Published in final edited form as:
Vaccine. 2011 September 2; 29(38): 6485–6497. doi:10.1016/j.vaccine.2011.06.088.

Immunotherapy in prostate cancer: emerging strategies against a formidable foe

Marijo Bilusic[a,b], **Christopher Heery**[a,b], and **Ravi A. Madan**[a,b,*]

[a]Laboratory of Tumor Immunology and Biology, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20892, USA

[b]Medical Oncology Branch, Center for Cancer Research, National Cancer Institute, National Institutes of Health, Bethesda, Maryland 20892, USA

Abstract

Recent clinical trials have shown therapeutic vaccines to be promising treatment modalities against prostate cancer. Unlike preventive vaccines that teach the immune system to fight off specific microorganisms, therapeutic vaccines stimulate the immune system to recognize and attack certain cancer-associated proteins. Additional strategies are being investigated that combine vaccines and standard therapeutics, including radiation, chemotherapy, targeted therapies, and hormonal therapy, to optimize the vaccines' effects. Recent vaccine late-phase clinical trials have reported evidence of clinical benefit while maintaining excellent quality of life. One such vaccine, sipuleucel-T, was recently FDA-approved for the treatment of metastatic prostate cancer. Another vaccine, PSA-TRICOM, is also showing promise in completed and ongoing randomized multicenter clinical trials in both early and late stage prostate cancer. Clinical results available to date indicate that immune-based therapies could play a significant role in the treatment of prostate and other malignancies.

Keywords

Prostate cancer; Immunotherapy; Cancer vaccines; Immune Checkpoint Inhibitors

1. Introduction

Prostate cancer is the most common malignancy in the Western world and ranks third in terms of mortality. In 2010, an estimated 217,730 new cases were anticipated in the United States, and about 32,050 men were expected to die from the disease (1). Although the majority of patients are treated successfully with radical prostatectomy or radiation therapy, approximately 30–40% of patients will ultimately develop recurrent disease (2). Of the many treatment approaches for recurrent prostate cancer that no longer responds to hormonal agents, immunotherapy is particularly promising, due to several unique characteristics of both the disease and the treatment. Prostate cancer is a relatively indolent disease, allowing time for the immune system to generate an immunologic response. Furthermore, since the prostate is a nonessential organ, targeting prostate cancer-associated

*Corresponding author: Building 10, Rm 8B09, 10 Center Dr., MSC 1750, Bethesda, MD 20892; phone: (301) 496-3493; fax: (301) 480-5094; madanr@mail.nih.gov.

antigens is unlikely to have significant negative side effects. Finally, therapeutic cancer vaccines have been shown to be much less toxic than chemotherapy, hormonal therapy or targeted therapies, thus significantly improving a patient's quality of life.

This review of immunotherapy for prostate cancer focuses on late-stage clinical trials, with an emphasis on treatment strategies, immunologic endpoints, and biomarkers.

2. Targets of therapeutic prostate cancer vaccines

The ultimate goal of any cancer immunotherapy is to induce a targeted immune response against cancer cells. Ideally, therapeutic prostate cancer vaccines should induce a focused antitumor immune response by targeting defined tumor-associated antigens (TAAs) through T-cell stimulation. Human cytotoxic T cells are able to recognize 9- to 15-mer antigenic peptides expressed within the major histocompatibility complex (MHC). These peptides are derived from endogenously expressed proteins, including TAAs that are processed by proteases within cells. When appropriately activated, T cells can detect specific TAAs within the MHC and initiate targeted, immune-mediated cancer cell killing (3, 4). To accelerate translational research, the National Cancer Institute (NCI) recently developed a system for prioritizing cancer antigens (5). Using paired comparisons, 75 "ideal" cancer antigen criteria were weighted, in descending order, as follows: (a) therapeutic function, (b) immunogenicity, (c) role of the antigen in oncogenesis, (d) specificity, (e) expression level and percent of antigen-positive cells, (f) stem cell expression, (g) number of patients with antigen-positive cancers, (h) number of antigenic epitopes, and (i) cellular location of antigen expression. None of the 75 antigens had all of the characteristics of an ideal cancer antigen. However, 46 were immunogenic in clinical trials and 20 of them had suggestive clinical efficacy in the "therapeutic function" category. Several prostate-associated TAAs have been identified (see Table 1), including prostate-specific antigen (PSA), prostate-specific membrane antigen (PSMA), prostatic acid phosphatase (PAP), T-cell receptor gamma chain alternate reading frame protein (TARP), new gene expressed in prostate (NGEP), prostate stem cell antigen (PSCA), six-transmembrane epithelial antigen of the prostate (STEAP), A-kinase anchor protein-4 (AKAP-4) and mucin 1 (MUC1) (6-16).

PSA, a 34-kD kallikrein-like serine protease, is expressed almost exclusively by prostate epithelial cells and is the most widely used serum marker for diagnosis and monitoring of prostate cancer (6, 7). PSMA is a transmembrane protein commonly found on the surface of late-stage, undifferentiated metastatic prostate cancer and is an imaging biomarker for staging and monitoring of therapy. This 100-kD transmembrane glycoprotein can also be induced to higher levels of expression by androgen-deprivation therapy (ADT), which is fundamental to the treatment of prostate cancer. It also represents an attractive antigen for antibody-based diagnostic and therapeutic intervention in prostate cancer, since it is highly restricted to the prostate and overexpressed in all tumor stages (8).

PAP is a well-known tumor marker and a major phosphatase enzyme of differentiated prostate epithelial cells. It is a secreted glycoprotein (50 kDa) consisting of 2 subunits and is biosynthesized by columnar secretory epithelia of the prostate gland (9). The primary biologic function of PAP is still unclear. Another potential target, TARP, is a protein expressed in patients with prostate and breast cancer and is present in both normal and malignant prostate cancer tissue. It is found in about 95% of prostate cancer specimens, making TARP a promising target antigen for cancer vaccines (10, 11).

NGEP is a prostate-specific gene encoding either a small cytoplasmic protein (NGEP-S) or a larger polytopic membrane protein (NGEP-L). NGEP-L expression is detectable only in prostatic tissue (prostate cancer, benign prostatic hyperplasia, and normal prostate). An increase in NGEP-specific T cells was observed in the peripheral blood mononuclear cells

(PBMCs) of prostate cancer patients after vaccination with a PSA-based vaccine, further demonstrating the immunogenicity of NGEP in prostate cancer patients (12).

PSCA is a cell surface antigen in the Ly-6/Thy-1 family of glycosylphosphatidylinositol-anchored proteins. PSCA has limited expression in normal tissues, but is highly overexpressed in human prostate cancer. This makes it excellent target for both diagnosis and therapy. Several studies have now clearly correlated expression of PSCA with relevant clinical benchmarks, such as Gleason score and metastasis, while other studies have demonstrated the efficacy of targeting PSCA in various treatment modalities (13).

Biochemical and secondary structural analyses of STEAP, identified in advanced human prostate cancer, suggested that this protein could function as a channel, receptor, or transporter protein; however, its function is currently unknown. Low levels of STEAP have been detected in normal prostate tissues, but it is highly overexpressed in human prostate cancer tissue. STEAP is not prostate specific, it has been detected in several bladder, pancreatic, ovarian, and colon cancer cell lines. In TRAMP mice, STEAP is expressed at high levels in malignant prostate tissue and has been used as a TAA (14).

AKAP-4 plays a critical role in prostate cancer cell invasiveness (15). Aberrant expression of AKAP-4 in LNCaP prostate cancer cells and prostate cancer biopsies was recently reported, as well as immunogenicity in patients and the successful generation of AKAP-4-specific cytotoxic T lymphocyte (CTL) responses able to selectively kill prostate cancer cells (16). MUC1 is a transmembrane glycoprotein expressed on the apical surface of normal ductal epithelial cells as well as on adenocarcinomas of various tissues (17). It was reported that MUC1 overexpression correlates with higher Gleason score, which indicate that increased expression of MUC1 is up-regulated during cancer progression (18).

3. Target population

To be effective, a therapeutic prostate cancer vaccine must achieve 2 goals. First, the vaccine must stimulate specific immune responses against prostate cancer cells. Second, the immune responses must be sufficient to overcome immunosuppressive mechanisms that can be employed by cancer tissue (19). Greater tumor burden has been associated with a proportional increase in regulatory T cells (Tregs) (20, 21), as well as increased levels of indoleamine-2,3-dioxygenase, IL-10, and TGF-β, all of which can further inhibit T-cell activation and tumor lysis by a cancer vaccine (22, 23).

A recently published retrospective study provides clinical support for the concept that patients with very aggressive cancer may not benefit from a cancer vaccine as monotherapy (24). A well-established predictive algorithm, the Halabi nomogram (25), is used to stratify patients and predict overall survival (OS) in patients treated with chemotherapy or second-line hormonal agents. The Halabi nomogram is derived from an analysis of 1101 patients with metastatic castration-resistant prostate cancer (mCRPC) who were treated with chemotherapy or second-line hormonal therapy in Cancer and Leukemia Group B studies between 1991 and 2001. The nomogram employs 7 significant baseline parameters: Gleason score, performance status, PSA, alkaline phosphatase, lactate dehydrogenase, hemoglobin, and the presence of visceral disease. In a recent NCI trial in patients with metastatic prostate cancer, patients with a Halabi-predicted survival (HPS) of < 18 months (median predicted survival 12.3 months) had an actual median OS of 14.6 months, while those with an HPS of ≥ 18 months (median predicted survival 20.9 months) have an OS that will meet or exceed 37.3 months, with 12/15 patients living longer than predicted ($P = 0.035$). Chemotherapy-naïve patients with mCRPC treated with docetaxel had a median OS of 15.5 months compared with a predicted survival of 16.5 months. There was only a slight difference in predicted versus actual median OS for patients treated with docetaxel, regardless of whether

they were in the < 18-month or ≥ 18-month HPS groups. These data suggest that patients with more indolent disease characteristics may benefit most from therapeutic cancer vaccines (24). (See Table 2 for an outline of the advantages and disadvantages of chemotherapy vs. immunotherapy.)

4. Prostate cancer immunotherapies

4.1. Sipuleucel-T (PROVENGE®)

Sipuleucel-T is the first therapeutic cancer vaccine approved by the U.S. Food and Drug Administration (FDA) for the treatment of cancer. The vaccine is uniquely generated from each patient's own PMBCs. PMBCs are collected by leukapheresis, isolated through centrifugation, then incubated in vitro with PA2024, a recombinant fusion protein composed of PAP and granulocyte-macrophage colony-stimulating factor (GM-CSF). After incubating for 36 to 48 hours, the PMBCs are returned to the patient intravenously. The goal of this process is to activate immune cells ex vivo, then reinfuse them to generate an immune response targeting prostate cancer cells expressing PAP (26).

In phase I trials of sipuleucel-T, the most common adverse event was mild fever (27, 28). A subsequent phase II trial demonstrated early evidence of efficacy, with 2 patients having transiently decreased serum PSA; another patient's PSA became undetectable for more than 4 years (29).

Results were recently published for the IMPACT trial, a randomized, double-blind, placebo-controlled, multicenter phase III trial of sipuleucel-T in 512 patients with asymptomatic or minimally symptomatic mCRPC. The study randomized patients 2:1 to receive sipuleucel-T or placebo, and demonstrated a significant improvement in OS for sipuleucel-T (25.8 months) vs. placebo (21.7 months) (30). This trial supported the findings of a previous, smaller phase III trial, D9901 (31), that enrolled 127 patients randomized in the same fashion as the IMPACT trial. Patients were given either sipuleucel-T or placebo every 2 weeks for 3 total doses. There was no improvement in time to progression (11.7 weeks for sipuleucel-T vs. 9.1 weeks for placebo), which was the primary endpoint of the study, but there was a significant improvement in OS (25.9 months for sipuleucel-T vs. 21.4 months for placebo; $P = 0.010$). A similar study, D9902A (32), followed the same trial design and enrolled 98 patients. D9902A was closed prematurely when D9901 failed to reach its primary endpoint (time to progression). As in D9901, time to progression did not improve (10.9 weeks for sipuleucel-T vs. 9.9 weeks for placebo). Because this study was closed before it met accrual goals, its power was reduced. For the patients who enrolled, OS favored sipuleucel-T relative to placebo. (19.0 vs. 15.7 months; $P = 0.331$). A combination analysis of the 2 trials again demonstrated a significant improvement in OS (23.2 months for sipuleucel-T vs. 18.9 months for placebo; $P = 0.011$).

A clinical trial of sipuleucel-T randomized 176 patients with rising PSA after definitive local therapy prior to ADT 2:1 to receive sipuleucel-T (n = 117) or control (n = 59). The study failed to meet its primary endpoint of improved time to biochemical failure (18.0 for sipuleucel-T vs. 15.4 months for control). Secondary endpoints included analysis of PSA doubling time (PSADT), time to distant failure, immune response, and safety. Patients in the sipuleucel-T group had a 48% increase in PSADT. However, it should be noted that the use of PSADT as a clinical trial endpoint has not been validated. There was increased T-cell proliferation in the sipuleucel-T group compared with control (Stimulation Index 13.193 vs. 118.50, $P < 0.001$). Interferon-gamma ELISPOT number also nearly doubled in the sipuleucel-T arm (79 spots at week 13 for sipuleucel-T vs. 43 spots for control; $P < 0.001$) (33). The most clinically relavent endpoints, time to distant failure and, perhaps, OS, will require long-term follow-up. Based on previous data indicating no improvement in time to

progression in patients with mCRPC who receive vaccine, OS data for these patients will be particularly interesting. A phase II trial of sipuleucel-T in the neoadjuvant setting is ongoing, with a primary endpoint of immunologic response (34).

4.2. PSA-TRICOM (PROSTVAC™)

Poxviral vectors have several advantages as platforms for immunotherapy: they are very immunogenic, they can carry a large amount of genetic material, and there is extensive clinical experience with the vaccinia poxviruses because of its use in the worldwide eradication of smallpox (35). PSA-TRICOM is a vector-based vaccine that uses recombinant poxviruses to target PSA and initiate an immune response against prostate cancer cells. This "off the shelf" vaccine also contains transgenes for 3 costimulatory molecules, designated TRICOM, that enhance T-cell activation: B7.1 (CD80), lymphocyte function-associated antigen (LFA)-3, and intracellular adhesion molecule (ICAM)-1 (36). As a vehicle for vaccine delivery, poxviruses have high cell infectivity rates and can infect antigen-presenting cells (APCs) when injected into subcutaneous tissue. A phase II Eastern Cooperative Oncology Group clinical trial (ECOG 7897) showed that targeting PSA with two different types of poxviruses provided optimal results. Using a vaccine regimen that included a priming dose of recombinant vaccinia (rV) followed by recombinant fowlpox (rF) boosts, 45.3% of men were PSA progression-free at 19.1 months, and 78.1% demonstrated clinical progression-free survival (PFS). There was a trend favoring this schedule relative to other treatment schedules (37). While vaccinia likely induces a strong initial immune response, subsequent doses are neutralized by anti-vaccinia antibodies. Fowlpox is an ideal boosting agent because it does not replicate in human cells and does not induce neutralizing antibodies (38). Based on these initial data, PSA-TRICOM includes both vaccinia- (rV-PSA-TRICOM) and fowlpox-based (rF-PSA-TRICOM) platforms in a prime-and-boost strategy.

A phase I study in patients with mCRPC demonstrated that PSA-TRICOM was well tolerated, with local injection-site reaction as the most common adverse event (39). Evaluable patients treated with PSA-TRICOM showed increases in PSA-specific T cells, and 9/15 patients had decreased serum PSA velocity post-vaccination. An ongoing phase II ECOG trial is evaluating PSA-TRICOM in patients with recurrent castration-sensitive prostate cancer but no metastasis (stage D0). Patients were given an initial dose of rV-PSA-TRICOM, followed by rF-PSA-TRICOM every 4 weeks for 2 months, then every 12 weeks until PSA progression. Of the 69 patients enrolled, 29 were evaluable for response at 6 months. Of those 29 patients, 66% had a > 6-month PSA PFS. In addition, the median on-study PSADT increased from 4.4 months to 7.7 months ($P = 0.002$) (40). Additional follow-up is ongoing and the ultimate impact and clinical relevance of the prolongation of PSADT will be evaluated in this population.

A 43 center randomized placebo (empty vector)-controlled phase II study of PSA-TRICOM in minimally symptomatic mCRPC randomized patients 2:1 to receive either vaccine (n = 84) or placebo (n = 41). The primary endpoint was PFS, with a secondary endpoint of OS, similar to sipuleucel-T trials. There was no difference in PFS between the 2 groups (3.8 vs. 3.7 months). However, at 3 years post-study, patients treated with PSA-TRICOM had greater OS, with 25/82 (30%) patients alive vs. 7/40 (17%) controls. The median OS improved by 8.5 months (25.1 vs. 16.6 months for controls), an estimated hazard ratio (HR) of 0.56 (95% CI, 0.37 to 0.85), and stratified log-rank $P = 0.0061$ (36).

A trial conducted at the NCI in 32 patients with minimally symptomatic mCRPC provided additional evidence of immune response in vaccinated patients. All patients received an rV-PSA-TRICOM prime and monthly boosts of rF-PSA-TRICOM, resulting in declines in PSA (38% of patients) and PSA velocity (47% of patients). Median OS among all patients was

26.6 months (24). Immune analysis of 29 evaluable patients indicated a > 2-fold increase in PSA-specific T cells in 13 patients; 5 who had a > 6-fold increase in PSA-specific T cells were associated with a trend to improved OS ($P = 0.055$). Because of the possibility that a PSA antibody response could artificially lower PSA, the authors have analyzed this and other studies of poxviruses expressing PSA in prostate cancer and have seen only 1 of 269 patients with any evidence of free PSA antibody induction in serum post-vaccine. In addition, patients with PSA declines did not have a worsening of PAP, imaging, or clinical picture, suggesting that PSA is a reasonable marker for disease in patients treated with PSA-TRICOM (36, 37, 41-45). Because PSA-TRICOM is an “off the shelf” vaccine it can be, and is being widely evaluated at numerous cancer centers as both a monotherapy and in combination therapies.

These are promising data, and PSA-TRICOM’s beneficial impact on mCRPC patients will be further evaluated in a larger phase III study set to begin in late 2011 (46).

4.3. Ipilimumab

Ipilimumab is a fully humanized monoclonal antibody against cytotoxic T lymphocyte antigen (CTLA)-4, an immune checkpoint molecule expressed by CTLs after activation by an APC. The APC binds to the CTLA-4 molecule 24 to 48 hours after activation, which inhibits the CTL as a means of moderating the body’s immune response (47). Binding of ipilimumab to CTLA-4 blocks transmission of the regulatory signal and may increase the immune response of CTLs against tumor cells (48, 49).

Blockade of this signal, however, may result in increased nonspecific T-cell autoreactivity, which most commonly manifests as autoimmune-mediated colitis, endocrinopathies, and rash (49, 50). Several trials have confirmed the finding of severe rash in up to 50% of patients, and hypophysitis and hepatitis in around 5% of patients. Rarely, uveitis, pancreatitis, leukopenia, red cell aplasia, and neuropathy can occur (51-57). There also appears to be an association between immune-related adverse events and tumor response (56).

Ipilimumab recently demonstrated a survival benefit in a phase III trial in metastatic melanoma, in which patients were randomized to receive ipilimumab alone, a peptide-based vaccine (GP100), or the combination of both. A significant OS advantage was seen in patients who received ipilimumab, either alone (10.1 months; $P = 0.003$) or in combination with GP100 (10.0 months; $P < 0.001$) compared to GP100 alone (6.4 months) (58). This study led to FDA approval of ipilimumab in March 2011 for second-line treatment of advanced melanoma. These encouraging trial results have also bolstered interest in the use of ipilimumab to treat other tumor types. It is interesting to note that in the ipilimumab trial, an OS advantage was seen with no significant advantage in time to progression.

In 2 phase I trials of ipilimumab in prostate cancer, grade 3 autoimmune colitis was again the most serious toxicity, at times requiring systemic corticosteroid treatment (50). The first phase I trial demonstrated a PSA decline in one patient and a partial response by RECIST criteria in another. Toxicities were similar in this and a second phase I trial (59). Interestingly, systemic corticosteroids effectively blunted autoimmune toxicities in most cases, without diminishing the antitumor effect of ipilimumab (57, 60, 61). However, prophylactic budesonide does not appear to prevent ipilimumab-related autoimmune colitis (61).

Phase III trials of ipilimumab in prostate cancer are ongoing. One trial compares ipilimumab vs. placebo in mCRPC, with a primary endpoint of OS (62). Another large randomized phase III trial compares ipilimumab vs. placebo following radiation to bone metastases (63).

Combination with a cytotoxic therapy that results in cell death and subsequent antigen presentation may enhance the antitumor immune response induced by ipilimumab. This trial also has a primary endpoint of OS.

4.4. GVAX

GVAX is a cell-based immunotherapeutic vaccine consisting of a GM-CSF-transduced androgen-sensitive prostate cancer cell line (LNCaP) and a castration-resistant prostate cancer cell line (PC3). This vaccine was designed to secrete GM-CSF as an immune adjuvant to enhance immune stimulation, an approach that has been used in pancreatic, breast, lung, and hematologic cancers (64). Two phase III trials, VITAL-1 and VITAL-2, evaluated GVAX for prostate cancer in chemotherapy-naive mCRPC patients. VITAL-2 enrolled taxane-naive mCRPC patients with pain requiring opioid analgesics and randomized them to a treatment arm and a control arm. Both arms received 75 mg/m^2 of docetaxel every 3 weeks. The control arm was also given 10 mg/day of prednisone. In the treatment arm, 2 days after each docetaxel infusion patients were given a 500 million-cell priming dose of GVAX and 300 million-cell boosts every 3 weeks for 10 cycles. After that, patients received maintenance immunotherapy alone every 4 weeks. The study was prematurely terminated in August 2008 after accrual of 408 patients due to an imbalance in deaths between the 2 treatment arms (67 deaths in the docetaxel/vaccine arm vs. 47 deaths in the control arm). No significant toxicities were observed in the docetaxel/vaccine arm that could explain the imbalance in deaths. In both arms, 85% of deaths were attributed to prostate cancer. OS was shorter in the docetaxel/vaccine arm, with a median survival of 12.2 vs. 14.1 months in the docetaxel/prednisone arm ($P = 0.0076$) (65).

In VITAL-1, 626 patients were randomized to receive GVAX (500 million-cell prime/300 million-cell boosts every 2 weeks for 13 cycles, followed by maintenance every 4 weeks) or docetaxel (75 mg/m^2 every 3 weeks) with prednisone (10 mg/day). The study completed accrual in 2007, and all 626 patients completed the initial 6-month treatment period. The study was prematurely terminated in October 2008 based on the results of a futility analysis conducted by the study's Independent Data Monitoring Committee, which determined that the study had a < 30% chance of meeting its predefined primary endpoint of improved OS., The median follow-up was 66 weeks (at the time of study termination). Grade ≥ 3 adverse events were seen in 8.8% of patients in the vaccine arm vs. 43% in the docetaxel arm. No difference in median OS was observed (20.7 vs. 21.7 months; $P = 0.78$). In the subset of men with an HPS of > 18 months, median survival was prolonged on GVAX compared to docetaxel, but it was not statistically significant (29.7 vs. 27.1 months; $P = 0.60$) (66).

The failure to demonstrate clinical benefit for GVAX in VITAL-2 may be attributed to patient selection, since only 18% of patients had an HPS of ≥ 18 months. In that select subgroup, GVAX plus docetaxel appeared to do better than docetaxel with prednisone (HR 0.8). The survival advantage (demonstrated by separation of the Kaplan-Meier survival curves) in the randomized trials of sipuleucel-T and PSA-TRICOM did not become apparent for > 12 months (30, 36). Perhaps with longer follow-up, a similar trend will be seen in the VITAL-1 data, especially in the subgroup with longer predicted survival.

Overall, one of the key lessons learned from the GVAX trials is the importance of appropriate patient selection. In a previous phase II study of GVAX, the 22 patients treated at the highest dose of vaccine (also used in the VITAL-1 and -2 studies) had an HPS of 22 months, compared with an actual OS of 35 months (67). In contrast, of the 626 patients enrolled in VITAL-1, only 264 (42%) had an HPS of ≥ 18 months. Notably, within that subgroup, patients randomized to treatment with vaccine showed a trend toward improved survival compared to patients treated with docetaxel (HR 0.9) (66). Also, the possible impact of GM-CSF and lack of prednisone on the outcome in both phase III studies is

unclear. Exploratory analyses to identify subgroups of patients with preferential benefit from the investigational therapy are currently underway. This outcome has yet to be explained by immunologic or other mechanisms.

In May 2010, the manufacturer of GVAX, announced plans to develop and manufacture a new version of the vaccine; a new phase II clinical trial is under consideration (68).

4.5. DNA vaccine encoding PAP

DNA-based vaccines are another antigen-specific approach to immunotherapy. DNA can be rapidly and precisely synthesized to target nearly any selected antigen (69). The main disadvantage of DNA-based vaccines is their low level of immunogenicity. To improve their effect, several proinflammatory molecules, such as herpes simplex virus type 1 tegument protein VP22 or Toll-like receptor agonists, have been incorporated into DNA-based vaccines (70). In another approach, the vectors have been coadministered with GM-CSF to recruit APCs, particularly dendritic cells (DCs), to the vaccine site. DNA vaccines are usually injected intramusclulary or intradermally. To activate T cells, antigen must be transferred to a professional APC, such as a DC. This indirect transfer of antigenic material, possibly as apoptotic vesicles, is termed cross-presentation. A small proportion of DNA is also taken up directly by DCs, where the encoded antigen can be processed and presented endogenously (69).

A recent clinical study highlights the potential utility of DNA vaccines in prostate cancer. A phase I/IIa dose-escalation trial was conducted with a DNA vaccine encoding human PAP in 22 patients with stage D0 prostate cancer. Patients were treated with 100, 500, or 1500 g of plasmid DNA, coadministered intradermally with 200 g of GM-CSF as a vaccine adjuvant, 6 times at 14-day intervals. Patients were followed for one year after treatment, with no significant adverse events observed. Three of 22 patients (14%) developed PAP-specific IFN-secreting CD8 T cells immediately after the treatment course, as determined by ELISPOT. Nine of 22 patients developed PAP-specific CD4 and/or CD8 T-cell proliferation. Antibody responses to PAP were not detected. Overall, PSADT increased from a median 6.5 months pretreatment to a median 8.5 months on treatment ($P = 0.033$), and 9.3 months in the one-year post-treatment period ($P = 0.054$) (71). Quantifying clinical benefit in this patient population is especially difficult, since vaccine approaches in prostate cancer generally have not resulted in decreases in PSA. Here, an improvement in PSADT was noted, but the clinical benefit of such changes has not been established. Perhaps due to the relatively small number of patients studied, a correlation between clinical parameters and immunologic readouts was not significant, but the available data support the notion that this DNA vaccine induced detectable responses in patients with early-stage prostate cancer. It appears that DNA vaccine encoding PAP is safe, elicits an antigen-specific T-cell response, and may be associated with increased PSADT. Additional investigations designed to further evaluate clinical efficacy are warranted.

4.6. Other prostate cancer vaccines (TARP, Stimuvax®)

Patients with stage D0 prostate cancer have no evidence of metastatic disease on imaging, but have persistently elevated or rising PSA levels and are at increased risk for disease progression. Since they lack much of the tumor-associated immune suppression associated with the high tumor burden characteristic of end-stage metastatic disease, and they are chemotherapy-naive, they are an ideal population in which to study the ability of therapeutic vaccines to slow or prevent disease recurrence and progression (72). A novel 58 amino acid protein, TARP, expressed in patients with prostate and breast cancer, has recently been described (10). The protein is expressed by both normal and malignant prostate tissue (about 95% of prostate cancer specimens are TARP-positive). Essand et al. demonstrated that

TARP originates from epithelial cells and not from infiltrating T lymphocytes, and that it is expressed in normal prostate epithelium, adenocarcinoma of the prostate, and the prostatic adenocarcinoma cell line LNCaP (73). Wolfgang et al. showed that TARP was expressed in androgen-sensitive (LNCaP) but not in androgen-independent (PC3) prostate cancer cell lines, implicating the role of TARP in prostate cancer progression (74). TARP is immunogenic and hence a target antigen for therapeutic vaccines. Furthermore, the immunogenicity of TARP peptides can be augmented through epitope enhancement achieved through amino acid substitutions, resulting in increased peptide binding affinity. An ongoing phase I randomized study at the NCI is evaluating vaccination with TARP peptides in HLA-A*0201 stage D0 prostate cancer, using Montanide® ISA 51 VG (an off-the-shelf vaccine adjuvant) plus sargramostim vs. autologous TARP peptide-pulsed DCs.

BLP25 liposome vaccine (L-BLP25, Stimuvax) targets the exposed core peptide of the MUC1 TAA. More than 60% of primary prostate cancers express MUC1, as do > 90% of metastatic lesions in lymph nodes (18). More aggressive disease (higher Gleason score) has also been associated with higher MUC1 expression (75). Stimuvax is a lyophilized preparation consisting of BLP25 lipopeptide, immunoadjuvant monophosphoryl lipid A, and 3 lipids (cholesterol, dimyristoyl phosphatidylglycerol, and dipalmitoyl phosphatidylcholine), forming a liposomal product (76). A single-institution pilot trial was performed using Stimuvax in 16 ADT-naïve patients with biochemical progression after radical prostatectomy (77), with primary endpoints of safety and clinical activity. Patients received a single intravenous dose of cyclophosphamide, followed by treatment with Stimuvax for up to one year. There were no serious adverse events reported. After the 8-week primary treatment period, 8/16 patients had stable or decreased PSA. At the last on-study PSA measurement, one patient maintained stable PSA, but all others had PSA progression. However, 6/16 patients had > 50% prolongation of PSADT compared to pre-study PSADT. (As mentioned earlier, the clinical benefit of increased PSADT has not been established and is merely suggestive of a clinical benefit.) These data indicate a good safety profile in patients with prostate cancer and possible evidence of clinical activity. A pilot study of Stimuvax in combination with standard ADT and radiation therapy for newly diagnosed, high-risk prostate cancer patients is planned.

5. Treatment strategies

Therapeutic cancer vaccines as monotherapy have demonstrated varying levels of efficacy in clinical trials; however, their ultimate role may be in combination with other standard and experimental approaches. Emerging data suggest that the immune-mediated tumor-cell killing induced by cancer vaccines can be augmented by some conventional anticancer therapies (78). Certain chemotherapeutic agents and radiation therapy may up-regulate MHC molecules, enhance TAA expression, and induce apoptosis by increasing the expression of cell-death receptors such as TNF receptor, TNF-related ligand receptors, and Fas (79). Regulators of immune response, such as certain cytokines and immune regulatory cells, may decrease the immune response that can be generated against a tumor (80-84). Agents that suppress these barriers to immune activation may thus enhance immune response to cancer vaccines.

5.1. Vaccine plus radiotherapy

In addition to killing tumor cells, radiation may alter their phenotypic expression. Preclinical data in several murine models indicate that nonlethal levels of radiation may induce phenotypic changes in tumor cells and can up-regulate gene expression of cell markers such as MHC, Fas, ICAM-1 and TAAs, rendering tumor cells more susceptible to T cell-mediated immune attack (85, 86). Murine studies have also demonstrated that radiation

combined with a therapeutic cancer vaccine enhances tumor-cell killing compared to either modality alone (87).

Initial clinical data involving a first-generation poxviral vaccine provided a clinical proof of concept. In a phase I trial, 19 patients received vaccine plus radiation, and 11 patients were given radiation alone. Patients in the combination arm received a priming dose of rV-PSA admixed with rV-B7.1, followed by monthly boosts of rF-PSA, for a total of 8 vaccinations. The combination regimen was well tolerated, with no reported grade 3 toxicities to vaccine. Of the 17 patients in the combination arm who completed all scheduled vaccinations, 13 had a ≥ 3-fold increase in PSA-specific T cells, while patients in the radiotherapy-only arm had no detectable increases in PSA-specific T cells ($P < 0.0005$) (42). Patients in the combination arm also showed evidence of de novo generation of T cells to prostate-associated antigens not present in the vaccine (a phenomenon known as antigen cascade), providing indirect evidence of immune-mediated tumor killing. In short-term follow-up after a median of 20 to 25 months, 2/17 patients in the combination arm had recurrence compared to 2/9 patients in the radiation-only arm (42).

Nesslinger et al. reported that vaccine plus radiation therapy induced antigen spreading in a large proportion of patients with localized prostate cancer who were treated as part of a phase II clinical trial with a recombinant poxvirus-based vaccine targeting PSA (88). In addition to inducing immunity to PSA, as intended, vaccination induced autoantibody responses to a panel of 4 self-antigens in a significant proportion of patients, which seemed to be associated with inferior outcomes. Tumor-specific vaccines can induce immune responses to self-proteins, which may compromise therapeutic efficacy by provoking regulatory responses in the host to minimize autoimmune damage.

5.2. Vaccine plus chemotherapy

Although it is generally believed that chemotherapy mutes immune responses, both preclinical and clinical data have shown that docetaxel can be combined with vaccine for enhanced outcomes. Preclinical data have shown that docetaxel increases antigen presentation and improves immune response directed against tumor-cell TAAs and cascade antigens. In the same model, antitumor effect was greater in mice that received vaccine followed by docetaxel than in mice given either agent alone or the same agents in the opposite order (89). Thus, chemotherapy may create an immunologic milieu in which vaccine is better able to take effect (89, 90). The direct antitumor effect of chemotherapy agents may decrease tumor burden or slow disease progression, allowing immunotherapy to be more effective (24, 89). Furthermore, Von Mehren et al. demonstrated that the number of prior chemotherapy regimens was inversely correlated with the ability to generate a T-cell response, suggesting that patients with the most extensive chemotherapy pretreatment were least likely to have immunological response to vaccination (91).

Matzinger recently proposed that direct tumor killing by chemotherapy agents may induce molecular “danger signals” that can result in a more active immune response against antigens found on dying cancer cells. Antigen presentation initiates a chain of events that educates effector T cells to destroy TAA-bearing cells, but those immune responses are triggered more powerfully by entities that cause damage than by those that do not (92-94).

A phase II clinical trial evaluated 28 mCRPC patients randomized to receive either vaccine plus weekly docetaxel plus prednisone or vaccine alone. Patients on the vaccine-alone arm were allowed to cross over to receive docetaxel alone at time of progression. After 3 months of treatment, the median increase in T-cell precursors activated against PSA was 3.3-fold in both arms as measured by ELISPOT. Evidence of antigen cascade was also seen in both arms. These immunologic data suggest that vaccine combined with docetaxel plus

prednisone did not inhibit a prostate cancer-specific immune response. Median time to progression for the 11 patients who crossed over to docetaxel from vaccine alone was 6.1 months compared with 3.7 months for historical controls (95). Time to progression in the combination arm (3.2 months) was similar to historical controls (90).

The 2 most fully developed prostate cancer vaccines, sipuleucel-T and PSA-TRICOM, have both demonstrated a survival benefit without affecting short-term disease progression (30, 36). Therefore, using standard chemotherapy as a bridge to immune response may be a reasonable approach. Vaccine therapy followed by docetaxel could delay short-term disease progression until the vaccine can take effect. Clinical trials are being planned to prospectively validate the benefits of vaccine followed by chemotherapy. One such trial is ECOG E1809, a multicenter randomized phase II trial of docetaxel with or without PSA-TRICOM vaccine in patients with mCRPC. Patients are randomized to 3 months of PSA-TRICOM vaccine followed by docetaxel and prednisone vs. docetaxel and prednisone up front, with median OS as the primary endpoint. This trial was initiated in late 2010 and will enroll 135 patients.

5.3. Vaccine plus hormonal therapy

Combining therapeutic cancer vaccines with hormonal therapies is a potential approach for hormone-sensitive tumors, such as breast and prostate cancer. Preclinical data indicate that testosterone suppression affects not only prostate tumors, but also the immune system (96). Increasingly, data suggest that ADT in prostate cancer can augment the immune response by increasing T-cell infiltration into the prostate (97). Furthermore, ADT has been shown to decrease immune tolerance of TAAs, increase the size of the thymus (where CTL precursors proliferate and differentiate), and enhance the T-cell repertoire (96, 98-100). It may also stimulate CTLs by reducing the number of Tregs and thus improving immune-mediated tumor-specific response (101).

Hormonal therapy may have additional benefit when combined with therapeutic cancer vaccines. Koh et al. showed that androgen ablation increased DC maturation and costimulatory marker expression, but had no effect on DC costimulatory function. In adition, DCs isolated from castrated mice increased the expression of key cytokines by antigen-experienced T cells, while decreasing their expression in naïve cells. Finally, androgen ablation improved immune responses to vaccination only when employed after immunization (102).

A clinical study in men with nonmetastatic CRPC has also suggested a possible beneficial effect using vaccines with combined androgen blockade (CAB), where patients are treated with both ADT and an androgen receptor antagonist. Patients were randomized to either vaccine or standard CAB that included nilutamide, an FDA-approved androgen receptor antagonist. After 6 months, patients with rising PSA but no metastasis could cross over to receive both therapies. The median time to treatment failure (defined by rising PSA or development of a metastatic lesion) with the combined therapy was 13.9 months for patients who started on the vaccine arm and added nilutamide at PSA progression. In contrast, patients who started on nilutamide and added vaccine at PSA progression had a median time to treatment failure of 5.2 months. This study suggested that vaccine given in early-stage disease followed by nilutamide may have greater clinical efficacy than nilutamide followed by vaccine (43). A subsequent follow-up survival analysis of these patients revealed a 75% 5-year survival rate for patients who were treated first with vaccine then had nilutamide added, compared to a 43% 5-year survival rate for patients who received nilutamide first and added vaccine later (103). As in other trials in mCRPC, patients with more indolent disease (Gleason score < 7; $P = 0.033$) and lower disease volume (PSA < 20 mcg/ml; $P = 0.013$) had

the most significant survival advantage if treated with vaccine prior to hormone vs. the converse.

Evaluation of the first 26 patients enrolled in an ongoing randomized phase II study at the NCI of flutamide with or without PSA-TRICOM in nonmetastatic CRPC revealed a median time to progression of 223 days (range, 70 to 638) for flutamide plus PSA-TRICOM (n = 13) vs. 85 days (range, 56 to 372) for flutamide alone (n = 13) (104).

5.4. Treatment earlier in the disease course – intraprostatic vaccination

As mentioned earlier, preclinical data support the concept that immunotherapy provides the most benefit earlier in the course of disease (21, 105-109). Newly diagnosed patients, then, might be ideal candidates for vaccination. Preclinical data suggest that, for this patient population, neoadjuvant intratumoral vaccination prior to definitive therapy may improve immune responses and decrease tumor burden more effectively (110, 111). To determine whether intratumoral vaccination of the prostate could be done safely, a phase I trial was conducted in patients with locally recurrent prostate cancer. In all, 21 patients, 19 of whom had undergone only previous radiation therapy (and thus had intact prostates), were enrolled and given subcutaneous priming doses followed by intraprostatic booster doses of PSA-TRICOM. No patients had local inflammatory responses that would have precluded surgery, and only one patient had a grade 3 toxicity (transient fever, which resolved) (112). These safety data may provide the basis for a phase II trial evaluating the efficacy of intraprostatic vaccination in earlier-stage disease.

6. Changing Tumor Growth Rate Kinetics

Since 1989, PSA has been used in the United States as a marker of tumor response in prostate cancer (113). Approximately 95% of patients with mCRPC will have elevated PSA (114). Multiple trials have shown a significant OS benefit in patients who had a serum PSA decline of ≥ 50% (115-117). This correlation established the standard of judging new prostate cancer treatments by their ability to affect serum PSA values. Data analyses from numerous trials in prostate cancer performed at the NCI have suggested that the use of PSA values in a bivariate equation can identify a tumor growth constant. This model was created based on tumor markers or radiographic findings in multiple myeloma (M protein), renal cell carcinoma (radiographic changes), and prostate cancer (PSA). In turn, that growth constant can be used to predict survival based on expected tumor volume. When the model was applied in a trial where patients are being treated with cytotoxic therapies (chemotherapy or tyrosine kinase inhibitors), it accurately predicted outcomes (even while patients are still responding to therapy). However, analysis of a vaccine study showed no initial responses in tumor growth, while survival substantially exceeded predictions based on short-term growth rates. One likely explanation is that vaccines can alter tumor biology in a way that ultimately alters growth rate, even when patients are no longer receiving vaccine. By contrast, cytotoxic therapies can cause a temporary decrease in tumor volume, but growth rate reverts to pretreatment levels when therapy is discontinued. Vaccines probably do not decrease tumor volume, but they may slow growth rate after the vaccine has been discontinued (118-120).

This model may help to explain the phenomenon described above in which patients with the least disease appear to derive the most benefit from vaccine therapy. In a patient with a large tumor burden of disease, slowing the growth rate will not have much impact. But a patient with minimal disease whose tumor growth rate decreases may live much longer as a result. Taking that a step further, each subsequent therapy the patient receives after vaccine may provide more benefit than it would have done alone, as was seen in the nilutamide and

flutamide studies described above (103, 104). Those subsequent therapies could also act as a boost to vaccine if some tumor cell lysis occurred.

7. Appropriate trial endpoints

PFS vs. OS as an appropriate endpoint in clinical trials has been debated in the literature for several years (121). PFS is measured as the time between initiation of treatment and tumor progression or death from any cause, with censoring of patients who are lost to follow-up. PFS is often deemed a more attractive endpoint than OS for clinical trials because it can be determined earlier than OS, is less influenced by competing causes of death, and is not influenced by second-line treatments. However, unlike OS, which is an objective measurement, disease progression may be subject to measurement errors. Accurate determination of the starting point of disease progression can be problematic, and the quality of PFS measurement can vary among centers and investigators. In addition, the date of progression is in fact a proxy for the true time of progression, which occurs at an unknown point between 2 successive radiological assessments (122). Finally, while RECIST is a useful tool for determining antitumor activity, improving survival should be the ultimate goal of all cancer therapeutics (123).

We have seen that cancer vaccines can improve OS without significant changes in PFS. Several mechanisms have been proposed for this phenomenon (78, 124, 125). Subsequent therapies may alter the expression of TAAs on tumor cells, making them more susceptible to immune-mediated tumor-cell killing, or may enhance the immune response by depleting immune regulatory mechanisms. Chemotherapy-induced cytolysis may expose an activated immune response to additional antigens that can then be targeted in a broader immune response, or may trigger a molecular danger signal that leads to an enhanced immune response.

A randomized placebo-controlled phase III trial of sipuleucel-T and a randomized placebo-controlled phase II study of the vector-based vaccine PSA-TRICOM are good examples of the importance of appropriate endpoints. The sipuleucel-T trial initially failed to meet its primary endpoint of PFS; however, it did provide evidence of longer OS (25.8 vs. 21.7 months; $P = 0.032$) (27-29, 31). This advantage was confirmed in a larger OS endpoint study (30). Likewise, the PSA-TRICOM study failed to meet its primary endpoint of improved time to progression as determined by new or enlarging soft tissue tumors or bone metastasis, but a survival analysis indicated a clear clinical benefit for the vaccine. The median OS was 8.5 months longer in the vaccine arm than in the control arm ($P = 0.016$), suggesting that despite a lack of improved time to progression, there was a long-term survival advantage for patients treated with PSA-TRICOM (36).

Unlike chemotherapy, which may have an early clinical effect, immunotherapies often demonstrate delayed clinical effects. As seen in the IMPACT trial of sipuleucel-T, the separation of Kaplan-Meier curves occurs approximately 8 months after randomization. In randomized trials in which immunotherapies are compared with either placebo or inactive controls, Kaplan-Meier survival curves may be superimposable for a time before separation is observed. Generally, if there is a delayed separation, the statistical power to differentiate the complete curves is reduced (126, 127). It is therefore recommended that computations of the required events be based on a plausible specification of the timing of the delayed separation, the desired statistical power, and the understanding that the trial will be overpowered if delayed separation is not observed or is less than that specified. The log-rank test is recommended to avoid deviation from conventional methods and to avoid prespecification of parameters describing the delay, and as a hedge against absence of a

delay. The cost of using the log-rank statistic is some loss of optimal conditions should a delay occur (68, 126-128).

The vast majority of clinical trials rely on RECIST criteria, which were developed in 2000 through a collaboration of the European Organization for Research and Treatment of Cancer, the National Cancer Institute of Canada, and the U.S. NCI (123, 129). RECIST evaluates clinical benefit strictly in terms of reduction in size of soft tissue tumors, and defines progressive disease as a 20% increase in the cumulative size of target lesions or development of any new lesions (123, 129). However, the value of RECIST in immunotherapy trials is questionable. A significant and potentially beneficial immune response may cause transient increases in the size of lymph nodes, which RECIST criteria could identify as progressive disease (130-132). Indeed, immunotherapy trials in melanoma have suggested that the disease may initially flare in some areas before more clinically beneficial results are seen (133).

New response criteria based on WHO and RECIST were evaluated in a few recent studies with ipilimumab (53, 61) in which 4 distinct response patterns were described: immediate response, durable stable disease, response after tumor burden increase, and response in the presence of new lesions. All patterns appear to be associated with favorable survival compared to patients with progressive disease by WHO criteria.

To evaluate all observed response patterns, a new set of immune-related response criteria (irRC) for tumor immunotherapy was proposed (53, 134). Response categories defined as immune-related complete response (irCR), immune-related partial response (irPR), immune-related stable disease (irSD), and immune-related progressive disease (irPD) are considered clinically meaningful because they appear to be associated with favorable survival. By irRC, new lesions alone do not constitute irPD if they do not increase tumor burden by ≥ 25%. Appearance of new lesions accompanied by an overall decrease in tumor burden of ≥ 50% is defined as irPR, while a < 50% decrease to ≤ 25% increase in tumor burden is defined as irSD. Importantly, an early increase in the size of lesions, which may be attributable to inflammation, does not mean that irCR, irPR, or irSD may not be achieved at the next consecutive time point.

Confirmation of irPD by a second scan in the absence of rapid clinical deterioration is required. Thus, disease progression is confirmed by an increase in tumor burden of ≥ 25% over baseline at 2 consecutive time points ≥ 4 weeks apart.

Appropriate endpoints in clinical studies are vital to understanding the benefits of emerging immunotherapeutic agents used alone or in combination with cytoreductive therapies. The final results of these trials will give added insight into appropriate endpoints for vaccine combination studies.

8. Immunologic endpoints and tumor biomarkers

The recent clinical successes of modern immunotherapeutics have posed new dilemmas. As noted, ipilimumab and sipuleucel-T, among other therapeutic cancer vaccines, have demonstrated an OS benefit without changes in time to progression (30, 58). Although there may be rational immunologic and clinical explanations for this phenomenon, clinicians charged with making treatment decisions are left to wonder how to assess long- and short-term benefits (125). There is an obvious role for standardized immune response biomarkers to help determine clinical benefit soon after immunotherapy. These readouts would determine if additional therapy is required and when it should commence. Many assays have been described in the literature, but none has found a standardized role. Some are very specific, such as the ELISPOT assay, which determines antigen-specific T-cell activation

through individual cell IFN-γ production in response to an APC expressing the specific TAA (135). The major shortcoming with ELISPOT assays is reproducibility, which may vary from reader to reader or institution to institution (136, 137). Even if assays were standardized, allowing for more uniform readings, they would not account for antigen cascade, whereby the most relevant immune response may not be targeting the TAA specified by a given vaccine. As previously demonstrated, an antigen cascade following a vaccine-mediated immune response may result in the targeting of multiple antigens not specified by that particular vaccine (42). Furthermore, the most relevant TAA may vary among patients treated with the same therapeutic cancer vaccine. With agents such as ipilimumab, which allow for a more nonspecific, less targeted immune response, it is less clear which specific TAAs are most likely to be targeted. Thus, while evidence of a specific immune response against a specified TAA may support the efficacy of an immune-based treatment, the absence of that response may not preclude an immune response to a more relevant secondary antigen as an indicator of potent antitumor immune effect. This perspective suggests the wisdom of identifying a more generalized marker of immune response.

Many methods have been employed to evaluate cytokine production in response to immunotherapy. However, cytokine detection by ELISA assays is highly variable among patients, and the overall sensitivity of this test is low. Conversely, real-time polymerase chain reaction (RT-PCR) analysis, which is highly sensitive and reproducible, can evaluate cytokine production by measuring mRNA. The drawback of this technique is that mRNA analysis necessitates destruction of the immune cell, which prevents determination of T-cell specificity (138). These and other techniques for assessing immune biomarkers need further prospective evaluation in patients treated with modern immunotherapeutics. After decades of research devoted to demonstrating the clinical efficacy of therapeutic cancer vaccines, their ultimate utility could be substantially limited by a lack of useful biomarkers. Clearly, this is the most pressing need for clinicians as these agents evolve from experimental to standard therapies.

A new analysis of a phase III abiraterone acetate trial (COU-AA-301) supports previous research suggesting that levels circulating tumor cells (CTCs) could be a predictor of survival and an intermediate endpoint in randomized phase III trials. CTC levels at baseline and 4, 8, and 12 weeks follow-up were correlated with OS, demonstrating their potential as a surrogate biomarker. Although the initial data are encouraging, use of CTCs as a surrogate endpoint will require prospective evaluation in clinical trials employing immune-based therapies. (139).

The serological analysis of recombinant cDNA expression library (SEREX) offers another approach for detecting novel tumor antigens serologically (140). SEREX, which is generated from various tumor tissues or from cancer cell lines, provides a molecular definition of immunogenic tumor proteins based on their reactivity with autologous patient sera. It has been used to identify new tumor antigens in several malignancies (62, 63). However, SEREX has limited application because antibodies to most antigens can be detected in only 10% to 30% of patients bearing a tumor expressing the respective antigen, and has no correlation to any obvious clinical parameter (46).

The development of intermediate endpoint biomarkers is an absolute requirement for accelerating the development of novel treatments for all cancers, but particularly cancer vaccines. Intermediate endpoint biomarkers have been pivotal in the approval of drugs for diabetes and chronic myeloid leukemia (CML), where blood glucose levels and circulating Philadelphia chromosome-positive CML cells, cytogenetic response, and molecular monitoring by RT-PCR of BCR-ABL transcripts have proven utility. The rapid development

of ABL inhibitors and their expedited regulatory approval was also due, at least in part, to the availability of highly specific and sensitive biomarkers of response and clinical outcome. Unfortunately, unlike CML, most malignancies do not yet have such easily measurable biomarkers (141).

There has been a significant effort to define pathways and harmonize methods for translational cancer immunotherapy. The Translational Research Working Group published a recommended pathway incorporating various milestones in a flowchart algorithm for translating potential immunologic therapies from the bench to use in clinical trials (142). In 2007, the Cancer Vaccine Clinical Trial Working Group published a useful guide for appropriate design of trials for vaccines that are ready for clinical testing (143, 144). Instead of the usual phase I to III trials, the recommendations include proof-of-principle trials followed by efficacy trials for promising candidates. A proof-of-principle trial would enroll 20 to 30 patients to test for safety, limited dose ranging, and immunologic efficacy. An efficacy trial would be a randomized hybrid phase II/III trial, with well-defined time-to-event endpoints such as overall, disease-free, and progression-free survival. Until this work is finalized, published recommendations on harmonization of ELISPOT and tetramer methods are available that provide a good foundation for conducting correlative immunologic testing (143, 145).

In summary, improved biomarkers that can serve as generalized markers of immune response or as intermediate and surrogate endpoints are urgently needed. These biomarkers would help to identify patient benefit earlier in treatment, guide decisions to discontinue ineffective strategies, and identify active anticancer drugs more efficiently. Ideally, such biomarkers could be measured easily, rapidly, and frequently.

9. Future directions

The efficacy of prostate cancer vaccines will likely increase when the vaccines are combined with agents that favorably alter the tumor microenvironment, leading to enhanced tumor-cell killing by T cells. Combining vaccines that target different TAAs and use different mechanisms to induce an immune response may also create more potent tumor destruction. Boehm et al. have shown that using 2 vaccine platforms that target the same antigen may induce shared and distinct antigen-specific T-cell populations. They compared the T-cell populations induced by recombinant poxvirus and yeast vaccines in terms of serum cytokine response, T-cell gene expression, T-cell receptor phenotype, antigen-specific cytokine expression, T-cell avidity, and T-cell antigen-specific tumor-cell lysis. They demonstrated for the first time that vaccination with a recombinant poxvirus vaccine (rV/F-CEA/TRICOM) and a heat-killed yeast vaccine (yeast-CEA) elicits more diverse T-cell populations, resulting in enhanced antitumor efficacy. These studies provide the rationale for future clinical studies investigating concurrent administration of vaccine platforms targeting a single antigen to enhance the antigen-specific immune response (146). As described above, using vaccine therapies at different stages of disease and in combination with standard therapies may provide additional benefit to patients. Rappuoli and Aderem have proposed some novel principles for improving vaccines in general (147). An individual's response to vaccination depends on a multitude of interacting genetic, molecular, and environmental factors spanning numerous temporal and spatial scales. These data sets include molecular measurements such as DNA sequences, RNA and protein expression levels, microRNAs, protein-protein and protein-DNA interactions, and metabolite biology. Thus, the tools of systems biology are particularly well suited for analyzing vaccine studies, with computation as an essential element of the systems biology approach. Computational analysis may facilitate the development of detailed computational models that directly link system phenotype to the behavior of the protein and gene regulatory networks. Once the

model is sufficiently developed, it may have the potential to identify biomarkers that could be used to predict the efficacy of vaccines.

10. Conclusions

Therapeutic cancer vaccines have been in development for several decades, initially with disappointing results. But those failures increased our understanding of the immune antitumor response and prompted the development of more potent vaccines and other immunotherapeutic agents that are considerably less toxic than chemotherapies or targeted therapies. Recent trials in prostate cancer have renewed hope that initiating an active immune response with a therapeutic cancer vaccine can have long-term clinical benefit for cancer patients. The generally indolent nature of prostate cancer, and the dearth of effective systemic treatments for metastatic disease, may explain why vaccines have been more successful in prostate cancer than in other types of cancer. Prostate cancer vaccines' ability to generate a cellular immune response that results in altered tumor growth curves and prolonged survival has changed our understanding of the kinetics of immune response.

However, much work remains to be done. First, cellular immune response assays must be standardized and validated as reproducible biomarkers that can be correlated with clinical outcomes. Second, the new irRC, which can capture more complex response patterns, should replace RECIST in studies of immunotherapies. irRC assess tumor burden as a continuous variable that considers index lesions identified at baseline and new lesions that occur after treatment is initiated, based on bidimensional measurements of each lesion. Third, new statistical models describing hazard ratios as a function of time and recognizing differences before and after the separation of Kaplan-Meier curves should be developed to evaluate phase III trials.

The initial successes in prostate cancer may allow investigators to optimize vaccine therapies for other diseases as well. Additional strategies are being investigated, many in late clinical development. Combinations of vaccines, standard therapeutics, and other immune-regulating agents are also under investigation.

Therapeutic prostate cancer vaccines, which have few side effects and the potential to generate long-term immune responses that add clinical benefit to subsequent therapies are in process of transforming cancer care and hold the promise of revolutionizing future cancer treatments. As medical oncologists become more familiar with immune-based strategies, these vaccines may well play a more dominant role in cancer treatment.

Acknowledgments

The authors thank Dr. Jeffrey Schlom and Dr. James L. Gulley for their review of this manuscript and the Intramural Research Program of the NIH, National Cancer Institute, Center for Cancer Research for their support of this study. We also thank Bonnie L. Casey for editorial assistance in the preparation of this manuscript.

References

1. Jemal A, Siegel R, Xu J, Ward E. Cancer statistics, 2010. CA Cancer J Clin. 2010; 60:277–300. [PubMed: 20610543]
2. Roehl KA, Han M, Ramos CG, Antenor JA, Catalona WJ. Cancer progression and survival rates following anatomical radical retropubic prostatectomy in 3,478 consecutive patients: long-term results. J Urol. 2004; 172:910–4. [PubMed: 15310996]
3. Tanaka K, Tanahashi N, Tsurumi C, Yokota KY, Shimbara N. Proteasomes and antigen processing. Adv Immunol. 1997; 64:1–38. [PubMed: 9100978]

4. Hammer GE, Kanaseki T, Shastri N. The final touches make perfect the peptide-MHC class I repertoire. Immunity. 2007; 26:397–406. [PubMed: 17459809]
5. Cheever MA, Allison JP, Ferris AS, Finn OJ, Hastings BM, Hecht TT, et al. The prioritization of cancer antigens: a national cancer institute pilot project for the acceleration of translational research. Clin Cancer Res. 2009; 15:5323–37. [PubMed: 19723653]
6. Freedland SJ, Hotaling JM, Fitzsimons NJ, Presti JC Jr, Kane CJ, Terris MK, et al. PSA in the new millennium: a powerful predictor of prostate cancer prognosis and radical prostatectomy outcomes--results from the SEARCH database. Eur Urol. 2008; 53:758–64. discussion 65-6. [PubMed: 17868976]
7. Madan RA, Arlen PM, Mohebtash M, Hodge JW, Gulley JL. Prostvac-VF: a vector-based vaccine targeting PSA in prostate cancer. Expert Opin Investig Drugs. 2009; 18:1001–11.
8. Fishman M. A changing world for DCvax: a PSMA loaded autologous dendritic cell vaccine for prostate cancer. Expert Opin Biol Ther. 2009; 9:1565–75. [PubMed: 19916735]
9. Becker JT, Olson BM, Johnson LE, Davies JG, Dunphy EJ, McNeel DG. DNA vaccine encoding prostatic acid phosphatase (PAP) elicits long-term T-cell responses in patients with recurrent prostate cancer. J Immunother. 2010; 33:639–47. [PubMed: 20551832]
10. Maeda H, Nagata S, Wolfgang CD, Bratthauer GL, Bera TK, Pastan I. The T cell receptor gamma chain alternate reading frame protein (TARP), a prostate-specific protein localized in mitochondria. J Biol Chem. 2004; 279:24561–8. [PubMed: 15150260]
11. Epel M, Carmi I, Soueid-Baumgarten S, Oh SK, Bera T, Pastan I, et al. Targeting TARP, a novel breast and prostate tumor-associated antigen, with T cell receptor-like human recombinant antibodies. Eur J Immunol. 2008; 38:1706–20. [PubMed: 18446790]
12. Cereda V, Poole DJ, Palena C, Das S, Bera TK, Remondo C, et al. New gene expressed in prostate: a potential target for T cell-mediated prostate cancer immunotherapy. Cancer Immunol Immunother. 2010; 59:63–71. [PubMed: 19495750]
13. Raff AB, Gray A, Kast WM. Prostate stem cell antigen: a prospective therapeutic and diagnostic target. Cancer Lett. 2009; 277:126–32. [PubMed: 18838214]
14. Garcia-Hernandez M de L, Gray A, Hubby B, Kast WM. In vivo effects of vaccination with six-transmembrane epithelial antigen of the prostate: a candidate antigen for treating prostate cancer. Cancer Res. 2007; 67:1344–51. [PubMed: 17283172]
15. Sabbisetti VS, Chirugupati S, Thomas S, Vaidya KS, Reardon D, Chiriva-Internati M, et al. Calcitonin increases invasiveness of prostate cancer cells: role for cyclic AMP-dependent protein kinase A in calcitonin action. Int J Cancer. 2005; 117:551–60. [PubMed: 15929083]
16. Chiriva-Internati M, Yu Y, Mirandola L, D'Cunha N, Hardwicke F, Cannon MJ, et al. Identification of AKAP-4 as a new cancer/testis antigen for detection and immunotherapy of prostate cancer. Prostate. 2011
17. Finn OJ, Jerome KR, Henderson RA, Pecher G, Domenech N, Magarian-Blander J, et al. MUC-1 epithelial tumor mucin-based immunity and cancer vaccines. Immunol Rev. 1995; 145:61–89. [PubMed: 7590831]
18. Cozzi PJ, Wang J, Delprado W, Perkins AC, Allen BJ, Russell PJ, et al. MUC1, MUC2, MUC4, MUC5AC and MUC6 expression in the progression of prostate cancer. Clin Exp Metastasis. 2005; 22:565–73. [PubMed: 16475027]
19. Parmiani G, Russo V, Marrari A, Cutolo G, Casati C, Pilla L, et al. Universal and stemness-related tumor antigens: potential use in cancer immunotherapy. Clin Cancer Res. 2007; 13:5675–9. [PubMed: 17908956]
20. Fu T, Shen Y, Fujimoto S. Tumor-specific CD4(+) suppressor T-cell clone capable of inhibiting rejection of syngeneic sarcoma in A/J mice. Int J Cancer. 2000; 87:680–7. [PubMed: 10925362]
21. Ghiringhelli F, Larmonier N, Schmitt E, Parcellier A, Cathelin D, Garrido C, et al. CD4+CD25+ regulatory T cells suppress tumor immunity but are sensitive to cyclophosphamide which allows immunotherapy of established tumors to be curative. Eur J Immunol. 2004; 34:336–44. [PubMed: 14768038]
22. Teicher BA. Transforming growth factor-beta and the immune response to malignant disease. Clin Cancer Res. 2007; 13:6247–51. [PubMed: 17975134]

23. Muller AJ, Prendergast GC. Indoleamine 2,3-dioxygenase in immune suppression and cancer. Curr Cancer Drug Targets. 2007; 7:31–40. [PubMed: 17305476]
24. Gulley JL, Arlen PM, Madan RA, Tsang KY, Pazdur MP, Skarupa L, et al. Immunologic and prognostic factors associated with overall survival employing a poxviral-based PSA vaccine in metastatic castrate-resistant prostate cancer. Cancer Immunol Immunother. 2010; 59:663–74. [PubMed: 19890632]
25. Halabi S, Small EJ, Kantoff PW, Kattan MW, Kaplan EB, Dawson NA, et al. Prognostic model for predicting survival in men with hormone-refractory metastatic prostate cancer. J Clin Oncol. 2003; 21:1232–7. [PubMed: 12663709]
26. Peshwa MV, Shi JD, Ruegg C, Laus R, van Schooten WC. Induction of prostate tumor-specific CD8+ cytotoxic T-lymphocytes in vitro using antigen-presenting cells pulsed with prostatic acid phosphatase peptide. Prostate. 1998; 36:129–38. [PubMed: 9655265]
27. Small EJ, Fratesi P, Reese DM, Strang G, Laus R, Peshwa MV, et al. Immunotherapy of hormone-refractory prostate cancer with antigen-loaded dendritic cells. J Clin Oncol. 2000; 18:3894–903. [PubMed: 11099318]
28. Burch PA, Breen JK, Buckner JC, Gastineau DA, Kaur JA, Laus RL, et al. Priming tissue-specific cellular immunity in a phase I trial of autologous dendritic cells for prostate cancer. Clin Cancer Res. 2000; 6:2175–82. [PubMed: 10873066]
29. Burch PA, Croghan GA, Gastineau DA, Jones LA, Kaur JS, Kylstra JW, et al. Immunotherapy (APC8015, Provenge) targeting prostatic acid phosphatase can induce durable remission of metastatic androgen-independent prostate cancer: a phase 2 trial. Prostate. 2004; 60:197–204. [PubMed: 15176049]
30. Kantoff PW, Higano CS, Shore ND, Berger ER, Small EJ, Penson DF, et al. Sipuleucel-T immunotherapy for castration-resistant prostate cancer. N Engl J Med. 2010; 363:411–22. [PubMed: 20818862]
31. Small EJ, Schellhammer PF, Higano CS, Redfern CH, Nemunaitis JJ, Valone FH, et al. Placebo-controlled phase III trial of immunologic therapy with sipuleucel-T (APC8015) in patients with metastatic, asymptomatic hormone refractory prostate cancer. J Clin Oncol. 2006; 24:3089–94. [PubMed: 16809734]
32. Higano CS, Schellhammer PF, Small EJ, Burch PA, Nemunaitis J, Yuh L, et al. Integrated data from 2 randomized, double-blind, placebo-controlled, phase 3 trials of active cellular immunotherapy with sipuleucel-T in advanced prostate cancer. Cancer. 2009; 115:3670–9. [PubMed: 19536890]
33. Beer TM, Bernstein GT, Corman JM, Glode M, Hall SJ, Poll WL, et al. Randomized trial of autologous cellular immunotherapy with sipuleucel-T in androgen-dependent prostate cancer. Clin Cancer Res. 2011
34. Sipuleucel-T as Neoadjuvant Treatment in Prostate Cancer (NeoACT). Jun. 2011 Available from: http://clinicaltrials.gov/ct2/show/NCT00715104
35. Arlen PM, Kaufman HL, DiPaola RS. Pox viral vaccine approaches. Semin Oncol. 2005; 32:549–55. [PubMed: 16338420]
36. Kantoff PW, Schuetz TJ, Blumenstein BA, Glode LM, Bilhartz DL, Wyand M, et al. Overall survival analysis of a phase II randomized controlled trial of a poxviral-based PSA-targeted immunotherapy in metastatic castration-resistant prostate cancer. J Clin Oncol. 2010; 28:1099–105. [PubMed: 20100959]
37. Kaufman HL, Wang W, Manola J, DiPaola RS, Ko YJ, Sweeney C, et al. Phase II randomized study of vaccine treatment of advanced prostate cancer (E7897): a trial of the Eastern Cooperative Oncology Group. J Clin Oncol. 2004; 22:2122–32. [PubMed: 15169798]
38. Harrington LE, Most Rv R, Whitton JL, Ahmed R. Recombinant vaccinia virus-induced T-cell immunity: quantitation of the response to the virus vector and the foreign epitope. J Virol. 2002; 76:3329–37. [PubMed: 11884558]
39. Arlen PM, Skarupa L, Pazdur M, Seetharam M, Tsang KY, Grosenbach DW, et al. Clinical safety of a viral vector-based prostate cancer vaccine strategy. J Urol. 2007; 178:1515–20. [PubMed: 17707059]

40. DiPaola R, Chen Y, Bubley G, Hahn N, Stein M, Schlom J, et al. A phase II study of PROSTVAC-V (vaccinia)/TRICOM and PROSTVAC-F (fowlpox)/TRICOM with GM-CSF in patients with PSA progression after local therapy for prostate cancer: results of ECOG 9802. ASCO Genitourinary Cancers Symposium. 2009; 108 abstract.
41. Gulley J, Chen AP, Dahut W, Arlen PM, Bastian A, Steinberg SM, et al. Phase I study of a vaccine using recombinant vaccinia virus expressing PSA (rV-PSA) in patients with metastatic androgen-independent prostate cancer. Prostate. 2002; 53:109–17. [PubMed: 12242725]
42. Gulley JL, Arlen PM, Bastian A, Morin S, Marte J, Beetham P, et al. Combining a recombinant cancer vaccine with standard definitive radiotherapy in patients with localized prostate cancer. Clin Cancer Res. 2005; 11:3353–62. [PubMed: 15867235]
43. Arlen PM, Gulley JL, Todd N, Lieberman R, Steinberg SM, Morin S, et al. Antiandrogen, vaccine and combination therapy in patients with nonmetastatic hormone refractory prostate cancer. J Urol. 2005; 174:539–46. [PubMed: 16006888]
44. Eder JP, Kantoff PW, Roper K, Xu GX, Bubley GJ, Boyden J, et al. A phase I trial of a recombinant vaccinia virus expressing prostate-specific antigen in advanced prostate cancer. Clin Cancer Res. 2000; 6:1632–8. [PubMed: 10815880]
45. Sanda MG, Smith DC, Charles LG, Hwang C, Pienta KJ, Schlom J, et al. Recombinant vaccinia-PSA (PROSTVAC) can induce a prostate-specific immune response in androgen-modulated human prostate cancer. Urology. 1999; 53:260–6. [PubMed: 9933036]
46. Sahin U, Tureci O, Pfreundschuh M. Serological identification of human tumor antigens. Curr Opin Immunol. 1997; 9:709–16. [PubMed: 9368781]
47. Melero I, Hervas-Stubbs S, Glennie M, Pardoll DM, Chen L. Immunostimulatory monoclonal antibodies for cancer therapy. Nat Rev Cancer. 2007; 7:95–106. [PubMed: 17251916]
48. Fong L, Small EJ. Anti-cytotoxic T-lymphocyte antigen-4 antibody: the first in an emerging class of immunomodulatory antibodies for cancer treatment. J Clin Oncol. 2008; 26:5275–83. [PubMed: 18838703]
49. O'Day SJ, Hamid O, Urba WJ. Targeting cytotoxic T-lymphocyte antigen-4 (CTLA-4): a novel strategy for the treatment of melanoma and other malignancies. Cancer. 2007; 110:2614–27. [PubMed: 18000991]
50. Small EJ, Tchekmedyian NS, Rini BI, Fong L, Lowy I, Allison JP. A pilot trial of CTLA-4 blockade with human anti-CTLA-4 in patients with hormone-refractory prostate cancer. Clin Cancer Res. 2007; 13:1810–5. [PubMed: 17363537]
51. Hersh EM, O'Day SJ, Powderly J, Khan KD, Pavlick AC, Cranmer LD, et al. A phase II multicenter study of ipilimumab with or without dacarbazine in chemotherapy-naive patients with advanced melanoma. Invest New Drugs. 2010 Jan 16. Epub ahead of print.
52. O'Day SJ, Maio M, Chiarion-Sileni V, Gajewski TF, Pehamberger H, Bondarenko IN, et al. Efficacy and safety of ipilimumab monotherapy in patients with pretreated advanced melanoma: a multicenter single-arm phase II study. Ann Oncol. 2010; 21:1712–7. [PubMed: 20147741]
53. Wolchok JD, Neyns B, Linette G, Negrier S, Lutzky J, Thomas L, et al. Ipilimumab monotherapy in patients with pretreated advanced melanoma: a randomised, double-blind, multicentre, phase 2, dose-ranging study. Lancet Oncol. 2010; 11:155–64. [PubMed: 20004617]
54. Fong L, Kwek SS, O'Brien S, Kavanagh B, McNeel DG, Weinberg V, et al. Potentiating endogenous antitumor immunity to prostate cancer through combination immunotherapy with CTLA4 blockade and GM-CSF. Cancer Res. 2009; 69:609–15. [PubMed: 19147575]
55. Ansell SM, Hurvitz SA, Koenig PA, LaPlant BR, Kabat BF, Fernando D, et al. Phase I study of ipilimumab, an anti-CTLA-4 monoclonal antibody, in patients with relapsed and refractory B-cell non-Hodgkin lymphoma. Clin Cancer Res. 2009; 15:6446–53. [PubMed: 19808874]
56. Yang JC, Hughes M, Kammula U, Royal R, Sherry RM, Topalian SL, et al. Ipilimumab (anti-CTLA4 antibody) causes regression of metastatic renal cell cancer associated with enteritis and hypophysitis. J Immunother. 2007; 30:825–30. [PubMed: 18049334]
57. Beck KE, Blansfield JA, Tran KQ, Feldman AL, Hughes MS, Royal RE, et al. Enterocolitis in patients with cancer after antibody blockade of cytotoxic T-lymphocyte-associated antigen 4. J Clin Oncol. 2006; 24:2283–9. [PubMed: 16710025]

58. Hodi FS, O'Day SJ, McDermott DF, Weber RW, Sosman JA, Haanen JB, et al. Improved survival with ipilimumab in patients with metastatic melanoma. N Engl J Med. 2010; 363:711–23. [PubMed: 20525992]
59. Beer T, Slovin S, Higano C, Tejwani S, Dorff T, Stankevich E, et al. Phase I trial of ipilimumab (IPI) alone and in combination with radiotherapy (XRT) in patients with metastatic castration resistant prostate cancer (mCRPC). J Clin Oncol. 2008; 26(15S):5004. abstract.
60. Weber J. Ipilimumab: controversies in its development, utility and autoimmune adverse events. Cancer Immunol Immunother. 2009; 58:823–30. [PubMed: 19198837]
61. Weber J, Thompson JA, Hamid O, Minor D, Amin A, Ron I, et al. A randomized, double-blind, placebo-controlled, phase II study comparing the tolerability and efficacy of ipilimumab administered with or without prophylactic budesonide in patients with unresectable stage III or IV melanoma. Clin Cancer Res. 2009; 15:5591–8. [PubMed: 19671877]
62. Chen YT, Gure AO, Tsang S, Stockert E, Jager E, Knuth A, et al. Identification of multiple cancer/testis antigens by allogeneic antibody screening of a melanoma cell line library. Proc Natl Acad Sci U S A. 1998; 95:6919–23. [PubMed: 9618514]
63. Scanlan MJ, Chen YT, Williamson B, Gure AO, Stockert E, Gordan JD, et al. Characterization of human colon cancer antigens recognized by autologous antibodies. Int J Cancer. 1998; 76:652–8. [PubMed: 9610721]
64. Ward JE, McNeel DG. GVAX: an allogeneic, whole-cell, GM-CSF-secreting cellular immunotherapy for the treatment of prostate cancer. Expert Opin Biol Ther. 2007; 7:1893–902. [PubMed: 18034654]
65. Small E, Demkow T, Gerritsen W, Rolland F, Hoskin P, Smith D, et al. A phase III trial of GVAX immunotherapy for prostate cancer in combination with docetaxel versus docetaxel plus prednisone in symptomatic, castration-resistant prostate cancer (CRPC). ASCO Genitourinary Cancers Symposium. 2009; 7 abstract.
66. Higano C, Saad F, Somer B, Curti B, Petrylak D, Drake C, et al. A phase III trial of GVAX immunotherapy for prostate cancer versus docetaxel plus prednisone in asymptomatic, castration-resistant prostate cancer (CRPC). ASCO Genitourinary Cancers Symposium. 2009 abstract LBA150.
67. Higano CS, Corman JM, Smith DC, Centeno AS, Steidle CP, Gittleman M, et al. Phase 1/2 dose-escalation study of a GM-CSF-secreting, allogeneic, cellular immunotherapy for metastatic hormone-refractory prostate cancer. Cancer. 2008; 113:975–84. [PubMed: 18646045]
68. Lakatos E. Sample sizes based on the log-rank statistic in complex clinical trials. Biometrics. 1988; 44:229–41. [PubMed: 3358991]
69. Rice J, Ottensmeier CH, Stevenson FK. DNA vaccines: precision tools for activating effective immunity against cancer. Nat Rev Cancer. 2008; 8:108–20. [PubMed: 18219306]
70. Tsen SW, Paik AH, Hung CF, Wu TC. Enhancing DNA vaccine potency by modifying the properties of antigen-presenting cells. Expert Rev Vaccines. 2007; 6:227–39. [PubMed: 17408372]
71. McNeel DG, Dunphy EJ, Davies JG, Frye TP, Johnson LE, Staab MJ, et al. Safety and immunological efficacy of a DNA vaccine encoding prostatic acid phosphatase in patients with stage D0 prostate cancer. J Clin Oncol. 2009; 27:4047–54. [PubMed: 19636017]
72. Madan RA, Mohebtash M, Schlom J, Gulley JL. Therapeutic vaccines in metastatic castration-resistant prostate cancer: principles in clinical trial design. Expert Opin Biol Ther. 2010; 10:19–28. [PubMed: 19857185]
73. Essand M, Vasmatzis G, Brinkmann U, Duray P, Lee B, Pastan I. High expression of a specific T-cell receptor gamma transcript in epithelial cells of the prostate. Proc Natl Acad Sci U S A. 1999; 96:9287–92. [PubMed: 10430935]
74. Wolfgang CD, Essand M, Lee B, Pastan I. T-cell receptor gamma chain alternate reading frame protein (TARP) expression in prostate cancer cells leads to an increased growth rate and induction of caveolins and amphiregulin. Cancer Res. 2001; 61:8122–6. [PubMed: 11719440]
75. Kirschenbaum A, Itzkowitz SH, Wang JP, Yao S, Eliashvili M, Levine AC. MUC1 expression in prostate carcinoma: correlation with grade and stage. Mol Urol. 1999; 3:163–8. [PubMed: 10851319]

76. Butts C, Murray N, Maksymiuk A, Goss G, Marshall E, Soulieres D, et al. Randomized phase IIB trial of BLP25 liposome vaccine in stage IIIB and IV non-small-cell lung cancer. J Clin Oncol. 2005; 23:6674–81. [PubMed: 16170175]
77. North SA, Graham K, Bodnar D, Venner P. A pilot study of the liposomal MUC1 vaccine BLP25 in prostate specific antigen failures after radical prostatectomy. J Urol. 2006; 176:91–5. [PubMed: 16753376]
78. Gulley JL, Madan RA, Arlen PM. Enhancing efficacy of therapeutic vaccinations by combination with other modalities. Vaccine. 2007; 25(Suppl 2):B89–96. [PubMed: 17573164]
79. Ozoren N, El-Deiry WS. Cell surface death receptor signaling in normal and cancer cells. Semin Cancer Biol. 2003; 13:135–47. [PubMed: 12654257]
80. Piccirillo CA, Shevach EM. Cutting edge: control of CD8+ T cell activation by CD4+CD25+ immunoregulatory cells. J Immunol. 2001; 167:1137–40. [PubMed: 11466326]
81. Woo EY, Yeh H, Chu CS, Schlienger K, Carroll RG, Riley JL, et al. Cutting edge: regulatory T cells from lung cancer patients directly inhibit autologous T cell proliferation. J Immunol. 2002; 168:4272–6. [PubMed: 11970966]
82. Sakaguchi S. Regulatory T cells: key controllers of immunologic self-tolerance. Cell. 2000; 101:455–8. [PubMed: 10850488]
83. Mule JJ, Schwarz SL, Roberts AB, Sporn MB, Rosenberg SA. Transforming growth factor-beta inhibits the in vitro generation of lymphokine-activated killer cells and cytotoxic T cells. Cancer Immunol Immunother. 1988; 26:95–100. [PubMed: 2452015]
84. Farsaci B, Sabzevari H, Higgins JP, Di Bari MG, Takai S, Schlom J, et al. Effect of a small molecule BCL-2 inhibitor on immune function and use with a recombinant vaccine. Int J Cancer. 2010; 127:1603–13. [PubMed: 20091862]
85. Chakraborty M, Abrams SI, Camphausen K, Liu K, Scott T, Coleman CN, et al. Irradiation of tumor cells up-regulates Fas and enhances CTL lytic activity and CTL adoptive immunotherapy. J Immunol. 2003; 170:6338–47. [PubMed: 12794167]
86. Ferrara TA, Hodge JW, Gulley JL. Combining radiation and immunotherapy for synergistic antitumor therapy. Curr Opin Mol Ther. 2009; 11:37–42. [PubMed: 19169958]
87. Chakraborty M, Abrams SI, Coleman CN, Camphausen K, Schlom J, Hodge JW. External beam radiation of tumors alters phenotype of tumor cells to render them susceptible to vaccine-mediated T-cell killing. Cancer Res. 2004; 64:4328–37. [PubMed: 15205348]
88. Nesslinger NJ, Ng A, Tsang KY, Ferrara T, Schlom J, Gulley JL, et al. A viral vaccine encoding prostate-specific antigen induces antigen spreading to a common set of self-proteins in prostate cancer patients. Clin Cancer Res. 2010; 16:4046–56. [PubMed: 20562209]
89. Garnett CT, Schlom J, Hodge JW. Combination of docetaxel and recombinant vaccine enhances T-cell responses and antitumor activity: effects of docetaxel on immune enhancement. Clin Cancer Res. 2008; 14:3536–44. [PubMed: 18519787]
90. Arlen PM, Gulley JL, Parker C, Skarupa L, Pazdur M, Panicali D, et al. A randomized phase II study of concurrent docetaxel plus vaccine versus vaccine alone in metastatic androgen-independent prostate cancer. Clin Cancer Res. 2006; 12:1260–9. [PubMed: 16489082]
91. von Mehren M, Arlen P, Tsang KY, Rogatko A, Meropol N, Cooper HS, et al. Pilot study of a dual gene recombinant avipox vaccine containing both carcinoembryonic antigen (CEA) and B7.1 transgenes in patients with recurrent CEA-expressing adenocarcinomas. Clin Cancer Res. 2000; 6:2219–28. [PubMed: 10873071]
92. Matzinger P. The danger model: a renewed sense of self. Science. 2002; 296:301–5. [PubMed: 11951032]
93. Zitvogel L, Apetoh L, Ghiringhelli F, Andre F, Tesniere A, Kroemer G. The anticancer immune response: indispensable for therapeutic success? J Clin Invest. 2008; 118:1991–2001. [PubMed: 18523649]
94. Aymeric L, Apetoh L, Ghiringhelli F, Tesniere A, Martins I, Kroemer G, et al. Tumor cell death and ATP release prime dendritic cells and efficient anticancer immunity. Cancer Res. 2010; 70:855–8. [PubMed: 20086177]

95. Dahut WL, Gulley JL, Arlen PM, Liu Y, Fedenko KM, Steinberg SM, et al. Randomized phase II trial of docetaxel plus thalidomide in androgen-independent prostate cancer. J Clin Oncol. 2004; 22:2532–9. [PubMed: 15226321]

96. Aragon-Ching JB, Williams KM, Gulley JL. Impact of androgen-deprivation therapy on the immune system: implications for combination therapy of prostate cancer. Front Biosci. 2007; 12:4957–71. [PubMed: 17569623]

97. Mercader M, Bodner BK, Moser MT, Kwon PS, Park ES, Manecke RG, et al. T cell infiltration of the prostate induced by androgen withdrawal in patients with prostate cancer. Proc Natl Acad Sci U S A. 2001; 98:14565–70. [PubMed: 11734652]

98. Goldberg GL, Sutherland JS, Hammet MV, Milton MK, Heng TS, Chidgey AP, et al. Sex steroid ablation enhances lymphoid recovery following autologous hematopoietic stem cell transplantation. Transplantation. 2005; 80:1604–13. [PubMed: 16371932]

99. Drake CG, Doody AD, Mihalyo MA, Huang CT, Kelleher E, Ravi S, et al. Androgen ablation mitigates tolerance to a prostate/prostate cancer-restricted antigen. Cancer Cell. 2005; 7:239–49. [PubMed: 15766662]

100. Sutherland JS, Goldberg GL, Hammett MV, Uldrich AP, Berzins SP, Heng TS, et al. Activation of thymic regeneration in mice and humans following androgen blockade. J Immunol. 2005; 175:2741–53. [PubMed: 16081852]

101. Wang J, Zhang Q, Jin S, Feng M, Kang X, Zhao S, et al. Immoderate inhibition of estrogen by anastrozole enhances the severity of experimental polyarthritis. Exp Gerontol. 2009; 44:398–405. [PubMed: 19298850]

102. Koh YT, Gray A, Higgins SA, Hubby B, Kast WM. Androgen ablation augments prostate cancer vaccine immunogenicity only when applied after immunization. Prostate. 2009; 69:571–84. [PubMed: 19143030]

103. Madan RA, Gulley JL, Schlom J, Steinberg SM, Liewehr DJ, Dahut WL, et al. Analysis of overall survival in patients with nonmetastatic castration-resistant prostate cancer treated with vaccine, nilutamide, and combination therapy. Clin Cancer Res. 2008; 14:4526–31. [PubMed: 18628467]

104. Bilusic M, Gulley J, Heery C, Apolo A, Arlen P, Rauckhorst M, et al. A randomized phase II study of flutamide with or without PSA-TRICOM in nonmetastatic castration-resistant prostate cancer (CRPC). J Clin Oncol. 2011; 29(7S):163. abstract.

105. Yokokawa J, Cereda V, Remondo C, Gulley JL, Arlen PM, Schlom J, et al. Enhanced functionality of CD4+CD25(high)FoxP3+ regulatory T cells in the peripheral blood of patients with prostate cancer. Clin Cancer Res. 2008; 14:1032–40. [PubMed: 18281535]

106. Gray A, Raff AB, Chiriva-Internati M, Chen SY, Kast WM. A paradigm shift in therapeutic vaccination of cancer patients: the need to apply therapeutic vaccination strategies in the preventive setting. Immunol Rev. 2008; 222:316–27. [PubMed: 18364011]

107. Garcia-Hernandez, MdeL; Gray, A.; Hubby, B.; Klinger, OJ.; Kast, WM. Prostate stem cell antigen vaccination induces a long-term protective immune response against prostate cancer in the absence of autoimmunity. Cancer Res. 2008; 68:861–9. [PubMed: 18245488]

108. Gray A, Yan L, Kast WM. Prevention is better than cure: the case for clinical trials of therapeutic cancer vaccines in the prophylactic setting. Mol Interv. 2010; 10:197–203. [PubMed: 20729485]

109. Gray A, de la Luz Garcia-Hernandez M, van West M, Kanodia S, Hubby B, Kast WM. Prostate cancer immunotherapy yields superior long-term survival in TRAMP mice when administered at an early stage of carcinogenesis prior to the establishment of tumor-associated immunosuppression at later stages. Vaccine. 2009; 27(Suppl 6):G52–9. [PubMed: 20006141]

110. Kudo-Saito C, Schlom J, Hodge JW. Induction of an antigen cascade by diversified subcutaneous/intratumoral vaccination is associated with antitumor responses. Clin Cancer Res. 2005; 11:2416–26. [PubMed: 15788693]

111. Kudo-Saito C, Schlom J, Hodge JW. Intratumoral vaccination and diversified subcutaneous/intratumoral vaccination with recombinant poxviruses encoding a tumor antigen and multiple costimulatory molecules. Clin Cancer Res. 2004; 10:1090–9. [PubMed: 14871989]

112. Heery C, Pinto P, Schlom J, Tsang K, Madan R, Poole D, et al. Intraprostatic vaccine administration in patients with locally recurrent prostate cancer. J Clin Oncol. 2011; 29(7S):141. abstract.
113. Ferro MA, Gillatt D, Symes MO, Smith PJ. High-dose intravenous estrogen therapy in advanced prostatic carcinoma. Use of serum prostate-specific antigen to monitor response. Urology. 1989; 34:134–8. [PubMed: 2476882]
114. Figg WD, Ammerman K, Patronas N, Steinberg SM, Walls RG, Dawson N, et al. Lack of correlation between prostate-specific antigen and the presence of measurable soft tissue metastases in hormone-refractory prostate cancer. Cancer Invest. 1996; 14:513–7. [PubMed: 8951355]
115. Smith DC, Dunn RL, Strawderman MS, Pienta KJ. Change in serum prostate-specific antigen as a marker of response to cytotoxic therapy for hormone-refractory prostate cancer. J Clin Oncol. 1998; 16:1835–43. [PubMed: 9586898]
116. Kelly WK, Scher HI, Mazumdar M, Vlamis V, Schwartz M, Fossa SD. Prostate-specific antigen as a measure of disease outcome in metastatic hormone-refractory prostate cancer. J Clin Oncol. 1993; 11:607–15. [PubMed: 7683043]
117. Scher HI, Kelly WM, Zhang ZF, Ouyang P, Sun M, Schwartz M, et al. Post-therapy serum prostate-specific antigen level and survival in patients with androgen-independent prostate cancer. J Natl Cancer Inst. 1999; 91:244–51. [PubMed: 10037102]
118. Stein WD, Yang J, Bates SE, Fojo T. Bevacizumab reduces the growth rate constants of renal carcinomas: a novel algorithm suggests early discontinuation of bevacizumab resulted in a lack of survival advantage. Oncologist. 2008; 13:1055–62. [PubMed: 18827177]
119. Stein WD, Figg WD, Dahut W, Stein AD, Hoshen MB, Price D, et al. Tumor growth rates derived from data for patients in a clinical trial correlate strongly with patient survival: a novel strategy for evaluation of clinical trial data. Oncologist. 2008; 13:1046–54. [PubMed: 18838440]
120. Stein WD, Gulley JL, Schlom J, Madan RA, Dahut W, Figg WD, et al. Tumor regression and growth rates determined in five intramural NCI prostate cancer trials: the growth rate constant as an indicator of therapeutic efficacy. Clin Cancer Res. 2011; 17:907–17. [PubMed: 21106727]
121. Tang PA, Bentzen SM, Chen EX, Siu LL. Surrogate end points for median overall survival in metastatic colorectal cancer: literature-based analysis from 39 randomized controlled trials of first-line chemotherapy. J Clin Oncol. 2007; 25:4562–8. [PubMed: 17876010]
122. Panageas KS, Ben-Porat L, Dickler MN, Chapman PB, Schrag D. When you look matters: the effect of assessment schedule on progression-free survival. J Natl Cancer Inst. 2007; 99:428–32. [PubMed: 17374832]
123. Therasse P, Eisenhauer EA, Verweij J. RECIST revisited: a review of validation studies on tumour assessment. Eur J Cancer. 2006; 42:1031–9. [PubMed: 16616487]
124. Schlom J, Arlen PM, Gulley JL. Cancer vaccines: moving beyond current paradigms. Clin Cancer Res. 2007; 13:3776–82. [PubMed: 17606707]
125. Madan RA, Gulley JL, Fojo T, Dahut WL. Therapeutic cancer vaccines in prostate cancer: the paradox of improved survival without changes in time to progression. Oncologist. 2010; 15:969–75. [PubMed: 20798195]
126. Hoos, A. Proposal of a clinical development paradigm for cancer immunotherapy: novel endpoints. Presented at ASCO 2008 Annual Meeting; June 2; Chicago, IL. Jun. 2011 Available from: http://www.asco.org/ascov2/MultiMedia/Virtual+Meeting?&vmview=vm_session_presentations_view&confID=55&sessionID=2624
127. Fine GD. Consequences of delayed treatment effects on analysis of time-to-event endpoints. Drug Inf J. 2007; 41:535–9.
128. Sposto R, Stablein D, Carter-Campbell S. A partially grouped logrank test. Stat Med. 1997; 16:695–704. [PubMed: 9131757]
129. Therasse P, Arbuck SG, Eisenhauer EA, Wanders J, Kaplan RS, Rubinstein L, et al. New guidelines to evaluate the response to treatment in solid tumors. European Organization for Research and Treatment of Cancer, National Cancer Institute of the United States, National Cancer Institute of Canada. J Natl Cancer Inst. 2000; 92:205–16. [PubMed: 10655437]

130. Gulley JL, Arlen PM, Tsang KY, Yokokawa J, Palena C, Poole DJ, et al. Pilot study of vaccination with recombinant CEA-MUC-1-TRICOM poxviral-based vaccines in patients with metastatic carcinoma. Clin Cancer Res. 2008; 14:3060–9. [PubMed: 18483372]

131. Jones RL, Cunningham D, Cook G, Ell PJ. Tumour vaccine associated lymphadenopathy and false positive positron emission tomography scan changes. Br J Radiol. 2004; 77:74–5. [PubMed: 14988145]

132. Loveland BE, Zhao A, White S, Gan H, Hamilton K, Xing PX, et al. Mannan-MUC1-pulsed dendritic cell immunotherapy: a phase I trial in patients with adenocarcinoma. Clin Cancer Res. 2006; 12:869–77. [PubMed: 16467101]

133. Saenger YM, Wolchok JD. The heterogeneity of the kinetics of response to ipilimumab in metastatic melanoma: patient cases. Cancer Immun. 2008; 8:1. [PubMed: 18198818]

134. Wolchok JD, Hoos A, O'Day S, Weber JS, Hamid O, Lebbe C, et al. Guidelines for the evaluation of immune therapy activity in solid tumors: immune-related response criteria. Clin Cancer Res. 2009; 15:7412–20. [PubMed: 19934295]

135. Czerkinsky C, Andersson G, Ekre HP, Nilsson LA, Klareskog L, Ouchterlony O. Reverse ELISPOT assay for clonal analysis of cytokine production. I. Enumeration of gamma-interferon-secreting cells. J Immunol Methods. 1988; 110:29–36. [PubMed: 3131436]

136. Ryan JE, Ovsyannikova IG, Dhiman N, Pinsky NA, Vierkant RA, Jacobson RM, et al. Inter-operator variation in ELISPOT analysis of measles virus-specific IFN-gamma-secreting T cells. Scand J Clin Lab Invest. 2005; 65:681–9. [PubMed: 16319042]

137. Cox JH, Ferrari G, Kalams SA, Lopaczynski W, Oden N, D'Souza MP. Results of an ELISPOT proficiency panel conducted in 11 laboratories participating in international human immunodeficiency virus type 1 vaccine trials. AIDS Res Hum Retroviruses. 2005; 21:68–81. [PubMed: 15665646]

138. Hernandez-Fuentes MP, Warrens AN, Lechler RI. Immunologic monitoring. Immunol Rev. 2003; 196:247–64. [PubMed: 14617209]

139. Scher HI, Heller G, Molina A, Kheoh T, Attard G, Moreira J, et al. Evaluation of circulating tumor cell (CTC) enumeration as an efficacy response biomarker of overall survival (OS) in metastatic castration-resistant prostate cancer (mCRPC): Planned final analysis (FA) of COU-AA-301, a randomized double-blind, placebo-controlled phase III study of abiraterone acetate (AA) plus low-dose prednisone (P) post docetaxel. J Clin Oncol. 2011; 29:LBA4517. abstract.

140. Sahin U, Tureci O, Schmitt H, Cochlovius B, Johannes T, Schmits R, et al. Human neoplasms elicit multiple specific immune responses in the autologous host. Proc Natl Acad Sci U S A. 1995; 92:11810–3. [PubMed: 8524854]

141. de Bono J, Ashworth A. Translating cancer research into targeted therapeutics. Nature. 2010; 467:543–9. [PubMed: 20882008]

142. Cheever MA, Schlom J, Weiner LM, Lyerly HK, Disis ML, Greenwood A, et al. Translational Research Working Group developmental pathway for immune response modifiers. Clin Cancer Res. 2008; 14:5692–9. [PubMed: 18794077]

143. Britten CM, Janetzki S, van der Burg SH, Gouttefangeas C, Hoos A. Toward the harmonization of immune monitoring in clinical trials: quo vadis? Cancer Immunol Immunother. 2008; 57:285–8. [PubMed: 17721782]

144. Hoos A, Parmiani G, Hege K, Sznol M, Loibner H, Eggermont A, et al. A clinical development paradigm for cancer vaccines and related biologics. J Immunother. 2007; 30:1–15. [PubMed: 17198079]

145. Janetzki S, Panageas KS, Ben-Porat L, Boyer J, Britten CM, Clay TM, et al. Results and harmonization guidelines from two large-scale international ELISPOT proficiency panels conducted by the Cancer Vaccine Consortium (CVC/SVI). Cancer Immunol Immunother. 2008; 57:303–15. [PubMed: 17721781]

146. Boehm AL, Higgins J, Franzusoff A, Schlom J, Hodge JW. Concurrent vaccination with two distinct vaccine platforms targeting the same antigen generates phenotypically and functionally distinct T-cell populations. Cancer Immunol Immunother. 2010; 59:397–408. [PubMed: 19756595]

147. Rappuoli R, Aderem A. A 2020 vision for vaccines against HIV, tuberculosis and malaria. Nature. 2011; 473:463–9. [PubMed: 21614073]

148. Phase 3 Study of Immunotherapy to Treat Advanced Prostate Cancer. Apr. 2011 Available from: http://www.clinicaltrials.gov/ct2/show/NCT01057810?term=NCT01057810&rank=1

149. Study of Immunotherapy to Treat Advanced Prostate Cancer. Apr. 2011 Available from: http://www.clinicaltrials.gov/ct2/show/NCT00861614?term=NCT00861614&rank=1

Table 1

Targets of therapeutic prostate cancer vacciness

Target antigen	Characteristics	Expression
PSA (prostate-specific antigen)	238 AA serine protease enzyme	prostate and semen
PSMA (prostate-specific membrane antigen)	238 AA serine protease enzyme	Prostate and neovasculature of other solid tumors; overexpressed in PCa
PAP (prostatic acid phosphatase)	386 AA secreted glycoprotein	Widely expressed in many nonprostatic cells (lung, kidney, brain, prostate)
TARP (T-cell receptor gamma chain alternate reading frame protein)	58 AA protein	Normal prostate, prostate, and breast Ca
NGEP (new gene expressed in prostate)	179 AA protein	Prostate only; overexpressed in PCa
PSCA (prostate stem cell antigen)	123 AA membrane glycoprotein	Prostate, bladder, colon, kidney, stomach; overexpressed in PCa and bladder Ca
STEAP (six-transmembrane epithelial antigen of the prostate)	339 AA protein	Prostate only; overexpressed in PCa
AKAP-4 (A kinase [PRKA] anchor protein 4)	854 AA protein	Multiple myeloma, prostate, testes; over-expressed in PCa
MUC 1 (mucin 1)	1255 AA protein	Colon, breast, ovarian, lung, pancreatic cancers and prostate, overexpressed in metastatic disease

AA = amino acid
Ca = cancer
PCa = prostate cancer

Table 2

Advantages and disadvantages of chemotherapy vs. active immunotherapy

	Immunotherapy
Advantages	Minimal toxicity *
	Indirect, immune-mediated antitumor effect.
	May work best in patients with minimal tumor burden.
	May be effective after completion of treatment.
Disadvantages	Delayed effect; may take months.
	Unlikely to relieve symptoms.
	No intermediate markers of benefit.
	Response assessment unclear (irRC in development).
	Chemotherapy
Advantages	Improved symptoms.
	Direct cytotoxic effect.
	Potential short-term benefit.
	Reliable response criteria (PFS, RECIST).
Disadvantages	Toxicity.
	Drug resistance may develop.
	Tumor growth may resume when therapy is discontinued.

* Immune-related toxicity can be seen with immune-checkpoint inhibitors like anti-CTLA4 monoclonal antibodies, however therapeutic cancer vaccines have minimal toxicity.

Table 3

Completed randomized trials of prostate cancer vaccines

Immunotherapy agent (trial)	Endpoint	# of patients	Outcome	Ref.
Sipuleucel-T (IMPACT)	OS	512	Prolonged OS (25.8 vs. 21.7 months for placebo ($P = 0.03$), no change in time to progression	(30)
Sipuleucel-T (D9901)	TTP	127	Prolonged OS (25.9 vs. 21.4 months for placebo ($P = 0.01$), no change in time to progression	(31)
PSA-TRICOM	TTP	125	Prolonged OS (25.1 vs. 16.6 months for placebo ($P = 0.0061$), no change in time to progression	(36)
GVAX (VITAL-1)	OS	626	No difference in median OS (20.7 vs. 21.7 months, $P = 0.78$)	(66)
GVAX (VITAL-2; prematurely terminated)	OS	408	OS shorter in docetaxel/vaccine arm vs. docetaxel/prednisone arm (12.2 vs. 14.1 months, $P = 0.0076$)	(65)

Table 4

Ongoing randomized phase III trials of prostate cancer immunotherapy

Immunotherapy agent (trial)	# of patients	Primary endpoint	Design	Ref.
Ipilimumab	600	OS	Chemotherapy-naive; ipilimumab 10 mg/kg IV or placebo	(148)
PSA-TRICOM	1200	OS	Chemotherapy-naive; vaccine alone or vaccine with adjuvant doses of GM-CSF	(46)
Ipilimumab	800	OS	Post-docetaxel; ipilimumab 10 mg/kg IV or placebo	(149)