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COMMENTARY

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Is there a role for therapeutic cancer vaccines in the age of checkpoint inhibitors?

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ABSTRACT

Because of the recent success of monoclonal antibody checkpoint inhibitors, and the disappointing results of most therapeutic cancer vaccine trials, it has been questioned whether there is any potential role for such products going forward. In my opinion the answer is "yes" based on the following: [1] there is a persistent unmet clinical need because the majority of patients do not benefit from anti-checkpoint therapy, [2] there is evidence that not all patients make immune responses to their tumors, [3] there is evidence that immune responses to autologous tumor antigens can be induced by patient-specific vaccines, [4] there is clinical evidence from the pre-checkpoint era that suggests survival can be positively impacted by such patient-specific vaccines, and [5] the 2 available therapeutic vaccines that have received regulatory approval are quite limited in terms of their therapeutic benefit.

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Introduction

There has been long-standing interest in anti-cancer vaccines. The term "vaccine" is derived from *Variolae vaccinae*, the Latin name for cowpox which was the first documented efficacious vaccine in that it prevented small pox. A vaccine is typically a biological preparation that induces an acquired immunity to a particular disease. Cancer is such a disease. More specifically, a vaccine induces immune B and T lymphocyte recognition and memory of specific molecules (antigens) expressed by cells. Unlike most anti-cancer therapies that act directly on cancer cells, vaccines work indirectly by activating cells of the endogenous adaptive immune system to induce a response that targets antigens on the cancer cells. However, not every agent that acts indirectly through the immune system is a vaccine. For instance, the cytokines interferon-α and interleukin-2 (IL-2) drive existing immune responses, and the monoclonal antibody checkpoint inhibitors (e.g. ipilimumab, nivolumab, pembrolizumab, atezolizumab) release existing immune responses that are being actively suppressed.

Vaccines are often classified as preventive, or therapeutic, based on whether the intent is to decrease the risk of disease or to treat active disease. Examples of efficacious prophylactic vaccines that may prevent cancer, and are in standard prophylactic use, are those that target human papilloma virus (HPV)[1] and hepatitis B virus (HBV).[2] These vaccines prevent viral infections that are associated with an increased risk of certain cancers that occur in tissues infected by these viruses. However, these vaccines are ineffective against the cancers once they have occurred, and have nothing to do with the antigens expressed on the cancer cells.

Examples of therapeutic anti-cancer vaccines that have been granted regulatory approval are sipuleucel-T for metastatic prostate cancer,[3] and talimogene laherparepvec for metastatic melanoma.[4] Both are biological preparations, but they are quite different. Sipuleucel-T is a cellular immunotherapy administered intravenously, and characterized as a vaccine because it induces immune responses to prostatic acid phosphatase, which was intended to be its mechanism of action, but the product itself largely consists of immune cells that may be a form of adoptive cell therapy. In contrast, talimogene laherparepvec consists of oncolytic *Herpes simplex* virus encoding genes to secrete the cytokine granulocyte-macrophage colony-stimulating factor (GM-CSF). This product is injected into tumor nodules where cancer cells can be killed directly by the virus, but may also lead to attraction and differentiation of dendritic cells and induction of an immune response that can result in tumor effects on distant non-injected lesions, often called an "abscopal" effect.

There have been many failed attempts to create therapeutic anti-cancer vaccines, and most of that experience has been in melanoma.[5] Arguably the most important characteristic of a vaccine is its antigens that are serving as immunogens. The simplest anti-cancer vaccines have included one or a few well characterized antigens. Trials of these products have been useful to prove that cancer patients can make immune responses to specific antigens that have been injected with the intent of inducing such responses. However, significant clinical benefit is uncommon, and this approach has yet to succeed in randomized trials testing single or combinations of such characterized antigens.[6-8] Another "off-the-shelf" approach has been to use allogeneic cell lines as a source of tumor associated antigens (TAA), but randomized trials with this approach have also been negative or unconvincing.[9,10] Given inter-patient heterogeneity, it should not be a surprise that "one-size fits all" vaccines using well-characterized common antigens or allogeneic tumor cells as TAA sources, have not succeeded when tested in a large pools of cancer patients. Theoretically the ideal source

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of TAA should be a patient's own tumor if one wants to exploit the entire repertoire of potential TAA in that patient. Obviously there are technical challenges with such approaches whether one tries to use fresh tumor as the TAA source,[11,12] or tries to establish autologous tumor cell lines for each patient.[13]

Exomic analyses have confirmed that cancers from each patient contain tens to thousands of non-synonymous mutations that might result in translation of immunogenic proteins that could be targeted by the host immune system.[14] Most of these are passenger mutations rather than driver mutations in that most result in proteins that do not facilitate malignancy by increasing proliferation or inhibiting apoptosis or related processes that suppress cancer cells. Therefore each patient could be making an immune response to several non-self proteins that are expressed on each patient's cancer cells, but not on their normal cells. Over the years evidence accumulated indicating that such anti-cancer immune responses exist, but they obviously were not continually effective, resulting in appearance and/or persistence of the cancer.

The biggest therapeutic breakthrough in immunotherapy to date did not occur because of vaccines or targeting TAA with patient-specific tumor infiltrating lymphocytes (TIL), but rather by the use of monoclonal antibodies that block molecules that suppress immune responses at the level of antigen-presenting cells, T lymphocytes, and tumor cells. Collectively these are referred to as check-point inhibitors. The first success was in melanoma with the anti-cytotoxic –T lypmphocyte-4 (CTLA-4) antibody ipilimumab which prolonged survival in melanoma despite no substantial impact on tumor response rates or relapse free survival.[15] Of note, so far, this product has yet to find a second cancer for a marketing indication. Even more dramatic has been the broader success of inhibitors of programmed death-1 (PD-1) and its ligand (PDL-1) by monoclonal antibodies nivolumab and pembrolizumab (anti-PD1) and atezolizumab (anti-PDL-1). These products all "take the brakes off" of existing anti-TAA responses that are being inhibited via these molecules. These anti-checkpoint antibodies have received regulatory approval based on high objective response rates and/or increases in progression free survival and/or overall survival in melanoma,[16,17] renal cell cancer,[18] non-small cell lung cancer,[19-21] and bladder cancer,[22] and have shown promise in other cancers including Hodgkins,[23] ovarian,[24] head and neck,[25] and neuroendocrine.[26]

Because of the high profile failure in clinical trials of vaccines based on peptide TAA, and allogeneic cell lines, and the great clinical success of checkpoint inhibitors that succeed through patient-specific TAA and existing immune responses in each patient, is there any reason to pursue development of anti-cancer vaccines? In my opinion, from a scientific perspective, and therefore eventually from an unmet clinical need and commercial perspective, the answer is "yes."

Why do I say, yes, indeed there is a role for therapeutic vaccines in the age of checkpoint inhibitors? Because [1] there is a persistent unmet clinical need in the universe of patients with metastatic cancer despite anti-checkpoint therapy, [2] there is evidence that not all patients make immune responses to their TAA, [3] there is evidence that immune responses to autologous TAA can be induced by patient-specific vaccines, [4] there is clinical evidence in the pre-checkpoint era that suggests survival can be positively impacted by such patient-specific vaccines, and [5] existing therapeutic vaccines that have received regulatory approval, are quite limited in terms of their therapeutic benefit; therefore, there is room for introduction of more effective vaccine products.

[1] Unmet clinical need. At this time the best results with the anti-checkpoint antibodies in any cancer are those resulting from the combination of nivolumab and ipilimumab in patients with metastatic melanoma.[27] The response rate was nearly 60% and median overall survival probably around 2 years, and 5-year survival rate that almost certainly will be over 40%, and may be as high as 50%. Thus, without even taking into account the considerable high-grade toxicity associated with this regimen, there is no or only limited benefit for at least half the patients who develop metastatic melanoma and are treated with the best anti-checkpoint combination tested to date.

[2] No response to TAA. Tumor samples from patients with metastatic cancer have been grossly lumped into 3 groups.[28] These are: Group 1: no tumor infiltrating lymphocytes (40%), Group 2: tumor infiltrating lymphocytes without elevated PD-1/ PDL-1 [20%], and [3] tumor infiltrating lymphocytes with elevated PD-1/PDL-1 (40%). The proportions of each are estimations based on observations in melanoma, and almost certainly vary among tumor types. One hypothesis is that Group 2 are the ones that respond to IL-2 or TIL therapy with IL-2, that Group 3 are the ones who respond to anti-checkpoint therapy, and that Group 1 could benefit from vaccine therapy alone, or result in the patient moving into Group 2 or Group 3. For non-melanoma cancers, the estimates may be more along the line of 70%, 10%, and 20%.

[3] Inducing patient-specific immune responses. Elegant work in this regard has included exomic analysis of cancer samples to identify mutated proteins, then use of mRNA to see which proteins are translatable, then computer programs to predict antigenicity of those proteins, then selection of a few for testing, then loading those TAA onto autologous dendritic cells, then injecting patients and collecting immune modulation data.[29] When such antigen-specific products were injected into patients, there was evidence of induction of immune responses to certain TAA, and enhancement of weak existing immune responses for other TAA.

[4] Evidence of clinical benefit. The best results with therapeutic cancer vaccines appear to be achieved using dendritic cells and whole tumor cell antigens, especially autologous TAA. Despite the limitations of injecting dendritic cells loaded with TAA consisting of only one or a few peptides, or TAA from allogeneic cell lines, rare strong durable clinical remissions have been repeatedly documented with such products. Crude meta-analyses showed that the objective response rates were higher for vaccines using whole tumor cell TAA sources (autologous or allogeneic) than defined TAA sources (140/1736, 8.1% vs 62/1711 3.6% $p<0.001$).[30] In similar analysis of metastatic melanoma patients who were treated with dendritic cell vaccines, the response rate for defined TAA was 11/201 (5.5%) for 9 studies in which defined TAA were loaded onto DC, and 14/115 (12.2%) for 7 studies utilizing autologous DC loaded with autologous TAA ($p=0.034$), but only 8/116 (6.9%) for 6 studies in which DC or tumor cell sources were allogeneic.[31] In a randomized phase II trial in metastatic melanoma, patients injected with DC loaded with TAA by phagocytosis of irradiated autologous tumor cells had an improved survival at the

time of initial analysis,[32] and eventually showed a median survival of 42.2 months and 3-year survival of 61%, compared to a median survival of 19.9 months and 3-year survival of 25% for patients treated with injections of the irradiated tumor cells as the source of TAA.[33] Pooled data confirmed therapeutic benefit regardless of whether patients had no evidence of disease at the time of treatment.[34] In that trial each dose of vaccine was given weekly for 3 weeks, then monthly for 5 months, and each dose was administered in 500 microgram GM-CSF. These trials were conducted before checkpoint inhibitors became widely available; so, they are supportive of independent single-agent benefit. It is important to note that none of these vaccine therapy products has been associated with significant toxicity other than local injections site reactions and mild flu-like symptoms.

[5] Limitations of existing therapeutic vaccines. As mentioned earlier in this commentary, there are currently 2 FDA-approved products, sipileucel-T (2010 for metastatic prostate cancer),[3] and talimogene laherparepvec for metastatic melanoma (2015).[4] Neither of these appears to represent an ideal prototype for a therapeutic anti-cancer vaccine. Sipuleucel-T is a vaccine/adoptive cell product that is produced by incubating peripheral blood monocytes with a fusion protein that encodes GM-CSF and PAP. Regulatory approval was based on a median, survival advantage of 4.1 months, which represented a 22% increase (21.7 to 25.8 months). The rationale for this product was that GM-CSF would drive the differentiation of peripheral blood monocytes obtained by leukapheresis into dendritic cells, and that PAP would be presented by these dendritic cells, perhaps preferentially via class I molecules because of internalization of the fusion protein by monocytes. The limitations of this product include [1] the need to perform a leukapheresis to make a new product for each treatment every 2 weeks, [2] PAP is not an ideal TAA because of limited expression in prostate cancer, [3] the product is only patient-specific because of the autologous DC, not because of the TAA target, [4] GM-CSF alone is not ideal for inducing differentiation of monocytes into dendritic cells or maturation of dendritic cells for antigen presentation to lymphocytes, [5] animal models suggest that intravenous is a poor route for getting dendritic cells to lymphatic tissues for antigen presentation, [6] the mechanism of action may actually be as an adoptive cell therapy rather than vaccination against PAP. Talimogene laherparepvec also features transfection, in this case with oncolytic *Herpes simplex* virus encoding genes for GM-CSF. This product is injected into tumor nodules where cancer cells can be killed directly by the virus resulting in the release of autologous TAA. The GM-CSF is intended to facilitate the differentiation of local monocytes into dendritic cells that may take up the TAA and then migrate to regional lymph nodes to present TAA to T lymphocytes. If that occurs, then there could be anti-tumor effects throughout the body. This product was approved based on a higher durable response rate (16% vs 2%) and was associated with a higher objective response rate (26% vs 6%), and longer median overall survival (23.3 vs 18.9 months) versus every 4-week cycles of subcutaneous GM-CSF, 125 microgram weekly for 2 weeks, then off 2 weeks.[4] The limitations of this product include [1] the environment within tumor nodules is often highly immunosuppressive which would limit the ability to induce an immune response in that site, [2] GM-CSF alone is not ideal for differentiating monocytes into dendritic cells or inducing maturation of antigen-loaded dendritic cells [3] most of the responses observed were in the nodules that were injected rather than distant lesions, [4] the patient population was highly selected based on the ready availability of nodules for injection and included very few patients with visceral metastases, [5] the control arm for the study that led to regulatory approval arguably should have been GM-CSF injected directly into lesions rather than a non-intralesional regimen whose efficacy was not validated in a randomized trial,[7] especially since intralesional injections of many different cytokines are known to cause regression of the lesions into which they are injected. Given that other vaccine approaches could improve the clinical results associated with vaccines, there is the potential for approval of more effective vaccine products.

It has been more than 3 decades since it was proclaimed that biotherapy would join surgery, radiation therapy, and chemotherapy as the 4th modality of cancer treatment.[35] That prediction became a reality during the past 20 years with the introduction into the cancer therapy armamentarium of effective monoclonal antibodies and enzyme inhibitors. Immunotherapy is typically classified as a form of biotherapy as well.[36] Even if one prefers to classify antibodies and tyrosine kinase inhibitors as "targeted therapy" rather than "biotherapy," immunotherapy could still be classified with this cohort because TAA are ultimately the target of therapy. No matter the terminology one prefers, immunotherapy has truly emerged as an effective therapeutic modality for the treatment of metastatic cancer, but there is still plenty of clinical space for additional therapeutic vaccines to impact this field.

In this commentary I have made the case that despite the success of immune checkpoint inhibitors, there are unmet clinical needs and therapeutic potential that clearly justify continued development of therapeutic cancer vaccines. I believe the focus for that approach needs to be on patient-specific vaccines using autologous TAA. This may be accomplished in 2 ways: [1] TAA derived from exomic analysis of each individual followed by synthesis of patient-specific TAA, or [2] the use of autologous tumor as the TAA source. With regard to the latter, for many years I have suggested that short-term autologous tumor cell lines may be the best source of TAA to avoid issues associated with non-cancer cells when one is using autologous tumor as the TAA source. Such short-term cultures result in the elimination of haematopoietic cells, immune cells, and mesenchymal cells resulting in a relatively pure sample of autologous tumor as the TAA source. This approach also offers advantages for exomic analysis of tumor cells rather than having to account for the contamination of non-cancer cells in a tumor samples. It may also favor inclusion of certain TAA that are only expressed in cancer stem cells.[37]

Disclosure of potential conflicts of interest

The author is employed by AiVita Biomedical, Inc.

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