

Prostate Cancer Vaccines: Maximizing a Suboptimal Immune Response for Improved Outcome

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Abstract: As approaches toward isolating tumor-associated antigens have become more refined, vaccines composed of novel cell surface proteins, peptides, or DNA encoding the molecule of interest have been developed. All of these approaches have in some way been shown to break immunologic tolerance, either by the generation of high-titer antibodies against the immunogen or by eliciting T-cell responses as shown by T-cell proliferation assays or specific cytokine release via enzyme-linked immunosorbent spot. No single immunologic approach to date has successfully shown significant durable disease remission in prostate cancer, albeit clinical trials using immune modulators together with vaccines have suggested that radiographic antitumor responses are feasible. This review updates the status of prostate cancer vaccines as tools for induction of active immunity and discusses the issues relevant to their clinical trial development.

Background

The concept of using the immune system to fight cancer is not novel. Efforts toward developing vaccines for a variety of solid tumor malignancies have included tumor cell suspensions made by homogenizing pieces of surgically resected tumors or tumor cell lysates, which were thought to provide pieces of cell membranes that express tumor antigens. As approaches toward isolating tumor-associated antigens have become more refined, vaccines composed of novel cell surface proteins, peptides, or DNA encoding the molecule of interest have been developed.

Prostate cancer has evolved as a target for vaccine and other immunotherapeutic approaches for several reasons. First, prostate cancer can be monitored by two biomarkers, prostate-specific antigen (PSA) and prostatic acid phosphatase (PAP), which can be used to follow disease activity and progression. Second, in addition to these antigens, there is diffuse expression on the cell surface of a variety of carbohydrate,¹⁻³ mucin, and mucin-related molecules⁴⁻⁸ and glycoprotein antigens (prostate-specific membrane antigen [PSMA],⁹ prostate stem cell antigen¹⁰) to which immune therapies can be targeted. Third, there are different disease states for which specific vaccines may be directed. There is also an unmet need to develop nontoxic therapies, particularly for those men

Keywords

Antibody, CTLA-4, prostate cancer, PSA, T cell, vaccines.

who have failed primary therapies such as surgery and radiation and whose sole manifestation of disease relapse is rising PSA level. Though many of these men have a long natural history of disease progression, there is a subset of patients at high risk for recurrence (based on time to PSA relapse, Gleason grade, and PSA doubling times of <6 months) for which immunologic approaches may offer an alternative approach to hormonal ablation.¹¹

Defining Tumor Antigens

Many of the molecules identified on prostate cancer cells and cell lines are self antigens, meaning they appear on the normal counterparts of the tumor cells derived from the organ in question but change in very subtle ways with malignant transformation.¹² This is particularly true of the mucin family—ie, MUC-1 and MUC-2—which are present normally within glandular elements and become overexpressed and underglycosylated with malignancy. It is unclear why the immune system does not recognize the altered self antigens expressed on new tumor cells as foreign and allows them to grow. Strategies have focused on means of educating the immune system to recognize these molecules as foreign and thereby break immunologic tolerance.

Augmenting Immunogenicity

Active Immunization—Generation of Antitumor Antibodies

Vaccines designed to induce an optimal antibody response have several components, each of which must be optimized.¹²⁻¹⁴ The first component is the antigen itself, which must closely resemble its expression on the target—for example, Thomsen-Friedenreich (TF) antigen expression on tumor mucins. Although the TF antigen is a disaccharide covalently attached to serine or threonine, akin to the closely associated disaccharide sTn, monoclonal antibodies and sera selected for preferential reactivity with cancer cells (as opposed to normal tissue) react with clusters (c) of three such disaccharide antigens rather than a single antigen.⁸

The second component necessary for an optimal antibody response is an immunogenic carrier protein, to which the antigen is covalently conjugated. Several studies have shown keyhole limpet hemocyanin (KLH) to be the optimal carrier for antibody induction.^{13,14} The conjugation must be achieved in a manner that does not interfere with the antigenic epitope itself (TF[c]) and that maintains as high an antigen/carrier ratio as possible (in this case >466 TF[c] molecules per KLH molecule).⁸ This was made feasible using a heterobifunctional cross-linker that links the terminal cysteine group of the cluster backbone to amino groups on KLH. The final necessary component

is the immunological adjuvant. In our experience, saponin adjuvants such as QS21 have been the most potent for augmenting the antibody response against conjugate vaccines. The 100 mg dose level of QS21 used here has been found to be optimal, with higher doses resulting in excessive local and systemic toxicity and lower doses resulting in decreased immunogenicity.^{6,13}

Impact of Antibodies on Disease Status The induction of the high-titer immunoglobins M (IgM) and G (IgG) antibodies specific for the immunogen used within the vaccine suggests that immunologic tolerance can be broken. However, the antibodies' impact on the actual biology of the tumor remains unclear, which has been particularly true with carbohydrate vaccines used in prostate cancer patients with widespread high tumor burden. Despite the induction of antibody titers in excess of 1/10,240 against a particular antigen(s), there was no impact on disease growth.⁵ The same was true of antibodies that could induce antibody-dependent cell-mediated lysis.² What remains of continued interest is whether induction of antibodies can predict for a change in PSA level, and as such, whether this inherently can reflect change in the biology of the tumor. Several vaccine trials to date have shown either changes in slope¹ or “broken arrow”¹⁵ changes in PSA direction, suggesting induction of humoral immunity might play a role in modulating PSA and, thus, potentially in the disease itself. However, the use of PSA as a disease surrogate remains controversial.¹⁶

Active Immunization—Induction of T-cell Immunity

Recombinant approaches have afforded the opportunity to test the hypothesis that immunologic tolerance can be disrupted with vaccines that target molecules such as PSMA and PSA. Plasmids encoding the sequence of molecules such as PSMA and DNA have been used.¹⁷⁻²¹ The main effectors in antitumor immunity after DNA immunizations are CD8+ cytotoxic T cells that recognize tumor or tumor-associated antigen-derived peptides or proteins expressed in the context of the class I major histocompatibility complex (MHC) molecules. The benefits of a DNA vaccine are many: 1) it is relatively inexpensive and simple to purify in large quantities; 2) complex *ex vivo* expansion and manipulation of patient cells is avoided; 3) the antigen of interest is cloned into a bacterial expression plasmid with a constitutively active promoter; 4) the bacterial plasmid DNA contains immunostimulatory sequences (CpG motifs) that act as an immunologic adjuvant; and 5) there is direct entry of the antigen into the intracellular MHC class I pathway.

Mincheff and colleagues²⁰ studied two plasmid DNA vaccines encoding either products that are retained in the

cytosol and degraded in the proteasome (tVacs; hPSMA_t) or secreted proteins (sVacs; hPSMA_s). Immunization with both vectors given in combination with the cytokine granulocyte-macrophage colony-stimulating factor (GM-CSF) led to the generation of T cell-mediated cytotoxicity in preclinical studies, supporting the use of a strategy that could induce a strong cellular cytotoxic response. The authors demonstrated that priming with tVacs and boosting with protein could induce antibody formation of the cytotoxic Th1 isotype. This model seems to suggest that the best strategy in gene-based vaccination is to prime with the xenogeneic and boost with the autologous constructs. This approach has also been shown to be feasible in patients.²¹

Studies in Man Todorova and coworkers¹⁹ immunized prostate patients with plasmid and adenoviral vectors encoding for the extracellular portion of human PSMA, then tested for anti-PSMA antibodies by western blot. PSMA-producing LNCaP cells were used as a control. Using these multiple gene-based vaccinations induced an anti-PSMA humoral immune response: specific anti-PSMA antibodies were detected in the immunized patients' sera, mainly against the PSMA protein core. Gregor and associates¹⁸ used human and mouse PSMA DNA inserted into a pING vector containing a cytomegalovirus promoter and kanamycin resistance selection marker. The vaccine, which was administered to human leukocyte antigen (HLA)-A02.01+ patients in both the noncastrate and castrate metastatic states, was safe when administered in a dose-escalating manner via a high-powered carbon dioxide delivery system. This xenogeneic approach was a proof of principle using murine-followed-by-human or human-followed-by-murine PSMA DNA. Although selected patients generated only a modest antibody response by enzyme-linked immunosorbent assay and flow cytometry, nevertheless impact on PSA doubling time was observed at the 4,000- μ g dose of vaccine. No difference was seen in benefit at this level if the patient received the murine or human vaccine first.

An alternative strategy was shown by Dunphy and McNeel, who identified prostate-associated antigens that were immunologically recognized in 13 prostate cancer patients treated with multiple cycles of flt3 ligand, a potent growth and differentiation factor for dendritic cells.²² Flt3 ligand has been shown in murine tumor models to induce dendritic cells systemically, which can lead to the eradication of established solid tumors such as murine melanoma and lymphoma. A prior vaccine, E75 HLA-A2 epitope from *HER2/neu*, was given with flt3 ligand as a systemic vaccine adjuvant for a peptide vaccine to patients with advanced hormone-resistant prostate cancer.²³ Using a normal prostate cDNA expression library and sera from

subjects before and after treatment with flt3 ligand, a modified SEREX approach was used to identify six proteins to which IgG antibody responses were augmented posttreatment versus pretreatment with flt3. This research resulted in the identification of a protein, MAD-CaP-5, that is now the target of increased interest as a novel prostate-associated antigen. This molecule was found to encode a protein of unknown function (KIAA1404) that has also been identified in other tumor types.

PSA remains a target of interest for immune therapy. In a recent phase I trial, 6 patients with advanced hormone-refractory prostate cancer were studied for their ability to elicit PSA-specific cytotoxic T-cell responses following a pVAX/PSA DNA vaccine given at doses of 100 and 900 μ g.^{24,25} The vaccine was produced from a gene coding for the full-length human PSA protein, which was inserted into the pVAX1 vector. The pVAX/PSA vaccine was administered together with GM-CSF and interleukin (IL)-2 as vaccine adjuvants. Initial studies showed this construct to be safe, and preliminary results suggested that the 900- μ g dose could induce cellular and humoral immune responses against PSA protein. Additional analyses suggested that a cellular immune response could be induced with the production of interferon- γ as well as IL-4 and IL-6 using enzyme-linked immunosorbent spot.

Are we successfully inducing T-cell responses based on the studies presented to date? One would argue that despite the identification of numerous antigens that may be preferentially expressed on the tumor cell surface, improved vaccine delivery, and novel strategies to prime robust T-cell effector function and memory T-cell responses, clinical trials thus far have fallen short of significant success. Therefore we continue in our effort to improve T-cell responsiveness. Recent data from Gulley and colleagues suggest that vaccines might maintain efficacy even when given with treatment modalities, such as radiation, that were thought to decrease nonspecific T-cell responses.²⁶ They conducted a phase II trial designed to determine if a poxviral vaccine encoding PSA can induce a PSA-specific T-cell response when combined with radiotherapy in patients with clinically localized prostate cancer. Patients were randomized into vaccine-plus-radiotherapy or radiotherapy-only arms. Patients in the combination arm received a priming vaccine with recombinant vaccinia (rV) PSA plus rV containing the T-cell costimulatory molecule B7.1 followed by monthly booster vaccines with recombinant fowlpox PSA. They also received GM-CSF and low-dose systemic IL-2 following their vaccines. The vaccine regimen was found to be safe, and a majority of patients were able to generate a PSA-specific cellular immune response to vaccine. A different approach was used by Thomas-Kaskel and coworkers in a phase I/II trial that demonstrated the safety and

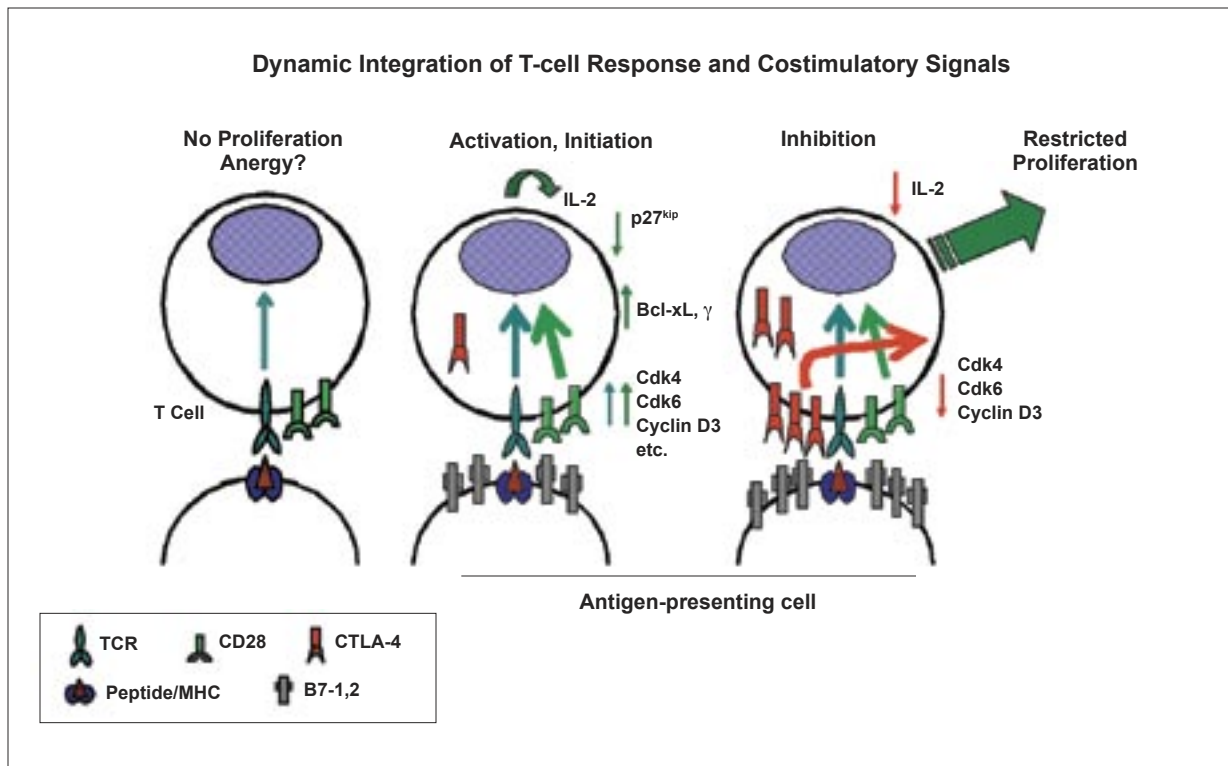


Figure 1. The complex interaction of costimulatory molecules in causing T-cell proliferation and interruption of effect by MDX-010, a monoclonal antibody against CTLA-4. Kindly provided by Dr. James Allison.

feasibility of inducing antigen-specific immunity by vaccination with dendritic cells presenting prostate stem cell antigen and PSA peptides in HLA-A02.01+ patients with hormone- and chemotherapy-refractory prostate cancer.²⁷ Delayed-type hypersensitivity responses were correlated with superior responses ($P=0.003$). HLA tetramer analysis detected high frequencies of peptide-specific T cells after two of the planned four vaccinations in 1 patient.

Novel T-cell Molecules: Can T-cell Function Be Modulated to Enhance Immune Responsiveness?

Combination approaches have been shown to play an important role in targeting both extracellular and intracellular pathways in prostate cancer growth. It is well known that T-cell receptor engagement with the antigen presented on the antigen-presenting cell (APC) is based on several interactions, including but not limited to the T cell perceiving the tumor antigen in the context of the MHC. This alone is insufficient for T-cell recognition of the tumor antigen. Engagement occurs when a signal is generated by CD28 on the T cell to bind to another mol-

ecule, B7, on the APC (Figure 1). CTL-associated antigen 4 (CTLA-4) is expressed on the surface of the T cell following activation and also binds to B7.²⁸ This binding of CTLA-4 to B7 provides an inhibitory signal that prevents an immune response. This response is particularly helpful in stopping untoward proliferation of T cells against self and thus inducing severe autoimmunity.

Although initially thought to be a costimulatory molecule, Allison's group showed that CTLA-4 is an inhibitory molecule, functioning as a checkpoint that limits T-cell activation and expansion.²⁹⁻³⁷ CTLA-4 has also been shown to play a critical role in preventing or enhancing autoimmunity in several animal systems. Antagonistic antibodies to CTLA-4 were shown by the same group to cause rejection of established tumors in murine models.^{32,34,35} This activity is done at the potential risk of inducing inhibitory T regulatory cells (Tregs). Using an in vivo model of B16/BL6 melanoma, Quezada and colleagues showed that anti-CTLA-4 treatment with a directed monoclonal antibody increases Tregs and T effector cells within the tumor.³⁸ When CTLA-4 blockade was used in combination with a vaccine, there was a change in the intratumor balance of Tregs and T effector

cells that directly correlated with tumor rejection. This combination induced tumor cell rejection unlike CTLA-4 alone.

Although T-cell responses are initiated by T-cell antigen-receptor signaling in the context of an antigen within the pocket of MHC on the cell surface, multiple cell surface molecules also participate in a complex interplay with cytokines, which can act in a stimulatory or inhibitory manner. Our understanding of the complexity of these regulatory pathways was increased by the demonstration by several groups that B7-1 and B7-2, whose expression is limited to "professional" APCs, can also interact with another molecule, CTLA-4.²⁹ Prior studies using multiple experimental tumors in mice showed that CTLA-4 blockade can enhance antitumor responses either as a single agent or in combination with a vaccine.^{36,37,39} CTLA-4 blockade can synergize with conventional cytotoxic therapies, including vaccines in patients with prostate, renal, and ovarian cancer and melanoma. One study demonstrated significant synergy when given with GVAX to a patient with recurrent ovarian cancer.⁴⁰ Rechallenge of the patient with CTLA-4 after receiving prior vaccine resulted in a decline in CA-125 for over 2 years with stabilization of disease. Although there have been instances of autoimmune breakthrough events—characterized in rare instances by hypophysitis, colitis, and pancreatitis—they have been seen mainly in what are thought to be immunologically driven malignancies such as melanoma and renal cell carcinoma. Clinical trials are currently underway in patients with metastatic prostate cancer. Though CTLA-4 is the prototype of a checkpoint blockade, there are other molecules that may offer additional targets for blockade. There are at least seven members of the extended B7 family of molecules. Three of these, B7-H1, B7-H3, and B7x (the latter identified by Allison's group), also inhibit T-cell responses but do so at later stages than CTLA-4.

Cytokines and Whole-cell Vaccines:

The GVAX Approach

Early vaccine trials made use of manipulated autologous tumor cell vaccines; however, it soon became clear that something more than the introduction of these cells was needed. Allogeneic tumor cells or cell lines were considered but concern arose about HLA histocompatibility and the possibility of lack of T-cell recognition in the absence of a recognized compatible HLA haplotype. Even if a short-lived antitumor response were feasible, it was unclear how to better sustain the antitumor effect, that is, how to keep the immune-generated cells at the tumor site to maintain the antitumor effect. Gene transfer technology has enabled the enhancement of tumor cell immunogenicity when used as vaccines. This process was facilitated by the introduction of cytokine genes into cell lines with the idea

of having the tumor cell act as the immunogen or stimulator and actively secrete a factor that would favor recruitment of other effector cells systemically. The cytokine, GM-CSF, was shown by Dranoff and colleagues⁴¹ to provide potent antitumor immunity when transduced into B16 melanoma cells, a weakly immunogenic tumor cell line. Compared with other immune-modulating cytokines, GM-CSF provided the most specific, potent, and durable antitumor responses. This was due to the recruitment of granulocytes, macrophages, and APCs such as dendritic cells (Langerhans cells) within the skin. The recruitment of APCs suggested that GM-CSF could create an environment ripe for antigen presentation.^{15,42}

Simons and associates initially studied the immune response preclinically in animals injected with either LNCaP or PC-3 prostate cancer cell lines transduced with the gene for the production of GM-CSF.^{15,43} Simons and colleagues extended this approach further in a phase I clinical trial by inserting the GM-CSF gene into prostate cancer cells derived from individual patients' prostatectomy specimens.⁴⁴ Autologous prostate cancer cells were established as primary cultures, transduced with the replication-defective retrovirus containing cDNA encoding GM-CSF (MFG-GM-CSF), as described previously.^{15,43,45} These genetically modified prostate cancer cells (GVAX) were then lethally irradiated and assessed for GM-CSF secretion and MFG-GM-CSF integration. This vaccine was administered intradermally as two injections of 0.5×10^7 cells every 21 days until the patient's vaccine cell repository was exhausted. Vaccine-site skin biopsies showed infiltrates of dendritic cells and macrophages. Biopsy of the delayed-type hypersensitivity response skin sites showed infiltrates of effector cells consisting of CD45 RO+ T cells and degranulating eosinophils consistent with activation of both Th1 and Th2 T-cell responses. A unique kind of vasculitis was also seen near the autologous tumor cells at the vaccine site. The trial demonstrated not only the feasibility of this approach but that it was safe and could induce both humoral and T-cell responses against prostate cancer cell-associated antigens.

This proof of principle has led to the further development of this technology in more advanced phase trials. GVAX now uses allogeneic whole tumor cells from established prostate cancer cell lines LNCaP and PC-3 in lieu of autologous tumor cells derived from the patient. These cell lines were selected based on their complementary behavioral patterns, which represent the basic dynamics of prostate cancer growth: LNCaP is a hormone-sensitive line expressing a number of differentiation antigens, including PSA and PSMA, with a mutant androgen receptor similar to that found in the tumor of patients with prostate cancer; PC-3 represents a diametrically opposed aggressive cell line that is not hormone-sensitive

and is suggestive of an aggressive phenotype. PC-3 does not express PSA but expresses urine plasminogen activator, metalloproteinases, and neuroendocrine peptides, all of which are suggestive of a hormone-resistant state.

To date, five clinical trials with GVAX prostate cancer vaccine have been conducted in more than 200 patients with recurrent or hormone-resistant prostate cancer.⁴⁴ In an early trial of 34 patients with asymptomatic metastatic hormone-refractory prostate cancer, the median survival of patients treated with the vaccine was 26.2 months, consistent with findings of patients treated with standard-of-care chemotherapy. Another study enrolled 80 patients who received the vaccine at low (200×10^6 cells monthly), medium (500×10^6 cells biweekly), and high (500×10^6 cells as priming dose followed by 300×10^6 cells biweekly) doses.⁴⁴ Six of 19 patients (32%) in the high-dose group had PSA declines after several months of vaccinations. Almost all patients had an immune response irrespective of dose level, although the high-dose group had the greatest percentage of patients with an IgG response against the tumor cell line. The vaccine appears safe and thus far has had no dose-limiting toxicities. A phase III trial is comparing vaccine to docetaxel and prednisone; endpoints include the proportion of patients who develop skeletal-related events, progression of disease, and time to onset of bone pain. Recent data presented by Gerritsen and coworkers⁴⁶ suggest that an antitumor effect can be induced by combining GVAX with an immune-modulating agent. In a phase I study, patients given MDX-010 (ipilimumab), a humanized monoclonal antibody that blocks the inhibitory effects of CTLA-4, in combination with GVAX experienced regression of bone metastases.⁴⁶ Interestingly, a decline in PSA seemed to correspond with the development of autoimmune hypophysitis manifested as either adrenal insufficiency or autoimmune thyroiditis. This finding appeared to portend a more favorable response to treatment in a manner often seen with patients with melanoma who develop vitiligo following immune treatment with vaccine. Overall, the combination of the antibody and GVAX was safe. Skin biopsies confirmed inflammatory infiltrates. A phase III trial of GVAX and an anti-CTLA-4 monoclonal antibody, ipilimumab, in patients with castrate metastatic prostate cancer is planned for enrollment shortly.

CTLA-4 in the Clinical Arena: Can Immune Modulators Stand Alone?

Clinical trials with vaccine and docetaxel have shown safety without inhibiting specific T-cell responses.⁴⁷ Interestingly, even the addition of an immune modulator such as anti-CTLA-4 after cancer vaccine failure is under consideration as a salvage strategy.⁴⁸ Currently, two trials are ongoing in the United States with survival as the primary

endpoint: VITAL-1 is for patients without cancer-related pain and compares a cellular vaccine, GVAX, to docetaxel and prednisone. A second trial in prostate cancer, VITAL-2, is for patients with cancer-related pain and compares GVAX plus docetaxel to docetaxel and prednisone.

Anti-CTLA-4 monoclonal antibody has been used as a single agent at 3 mg/kg with 2 of 14 patients having greater than 50% declines in PSA. Although this finding suggested that anti-CTLA-4 antibody can activate and expand preexisting tumor-specific T cells,^{49,50} more interesting data are being culled from combination trials. In a phase I study of chemotherapy-naïve patients with androgen-independent prostate cancer, 14 once-daily doses of GM-CSF (250 µg subcutaneously) followed by a 14-day rest period plus ipilimumab every 28 days in a dose-escalating fashion was well-tolerated.^{28,51} Two of 3 patients who received ipilimumab at 3 mg/kg had PSA declines of greater than 50%. However, both these patients experienced an immune breakthrough event. As seen in the study by Gerritsen and coworkers, the development of an immune breakthrough event may portend both a biochemical and/or radiographic response.⁴⁶ Thus, combinations of immunotherapy with anti-CTLA-4 may be more beneficial than anti-CTLA-4 alone is expected to be.

More recently, another approach has demonstrated benefit using autologous leukapheresis cellular products incubated with a novel immunogen, a recombinant fusion protein consisting of PAP linked to GM-CSF.⁴⁹ Here, too, GM-CSF plays a critical role in the induction and recruitment of immune cells. This cellular product vaccine was another proof of principle utilizing a novel protein (PAP) to stimulate the immune system. Preclinical studies in rats were directed at eliciting prostate cancer-specific immunity, ie, breaking immune tolerance. This research was done *ex vivo* by loading purified dendritic cells, the most potent APCs, with an engineered antigen-cytokine fusion protein (PA2024) consisting of PAP and GM-CSF.⁴⁹ Delay of tumor development and improved survival were observed in tumor-prevention models. Dendritic cells pulsed with PAP alone generated inferior immune responses. This approach was brought to the clinical arena as an immunotherapy product (sipuleucel-T [Provenge, Dendreon]), which consisted of autologous dendritic cells loaded *ex vivo* with the recombinant fusion protein consisting of PAP and GM-CSF. Patients underwent leukapheresis to remove mononuclear cells without using any mobilizing cytokine. The product was immediately sent to the Dendreon Corporation for processing, where dendritic cell precursors were collected and purified, then incubated with either the fusion protein (PA2024) or placebo. The CD54+ cells were then returned to the patient within 24 hours and reinfused within 8 hours of formulation. Sequential phase I and phase II trials in patients with

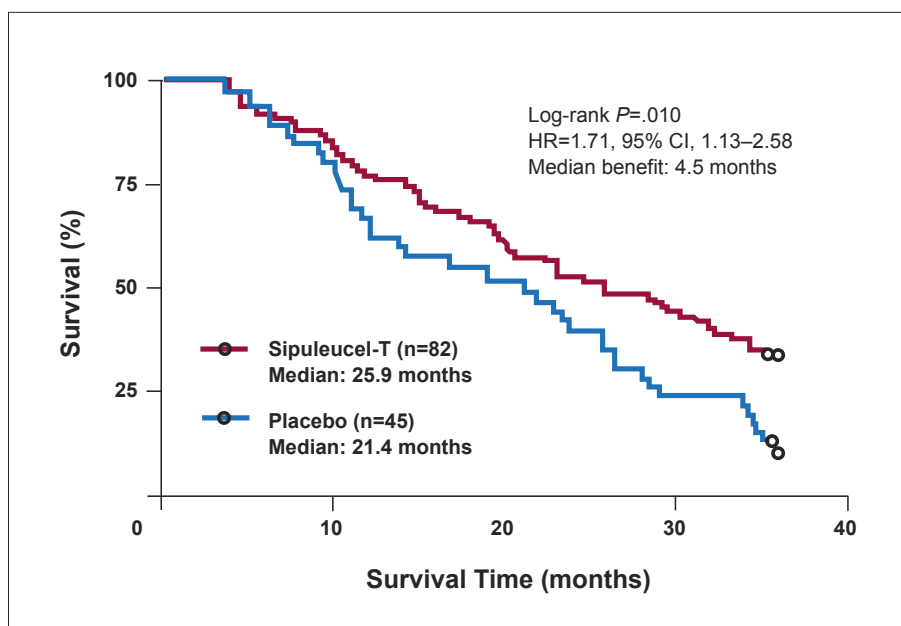


Figure 2A. Primary endpoint, time to disease progression (intent-to-treat population)—phase III multicenter, double-blind trial of sipuleucel-T versus placebo.

HR=hazard ratio.

Adapted from Small EJ et al.⁵⁰

hormone-resistant prostate cancer have been performed.⁴⁹ A total of 31 patients were treated: 12 patients with castrate metastatic disease were treated on the phase I portion and 19 patients who were hormone-resistant but had no radiographic evidence of disease were treated on the phase II portion. The immune product was found to be safe, and the most common side effect was a brief febrile reaction during the cell product reinfusion. Overall, no improvement in bone scans or soft-tissue disease was seen in the phase I trial. The median time to disease progression for the phase I patients was 12 weeks and the median time to progression for the phase II patients was 29 weeks. Seven of the 19 phase II patients had not progressed by the end of the planned 1-year follow-up. Cytokine production by T cells responding to the target antigen was assessed with the cytokine profile, revealing that T cells released interferon- γ but not IL-4. This suggested that the T-cell responses were more consistent with Th-1-type responses usually associated with host immunity to tumors.

These results established the framework for a recently reported inaugural phase III multicenter double-blind trial randomizing sipuleucel-T versus placebo.⁵⁰ This trial was designed to test the effect of sipuleucel-T on time to progression and survival in patients with hormone-refractory prostate cancer. A total of 127 patients with asymptomatic metastatic hormone-refractory prostate cancer were randomized in a 2:1 ratio to receive three infusions of sipuleucel-T or placebo every 2 weeks. The trial included a crossover to the treatment arm if placebo patients developed disease progression. As seen in earlier trials, the treat-

ment was safe, with infusion-associated rigors followed by pyrexia being the most common side effects. As early as 8 weeks posttreatment, the median ratio of the T-cell stimulation index, a measure of immunologic recognition of the immune product, was approximately 8-fold higher in the sipuleucel-T arm versus placebo ($P<.001$). A total of 115 patients contributed a progression event to the primary analysis of time to progression. It did not demonstrate an improvement in the primary endpoint of time to progression in patients treated with sipuleucel-T (Figure 2A).⁵⁰ Historically, the median time to progression is about 3 months; however, the current study, in an intent-to-treat analysis, demonstrated a 4.5-month improvement in overall survival ($P=.01$; Figure 2B). This seminal trial is the first phase III study to suggest that an immune strategy can lead to a modest improvement in overall survival. Although other confirmatory trials are ongoing, it remains unclear as to the nature of the effector population involved in eliciting the antitumor effects and the best way to standardize the minimal number of effector cells for treatment.

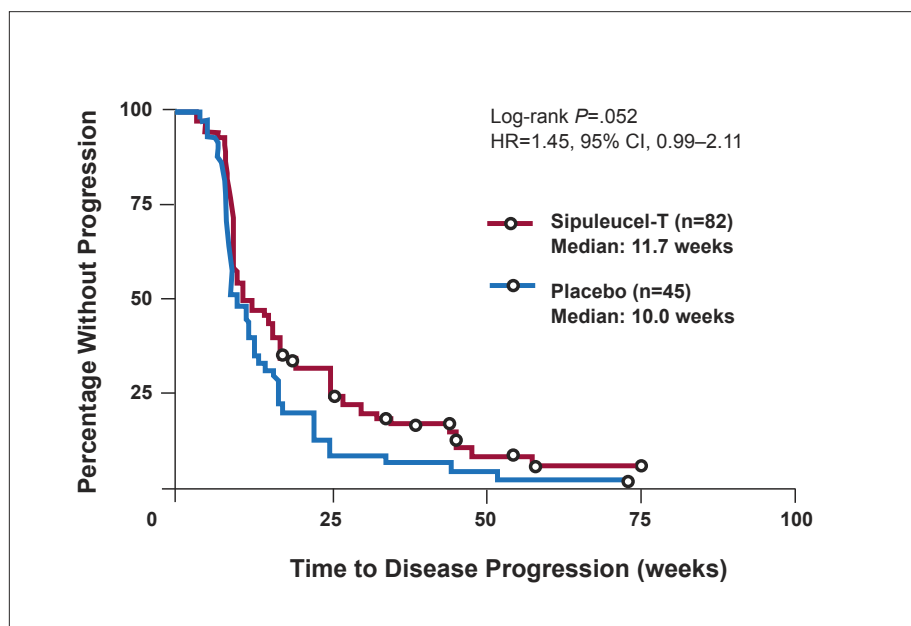
Conclusions

There are multiple approaches toward inducing both humoral and cellular immune responses that strongly suggest immunologic tolerance to tumor-associated antigens can be successfully disrupted. Clearly, the addition of cytokines or checkpoint inhibitors such as GM-CSF or anti-CTLA-4 have proven beneficial in enhancing anti-

Figure 2B. Final overall survival (intent-to-treat population)—phase III multicenter, double-blind trial of sipuleucel-T versus placebo.

HR=hazard ratio.

Adapted from Small EJ et al.⁵⁰



tumor responses both serologically and radiographically, which heretofore had not been successfully demonstrated. A remaining challenge is how to assess the impact of biologic therapies, such as vaccines, on endpoints such as PSA. To date, PSA has not been accepted as a surrogate endpoint, in part because a decline in PSA may not reflect the true biology of the tumor or demonstrate the impact of the agent on the course of the disease. Scher and colleagues¹⁶ brought this concept forth in the PSA Working Group, suggesting that specific endpoints be built into clinical trials that address the function of the agent being tested. Thus, biologic therapies may mandate different endpoints in clinical trial development compared with cytotoxic or cytostatic agents. Vaccines and other immunologic/biologic therapies are no different from cytotoxic agents in that specific endpoints to assess drug effectiveness should be tailored to the individual drug under evaluation.⁵² Enthusiasm for vaccines remains, irrespective of the clinical state of the disease, and the fact that investigators are starting to give serious consideration to appropriate clinical trial endpoints suggests that we are starting to appreciate the need for more rigorously designed trials, which will yield results that will allow the drug to go forward.

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