Therapeutic Vaccines for Prostate Cancer

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LEARNING OBJECTIVES
After completing this course, the reader will be able to:
1. List the different types of vaccines being studied clinically in prostate cancer.
2. Explain the basic concepts of generating an active immune response.
3. Discuss the clinical trials with prostate cancer vaccines including immunologic and clinical responses.

ABSTRACT
Prostate cancer is the most common, noncutaneous cancer for men in the U.S., leading to more than 30,000 deaths a year. Vaccines for prostate cancer, which for several years have been shown to generate immunologic responses, are beginning to show significant clinical promise. At present, numerous therapeutic options are being investigated, including autologous and allogeneic whole-tumor cell vaccines, dendritic cell vaccines, and poxvirus-based vaccines. Advances in basic immunology have translated into new, more complex therapeutic strategies. The findings from current trials and the demonstrated potential to combine vaccines with conventional therapies herald a promising future for the treatment of prostate cancer. This review highlights recent advances and clinical trials in immunotherapy for prostate cancer, along with current thoughts on immunologic and clinical monitoring of these trials. The Oncologist 2006;11:451–462

INTRODUCTION
It was estimated that 232,090 men would be diagnosed with prostate cancer and 30,350 would die from the disease during 2005 [1]. Prostate cancer is the most common noncutaneous cancer, and new cases now account for a full third of all male cancer diagnoses and 10% of all deaths [1]. Increased cancer screening with prostate-specific antigen (PSA) is in part responsible for the increased localized staging of disease at the time of diagnosis. For localized prostate cancer, treatment typically includes radical prostatectomy, external-beam radiation therapy (EBRT), brachytherapy, or watchful waiting. Unfortunately, up to 30%–40% of patients fail local therapy [2–4]. PSA monitoring following primary therapy is useful in detecting disease recurrence before the development of overt metastatic disease. In a study of almost 2,000 patients, albeit most with good to intermediate risk disease, the average time to development of metastatic disease for patients with a rising PSA level following radical prostatectomy was 8 years [5]. The standard of care for...
patients failing primary therapy is androgen-deprivation therapy (ADT). The majority of patients eventually become hormone refractory; however, the time in which androgen-insensitive clones emerge may range from months to years from initiation of ADT. Since the early 1990s, with the advent of routine monitoring of patients using PSA following local therapy, patients have often been treated with ADT prior to the onset of clinical symptoms. This has led to patients eventually developing rising PSA levels with castrate levels of testosterone, often with a relatively low tumor burden. This systemic treatment earlier in the disease course combined with effective palliative chemotherapy is implicated in the improvement in median survival time of patients with androgen-independent prostate cancer (AIPC) from an average of about 12 months to about 17–18 months [6, 7]. Because there is no standard treatment for patients failing chemotherapy, development of vaccine strategies designed to break tolerance and generate a sustained potent immune response against prostate cancer represents a novel therapeutic approach. Thus far, several different approaches to prostate cancer immunotherapy have been investigated. In this review, dendritic cell (DC), whole-tumor cell, and poxvirus vaccines as well as antibody therapy are examined (Table 1).

**DC Vaccines**

DCs are highly proficient antigen-presenting cells (APCs) that localize to multiple epithelial sites including the skin, gut, lung, and genitourinary tract [8]. Since their initial discovery more than 30 years ago, DCs have been widely studied and are currently being used in numerous cancer vaccine trials [9–12]. The appeal of DCs in vaccine therapy is attributable to the presence of both class I and class II major histocompatibility complexes (MHCs), which in contrast to other APCs, allows them to be capable of stimulating both naïve helper (CD4⁰) and cytotoxic (CD8⁰) T lymphocytes (CTLs) (Fig. 1), as well as B cells through other pathways [13, 14]. DCs are derived from the bone marrow but subsequently migrate to epithelia to await invading pathogens. Following exposure to antigens, DCs undergo maturation and migrate to regional lymph nodes. During maturation, antigen is processed and presented on both classes of MHC in a process known as cross priming [15, 16]. Several strategies have thus far been employed using DCs for cancer vaccines, including peptide-, mRNA-, and DNA-based approaches.

mRNA-pulsed DCs have been successful in preclinical animal studies [17, 18]. In a clinical trial involving patients with metastatic prostate cancer \( n = 20 \), vaccinations consisted of three or six weekly intradermal (i.d.) injections of \( 1 \times 10^7 \) DCs pulsed with telomerase reverse transcriptase (hTERT) mRNA with or without lysosome-associated membrane protein-1 (LAMP) mRNA [19]. The vaccines were capable of inducing expansion of hTERT-specific CTLs in 19 of 20 patients. Transfection of DCs was accomplished via electroporation. The vaccines were well tolerated and toxicities were limited to grade 1. Both vaccines induced CD8⁰ and CD4⁰ T-cell responses; however, patients receiving LAMP hTERT-transfected DCs \( n = 9 \) generated a significantly greater hTERT-specific CD4⁰ T-cell response than patients \( n = 11 \) receiving hTERT alone \( (p = .004) \). Furthermore, CTLs generated from patients receiving the LAMP hTERT vaccine killed tumor cells more proficiently than CTLs from patients receiving the hTERT vaccine. In five of six evaluated patients, transient clearance of PSA-expressing circulating tumor cells was observed. Additionally, a short-term improvement in PSA

| Table 1. Comparison of selected modalities of immunotherapy for prostate cancer |
|------------------|------------------|------------------|------------------|------------------|------------------|
| **Pros**          | **Cons**          | **Pros**          | **Cons**          | **Pros**          | **Cons**          |
| DC-based vaccine⁸ | • Good antigen-presenting cell | • Requirement for apheresis/in vitro manipulation (increased cost and time) | • Multiple tumor-associated antigens | • Technology may not be available in all areas |
|                   | • Generates active immune response | • Technology may not be available in all areas | • Generates active immune response | • Regulatory hurdles with individualized treatment (potency of lots, etc.) |
| Whole-tumor cell vaccine⁹ | • Multiple tumor-associated antigens | • Generating active immune response | • Difficult to monitor specific immunologic responses to vaccine | • Can add other immunostimulatory genes (cytokines, costimulatory molecules) |
|                   | • Generates active immune response | • Generating active immune response | • Multiple antigens may compete for strong immune response | • Can add other immunostimulatory genes (cytokines, costimulatory molecules) |
| Vector-based vaccines⁴ | • Generates active immune response | • Generating active immune response | • Limited number of tumor-associated antigens | • Can add other immunostimulatory genes (cytokines, costimulatory molecules) |
| Antibodies        | • Able to bring lethal payload to tumor (radionuclide or toxin) | • Generating passive immune response | • Tumor should express tumor-associated antigen found in vector | • Targets only surface antigens |

⁸Each of these has been shown to overcome tolerance and can target any antigen made by the cell (doesn’t need to be a surface antigen).
doubling time was noted in the six-cycle group (n = 5). Although these initial results appear promising, the limited number of patients tested and the design of the trials in this and the majority of other studies presented in this review preclude analysis for efficacy.

Other vaccines have generated similar immunologic responses but failed to yield substantial clinical benefit to patients. In a phase I study, autologous DCs were transfected with PSA mRNA and administered to 13 patients [20]. Patients were assigned to three dose levels: 1, 3, or 5 × 10^7 DCs administered i.v. (three cycles, biweekly) with an additional 1 × 10^7 DCs administered i.d. No dose-limiting toxicities (DLTs) were observed, and all patients had induction of PSA-specific T cells. No significant clinical benefit was observed in this small trial, but the log slope PSA was decreased in six of seven evaluated patients.

In another study, patients were infused with autologous DCs pulsed with recombinant PSA (Dendritophage-rPSA) [21]. In a series of nine injections (three weekly, three biweekly, three monthly), 24 patients received between 1.31 × 10^8 and 6.50 × 10^8 cells. No patient achieved a 50% PSA decline, but transient PSA declines ranged from 6%–39% at least once in 11 patients. Six patients had clearance of circulating tumor cells at 6 months on study.

The addition of proteins to DC/APCs ex vivo has been used successfully in a number of clinical trials (Table 2). Several trials in the late 1990s demonstrated interesting clinical results using prostate-specific membrane antigen (PSMA) [22–25]. In one phase II trial, patients with metastatic prostate cancer (n = 28) were administered 2.1 × 10^7 to 4.23 × 10^7 DCs pulsed with PSMA peptide per injection [22]. Three patients with metastatic AIPC had partial responses to treatment as indicated by a decreased PSA level, with two of them having improved bone scans. In fact, in another phase II study, 19 of 62 (31%) evaluable patients were reported to have a clinical response (based on PSA, bone scan, or ProstaScint scan) [24]. A randomized, double-blind, placebo-controlled phase III clinical trial (DCVax-Prostate vaccine; Northwest Biotherapeutics, Bothell, WA) of DCs pulsed with PSMA is currently in planning for patients with nonmetastatic AIPC.

Another vaccine, sipuleucel-T (APC8015, Provenge®, Dendreon, Seattle, WA), consists of an autologous APC-enriched product pulsed with a recombinant prostatic acid phosphatase (PAP) GM-CSF fusion protein (PA2024). PAP is expressed on more than 95% of prostate cancer cells [26]. In a combined phase I/II trial (n = 12/19), three patients demonstrated a >50% decrease in PSA and an additional three patients had PSA declines of 25%–49% [27]. Sipuleucel-T was associated with low toxicity. In 10 of 26 patients, new T cells specific for PAP were discovered. Furthermore, a trend toward delayed disease progression was associated with patients who developed an immune response to PAP. In the 20 patients who developed an immune response, the median time to progression (TTP) was 34 weeks, as compared with 13 weeks in the 11 patients who did not develop an immune response (p < .027). In another phase II sipuleucel-T trial for patients with metastatic AIPC (n = 21), one complete response was seen [28]. The patient’s PSA declined from 221 ng/ml to undetectable, where it has remained for 4 years. Furthermore, the patient’s retroperitoneal and pelvic adenopathy were reported to have resolved. Two other patients had transient PSA decreases of 25%–50%.

Figure 1. Antigen-presenting cells such as dendritic cells can process antigens such as peptides or whole-tumor cells, can become infected by viral vectors that then express tumor-associated antigens for presentation, or can be ex vivo pulsed with peptides then injected. These antigen-presenting cells present to the T cell, causing activation of the T cell. Upon activation, CTLA-4 is upregulated and inhibits activation of the T cell. Antibodies to CTLA-4 can block the inhibitory signals and cause prolonged activation. Abbreviations: CTLA-4, cytotoxic T-lymphocyte-associated protein 4; MHC, major histocompatibility complex; TCR, T-cell receptor.
A phase III trial \((n = 127)\) has recently been completed following these initial studies \([29, 30]\). The primary end point of that study, time to objective progression, did not achieve statistical significance \((p = .06)\); however, treatment with sipuleucel-T resulted in a statistically significant longer overall survival time \((25.9\ months in the treatment arm vs. 21.4\ months on placebo; \(p = .01)\). At 36 months, 34% of patients in the treatment arm were still alive, as compared with only 11% receiving placebo \((p = .0046)\) \([30]\). The authors report that the 4.5 months’ longer overall survival is the first survival advantage attributed to an immunotherapeutic product for prostate cancer. Because crossover to open-label vaccine was allowed for patients in the placebo arm, more patients in the vaccine arm may have ended the trial and started chemotherapy relatively earlier than those initially in the placebo arm. This raises the question of whether the earlier use of effective chemotherapy might have an impact on survival. In addition, the differences in both survival and TTP were seen largely in the subgroup of patients with Gleason scores of 7 or less. Given the heterogeneity of disease in this population along with the small patient numbers, interpretation of the findings of this trial is more difficult. A confirmatory phase III study sponsored by Dendreon \((D9902B)\) is currently under way.

**Whole-Tumor Cell Vaccines**

In vivo efficacy of whole-tumor cell vaccines was initially demonstrated in preclinical animal studies \([31–36]\). Several of these studies observed immunologic memory capable of protective immunity as well as curative effects against established s.c. tumors using Dunning rat prostatic carcinoma cells \((MAT-LyLu)\) \([34–36]\). Cells transduced with either interleukin-2 \((IL-2)\) or GM-CSF cDNA were subsequently irradiated prior to injection. In one study, animals injected with cells expressing GM-CSF performed significantly better than animals administered the MAT-LyLu with soluble GM-CSF \([35]\). Following these studies, several early clinical studies were successful in generating immune responses using novel vaccines \([37, 38]\).

Autologous vaccines for prostate cancer were initially investigated by Simons et al. \([39]\). Surgically harvested prostate tumor cells were irradiated and engineered to secrete GM-CSF via a replication-defective retrovirus. A small phase I study \((n = 11)\) demonstrated interesting immunologic response and confirmed the safety of this approach. The numerous technical difficulties involved in the preparation of autologous cells, however, represent a significant limitation. In this study, a sufficient volume of cells could not be harvested in three of 11 patients. In addition, the individualized preparation of the vaccine was labor intensive. These persistent problems have shifted the focus of research to allogeneic vaccines, which are readily available from established prostate cancer cell lines. This type of vaccine may be manufactured on a larger scale for distribution. In addition, patients do not need to be HLA matched for these vaccines because antigens can be presented by cross prim-

### Table 2. Selected clinical trials for whole-tumor cell and dendritic cell vaccines

<table>
<thead>
<tr>
<th>Vaccine</th>
<th>Immunologic adjuvant</th>
<th>Toxicity</th>
<th>Trial phase and participants</th>
<th>Stage</th>
<th>Immunologic response</th>
<th>Clinical response</th>
<th>Reference</th>
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</thead>
<tbody>
<tr>
<td><strong>Whole-tumor cell vaccines</strong></td>
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</tr>
<tr>
<td>Allogeneic GVAX</td>
<td>None</td>
<td>None &gt; grade 2</td>
<td>Phase II (n = 80)</td>
<td>M1, AIPC</td>
<td>Yes(^a)</td>
<td>None reported(^b)</td>
<td>Simons et al. ([41])</td>
</tr>
<tr>
<td>OnyVax-P</td>
<td>Bacillus adjuvant</td>
<td>None &gt; grade 2</td>
<td>Phase II (n = 28)</td>
<td>M1, AIPC</td>
<td>Yes(^c)</td>
<td>Yes(^d)</td>
<td>Michael et al. ([42])</td>
</tr>
<tr>
<td><strong>Dendritic cell vaccines</strong></td>
<td></td>
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</tr>
<tr>
<td>Provenge(^o) (APC8015)</td>
<td>None</td>
<td>None &gt; grade 2</td>
<td>Phase III (n = 127)</td>
<td>M1, AIPC</td>
<td>Yes(^e)</td>
<td>Yes(^f)</td>
<td>Small et al. ([30])</td>
</tr>
<tr>
<td>DC pulsed with PSMA</td>
<td>None</td>
<td>None &gt; grade 2</td>
<td>Phase II (n = 28)</td>
<td>M1, AIPC</td>
<td>Not assessed</td>
<td>Yes(^g)</td>
<td>Murphy et al. ([22])</td>
</tr>
</tbody>
</table>

\(^a\)The proportion of patients generating antibodies to one of the cells lines ranged from 40%–87%, with higher doses of vaccine associated with higher proportions of antibody response.

\(^b\)Trend toward longer survival in the high-dose arm of the study; median survival time had not yet been reached at 15 months.

\(^c\)PSA velocity responding patients showed titratable \(T_{h1}\) cytokine release profile suggestive of a better immune response.

\(^d\)Eleven of 26 patients had statistically significant prolonged decreases in PSA velocity.

\(^e\)Patients with Gleason scores ≤7 demonstrated a sevenfold greater T-cell-mediated immune response than patients with Gleason scores 8 or above \((p = .0065)\).

\(^f\)Median overall survival times were 25.9 months in treatment arm versus 21.4 months in the placebo arm.

\(^g\)Three patients with a 50% decline in PSA, two of these having improvements in bone scan.

Abbreviations: AIPC, androgen-independent prostate cancer; DC, dendritic cell; M1, metastatic; M0, nonmetastatic; PSA, prostate-specific antigen; PSMA, prostate-specific membrane antigen.
ing. The GM-CSF secreting vaccine GVAX (Cell GeneSys, South San Francisco, CA) is an admix of the prostate cancer cell lines PC-3 and LNCaP. The cells have been similarly transduced with a replication-defective retrovirus containing cDNA for GM-CSF [39, 40].

In a phase II study, 34 patients with metastatic AIPC were treated with irradiated GVAX [40]. Patients (n = 24) received a 5 × 10⁶ cell prime followed by 12 biweekly 100 million-cell boosters. Ten patients received a 300 million-cell booster. This vaccine was well tolerated. Since this study, GVAX has been re-engineered to secrete five- to tenfold higher levels of GM-CSF in an attempt to improve responses. A phase III randomized, open-label study (G-0029) is ongoing with 600 metastatic AIPC patients randomized to receive either vaccine or docetaxel and prednisone [41]. A second phase III trial, in planning, will compare GVAX plus chemotherapy with chemotherapy alone.

Another phase II trial using a different allogeneic whole-cell vaccine demonstrated a longer median TTP in patients with AIPC [42]. Onyvax-P (Onyvax Ltd, London) consists of the irradiated prostate cancer cell lines Ony-Cap23, LNCaP, and P4E6 administered at a dose of 8 × 10⁶ cells. Patients were administered the vaccine in three cycles biweekly then monthly up to 12 months from the initiation of vaccination. Eleven of 26 patients had decreased PSA velocities and a median TTP of 58 weeks, as compared with an historic value of 28 weeks [27, 43, 44]. No significant toxicities were observed. A randomized, placebo-controlled phase III trial is being planned for patients with non-metastatic AIPC. Time to metastatic disease is the primary end point.

### Table 3. Selected clinical trials for poxviral vaccines

<table>
<thead>
<tr>
<th>Vaccine</th>
<th>Immunologic adjuvant</th>
<th>Vaccine-induced toxicities</th>
<th>Trial phase and participants</th>
<th>Stage</th>
<th>Immunologic response</th>
<th>Clinical response</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prime/Boost cohorts:</td>
<td>VFFF, FFFF, or FFFFV (rV-PSA, rF-PSA)</td>
<td>None</td>
<td>None &gt; grade 2</td>
<td>Phase II</td>
<td>n = 64</td>
<td>All pts failed local therapy</td>
<td>Yes⁴</td>
</tr>
<tr>
<td>Prime:</td>
<td>rV-PSA, rF-PSA</td>
<td>Low-dose systemic IL-2 and GM-CSF</td>
<td>None &gt; grade 2</td>
<td>Phase II</td>
<td>n = 30</td>
<td>localized/locally advanced</td>
<td>Yes⁵</td>
</tr>
<tr>
<td>Boost:</td>
<td>rV-PSA, rF-PSA</td>
<td>Low-dose systemic IL-2 and GM-CSF</td>
<td>None &gt; grade 2</td>
<td>Phase II</td>
<td>n = 42</td>
<td>M₀, AIPC</td>
<td>Yes⁶</td>
</tr>
<tr>
<td>Prime:</td>
<td>rV-PSA (L155) TRICOM</td>
<td>GM-CSF or fowlpox-GM-CSF</td>
<td>None &gt; grade 2</td>
<td>Phase II</td>
<td>n = 32</td>
<td>M₁, AIPC</td>
<td>Yes⁷</td>
</tr>
</tbody>
</table>

⁴Fourteen of 30 patients had an increase in PSA-specific T cells by ELISPOT assay.

⁵Although not statistically significant, progression-free survival in the VFFF arm was nearly double the times observed in the FFFF and FFFFV arms of the study.

⁶Thirteen of 17 evaluable patients had a treethreefold increase in PSA-specific T cells by ELISPOT assay.

⁷Four of 9 had a twofold increase in PSA-specific T cells by ELISPOT assay.

⁸Two patients on vaccine had a sustained >50% decrease in PSA (one of these had a >95% decrease).

⁹Ten of 22 patients had a twofold increase in PSA-specific T cells (range, 2- to 7.7-fold) by ELISPOT assay. There was a significant difference in T-cell response after three vaccines in those with evidence of clinical benefit versus no evidence of clinical benefit (two-tailed p = 0.0003; exant Wilcoxon rank sum test).

⁰One of 10 evaluable patients had a partial response by Response Evaluation Criteria In Solid Tumors (RECIST) criteria. Another patient (1 of 23) had a sustained >50% decrease in PSA and 3 of 23 had prolonged stable disease for ≥12 months.

Abbreviations: AIPC, androgen-independent prostate cancer; F, fowlpox; IL-2, interleukin-2; L155, leucine 155; M₀, metastatic; M₁, nonmetastatic; PSA, prostate-specific antigen; r, recombinant; TRICOM, triad of costimulatory molecules; V, vaccinia.
Repeated administration of vaccinia-based vaccines resulted in the generation of neutralizing anti-vaccinia antibodies capable of preventing effective antigen presentation and subsequent T-cell proliferation. The inability of additional vaccinations to further enhance the immune response limited the efficacy of repeated administrations. Gulley et al. [47] noted that the rate of inoculation site reactions decreased dramatically from 74% of patients following the initial injection to 37% and 19% in subsequent injections. This finding led to the development of a “prime and boost” strategy. Following the initial vaccination with rV, subsequent vaccinations used rF, a similar but replication-defective avian poxvirus [49, 50]. A study conducted by the Eastern Cooperative Oncology Group (ECOG) determined the optimal vaccination strategy [51]. A priming dose with rV-PSA followed by booster vaccinations with rF-PSA was a more effective strategy than either fowlpox-PSA alone (FFFF) or fowlpox-PSA followed by vaccinia-PSA (FFEV). Patients in the VFFF arm of the study had the best overall biochemical response at 6 months. The median time to PSA progression-free survival (PFS) was about 9 months in both the FFFF and FFFV arms, but PFS was double at 18.2 months in the VFFF arm (p = .15). Differences among the groups were not statistically significant, but trends favored the VFFF arm [52].

Two signals are required for T-cell proliferation and cytokine response. The primary signal is the interaction of the T-cell receptor (TCR) with the MHC/TAA. This interaction triggers initiation of the cell cycle. The second costimulatory signal is essential in generating a potent T-cell response through the release of cytokines. Without either signal, T cells may undergo anergy, tolerance, or apoptosis [53–55]. One of the first costimulatory molecules studied was B7.1 (CD80) [56].

Three trials at the National Cancer Institute (NCI) have assessed priming with rV-PSA admixed with rV-B7.1 and boosting with rF-PSA in combination with radiation, ADT, or chemotherapy. The first trial randomized patients with localized or locally advanced prostate cancer (n = 30) 2:1 to receive either an rV-PSA/rV-B7.1 prime followed by an rF-PSA boost with local radiation therapy or radiation therapy alone [57]. Patients in the vaccine arm also received local GM-CSF (100 μg/day for 4 days given s.c.) and systemic IL-2 (4×10⁶ IU/m² given s.c.) It has been previously demonstrated that radiation may upregulate cell membrane proteins, of note Fas, MHC-I, and ICAM-1 [58–62]. Phenotypic modulation of cancer cells may allow vaccines to work more efficaciously by enhancing CTL binding as well as increasing the likelihood of inducing tumor cell apoptosis. In the vaccine plus radiation arm, 13 of 17 patients had at least a threefold increase in PSA-specific T cells at some point following vaccination. There was also evidence of immune-mediated tumor killing seen by de novo formation of T-cell responses to well-described prostate-associated antigens not found in the vaccine. No patient in the radiation-only arm had a PSA-specific T-cell response. Additional trials incorporating vaccine in conjunction with bone-targeting radiopharmaceuticals such as samarium-153-EDTMP (Quadramet®; Cytojen, Princeton, NJ) are currently in planning.

The second trial combined the same prime and boost vaccination strategy with 100 μg GM-CSF (s.c.) per day for 4 days and 6×10⁶ IU/m² IL-2 (s.c.) with the antiandrogen (AA) nilutamide [63]. Nonmetastatic AIPC patients (n = 42) were randomized 1:1 to receive either the vaccine or nilutamide with the option of receiving both treatments at 6 months if PSA was increasing without evidence of radiographically confirmed progressive disease. Induction of PSA-specific T cells was evaluated in HLA-A2–positive patients. No patients in the AA arm demonstrated induction of PSA-specific T cells, but four of eight patients in the vaccine arm had at least a twofold increase in PSA-specific T cells. Two patients had a 15- and 17-fold increase in PSA-specific T cells. Time to treatment failure was similar in the vaccine arm and the AA arm (9.9 months vs. 7.6 months, respectively; p = .28). After 6 months, 12 patients in the vaccine arm and eight patients in the AA arm initiated combination therapy. Secondary treatment failures on combined therapies were 5.2 and 13.9 months following the initial AA or vaccine therapies, respectively. It is of interest that patients receiving AA following treatment with vaccine had a median time to treatment failure that was 6.3 months longer (13.9 vs. 7.6 months).

There are mounting data in the literature that provide a rationale for this strategy. Mercader et al. [64] demonstrated that androgen ablative therapy induces profound T-cell infiltration of benign glands and tumors in human prostates. T-cell infiltration is readily apparent after 7–28 days of therapy and is comprised predominantly of a response by CD4+ T cells and comparatively fewer CD8+ T cells. Recruitment/activation of APCs in treated prostate tissues may contribute to local T-cell activation. The induction of T-cell infiltration in prostate tissues treated with androgen ablation may have implications for the immunotherapeutic treatment of prostate cancer. To understand the T-cell response to prostate cancer, Drake et al. [65] created transgenic mice that express a model antigen in a prostate-restricted pattern and crossed these animals with TRAMP mice that develop spontaneous prostate cancer. Adoptive transfer of prostate-specific CD4+ T cells showed that, in the absence of prostate cancer, the prostate gland is mostly ignored. Tumorigenesis allows T-cell recognition of the prostate gland, but this recognition is tolerogenic, resulting in abortive prolifera-
tion and ultimately in hyporesponsiveness at the systemic level. Androgen ablation was able to mitigate this tolerance, allowing prostate-specific T cells to expand and develop effector function after vaccination. These results suggest that immunotherapy for prostate cancer may be most efficacious when administered after androgen ablation.

The third study was a pilot trial \((n = 28)\) designed to assess the role of vaccine with docetaxel in patients with metastatic AIPC \([66]\). Prior preclinical studies had shown that taxanes could potentiate immune responses and augment the antitumor responses seen with vaccines \([67, 68]\). Patients in the combination arm were administered vaccine and GM-CSF as previously described, with docetaxel at a dose of 30 mg/m² weekly and with dexamethasone comedication for three consecutive weeks followed by 1 week off. Patients in the second arm received vaccine alone. Chemotherapy did not appear to blunt the immune response, as both cohorts demonstrated similar increases in PSA-specific T-cell precursors. Patients in the vaccine arm were allowed to go on to docetaxel alone at progressive disease. After receiving vaccine, the median TTP on docetaxel was 6.1 months. As an informal comparison, in a similar group of 25 patients with the same disease characteristics treated at the same institution using the same single-agent docetaxel regimen, the median PFS time was 3.7 months \([69]\). A larger randomized trial comparing docetaxel alone with the combination of docetaxel and vaccine with a primary clinical end point is needed to validate the significance of such observations.

Two additional costimulatory molecules have now augmented the vector containing B7.1: ICAM-1 (CD54) and leukocyte function-associated antigen (LFA)-3 (CD58). All three of these molecules, found on professional APCs, are capable of stimulating T cells \([56, 57, 70]\). When used in combination, TRICOM (TRiad of COstimulatory Molecules) has had a synergistic effect on T-cell activation and tumor treatment models, superior to those constructs that contain only one or two costimulatory molecules \([50, 71, 72]\).

A phase I/II study using PSA-TRICOM for patients with advanced metastatic AIPC demonstrated promising results: 3 of 23 evaluated patients had PSA declines during the study (two patients >30%, one patient >50%) \([73, 74]\). Furthermore, one patient had a partial response by Response Evaluation Criteria In Solid Tumors (RECIST) criteria, with three patients having radiographically stable or improving disease to 12 months or more on study \([73]\). Furthermore, there was a strong correlation between an increase in PSA-specific T cells of at least sixfold following vaccine and evidence of clinical benefit (decrease in PSA >50%, objective response by RECIST, and/or stable disease for at least 12 months). These studies also demonstrated the safety of recombinant fowlpox GM-CSF.

Cytotoxic T-lymphocyte-associated protein 4 (CTLA-4; CD152) is an important regulator of T-cell homeostasis. This inhibitory ligand becomes expressed about 2–3 days after T cells become activated and has a much higher affinity for B7.1 (CD80) and B7.2 (CD86) than their stimulatory ligand CD28 does. Antibodies that prevent CTLA-4 signaling enhance the level of T-cell expansions both in vitro and in vivo. In one clinical study of anti-CTLA-4 antibody alone in patients with AIPC, 2 of 14 patients exhibited a >50% decrease in serum PSA compared with baseline \([75]\). Other clinical studies using anti-CTLA-4 antibodies have shown rare but potentially life-threatening autoimmune breakthrough events with these drugs. These events, however, are associated with superior clinical responses \([76]\).

The combined use of poxviral vaccines containing multiple costimulatory molecules with GM-CSF and anti-CTLA-4 was shown in a murine model to enhance not only the quantity of, but to a greater magnitude, the avidity of T cells generated \([77]\). This combination strategy was also shown to enhance antitumor effects. Based on these studies, a clinical trial combining PSA-TRICOM with GM-CSF and an anti-CTLA-4 antibody, ipilimumab (MDX-010, Medarex, Princeton, NJ), has been initiated.

A double blind, placebo-controlled randomized phase III trial (ECOG-1805) is currently in the late stages of planning for patients with nonmetastatic AIPC. Patients will be randomized 2:1 to receive either an rV-PSA/TRICOM prime followed by an rF-PSA/TRICOM boost plus GM-CSF or placebo plus GM-CSF. It has been demonstrated that patients receiving GM-CSF have better immunologic and perhaps clinical responses \([70]\).

**Antibody Therapy**

Monoclonal antibodies for prostate cancer have targeted antigens that are either nonspecific for prostate cancer (e.g., cetuximab) or prostate specific (e.g., PSMA). Unlike PSA and PAP, PSMA is a cell-surface membrane antibody that is not secreted, thus making it an attractive candidate. Preliminary studies incorporating monoclonal antibodies have provided safety as well as clinical activity using radiolabeled and immunoconjugate forms of this therapy.

J591 is an anti-PSMA monoclonal antibody that binds to the extracellular domain of PSMA. The initial clinical trial of 111In-trace-labeled J591 was a phase I study in patients with progressive prostate cancer. No DLT was observed, and the maximum-tolerated dose (MTD) was not reached. Excellent tumor targeting was reported for all dose levels. One patient (of 14) showed a 50% decline in PSA \([78]\). Two independent phase I trials were then initiated with 90Y or 177L linked to J591. Twenty-nine patients with AIPC

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received $^{90}$Y-J591, four of whom were retreated. The DLT was hematologic, with two patients requiring platelet transfusions. Antitumor activity was seen in two patients who had significant declines in PSA associated with objective measurable disease response. Both patients were treated at doses higher than the MTD. Thirty-five patients received $^{177}$L-J591, 16 of whom received up to three doses. Myelosuppression was the DLT. PSA declines of $\geq 50\%$ were recorded in four patients. No patient developed an anti-J5951 antibody. Phase II trials of $^{90}$Y or $^{177}$L linked to J591 are planned [79, 80].

Further studies are required to determine the proper vaccine or monoclonal antibody dose and scheduling and, more importantly, to evaluate combination therapies using vaccines or monoclonal antibodies with standard of care therapies or other experimental therapies.

**Immunosuppression**

While studies have shown that we can overcome immune tolerance to tumor-associated antigens, this alone is not enough to induce significant tumor responses in the majority of patients with advanced cancer. These patients often have a profoundly suppressive milieu that abrogates this immune response. The tumor can produce a variety of proteins (e.g., transforming growth factor beta) that can inhibit the proliferation of antigen-specific T cells. In addition, tumors often downregulate expression of MHC molecules, making them invisible to T cells. Finally, regulatory T cells that downregulate cancer-specific cytotoxic T cells are abundant in both the peripheral blood and tumors of patients with cancer. To combat the immunosuppressive effects seen in patients with advanced cancer, several strategies have evolved. To decrease the effect of systemic immunosuppression by substances elaborated by tumor cells, many have suggested using vaccination in less advanced stages, such as in biochemical failure or in the adjuvant setting [52]. One drawback with this patient population is the long time to clinical events, leading to the need for larger (more expensive) trials with longer follow-up. Radiation therapy has emerged as an interesting strategy to change the phenotype of the tumor to make it more visible to the immune system (upregulate TAAs and MHC molecules) and facilitate immune-mediated killing (by upregulating Fas) [58–62]. A recent trial, mentioned above, demonstrated that this approach was safe and could generate vaccine-specific immune responses despite ongoing radiation therapy in patients with localized or locally advanced prostate cancer [57]. And finally, approaches targeting CD25$^+$ regulatory T cells are being employed [81]. These include the use of vaccine with denileukin, difitiox (Ontak, Seragen Inc., San Diego), a fusion protein of diphtheria toxin and IL-2 that binds to CD25 and leads to cell death following internalization. These and other strategies aim to maximize the effectiveness of vaccines by decreasing inhibitory immune regulatory signals or altering the phenotype of the tumor.

**Measuring Immunologic Response**

The optimal vaccination strategy in humans using specific TAAs against tumors expressing the antigen will not be determined until large clinical trials correlating survival, disease-free interval, or tumor regression are carried out. In the absence of such data, immunoassays (both T-cell- and antibody-based) may be useful to help determine (a) if a given vaccine can elicit any immune response and (b) the relative potency of such a response.

Early immunologic monitoring methods to determine the precursor frequency of specific T cells to a particular immunogen using cell lysis as a readout were labor intensive and required numerous in vitro stimulations of the cell lines [82]. The limiting dilution assay was used most frequently [83, 84]. However, this assay is time consuming and labor intensive. In addition to the in vitro stimulation, peripheral blood mononuclear cells (PBMCs) at different concentrations are needed to generate enough data points to allow for a quantitative analysis. Other methods, such as cytokine production assays, tetramer assays and the ELISA, measure cytokine production of mixed-cell populations; thus, the number of T cells that respond when a particular immunogen is administered cannot be calculated [85–90].

Newer techniques for the analysis of specific T-cell responses to vaccines include tetramer assays and the intracellular cytokine assay. Tetramers have been widely used to quantify the number of viruses and bacteria-specific T cells in animal models [91, 92]. However, the lower limit of detection for currently used tetramer assays is in the 1 in 10,000 precursor frequency range [93]. The intracellular cytokine FastImmune assay (BD Biosciences, Franklin Lakes, NJ) has also been used to evaluate the phenotype T-cell responses from vaccinated patients [94, 95]. This technique incorporates the use of cell flow cytometry to detect intracellular cytokines and allows the examination of multiple cytokines within individual cells. We have done a careful comparison of this assay with the ELISPOT (ELISA) assay and determined that the sensitivity of this assay is similar to that of the ELISPOT. However, the cost of second antibody reagents and extended fluorescence-activated cell sorting scanning time make this assay more expensive and time consuming than the ELISPOT assay.

The ELISPOT assay is relatively sensitive and quan-
titative. By measuring cytokine release on a single-cell basis, the assay can detect a peptide-specific T-cell response against specific HLA class I and class II binding peptides [88]. The level of cytotoxicity determined by the standard chromium release assay after in vitro expansion of specific T cells was shown to correlate with the number of interferon (IFN)-γ-releasing cells measured by the ELISPOT assay in a study of both healthy donors and melanoma patients [96]. We have shown that the ELISPOT assay can be used without prolonged ex vivo manipulation of a patient’s PBMCs to measure immunologic responses in patients receiving cancer vaccines [96]. Studies have demonstrated the ELISPOT assay for IFN-γ production to be reproducible as a measure of human T-cell responses to vaccination [45, 49, 96]. The continued use of one reproducible assay has been instrumental in our ability to evaluate and compare patients’ immune responses using different vaccines and vaccine strategies in the same institution, and among different cancer centers.

**Measuring Clinical Response**

Standard phase II clinical trials in solid tumors often have objective response with radiographically measurable disease as their primary end point. Because only approximately 40% of prostate cancer patients have soft tissue disease, and lesions seen by radioscintigraphy are not measurable, significant interest has been generated in using PSA as a marker for response to experimental agents. PSA is elevated in about 95% of patients with AIPC, and changes in PSA often predate changes seen on radiographic imaging. Several trials have shown that, in AIPC, a decline in PSA of at least 50% is associated with a statistically significantly longer overall survival [97–99]. A working group convened by the NCI proposed reporting a 50% decrease in PSA as a measurement of outcome in phase II clinical trials to guide the development of further randomized trials [100]. In addition, the correlation of PSA doubling time and PSA velocity with clinical events has raised the idea of not only stratifying patients based on PSA kinetics but also using PSA kinetics as an end point for clinical trials [101,102].

**Conclusion**

Many different treatment modalities have thus far been examined for prostate cancer. Each treatment has potential advantages as well as therapeutic limitations. Whole-tumor cell vaccines have multiple TAAs to stimulate the immune system; however, these cells often lack costimulatory molecules. DCS are optimal APCs, but add logistical complexity and cost to the treatment. For poxviral vectors, both TAAs and costimulatory molecules may be expressed following infection of APCs and other cells, but an immune response may be mounted against the delivery vehicle. Recent findings in immunology have translated into more complex mechanisms capable of achieving anticancer activity. Emerging clinical data regarding long-term safety and early evidence of clinical benefit raise hope that soon another modality of therapy will be widely available in the fight against prostate cancer. Future plans for the implementation of prostate cancer vaccines may include increased combination regimens designed to overcome immunosuppression or alter tumor phenotype making it more susceptible to immune-mediated killing. Vaccines or antibodies administered in combination with radiation, chemotherapy, small-molecule targeted therapy, or perhaps even other immunotherapies may generate substantial synergistic effects. Exploration of these new treatment strategies is already under way. For cancer patients in otherwise excellent health, vaccine therapy may become a promising therapeutic option that avoids toxicities associated with chemotherapy or radiation.

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**Disclosure of Potential Conflicts of Interest**

The authors indicate no potential conflicts of interest.

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