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Perspective

Recent Advances in Therapeutic Cancer Vaccines

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The Food and Drug Administration (FDA) approval of sipuleucel-T[1] as the first therapeutic cancer vaccine represents a major stride for this field. Sipuleucel-T consists of autologous peripheral blood mononuclear cells, including antigen-presenting cells that have been activated *ex vivo* with a recombinant fusion protein. In addition to this vaccine, numerous other vaccine platforms are currently demonstrating evidence of patient benefit in multicenter, randomized Phase II and Phase III studies in a range of human cancers.

This article will review several of these recent findings as well as describe those areas of research that will most impact on improving the ultimate use of cancer vaccines either as monotherapy or in combination therapy. Also described will be the hurdles that must be overcome in dealing with immune regulatory and inhibitory entities and the lessons learned for appropriate clinical trial design.

While the vast majority of clinical studies that involve therapeutic cancer vaccines have been performed in patients with metastatic melanoma, it is interesting to note that the first FDA-approved vaccine has shown benefit in patients with metastatic prostate cancer. Several characteristics render prostate cancer an excellent disease for evaluation of a cancer vaccine. Prostate cancer is generally an indolent disease with a long interval from disease detection to metastasis. The serum marker prostate-specific antigen (PSA) can also be used to help identify patients' response to therapy.

The initial Phase III study of sipuleucel-T[2] in patients with minimally symptomatic metastatic prostate cancer did not meet its primary end point of improved disease progression, but subsequently showed evidence of enhanced patient survival in the vaccine arm. A second Phase III trial[1] was then conducted with overall survival as the end point, which again demonstrated an improvement in median overall survival of 25.8 months in the vaccine arm *vs* 21.7 months in the control arm ($p = 0.03$). This vaccine platform involves three leukaphereses, each of which is shipped to a central facility where antigen-presenting cells are incubated with a prostate antigen (PAP)/granulocyte macrophage colony-stimulating factor (GM-CSF) fusion protein; the "vaccine" is then shipped back for infusion into patients.

A second prostate cancer vaccine has also shown promising results in the same patient population. This "off-the-shelf" vaccine platform (PROSTVAC) consists of recombinant viral vectors that contain transgenes for the prostate antigen PSA and three immune-stimulating molecules (designated TRICOM). A multicenter, placebo-controlled Phase II trial[3] demonstrated an improved median overall survival of 25.1 months in the vaccine arm *vs* 16.6 months in the control arm ($p = 0.006$). A Phase III study has been initiated with the PROSTVAC vaccine.

An important point to consider with both of the vaccines described above, as well as with other vaccines to be described below, is the extremely low level of toxicity. In addition to the quality of life consideration, this also renders vaccines amenable to combination therapies without the issue of compounding toxicities.

Successful Phase III studies have also recently been reported with two additional vaccine platforms. An anti-idiotype vaccine[4] in patients with follicular lymphoma demonstrated a median time to relapse of 44.2 months in the vaccine arm *vs* 30 months in the control arm ($p = 0.045$). Compared with two other anti-idiotype vaccine trials[5] that failed to meet end points, the patients in the successful trial had lower tumor burden. Vaccines in these trials were also produced differently. A modified GP100 peptide vaccine in adjuvant plus high-dose interleukin (IL)-2[6] demonstrated a longer median overall survival compared with the control IL-2 group only (17.8 months *vs* 11.1 months, $p = 0.06$). There are several other ongoing Phase III trials using additional vaccine platforms in a range of human cancers, including emepepimut-S (Stimuvax®)[7] (liposomal MUC-1 peptide) and belagenpumatucel-L (Lucanix™),[8,9] which is an allogeneic whole tumor cell vaccine that contains a transforming growth factor (TGF)-β antisense transgene, both in patients with non-small cell lung cancer, and a MAGE-A3 protein vaccine in metastatic melanoma.[10]

Several issues are emerging from experience with multiple randomized vaccine clinical studies. These include: (a) there appears to be a greater vaccine efficacy in patients with low grade or more indolent disease compared with what one would observe with other forms of therapy.[11] One potential

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reason for this is that it often takes multiple vaccinations and thus time to enhance the host T-cell response to tumor antigen(s) to a point at which it can effectively control tumor growth.[12] Often patients have been taken off vaccine therapy before receiving optimal boosting regimens. (b) Patients who have had a longer duration since their last chemotherapy or have received fewer regimens of chemotherapy will respond better to vaccine.[13,14] (c) The mechanism of action and kinetics of clinical response with vaccine compared with other forms of therapy appear to be quite different. Cytotoxic and even small molecule targeted therapies directly affect tumor only during the period of administration. If a drug is discontinued because of toxicity or drug resistance, all antitumor activity ceases and tumor growth rate will increase.

Recent vaccine studies have demonstrated that even in the absence of reduction of tumor size and/or time to progression, a slower growth rate of tumor can lead to enhanced survival.[12] This phenomenon is most likely because vaccines induce a dynamic process of host immunity that can persist long after vaccine therapy is terminated and thus continue to control tumor growth. The same phenomenon may also be associated with the still anecdotal findings that some patients for whom vaccine therapy has failed have better than expected responses to subsequent therapies. A multicenter, randomized trial has now been initiated to evaluate this hypothesis. Patients with metastatic prostate cancer will be randomized in a multicenter trial to receive docetaxel *vs* PROSTVAC vaccine followed by docetaxel.[15]

It is anticipated that patient benefit may well occur with the use of vaccine combination therapies. This area of research/clinical trial design can be divided into two major segments: Vaccines in combination with other immune therapies and in combination with nonimmune therapeutic modalities. Preclinical and early clinical studies have demonstrated that a variety of immune stimulants can enhance vaccine efficacy. These include the use of one of numerous cytokines such as GM-CSF, IL-2, IL-15, IL-7, and interferons, all of which can enhance different components of the immune response. A range of toll-like receptor agonists as well as more "classical" adjuvants, such as incomplete Freund's, Monophosphoryl Lipid A (MPL), and chitosan, all have the potential to enhance vaccine efficacy.

Vaccines may also enhance immune and antitumor responses to monoclonal antibodies that are mediated by antibody-dependent cell-mediated cytotoxicity, as well as following adoptive T-cell transfer regimens.[16] One area of investigation that is often overlooked is the use of two or more diverse vaccine platform combinations, either used concomitantly or in diversified prime-boost schemas. Preclinical studies have clearly demonstrated that diverse vaccine platforms can activate different components of the immune system and thus additively or synergistically enhance vaccine efficacy. Combination chemotherapy is standard of care for numerous tumor types, and combinations of small molecule targeted therapies are demonstrating enhanced patient benefit. There is also a strong rationale for the use of combinations of vaccines, and unlike combinations of chemotherapies and small molecule targeted therapies, there should be minimal issues with compounding toxicities.

Inhibitors of immune suppressive entities should also lead to enhanced vaccine efficacy. While the immune checkpoint inhibitor ipilimumab (anti-cytotoxic T-lymphocyte antigen (CTLA) 4 MAb) has now been approved by the FDA for use in metastatic melanoma,[17] it and other immune checkpoint inhibitors, such as antiprogrammed death (PD)1 and anti-PDL1,[18,19] will most certainly be evaluated in the future in combination with vaccines. Preclinical studies have demonstrated that the use of carcinoembryonic antigen (CEA)-TRICOM vaccine to enhance costimulation, in combination with the checkpoint inhibitor anti-CTLA4, can actually enhance T-cell avidity and subsequent antitumor activity.[20] A single arm clinical trial has recently provided some preliminary evidence[21,22] of patient benefit in the use of PROSTVAC vaccine with anti-CTLA4.

The impact of the tumor microenvironment remains an important consideration in obtaining optimal vaccine efficacy.[23–27] In addition to cell-associated immune checkpoint inhibitors, numerous soluble immune suppressive factors such as TGF-β and IL-8 are found in the tumor microenvironment. While inhibitors of some of these molecules are currently in development, control of the tumor microenvironment to enhance vaccine efficacy will undoubtedly require combination therapies.

The next frontier for vaccine therapy will be the use of vaccines in combination with certain chemotherapeutic agents, radiation, hormone therapy, and certain small molecule targeted therapies. It has been elegantly shown that certain chemotherapeutic agents such as oxaliplatin and doxorubicin can induce "immunologic cell death," which results in enhanced uptake of dead and dying tumor cells by dendritic cells resulting in activation of T cells to tumor antigens.[28–30] Other chemotherapeutic agents such as docetaxel, as well as radiation of tumor, have been shown to alter the phenotype of tumor cells in terms of enhancing expression of tumor antigens, peptide major histocompatibility complexes (MHC) and death receptors, to render tumor cells more susceptible to vaccine-mediated T-cell killing.[31–33] These two phenomena of immunologic tumor cell death and altering phenotype of tumor are not mutually exclusive, and both can lead to enhanced patient benefit.

Certain chemotherapeutic regimens such as cisplatin, vinorelbine, or cyclophosphamide have also been shown to preferentially reduce the number of immune suppressive cells such as regulatory T cells and myeloid-derived suppressor cells.[34,35] This has also been observed with the use of small molecule targeted therapies such as a BCL-2 inhibitor and the tyrosine kinase inhibitor sunitinib, both of which have been shown in preclinical models to enhance the ratio of tumor-specific T cells to regulatory cells, resulting in enhanced vaccine antitumor efficacy.[36–39]

Antiandrogen hormonal therapy is standard of care for several stages of prostate cancer and has been shown to induce thymic regeneration, resulting in the induction of naïve T cells[40,41]; this phenomenon could potentially render greater vaccine-induced T-cell responses during a period of hormone therapy.[42] Clinical trials have been completed, are ongoing, and are planned using vaccines in combination with hormonal therapy in different stages of prostate cancer.[43–45] The challenge in all of the above combination therapies will be the appropriate scheduling of vaccine in relationship to the other therapeutic modalities as well as the appropriate patient population and clinical trial end point.

There are some 14 different vaccine platforms currently in Phase II and Phase III clinical studies, each of which

encompasses a wide range of targeted tumor-associated antigens. One of the major findings in recent years is the realization that one can target, with vaccine-induced T cells, molecules that are responsible for tumor initiation or progression; these molecules need not be found on the tumor cell surface but are presented in peptide MHC complexes for vaccine-mediated T-cell recognition. Thus, one can potentially also target with vaccine therapy molecules involved in processes such as cancer cell "stemness," epithelial-to-mesenchymal transition, and drug resistance.[46–51] The era of therapeutic cancer vaccine therapy is thus now entering a new stage.

Disclosure Statement

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