute to the low magnitude and transience of CD8⁺ T cell responses to short peptide vaccines administered in IFA. An intriguing question also is whether the chronic inflammation induced by IFA can serve to attract antigen-specific T cells back to the vaccine site, thereby depleting them from circulation and reducing the number that may traffick to sites of metastasis⁷⁰. These findings together support seeking new adjuvants.

Toll-like receptor (TLR) agonists as vaccine adjuvants

The critical functions of vaccine adjuvants are not known, but may include activation of innate immunity, optimization of antigen presentation, recruitment of dendritic cells, and creating a cytokine environment that supports the desired immunologic outcome. Toll-like receptors are early mediators of innate immune responses to pathogens. They may improve vaccination efficacy, through activation of innate immune mechanisms, mediated in part by IFN- α signaling and by activating dendritic cells. Toll-like receptor (TLR) agonists offer the potential to improve the magnitude and persistence of antitumor T cell responses⁹; however, most trials of TLR agonists have been limited to use of one TLR agonist, and have not defined molecular and cellular effects at the vaccine site microenvironment (VSME). Several TLR agonists may be effective vaccine adjuvants, including agonists for TLR 3, 4, 7, 8, and 9. In murine models, the combination of TLR agonists and IFA has strong immunological adjuvant properties with peptide vaccines. Murine and human studies have also suggested value of combining agonists for 2-3 TLRs.^{71,72} Future human studies need to evaluate whether individual agonists for TLRs 3, 4, 7, 8, or 9 are better adjuvants than IFA alone, and also whether these TLR agonists alone or together may be more immunogenic with or without IFA. TLR agonists are likely to support a strong Th1 environment⁷³⁻⁷⁵. Some data suggest TLR8 agonists may decrease regulatory T cells⁷⁶, though these data have not been replicated. Vaccination of humans with the oligonucleotide CpG7909 (TLR9 agonist) increases CD8 T cell responses to a short peptide when combined with IFA⁹, and vaccination with peptides and the TLR3 agonist polyICLC also shows promise in murine and human studies⁷⁷. Current trials with a MAGE-A3 protein use a complex adjuvant system that includes the TLR4 agonist Monophosphoryl Lipid A and the TLR9 agonist CpG oligonucleotide, in addition to the saponin QS-21⁷⁸. It is not yet known if this approach is optimal, or if it will be feasible or effective also for peptide vaccines. However, it does seem likely that some use of TLR agonists will be helpful to increase the immunogenicity of peptide vaccines.

Ligation of CD40

TLR activation synergizes with ligation of CD40 on DC; combining TLR agonists and CD40 ligation may augment immune responses to vaccines⁷⁹. In humans, antibody to CD40 may induce CD40 ligation, but many cells express CD40 and thus serve as sinks for systemic CD40 antibody. Another possible approach to ligate CD40 specifically on DC in the vaccine-site microenvironment (VSME) and in vaccine-draining lymph nodes (VDLN) is to take a lesson from physiologic immune responses to pathogens. When pathogens are encountered naturally in the skin, CD4 T cells are activated and upregulate CD40L, which then binds and activates CD40 to license DC. Thus, activation of CD4⁺ cells in the VSME and VDLN will upregulate CD40L on those cells. CD40L⁺ CD4 cells in turn license professional APC (DC) in tissues where antigen is presented. Progress in cancer vaccine development thus likely requires optimizing approaches to induce CD4⁺ T cells in the VSME and VDLN. The encouraging results of vaccinating with melanoma helper peptides alone are in contrast to the disappointing findings after vaccinating with melanoma helper peptides mixed with peptides to stimulate CD8⁺ T cells. Alteration in adjuvants may be critical to improving the ability to combine approaches to stimulate CD4⁺ T cells and CD8⁺ T cells.

LONG VS SHORT PEPTIDES

Short peptides may bind directly to MHC molecules on cells that are not professional antigen-presenting cells (APC), thereby potentially inducing tolerance or anergy^{80,81}. In contrast, recent work with long (30-mer) peptides that encompass short minimal epitopes suggests that these longer peptides may be more effective immunogens than the minimal peptides. The extra length contributes to a tertiary structure that may protect from exopeptidase-mediated degradation, and they are too long to be presented directly on MHC; so they must be internalized by professional APC and processed for presentation (eg CD11c⁺ DC).⁸²⁻⁸⁵ Unlike short peptides, long peptides induce memory CD8⁺ T cell responses that are boosted dramatically on repeat vaccination in mice, and induce substantially improved tumor control compared to vaccination with short peptides.^{83,86} Induction of helper T cells reactive to epitopes within the long peptide have been implicated as necessary for longterm T cell memory⁸⁶. This is supported by the finding that the improved immunologic responses and tumor control are blocked in mice knocked out for CD4 or for CD40. A vaccine using long (30-mer) peptides from HPV-16 for squamous vulvar neoplasia has induced clinical regressions in most patients, supporting clinical activity of long peptide vaccines.⁸⁷ Using these long peptides promises to induce a broad and more durable adaptive immune responses against multiple antigens.

COMBINATION IMMUNE THERAPY

Even from the most optimistic perspective, it is unlikely that vaccines alone will provide durable clinical benefit for the majority of melanoma patients; however, it is likely that combination with other effective agents may lead to cumulative or synergistic benefit in large proportions of patients. New immunomodulatory agents with clinical activity are now available for use in melanoma. Among these, perhaps the most exciting are antibodies that block PD-1/PD-L1 interactions. The NCI's Immunotherapy Agent Workshop prioritized therapeutic agents for use in cancer immunotherapy. Anti-PD-1 agents were ranked #2⁸⁸. There are at least 3 antibodies to PD-1 in clinical trials (MDX-1106, CT-011, and MK-3475). Clinical experience with one of these is that it induces objective clinical responses in 30% of patients with advanced melanoma, with high durability, and a safety profile that may be better than that of CTLA-4 antibody, and with MTD not reached in initial studies^{89,90}. PD-1 is expressed by activated T cells, B cells, and some myeloid cells. Its ligand, PD-L1 is expressed on many peripheral tissues and cell types, including melanoma cells. Its unique effects (as compared to CTLA-4, for example) may be mediated in part by its expression in peripheral tissues, including the tumor.⁹¹ PD-L1's expression in peripheral tissues appears critical to maintaining peripheral tolerance⁹¹. PD-1/PD-L1 interaction can limit T cell reactivity even long after initial activation, and its blockade can restore immune function^{91,92}. PD-1 ligation inhibits signaling through TCR activation; it depends on stimulation of the TCR at the time of PD-1/PD-L1/L2 ligation⁹³. Thus, inhibition of PD-1/PD-L1 can be expected to improve dysfunction of tumor-reactive T cells in situ in the presence of tumor antigen, may improve reactivity to vaccines given simultaneously, or to chronic viral antigen (eg CMV) but may not affect memory responses to viral antigens in the absence of viral antigen (eg influenza). PD-L1 expression also is induced by antigen-mediated immune reactivity. PD-1 also can alter T cell movement⁹⁴; and blockade of PD-1 can increase T cell infiltration of peripheral tissues (eg in autoimmunity models)^{95,96} and thus may be expected to affect T cell homing receptor (HR) expression or expression of homing receptor ligands (HRL), and may be expected to augment T cell infiltration of melanoma metastases. Thus, PD-1/PD-L1 blockade has promise to increase T cell activation in the VSME and in the tumor microenvironment, while also enabling activated T cells to infiltrate metastases and to remain activated. Thus,

there is strong rationale to combine an optimized peptide vaccine with an inhibitor of PD-1/PD-L1 interactions.

SUMMARY

Prospects for improving peptide vaccines include use of long peptides, modification of adjuvants, inclusion of new antigens, and combination therapy with other immunologically active agents. Evidence suggests that long peptides may be more immunogenic than short peptides, and studies evaluating them are underway. Current data suggest that long peptides may overcome many of the challenges with short peptide vaccines, by inducing both CD4+ and CD8+ responses with more optimal antigen presentation, and by enabling continued presentation of immunogenic but low affinity peptides as antigen-loaded DC migrate to regional nodes. TLR agonists may be more effective adjuvants than IFA, local or systemic GM-CSF, or systemic interferon-gamma. Preclinical studies support the value of TLR agonists as vaccine adjuvants, and some clinical studies support the value of TLR3, TLR4, and TLR9 agonists, in particular. However, the optimal TLR agonist(s), dose, timing, and mode of delivery remain to be determined. Very little is known about the molecular and cellular effects of various adjuvants, at the vaccine site and in the vaccine-draining node; an important area of future studies is to understand the local effects of TLR agonists and how they mediate adjuvanticity. A large number of antigens are known, but few have been studied in vaccines. A new class of peptides being brought to the clinic in the near future are peptides that contain phosphoserine or phosphotyrosine residues, which represent biologically active phosphoproteins that may be critical to the transformed phenotype.

A major limitation of all immune therapy is the fact that the tumor microenvironment is hostile to T cell infiltration, function and survival. Thus, in addition to optimizing the immune response induced by peptide vaccines, there is a need to understand critical molecular mediators of T cell trafficking to the tumor microenvironment and mediators of immune dysfunction in the tumor microenvironment. As evidence begins to accumulate for the therapeutic value of defined-antigen vaccines^{87,97-99}, it seems likely that improvements in vaccine immunogenicity, in T cell persistence, and reversal of tumor-associated immune dysfunction, will lead to improved therapeutic value of peptide vaccines in combination with optimal adjuvants and systemic immune modulation. Combinations of vaccines with IFN-alpha, GM-CSF, and CTLA-4 antibodies have not improved immune responses or clinical outcome compared to vaccine alone^{8,16,100,101}. On the other hand, combination of a peptide vaccine with high-dose IL-2 improved clinical response rate and progression-free survival, and induced a strong trend to improved survival (p=0.06).⁹⁹ Other clinically active agents offer promise to improve T cell responses and clinical outcome. Candidates include antibodies to PD-1 or PD-L1, agonistic antibody to CD137, and cytokines that may support T cell expansion and persistence, such as IL-7 and IL-15. As all of these new immune modulators are explored to augment cancer immunotherapy, studies with peptide vaccines will be particularly helpful because they are ideal for correlative studies of immune response to enable an understanding of the effects of each intervention.

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Table 1
Advantages and Limitations of Peptide Vaccines

Advantages	Limitations
<ul style="list-style-type: none">• Readily synthesized and purified at low cost• Stable in many storage conditions• Off-the-shelf reagent• Safe• Very effective at inducing CD8 or CD4 T cell responses in vivo in humans• Enables direct monitoring of T cell responses induced by the vaccine.• Safety in many studies• Using defined epitopes avoids use of uncharacterized antigens that may have nontherapeutic autoimmune activity• Repeated booster vaccines feasible	<ul style="list-style-type: none">• Class I MHC restriction limits relevance of individual peptides to certain HLA types.• Short peptides may bind directly to MHC on non-professional APC[*], which may induce tolerance• Rapidly degraded by serum/tissue peptidases• Peptides with low affinity for MHC may be poorly immunogenic• Patients have variable repertoires for melanoma antigens: a peptide vaccine may have to include a large number of peptides to be useful across a wide range of patients.• Immune responses may be transient and/or of low magnitude

* APC = antigen-presenting cell