Published in final edited form as:
Expert Opin Investig Drugs. 2009 July ; 18(7): 1001–1011. doi:10.1517/13543780902997928.

Prostvac-VF: a vector-based vaccine targeting PSA in prostate cancer

Ravi A. Madan, **Philip M. Arlen**, **Mahsa Mohebtash**, **James W. Hodge**, and **James L. Gulley**[*]
Laboratory of Tumor Immunology and Biology, National Cancer Institute, National Institutes of Health, Bethesda, MD, USA

Abstract

Prostvac is a prostate cancer vaccine regimen consisting of a recombinant vaccinia vector as a primary vaccination, followed by multiple booster vaccinations employing a recombinant fowlpox vector. Both vectors contain the transgenes for prostate-specific antigen (PSA) and multiple T-cell costimulatory molecules (TRICOM). The PSA-TRICOM vaccines infect antigen-presenting cells (APCs) and generate proteins that are expressed on the surface of the APCs in an immune context. The interaction of these APCs with T cells initiates a targeted immune response and T cell-mediated tumor cell destruction. Preliminary clinical trials have indicated negligible toxicity and determined the optimal dosing schedule. In addition, these trials indicate that Prostvac may hold promise in the treatment of prostate cancer. Phase II trials have shown a survival benefit after treatment with Prostvac, especially in patients with indolent disease characteristics. Preclinical and clinical data indicate that radiation, hormonal therapy, and chemotherapy may be combined with Prostvac to enhance the vaccine's efficacy. Additional strategies are in development to further enhance the clinical benefits of Prostvac, and a phase III trial is being planned in metastatic castration-resistant prostate cancer.

Keywords

prostate cancer; therapeutic cancer vaccine; immunotherapy

1. Introduction

The last 2 decades have seen great advances in the treatment of cancer, beginning with monoclonal antibody (mAb)-based treatments such as rituximab and trastuzumab [1–3]. Shortly thereafter, imatinib mesylate ushered in targeted molecular therapies, and numerous similar agents are currently part of clinical practice or clinical trials [4]. Prostate cancer treatment has also made strides in the last decade. In 2004, the U.S. Food and Drug Administration (FDA) approved docetaxel, the first, and as yet only, chemotherapeutic agent demonstrating improved overall survival in metastatic castration-resistant prostate cancer (CRPC) [5, 6]. As with other malignancies, many molecular targeted agents have been investigated in prostate cancer, although none has been approved to date. However, one targeted treatment strategy that has shown promise in prostate cancer is the use of therapeutic cancer vaccines. One if these is Prostvac (developed by the National Cancer Institute and licensed to BN Immunotherapeutics, Mountain View, CA), which has been investigated in both early- (castration-sensitive) and late-stage (castration-resistant) disease.

[*]**Author for correspondence:** James L. Gulley, Laboratory of Tumor Immunology and Biology, Center for Cancer Research, National Cancer Institute, National Institutes of Health, 10 Center Drive, Room 8B09, Bethesda, MD 20892. Phone: (301) 435-2956; Fax: (301) 480-5094; gulleyj@mail.nih.gov.

2. Rationale for use of vaccines in prostate cancer

There may be several reasons why therapeutic cancer vaccines have shown more promise in prostate cancer than in other tumor types. Prostate cancer tumors grow more slowly than most other malignancies, potentially allowing more time to generate a targeted immune response and yield a clinical benefit [7]. Furthermore, prostate-specific antigen (PSA), a unique marker of recurrent or early-stage prostate cancer, allows for the detection of minimal tumor volume before it is evident on imaging studies [8]; moreover, patients with low tumor volume may offer the best setting for the use of cancer vaccines. Finally, and perhaps most critically, prostate cancer generates unique gene products that are potential targets for immunotherapy [9, 10]. In many patients with prostate cancer, cytolytic T cells mount a weak response to these tumor-associated antigens (TAAs), with levels of immune recognition that are clinically insignificant and do not affect tumor growth. The goal of therapeutic cancer vaccines is to enhance immune recognition so that T cells can target these prostate-specific TAAs, leading to tumor-specific T cell-mediated destruction [11].

Prostate cancer produces several unique TAAs that are overexpressed in the prostate (a nonessential organ) and prostate cancer cells. PSA is not merely a serum marker of disease in patients with prostate cancer. The 34-kD protein is distinctively expressed in virtually all prostate cancer cells and nonessential epithelial cells within the prostate, making it a potential target for many prostate cancer vaccines [12, 13]. As an added benefit, tumor cell lysis generated by a vaccine that targets PSA may expose the immune system to additional TAAs, such as prostate-specific membrane antigen, prostatic acid phosphatase, and MUC-1, leading to an immune response against TAAs not targeted specifically by the vaccine. This phenomenon, called antigen spreading or antigen cascade, ultimately results in the immune targeting of tumor cells via multiple TAAs [14, 15].

3. Development of poxviral vaccines and TRICOM

Recombinant poxviruses are developed by the insertion of a recombinant plasmid containing the transgenes that will code for selected proteins, in this case TAAs. Upon infection, the viruses then transfer the plasmid into a permissive eukaryotic cell line. Next, the cell line is reinfected by wild-type poxviruses, and as they reproduce within the cell, a small proportion of poxviruses will contain the recombinant plasmid. Expression of markers inserted into the plasmid identifies which viruses contain the recombinant plasmid. These particular poxviruses are then selected and amplified for use as a cancer vaccine [16].

Many advantageous characteristics of poxviruses have led to their use as a delivery mechanism for cancer vaccines (Table 1). The presence of viral proteins at the injection site causes an inflammatory response, drawing APCs to the injection site where they can be infected [17, 18]. The large poxviral genome allows for the inclusion of costimulatory molecules, in addition to TAAs, which enhance the efficacy of APCs [16, 19–21]. Vaccinia has been safely administered to over a billion people in the form of smallpox vaccines. Furthermore, there is no risk of disrupting host DNA as all poxviral replication occurs in the cell cytoplasm.

The goal of therapeutic vaccines is to generate an immune response to weakly immunogenic TAAs such as PSA. Poxviruses, which serve as a vehicle for vaccine delivery, have high infectivity rates and can infect APCs when injected into subcutaneous tissue. Once delivered inside the APC, transgenes for TAAs are then processed and expressed by the APC within the major histocompatibility complex (MHC), leading to T-cell activation.

Several important preclinical studies of poxviral vaccines targeting PSA illustrated methods for augmenting the vaccines' clinical efficacy. Initial development of the vaccine required

enhancement of the PSA peptide. *In vivo*, cytolytic T cells must recognize peptides bound to MHCs in order to become activated and initiate a targeted immune response. Computer imaging analysis and investigational peptide binding data have previously demonstrated this phenomenon [22, 23]. The alteration of even a single amino acid in a peptide has the potential to enhance T cell-MHC interaction. The more stable and thermodynamically favorable this interface is, the greater the likelihood that a T cell will bind to the MHC and initiate a tumor-specific immune response [24]. Early preclinical studies led to slight alterations in the PSA peptide to optimize T cell-MHC interactions and the subsequent immune response [25, 26].

Another vital factor in initiating an immune response is the interaction of costimulatory molecules on the surface of APCs with their corresponding ligands on T cells. This costimulation is of greater consequence when the antigen involved is a weakly immuogenic TAA such as PSA. An interaction that lacks costimulation could lead to T-cell anergy or apoptosis [27]. Although early poxviral vaccines targeting PSA did not contain costimulatory molecules, recombinant vaccinia (rV) containing the transgene for costimulatory molecule B7.1 (rV-B7.1) was often admixed with the priming dose of recombinant vaccinia-PSA (rV-PSA). When the next generation of poxviral vaccines was developed, however, a triad of costimulatory molecules called TRICOM, which included the transgenes for the costimulatory molecules B7.1, leukocyte function-associated antigen-3 (LFA-3), and intercellular adhesion molecule-1 (ICAM-1), were included. *In vitro* and *in vivo* studies have shown that TRICOM significantly enhances T-cell activation relative to just 1 or 2 costimulatory molecules [28–30]. Further benefits of TRICOM include enhancement of T-cell avidity, which ultimately leads to enhanced T cell-mediated tumor-cell killing [31–33]. Costimulation with TRICOM also increases the number of antigen-specific memory T cells, which may help sustain the immune response [34].

Many therapeutic cancer vaccines employ immune adjuvants to augment immune response. Preclinical studies demonstrated that GM-CSF at the inoculation site stimulates APCs and enhances the immune response to weakly immunogenic self-antigens [35]. Furthermore, administering GM-CSF to the vaccination site in combination with TRICOM-based vaccines resulted in enhanced immune response [29]. For this reason, GM-CSF was used as an adjuvant for most early trials involving poxviral vaccines. However, recent trial results may lead to a re-evaluation of its role as a vaccine adjuvant [36, 37].

4. Preliminary clinical studies demonstrate safety, efficacy, and optimal dosing schedule of vector-based prostate cancer vaccines

Initial poxviral-based vaccines contained only the transgene for PSA, with no costimulatory molecules. A phase I study of rV-PSA enrolled 42 patients with metastatic CRPC at 5 escalating dose levels; treatments were administered monthly for 3 months. GM-CSF was given as an immune adjuvant at the vaccination site in an extension phase of the study. The vaccine was well tolerated and no dose-limiting toxicity was seen. Common side effects included self-limited fever, fatigue, and injection-site reactions. Three of 5 evaluable patients were found to have increases in PSA-specific T cells, demonstrating the effects of the vaccine on the immune system [38].

Another early study evaluated the dosing sequence of vaccinia- and fowlpox-based vaccines. Although vaccinia is highly immunogenic and induces a vigorous immune response with a single vaccination, multiple exposures to vaccinia may lead to neutralization by host antibodies that target viral coat proteins and reduce efficacy [38–40]. Fowlpox-based vectors were thus studied as a platform for booster vaccinations to sustain the immune response after an initial prime with a vaccinia-based vaccine. Fowlpox can infect APCs, but in

mammalian cells it produces no late viral gene products, including viral coat proteins. Therefore, fowlpox does not elicit significant quantities of neutralizing antibodies from the host immune system [41, 42]. After initial proof of concept studies in preclinical models, phase I studies provided preliminary validation [42, 43]. Ultimately, a trial in prostate cancer patients demonstrated that an rV-PSA prime followed by recombinant fowlpox (rF)-PSA boosts resulted in increased time to PSA progression compared to patients treated with fowlpox before vaccinia or fowlpox alone [44, 45].

The prostate cancer vaccine Prostvac uses both vaccinia- and fowlpox-based platforms in a prime-and-boost strategy. Prostvac contains the transgenes for PSA and 3 human T-cell costimulatory molecules (TRICOM) with each dose. An initial priming dose of rV-PSA-TRICOM is followed by monthly boosts of rF-PSA-TRICOM. This vaccine platform has been investigated extensively in clinical trials (Table 2). A phase I study in patients with metastatic CRPC demonstrated that a recombinant poxviral vaccine targeting PSA and containing TRICOM was well tolerated. Furthermore, evaluable patients treated with Prostvac in the phase I study had increases in PSA-specific T cells, and 9 of 15 patients had decreases in PSA velocity after vaccination [46].

A phase II Eastern Oncology Cooperative Group trial evaluated Prostvac in patients with prostate cancer recurrence but no metastasis (D0 prostate cancer) [47]. Patients were treated with an initial dose of rV-PSA-TRICOM and then 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; 66% of those patients had a > 6-month PSA progression-free survival. In addition, the median on-study PSA doubling time increased from 4.4 months to 7.7 months ($P = 0.002$), suggesting that the vaccine may delay disease progression.

Two phase II studies have demonstrated the clinical potential of Prostvac in patients with metastatic CRPC. In an industry-sponsored phase II trial, 125 patients with metastatic CRPC and Gleason scores of ≤ 7 were randomized to receive either Prostvac or placebo. Patients treated with vaccine were given a priming dose of rV-PSA-TRICOM and monthly boosts of rF-PSA-TRICOM. Patients in the control group were given monthly subcutaneous injections of fowlpox which, as an empty vector, induced no focused immune response. The study's primary endpoint was time to disease progression, as determined by new or significantly enlarging lesions observed on routine imaging. Although the study did not meet its primary endpoint, a survival advantage ultimately became apparent. Initial analysis showed a median overall survival of 24.4 months for patients treated with the vaccine, compared to 16.3 months for patients in the control arm. This suggests that, even though there was no benefit in terms of time to progression, there was a long-term benefit for patients treated with Prostvac. A final analysis confirmed this survival advantage, demonstrating an 8.5-month increase in overall survival among patients treated with Prostvac over patients treated with placebo ($P = 0.015$) [48].

This phenomenon of no decrease in time to progression but an increase in overall survival was also observed in a phase III trial of the APC-based vaccine sipuleucel-T (Dendreon Corp., Seattle, WA) in metastatic CRPC patients. Study participants were randomized 2:1 in favor of sipuleucel-T in this placebo-controlled trial. Crossover was permitted after 8 weeks if there was clinical or symptomatic progression (new lesions on imaging or increased pain). The vaccine was administered at weeks 0, 2, and 4 [49, 50]. Like the phase II trial of Prostvac, this trial failed to meet its primary endpoint of decreased time to progression, although progression favored patients randomized to the sipuleucel-T arm (16.6 weeks vs. 10 weeks; $P = 0.052$). And again, an overall survival benefit was seen in patients randomized to the vaccine arm (25.9 months vs. 21.4 months in the placebo arm; $P = 0.01$).

Analysis at 36 months indicated a 34% overall survival in the vaccine arm compared to 11% in the placebo arm (P = 0.005) [49]. Both of these vaccine studies in metastatic CRPC illustrate potentially important considerations for future trials using vaccine alone. First, vaccines may improve overall survival without showing a benefit in time to progression, and second, future trials should use overall survival as an endpoint.

A smaller trial of Prostvac at the National Cancer Institute (NCI), conducted in 32 patients with metastatic CRPC, provided evidence of immune response in vaccinated patients as well as insight into the type of patients best suited for treatment with vaccines. 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). Immune analysis of 29 evaluable patients indicated a > 2-fold increase in PSA-specific T cells in 13 patients, 5 of whom had a > 6-fold increase in PSA-specific T cells, associated with a trend to improved overall survival (P = 0.055). Median overall survival among all patients was 26.6 months [51]. Since all patients were treated with vaccine, overall survival was compared to predicted survival based on the Halabi nomogram, developed from survival outcomes of 1,101 patients with metastatic CRPC treated between 1991 and 2001 in CALGB clinical trials. The nomogram employs 7 baseline characteristics that assess disease volume and aggressiveness, and predicts survival time based on data from patients treated with chemotherapy or second-line hormonal manipulation [52]. For all patients in the NCI trial, the predicted survival was 17.4 months, compared to the actual observed median survival of 26.6 months. However, a more striking outcome was seen among patients with more indolent disease characteristics. Patients with a Halabi predicted survival of < 18 months showed no significant improvement after treatment with Prostvac (median survival: 14.6 months; Halabi predicted survival: 12.3 months). Patients with a Halabi predicted survival of ≥ 18 months had the greatest benefit, with a median overall survival that will meet or exceed 37.3 months, compared to a predicted survival of 20.9 months for patients treated with chemotherapy or second-line hormonal manipulation [51]. These data suggest that, in addition to using overall survival as an endpoint, future trials employing vaccine alone should be conducted primarily in patients with more indolent disease characteristics. Follow-up studies are currently in development to further evaluate this hypothesis.

5. Prostvac in combination with standard therapies

Along with surgery, radiation and hormonal therapy are fundamental to the treatment of early-stage prostate cancer [8]. Preclinical data and early clinical trials have demonstrated that both radiation and hormonal manipulation may enhance the efficacy of therapeutic cancer vaccines, and trials are currently underway employing both modalities in combination with Prostvac (Table 3).

Preclinical data indicate that nonlethal levels of radiation may induce phenotypic changes in tumor cells [53–56] and can upregulate gene expression of cell markers such as MHC, Fas, and TAAs, rendering tumor cells more susceptible to T cell-mediated immune attack [57–59]. Initial clinical data involving a first-generation poxviral vaccine provided a clinical proof of concept. Thirty patients with localized prostate cancer were treated with standard radiation therapy, and two thirds of these patients were randomized to receive vaccine as well. The vaccine utilized in this study was a priming dose of rV-PSA admixed with rV-B7.1, followed by monthly boosts of rF-PSA for a total of 8 vaccinations. Seventeen of 19 patients randomized to radiation plus vaccine had a ≥ 3-fold increase in PSA-specific T cells after radiation, compared to no change in T cells in patients treated with radiation alone (P = 0.0005) [14]. A follow-up study confirmed a similar magnitude of response in a similar proportion of patients [60].

These studies and several preclinical studies were the basis for an ongoing randomized phase II study of Samarium-153 (Sm-153), an FDA-approved agent for palliation of pain in metastatic cancer patients [54, 61, 62]. Sm-153 is composed of radioactive samarium and a tetraphosphate chelator that binds to metastatic lesions in bone, targeting low levels of radiation to sites of disease. This study is designed to evaluate whether Prostvac in combination with Sm-153 can improve time to progression compared to Sm-153 alone in patients with CRPC metastatic predominantly to bone. The study will also evaluate the ability of low-level local radiation to generate specific immunologic responses [63].

Hormonal therapy may have a similar benefit in combination with therapeutic cancer vaccines. A growing volume of preclinical data demonstrates that androgen-deprivation therapy (ADT) affects not only prostate tumors, but also the immune system [64]. Samples of both benign prostate tissue and prostate cancer indicate that ADT can traffic T cells to the prostate within 3 weeks of implementing therapy. Furthermore, in patients with prostate cancer, T cells that infiltrated prostate tissue had characteristics of a specific, restricted response, likely indicating a targeted response to TAAs [65]. Testosterone may affect T cells by reducing T-cell proliferation. One study showed that flutamide, an FDA-approved androgen receptor antagonist commonly used as a second-line hormonal agent, diminishes the antiproliferative effects of testosterone on T cells [66]. ADT may have broader effects on the immune system by enlarging the thymus gland (where the body produces T cells), enriching the T-cell repertoire, and minimizing immune tolerance to prostate TAAs, all of which could enhance the immune response to therapeutic cancer vaccines [67–69].

Early trials of poxviral vaccines targeting PSA in combination with hormonal therapy have also provided evidence of clinical benefit. Forty-two patients with nonmetastatic CRPC were randomized to treatment with either nilutamide, an FDA-approved androgen receptor antagonist, or poxviral vaccine. The vaccine strategy employed in this trial consisted of a priming vaccination of rV-PSA admixed with rV-B7.1, followed by monthly boosts of rF-PSA. The trial also had a crossover component where patients with rising PSA on either nilutamide or vaccine therapy alone, but no metatstaic disease on scans, could receive both treatments. Preliminary findings suggested improved clinical benefit with the combination therapy (especially when vaccine was started earlier in the disease process) and were confirmed in a recent overall survival analysis [70]. Among all patients, there was a trend to improved overall survival in patients randomized to initial treatment with vaccine compared to initial treatment with nilutamide (median 5.1 years vs. 3.4 years; $P = 0.13$). The trend was more pronounced in patients receiving the combination. For the 12 patients who received vaccine first then had nilutamide added, median overall survival was 6.2 years; for the 8 patients who received nilutamide first then had vaccine added, median overall survival was 3.7 years ($P = 0.045$). As in the NCI trial in metastatic CRPC, 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 advantages [71]. A trial is currently accruing to extend these findings. In the ongoing study, nonmetastatic CRPC patients are treated with either a combination of flutamide and Prostvac or flutamide alone. The primary endpoint of the study is time to progression, but immune parameters will also be evaluated [72].

For metastatic CRPC patients, hormonal therapy and radiation provide no overall survival benefit, and docetaxel is the standard of care [5, 6]. In spite of initial concerns that chemotherapy may blunt an immune response, preclinical and clinical data indicate that this is not the case. In fact, like radiation, some chemotherapeutic agents may increase expression of TAAs and MHC class I, potentially leading to enhanced vaccine-induced T-cell cytoxicity [73–76]. These direct cytotoxic effects may lead to an antigen cascade as the activated immune system is exposed to TAAs in addition to PSA, which can then be targeted as well [14, 15]. Docetaxel in particular has shown some promise for use in

combination with Prostvac. *In vitro* studies of docetaxel have demonstrated increases in proinflammatory cytokines [77]. Docetaxel also enhances T-cell responses to vaccine by augmenting immune responses to TAAs specifically targeted by the vaccine. Furthermore, recent preclinical data in a murine tumor treatment model indicate that docetaxel combined with vaccine is more effective than either docetaxel or vaccine alone at limiting tumor growth [15].

Clinical evidence also indicates that vaccine and docetaxel can be administered in combination without adversely affecting the immune response. A phase II trial in patients with metastatic CRPC combined docetaxel with Prostvac and compared the combination to Prostvac alone. After 3 months of treatment, the 2 groups showed equivalent immune responses as measured by PSA-specific T cells, thereby indicating that chemotherapy did not diminish immune response to Prostvac [78]. Additional trials combining Prostvac and chemotherapy either simultaneously or sequentially are currently in development.

Clinical trials have also evaluated Prostvac in combination with nonstandard agents such as anti-CTLA-4, an mAb that binds to the CTLA-4 molecule of the T cell, potentially enhancing cytolytic T-cell activity. As previously discussed, B7.1 is an important costimulatory molecule on the APC that binds to CD28 on an activated T cell. A short time after T-cell activation, however, the T cell expresses the CTLA-4 surface glycoprotein. CTLA-4 binds B7.1 with greater affinity than CD28. This interaction ultimately results in a diminution of the T cell's immune response, a process believed to be the body's own brake on the immune system [79, 80]. The immunoregulatory role of CTLA-4 is illustrated in CTLA-4 knockout mice, which cannot autoregulate their immune responses. They live only 2 to 3 weeks before succumbing to massive autoreactive T-cell infiltration of organs [81]. An mAb that could bind to CTLA-4 would block the CTLA-4-B7.1 interaction, thereby prolonging and enhancing the immune response after treatment with vaccine.

Clinical trials have suggested the efficacy of anti-CTLA-4 mAb in combination with a whole tumor cell vaccine in prostate cancer [82, 83]. A trial combining Prostvac with escalating doses of ipilimumab (Medarex, Princton, NJ), an anti-CTLA-4 mAb, has also been carried out. Although autoimmune side effects typical of a CTLA-4 blockade were seen, clinical benefits included declines in PSA in 14 of 30 patients, and increased PSA doubling time. From an immunologic standpoint, 5 of 9 evaluable patients had 2.5- to 5-fold increases in PSA-specific T cells [84]. This study provided preliminary evidence of the efficacy of this combination. Further study is warranted.

6. Expert opinion and conclusions

Oncologists today have only a limited arsenal for treating prostate cancer, a disease that annually ranks first in cancer diagnoses and second in cancer deaths for men in the United States [85]. Clearly, additional therapies are needed. Prostate cancer patients have not as yet enjoyed the therapeutic benefits of targeted molecular agents and mAbs, as have patients whose malignancies are more amenable to these newer treatments. This lack of treatment options is one reason that cancer vaccines are becoming an attractive therapeutic strategy for prostate cancer, along with the fact that the indolent nature of the disease makes it an ideal candidate for vaccine-mediated therapy. With many unique TAAs to target, several therapeutic vaccines have been developed whose benefit may be enhanced in combination with standard treatments for prostate cancer, including radiation, hormonal therapy, and chemotherapy.

As the clinical development of trastuzumab demonstrated, selection of a patient population with a likelihood of response is the best way to assess the clinical utility of any novel agent.

While appropriate patient selection is a logical step, the fact that 2 relatively large vaccine trials have shown a survival advantage in metastatic CRPC, with no benefit in time to progression, creates a dramatic paradigm shift in oncology treatment [48, 49, 86]. Future trials of vaccines, including Prostvac, in metastatic CRPC should enroll patients with more indolent disease characteristics and use overall survival as the endpoint. Vaccine-mediated therapy lacks the immediate cytotoxicity and subsequent reduction in tumor size seen with conventional chemotherapy or radiation. Instead, the immune system requires time to generate an immune response. During this interval, there may be initial tumor progression, followed by a period of stability as the activated immune system holds tumor growth in check. Given that chemotherapy trials in prostate cancer show response rates of ≤ 17%, it is unrealistic to expect significant response rates with a prostate cancer vaccine [5, 6]. Perhaps the most important component of this new paradigm is that patients may benefit from vaccine therapy even after treatment has been discontinued. Once the immune system is activated, subsequent treatments may be more effective due to this active, ongoing immune response, resulting in a potential *de facto* combination treatment. This phenomenon has been observed in several cancer vaccine trials, including trials in prostate cancer [87].

A phase III trial of Prostvac in metasatic CRPC is in the development stage. Current and planned randomized phase II trials will yield further information on combination therapies employing radiation and hormonal agents. Combined and sequential use of Prostvac with chemotherapy is supported by preclinical and clinical data, and new trials are being planned.

The benefits of Prostvac may be enhanced for all patients by manipulating regulatory T cells (Tregs) that modulate the body's immune response by maintaining a degree of self-tolerance [88–90]. Tregs constitute 5% to 10% of circulating $CD4^+$ T cells and can decrease activation and expansion of cytolytic T cells, potentially diminishing the effects of vaccine treatments [91, 92]. In addition, animal studies have correlated higher numbers and greater activity of Tregs with increased tumor size [93, 94]. Similar findings are seen in cancer patients, where higher numbers of Tregs correspond with poor outcome [95–98]. Thus, reducing the number of Tregs may enhance vaccine-induced, tumor-specific immune response [94, 99, 100]. Several agents have been investigated to selectively reduce Tregs, including low-dose cyclophosphamide and anti-CD25 mAbs [94, 96, 101, 102]. Future trials may focus on the combination of Prostvac with similar agents that reduce the effectiveness of Tregs or the immunosuppressive cytokines they produce, thereby mitigating the effects of larger tumor burden and broadening the potential utility of Prostvac.

Other novel vaccine combinations require further investigation. An intriguing combination includes sunitinib malate (Pfizer Inc., New York, NY), a tyrosine kinase inhibitor FDA-approved for the treatment of renal cell cancer. Preclinical studies have indicated that sunitinib malate may have benefit in the treatment of prostate cancer, and a phase II trial is ongoing [103, 104]. There is also preclinical evidence that this agent may reduce the efficacy of Tregs in cancer patients [105]. These early findings are encouraging and warrant further investigation.

Although clinical trials have yet to show their definitive efficacy, therapeutic cancer vaccines now show promise for the treatment of prostate cancer. In preliminary trials, Prostvac has delivered clinical benefit, especially for patients with more indolent disease characteristics. Vaccines have the potential to generate an effective antitumor immune response without the toxicity of chemotherapy and targeted molecular agents. Ultimately, well designed clinical trials will determine the role of Prostvac in the treatment of prostate cancer patients, who are in need of additional options.

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Table 1

Advantages of using poxviral vectors in therapeutic cancer vaccines.

- **Safety:** poxviruses have been used to vaccinate patients against smallpox for more than 50 years.
- **High rate of infectivity:** increases likelihood of infecting APCs.
- **Presence of viral proteins:** generates inflammatory response, attracting immune cells to vaccination site.
- **Large genome:** allows for expression of several transgenes, including TAAs and costimulatory molecules to enhance T-cell immunity.
- **Cytoplasmic replication:** poses no risk of inducing a mutation through incorporation into human DNA.

Table 2

Pivotal clinical trials of Prostvac.

Type of trial	n	Results	Ref
Randomized phase II	64	Confirmed vaccinia prime and fowlpox boost strategy in administration of poxviral-based vaccines.	[44, 45]
Phase I	15	Confirmed safety of Prostvac; showed evidence of immune response and decreased PSA velocity.	[46]
Phase II	69	For 29 evaluable patients with castration-sensitive, non-metastatic disease, there was an increase in PSA doubling time from 4.4 to 7.7 months (P = 0.002). In addition, 66% had PSA progression-free survival at 6 months.	[47]
Phase II	32	Demonstrated immune response in 13 of 29 evaluable metastatic CRPC patients treated with Prostvac. Patients with indolent disease characteristics had substantially greater survival than predicted on chemotherapy and second-line hormonal therapy.	[51]
Randomized phase II	125	Showed no change in time to progression, but demonstrated significant survival advantage in metastatic CRPC patients treated with Prostvac.	[48]
Randomized phase II	28	In metastatic CRPC, demonstrated equivalent immune response in patients treated with Prostvac and patients treated with Prostvac and docetaxel.	[78]

CRPC: castration-resistant prostate cancer.

Table 3

Rationale for combining Prostvac with other cancer therapies.

Modality	Proposed mechanism	
Radiation therapy	-	Phenotypic alterations enhance T cell-mediated killing.
	-	Increased expression of MHC, Fas, and TAAs.
Hormonal therapy	-	Traffics T cells to prostate.
	-	Minimizes antiproliferative effects of testosterone on T cells.
	-	Enriches T-cell repertoire.
	-	Decreases immune tolerance to TAAs.
Chemotherapy	-	Cytolysis of tumor cells exposes activated immune system to new TAAs, which can then be targeted.
	-	Increases proinflammatory cytokines (docetaxel).
	-	Selectively reduces Tregs (cyclophosphamide).
anti-CTLA-4 antibody	-	Binds to CTLA-4 surface glycoprotein on T-cell surface, minimizing immune autoregulation and potentially enhancing antitumor activity.

MHC – major histocompatibility complex
TAAs – tumor-associated antigens
Tregs – regulatory T cells