OncoImmunology 2:5, e24523; May 2013; © 2013 Landes Bioscience

Prostate cancer vaccines
Update on clinical development

Sean M. Geary and Aliasger K. Salem*

Department of Pharmaceutical Sciences and Experimental Therapeutics; College of Pharmacy; University of Iowa; Iowa City, IA USA

Keywords: gene-mediated cytotoxic immunotherapy, DNA Vaccines, viral-based prostate cancer vaccines, cell-based prostate cancer vaccines, prostate cancer, clinical trials, Sipuleucil-T, GVAX-PCa, ProstVac VF

Prostate cancer is a common malignancy among elderly men and is essentially incurable once it becomes metastatic. Results from clinical trials testing a panel of specific vaccines in patients with castration-resistant prostate cancer (CRPC) suggest that alternative therapies may one day substitute or support the current gold standard (docetaxel plus prednisone). Here, we summarize the results of germane clinical trials completed during the last 12 y and provide updates on some currently ongoing studies. As it stands, prostate cancer vaccines appear to be safe and capable of generating prostate-specific T lymphocyte responses with potential antitumor activity.

Introduction

Prostate cancer is the second leading cause of cancer-related deaths among men in the United States and is one of the most (if not the most) prevalent forms of cancer in both the United States and Europe.[1,2] A recent systematic review of over 71,000 patients reveals that 10–20% of prostate cancer cases progress to castration-resistant prostate cancer (CRPC), for which there is no effective cure.[3] Previous studies indicate indeed that the vast majority (> 80%) of patients diagnosed with CRPC already have (bone) metastases and that one third of the remaining subjects is likely to develop metastases within 2 y.[4,5] The current standard chemotherapeutic treatment for CRPC patients is based on docetaxel plus prednisone. This approach only modestly enhances patient survival and, as with most (if not all) chemotherapeutics, has a wide range of undesirable side effects.[6] There is consequently a need for less toxic alternative or companion therapies, such as active immunotherapy. Prostate cancer is indeed a viable candidate for the development of anticancer vaccines, as current standard treatments for the clinical management of CRPC are inadequate. In addition, prostate cancer cells usually grow at a reduced pace, thus allowing for the elicitation of effective immune responses.[7] Prostate cells express many tissue-specific proteins that could act as therapeutic targets, including prostate-specific antigen (PSA), prostatic acid phosphatase (PAP) and many others.[8] In the last decade, a variety of vaccines against prostate cancer have been developed and tested in clinical trials for safety and therapeutic profile. Results from salient and novel vaccine formulations against prostate cancer are reviewed here.

*Correspondence to: Aliasger K. Salem; Email: aliasger-salem@uiowa.edu
Submitted: 03/29/13; Accepted: 04/02/13
Citation: Geary SM, Salem AK. Prostate cancer vaccines: Update on clinical development. OncoImmunology 2013; 2:e24523; http://dx.doi.org/10.4161/onci.24523

Cell-Based Prostate Cancer Vaccines

Sipuleucel-T. Sipuleucel-T (Dendreon Corporation) is a cell-based FDA-approved prostate cancer vaccine employing the patient's own antigen-presenting cells that have been treated (ex vivo) with a recombinant fusion protein, PA2024 (human PAP fused to granulocyte macrophage colony-stimulating factor, GM-CSF). This vaccine is available to patients with asymptomatic or minimally symptomatic metastatic CRPC and is administered as three intravenous infusions at biweekly intervals. Phase I and Phase I/II clinical trials demonstrated that the vaccination of CRPC patients with sipuleucel-T is generally well tolerated and reported the induction of PAP-specific T-lymphocyte responses in 75% and 38% of patients, respectively.[9,10] In both settings, a proportion (25% and 10%, respectively) of patients experienced a greater than 50% reduction in circulating PSA levels. Interestingly, in the Phase I/II studies (involving 31 patients) a correlation was noted between time to progression and the development of a (cellular or humoral) cancer-specific immune response ($p < 0.027$).[10] Of note, the vaccination protocol employed in the Phase I trial (involving 12 evaluable patients) was different than that used in subsequent studies (including the abovementioned Phase I/II trial), in that patients were given only two sipuleucel-T infusions (one month apart from each other), followed by three subcutaneous monthly doses of PA2024. Why this particular vaccination protocol was discontinued is unclear. Subsequently, randomized, double-blind, placebo-controlled Phase III clinical trials were performed, namely, an integrated study involving 147 patients and a multicenter trial involving 512 patients (known as IMPACT).[11,12] All of these patients had CRPC with no or minimal symptoms. In the integrated study, the median overall survival (OS) was 23.2 mo for patients vaccinated with sipuleucel-T and 18.9 mo for the control group ($p = 0.011$). In the IMPACT study, the median OS was 25.8 mo for patients receiving sipuleucel-T and 21.7 mo for patients who were treated with placebo ($p = 0.03$). Interestingly, no change in time to progression was observed after the administration of sipuleucel-T despite the increase in OS. The lack of measurable

tumor regression in spite of increased OS among sipuleucel-T-treated patients is enigmatic.[13] The actual mechanism of action of sipuleucel-T is unknown, yet some suggestions in this respect have been made.[14,15] A recent report presenting previously unpublished data from the IMPACT trial suggests that the increased OS observed among sipuleucel-T-treated patients could be an artifact generated by age-related differences in the placebo group.[16] These findings are contentious and have been refuted by a number of experts in the field.[17,18]

A number of clinical trials are currently investigating sipuleucel-T in cohorts of prostate cancer patients (**Table 1**). Although these trials are still in progress, some of the data accrued thus far have already been presented at specialized meetings or workshops. Thus, in the context of an open-label Phase II study (NCT00715104) enrolling patients with localized prostate cancer, the vaccination with sipuleucel-T prior to radical prostatectomy has been shown to result in increased levels of $CD4^+$ (but not $CD8^+$) T lymphocytes at the interface between benign and malignant tissue (the site at which pre-treatment biopsies were compared with post-treatment tissues obtained by radical prostatectomy).[35]

GVAX-PCa. GVAX-PCa (Biosante Inc.) is a prostate cancer vaccine comprising a mixture of two irradiated allogeneic prostate cancer cell lines, LNCaP and PC-3, which constitutively express GM-CSF.[36] One Phase I/II trial to evaluate the safety and immunogenicity of GVAX-PCa was performed on 55 chemotherapy naïve metastatic CRPC patients.[26] These subjects received an intradermal priming vaccination with GVAX-PCa (5×10^8 cells) followed by 12 biweekly boosts (for 6 mo). Patients were allocated to receive radiotherapy alone, GVAX-PCa with high dose boosts (3×10^8 cells) or GVAX-PCa with low dose boosts (1×10^8 cells), and median OS for these groups was 26.2, 34.9 and 24 mo, respectively. The vaccination protocol was well tolerated and no autoimmune toxicities were recorded. A subsequent Phase I/II study enrolling 80 patients with metastatic CRPC demonstrated that GVAX-PCa is generally safe and high dose boosts are most effective at extending patient survival.[27]

Oncoimmunologists are becoming increasingly more aware of the importance of dampening the immunosuppressive function of regulatory T lymphocytes (Tregs) along with the delivery of anticancer vaccines. One way of achieving this is through the use of antibodies targeting immunological checkpoint regulators such as ipilimumab and nivolumab, which are specific for cytotoxic T lymphocyte antigen 4 (CTLA4) and programmed cell death protein 1 (PD1), respectively. The combination of GVAX-PCa and ipilimumab has been investigated in an open-labeled, single-center, Phase I clinical trial, which was partially prompted by preclinical results showing that CTLA4-targeting agents and GM-CSF-secreting tumor (melanoma and mammary carcinoma) cells exert synergistic antineoplastic effects in mice.[37–39] In addition, ipilimumab alone has been shown in two randomized Phase III trials to improve the OS of patients with metastatic melanoma.[40,41] This Phase I study was performed on 28 patients with metastatic CRPC and no previous history of chemotherapy, who received intradermal primes with GVAX-PCa (5×10^8) cells followed by 12 boosts. Initially, 4 cohorts of 3 patients were treated with escalating doses of ipilimumab. As immune-related toxicities such as hypophysitis (inflammation of pituitary gland) and/or sarcoid alveolitis developed in individuals receiving high doses of ipilimumab (5 mg/kg), the remaining patients received GVAX-PCa together with 3 mg/kg ipilimumab, a combination that was safe and well tolerated. One of the secondary endpoints of this study was survival, and it was found that patients treated with GVAX-PCa plus ipilimumab had a median OS of 29.2 mo (95% CI 9.6–48.8), which compared favorably with the median OS of patients treated with sipuleucel-T (see above) or PostVac VF (see below). Still, it is yet to be determined whether this combinatorial treatment actually constitutes a significant improvement over GVAX-PCa alone in terms of OS increases.

Finally it should be noted that two Phase III trials involving GVAX-PCa, known as VITAL-1 and VITAL-2, were prematurely terminated due to a lack of therapeutic effect and increased mortality, respectively. The results from these trials have recently been presented at international meetings.[23,42] According to some experts in the field, these two trials were problematic since the optimal dosing of docetaxel for combinatorial regimens had not yet been determined (in the context of VITAL-2) and since a placebo control group was not used (in the context of VITAL-1).[43]

Viral Prostate Cancer Vaccines

ProstVac VF. ProstVac VF, or PSA-TRICOM, is an optimized heterologous vaccine that involves a prime and multiple boosts with attenuated strains of vaccinia and fowlpox viruses, respectively.[44] Both these recombinant viruses have been engineered to encode human PSA and the co-stimulatory proteins CD54, CD58 and CD80. Multiple Phase I studies have established the safety and tolerability of PSA-coding recombinant vaccinia vectors, as well as of the ProstVac VF protocol.[19,20,45] A randomized Phase II study reported that 82 patients with minimally symptomatic metastatic CRPC treated with ProstVac VF exhibited increased OS (25.1 mo) as compared with 40 patients receiving the control empty vector (16.6 mo; $p = 0.006$).[21] In a similar, but not randomized, Phase II trial, ProstVac VF again was shown to increase the OS of patients as compared with that defined by the Halabi nomogram prediction (26.6 mo vs. 17.4 mo).[22] The Halibi nomogram is a prognostic model that, based on historical data, is designed to predict disease outcome and survival among individual CRPC patients.[46] Of note, the OS improvement trend associated with the administration of ProstVac VF was particularly evident among patients with less advanced or less aggressive disease. It has subsequently been suggested by Gulley et al. that cancer vaccines in general are more likely to provide clinical benefit to patients with early-stage disease.[47] In addition, it was shown that patients developing robust PSA-specific T-lymphocyte responses manifested a trend toward improved survival. More recently, Gulley et al. have presented preliminary data at the 2013 Genitourinary Cancers Symposium (Feb 14th–16th, Orlando, FL, USA) indicating that, in the context of a multicenter Phase II trial involving 50 hormone-naïve patients with non-metastatic prostate cancer, ProstVac VF

resulted in a significant reduction of tumor growth rate within 3 mo.[48]

In a small but important clinical trial involving bladder cancer patients before surgery, ipilimumab as a monotherapy was shown to increase the ratio of effector:regulatory T lymphocytes present within the tumor microenvironment.[49] A Phase I dose-escalation trial on patients with metastatic CRPC has recently revealed that the combination of ProstVac VF and ipilimumab is safe and well tolerated.[50] In this study, 30 patients were vaccinated subcutaneously with 2×10^8 plaque-forming units (PFUs) ProstVac VF followed by monthly boosts with 10^9 PFUs (beginning on day 15). On day 15 (and monthly from then on), patients were also given varying doses (1, 3, 5 or 10 mg/kg) of ipilimumab, intravenously. Most patients experienced grade 1–2 reactions at the site of vaccination. Rashes were the most common immune-related adverse event, most notably in patients receiving 10 mg/kg ipilimumab. Other side effects included grade 2–3 colitis (or diarrhea) at all doses of ipilimumab > 1 mg/kg. Overall, 8/30 patients experienced grade 3–4 toxic effects. These findings were similar to those from previous Phase I trials testing ipilimumab alone in melanoma patients, suggesting that ProstVac VF does not increase the severity of ipilimumab-related side effects.[51,52] Interestingly, the six patients in this study who had previously been treated with chemotherapy experienced a median progression-free survival of 2.4 mo, as compared with 5.9 mo among 24 chemotherapy-naïve patients. The median OS for all the 30 patients who received ProstVac VF plus ipilimumab was 34.4 mo. Future randomized clinical studies may elucidate the OS benefit of ProstVac VF plus ipilimumab over ProstVac VF alone. However, due to the potential for ipilimumab to cause aggressive autoimmune responses, nivolumab is being investigated as a possibly safer alternative.[53] Results from a recent Phase I clinical trial indicate that nivolumab was well tolerated (up to 10 mg/kg) by CRPC patients but did promote lymphopenia and fatigue as well as mild immune-related adverse effects, such as grade 3 inflammatory colitis and grade 2 polyarticular arthropathies, in a proportion of patients.[54] Although it is still too early to make emphatic comparisons between the safety of ipilimumab and nivolumab, the latter might provoke less frequent and less severe adverse effects than the former.

There is accumulating evidence that combining chemotherapy with anticancer vaccines may drive additive or synergistic antitumor effects.[55,56] Randomized Phase II clinical trials enrolling metastatic CRPC patients were performed with an ancestor of ProstVac VF and docetaxel, suggesting indeed that the combinatorial regimen may result in improved tumor-specific immune responses.[24] Finally, although GM-CSF is usually co-delivered with ProstVac VF, the impact that this cytokine has per se on the survival of prostate cancer patients has not yet been determined. Hence, a global, randomized, controlled Phase III trial is currently recruiting patients to investigate the importance of co-administering GM-CSF with ProstVac VF (NCT01322490).

Ad5-PSA. Replication-deficient recombinant Ad type 5 (Ad5) vectors represent one of the most efficient methods for in vivo gene delivery.[57] Importantly, Ad5 vectors are also useful adjuvants for the delivery of tumor-associated antigen (TAA)-coding genes, owing to their elevated tropism for dendritic cells.[58,59] In preclinical studies, the administration of Ad5-PSA admixed with the collagen matrix Gelfoam® was shown to protect the viral vector from high-titer anti-adenovirus antibodies, hence resulting in stronger immune responses than the use of Ad5-PSA alone.[60] This finding is particularly significant since a large percentage of patients exhibits pre-existing immunity, which de facto neutralizes the infectivity of Ad5 vectors. A Phase I clinical trial has been performed to test Ad5-PSA in 32 CRPC patients with evidence of metastatic disease.[25,61] The primary endpoint of establishing safety was met in patients who received varying doses (10^6–10^8 PFUs) of Ad5-PSA, with or without Gelfoam®. All adverse events were mild (< grade 3) and most of them were temporary (< 2 d). In addition, 57% of patients vaccinated with aqueous Ad5-PSA manifested detectable PSA-specific T lymphocytes, while 77% of patients receiving Ad5-PSA/Gelfoam® did so. Finally, albeit not statistically significant, a correlation was seen between T-lymphocyte responses and patient survival. To determine if Ad5-PSA vaccines can yield therapeutic benefits, a Phase II study employing two separate vaccination protocols is in progress (NCT00583024).[62] Patients with newly recurrent prostate cancer (protocol 1) will be treated with Ad5-PSA/Gelfoam®, either as a standalone intervention or subsequent to hormone deprivation therapy, while individuals affected by CRPC with low disease burden (protocol 2) will be treated with Ad5-PSA/Gelfoam® alone. In contrast to the Phase I study mentioned above, in the context of which patients were treated with only one Ad5-PSA vaccination, all patients will receive three vaccinations (30 d apart from each other). The primary endpoints for CRPC patients (with low disease burden) will be PSA doubling time (PSADT), time to progression and OS, while the primary endpoint for patients with recurrent disease will be the development of anti-PSA immune responses.[62] According to a recent report on this ongoing trial, which thus far has accrued data from 44 patients, 100% of patients enrolled under protocol 1 and 67% of patients enrolled under protocol 2 have developed anti-PSA T-lymphocyte responses (as measured by ELISPOT assays).[63]

DNA-Based Vaccines

DNA-PAP and DNA-PSA. DNA-based vaccines are promising candidates for cancer immunotherapy for several reasons, including their safety and ease of manufacture. To date, a few DNA-based vaccines have progressed to small scale clinical trials, each comprising a genetic construct coding for a prostate-specific target protein. However, as it stands, no naked DNA-based vaccine has been tested in randomized clinical trials.[64] In an open label, single institution Phase I/IIa clinical study, 22 prostate cancer patients with biochemical recurrence and no evidence of metastatic disease were vaccinated intradermally with a plasmid encoding human PAP (pTVG-HP/PAP).[30] Patients were vaccinated six times with pTVG-HP/PAP and 200 μg GM-CSF at intervals of 14 d. No significant adverse effects were recorded for all pTVG-HP/PAP doses (max. = 1.5 mg). Of the 22 patients who were vaccinated in this setting, seven exhibited at least a doubling of PSADT. PAP-specific T-lymphocyte responses were detected

Table 1. Summary of recently completed and ongoing clinical trials

Vaccine	Description	Is it safe?	Latest completed clinical trials: (main findings)	Clinical trials active (a) or recruiting (r)
ProstVac VF (PSA-TRICOM)	Heterologous prime/multiple boosts vaccinia virus (PSA-CD54-CD58-CD80) – fowlpox virus (PSA-CD54-CD58-CD80)	Yes[19,20]	•**Phase II**: Enhanced OS over control group by a median 8.5 mo[21] •**Phase II**: Enhanced OS compared with Halabi nomogram prediction[22]	•**Phase III (r)**: Comparison of OS with or without GM-CSF (NCT01322490) •**Phase II (r)**: Comparison of disease progressions with flutamide and flutamide alone (NCT00450463)
ProstVac VF + ipilimumab (nivolumab)	ProstVac VF + dose-escalation trial of anti-CTLA4 antibody	Yes*[,23]	**Phase I**: Safe and tolerable*[,23]	
ProstVac VF/ProstVac VF-like + docetaxel	ProstVac VF-like (rV-PSA + rV-CD80) prime/rF-PSA boost)	Yes[24]	**Phase II:** Safe. Docetaxel does not inhibit vaccine specific T-lymphocyte responses[24]	
Ad5-PSA	PSA-encoding adenovirus 5– one administration with/without Gelfoam® (Phase I) and three administrations with Gelfoam® (Phase II)	Yes[25]	**Phase I**: Safe. In Ad5-PSA/Gelfoam® group 77% of PCa patients had detectable anti-PSA T cells[25]	**Phase II (r):** Assessment of effect of Ad5-PSA on PSADT (NCT00583024 and NCT00583752)
Sipuleucel-T	Leukopheresed patients PBMCs transduced ex vivo with PAP-GM-CSF construct (PAP2024) an then reintroduced into patients	Yes[9,10]	**Phase III**: IMPACT trial: Enhanced OS over placebo group by a median of 4.1 mo[12]	**Phase II (a):**NCT00715078[a] **Phase II (a):**NCT00715104[b] **Phase II (a):**NCT00901342[c] **Phase IIIB (a):**NCT00779402[d] **Phase II (r):**NCT01306890[e] **Phase II (a):**NCT01487863[f] **Phase II (a):**NCT01431391[g]
GVAX-PCa	Irradiated PCa cell lines, LNCaP and PC-3, that constitutively express GM-CSF – Prime/Boosts	Yes[26,27]	**Phase I/II (×2):** Safe. Enhanced median survival (for high dose boosts) over radiotherapy and low dose boosts by 8.7 mo[26] and 11.9 mo[27] respectively	
GVAX-PCa + docetaxel	GVAX-PCa + docetaxel compared with docetaxel alone in PCa patients with symptomatic metastatic CRPC	No - imbalance in deaths in the combined treatment group[28]	**Phase III:** VITAL-2:† terminated due to safety concerns.	
GVAX-PCa + ipilimumab	GVAX-PCa (described above) + dose-escalation trial anti-CTLA4 antibody	Yes††[,29]	**Phase I:** Safe†† (up to 3 mg/kg ipilimumab safe and well tolerated)	
DNA-PAP	Multiple intradermal vaccinations of rhGM-CSF with a plasmid (pTVG-HP) encoding PAP	Yes[30]	**Phase I/II:** Safe. 7/22 patients had ≥2-fold increase in PSADT	•**Phase II (r):** Comparison of GM-CSF ± DNA-PAP (NCT01341652) •**Phase II (a):** Determine safety and immunogenicity (NCT00849121)
DNA-PSA	Multiple intradermal vaccinations of GM-CSF + IL-2 with a plasmid (pVAX) encoding PSA	Yes[31]	**Phase I:** Safe. 2/3 patients (given high dose pVax/PSA) had significantly elevated levels of PSA-specific IFNγ^+ T cells	

*Grade 3–4 side effects observed with 3–10 mg/kg ipilimumab (colitis and neutropenia); †Phase III trials (×2) with GVAX-PCa were terminated, as discussed in the main text; ††Hypophysitis and/or sarcoid alveolitis diagnosed in patients receiving 5 mg/kg ipilimumab; [a]Aims to assess CD54 upregulation with varying fusion protein (PAP2024) concentrations; [b]Aims to assess the immune response within prostate tissue following the neo-adjuvant administration of sipuleucel-T (prostatectomy specimens taken after sipuleucel-T vaccinations will be compared with tissue from the core biopsy specimen obtained prior to treatment); [c]Aims to evaluate the magnitude of immune responses to sipuleucel-T in patients with metastatic prostate cancer; [d]Aims to determine if sipuleucel-T is effective in early stage, non-metastatic prostate cancer patients (end-point: biochemical failure); [e]Aims to quantify the risk of cerebrovascular events following sipuleucel-T therapy for all subjects with CRPC; [f]Aims to evaluate the impact of concurrent or sequential administration of abiraterone acetate plus prednisone on product parameters of sipuleucel-T; [g]Aims to evaluate immune responses in patients with non-metastatic prostate cancer when androgen deprivation therapy is started before or after sipuleucel-T. CRPC, castration-resistant prostate cancer; GCV, ganciclovir; GMCI, gene-mediated cytotoxic immunotherapy; GM-CSF, granulocyte macrophage colony-stimulating factor; IFNγ, interferon γ; IL-2, interleukin-2; OS, overall survival; PAP, prostate acid phosphatase; PBMC, peripheral blood mononuclear cell: PSA, prostate-specific antigen; PSADT, PDA doubling time; RT, radiotherapy.

Table 1 (Continued). Summary of recently completed and ongoing clinical trials

Vaccine	Description	Is it safe?	Latest completed clinical trials: (main findings)	Clinical trials active (a) or recruiting (r)
GMCI	AdV-tk/anti-herpetic prodrug (GCV) prior to prostatectomy	Yes[32,33]	**Phase I–II:** Significant influx of CD^{8+} T cells No improvement in patient prognoses[34]	
GMCI + RT	AdV-tk/anti-herpetic prodrug (GCV) prior to prostatectomy + RT	Yes[74]	**Phase I/II:** Safe. Good locoregional control but inadequate systemic control[72]	**Phase III (r):** Comparing disease-free survival: GMCI + RT vs. placebo (NCT01436968)

*Grade 3–4 side effects observed with 3–10 mg/kg ipilimumab (colitis and neutropenia); †Phase III trials (×2) with GVAX-PCa were terminated, as discussed in the main text; ††Hypophysitis and/or sarcoid alveolitis diagnosed in patients receiving 5 mg/kg ipilimumab; [a]Aims to assess CD54 upregulation with varying fusion protein (PAP2024) concentrations; [b]Aims to assess the immune response within prostate tissue following the neo-adjuvant administration of sipuleucel-T (prostatectomy specimens taken after sipuleucel-T vaccinations will be compared with tissue from the core biopsy specimen obtained prior to treatment); [c]Aims to evaluate the magnitude of immune responses to sipuleucel-T in patients with metastatic prostate cancer; [d]Aims to determine if sipuleucel-T is effective in early stage, non-metastatic prostate cancer patients (end-point: biochemical failure); [e]Aims to quantify the risk of cerebrovascular events following sipuleucel-T therapy for all subjects with CRPC; [f]Aims to evaluate the impact of concurrent or sequential administration of abiraterone acetate plus prednisone on product parameters of sipuleucel-T; [g]Aims to evaluate immune responses in patients with non-metastatic prostate cancer when androgen deprivation therapy is started before or after sipuleucel-T. CRPC, castration-resistant prostate cancer; GCV, ganciclovir; GMCI, gene-mediated cytotoxic immunotherapy; GM-CSF, granulocyte macrophage colony-stimulating factor; IFNγ, interferon γ; IL-2, interleukin-2; OS, overall survival; PAP, prostate acid phosphatase; PBMC, peripheral blood mononuclear cell: PSA, prostate-specific antigen; PSADT, PDA doubling time; RT, radiotherapy.

in 10/22 patients. At least a 3-fold increase in PAP-specific proliferating $CD8^+$ T lymphocytes (which produced interferon γ) was detected in 3/22 patients. In addition, 41% (9/22) of patients developed PAP-specific ($CD4^+$ or $CD8^+$) T-lymphocyte proliferative responses. Interestingly no humoral responses to PAP were detected. A subsequent longitudinal analysis of data from this clinical trial concluded that increasing the number of vaccinations correlated with an increased probability of generating PAP-specific T-lymphocyte responses.[65] In addition, it was shown that 6/8 and 1/14 patients with long-term PAP-specific T-lymphocyte responses had a > 200% increase in PSADT and no change in PSADT, respectively ($p = 0.001$).

In a Phase I trial to evaluate safety, feasibility and immunogenicity, a plasmid encoding PSA (pVAX/PSA) was administered intradermally (5× at 4 week intervals) to nine patients with CRPC.[31] In addition, subcutaneous administrations of GM-CSF (40 μg/day for 3 d, starting 2 d prior to vaccination) and IL-2 (75 μg/day for 7 d, starting one day after vaccination) were performed at the site of vaccination. The presence of these adjuvants was shown to be beneficial in a preclinical study.[66] Amounts of pVax/PSA ranging from 100 to 900 μg were tested and no dose-limiting toxicities were observed, while treatment-related adverse effects did not exceed WHO grade 2. In addition, there was no evidence of vaccination-related autoimmune diseases. pVAX/PSA was capable of inducing PSA-specific humoral and cellular immune responses. Three out of 3 patients treated with the highest dose of pVAX/PSA (900 μg) manifested increased levels of PSA-specific interferon γ-producing T lymphocytes, and 2/3 of these patients also exhibited increased PSADT and disease stabilization.

The use of naked DNA-based vaccines is potentially limited by low transfection efficiencies. In this respect, it has recently been shown that in vivo electroporation significantly improves the ability of pVAX/PSA to elicit PSA-specific CD8+ T-lymphocyte responses (in preclinical models).[67] A clinical trial was initiated in 2009 to examine the effects of pVAXrcPSAv53l (pVAX encoding rhesus monkey PSA) delivered by in vivo electroporation, but its current status is unknown (NCT00859729).

Gene-Mediated Cytotoxic Immunotherapy

Gene-mediated cytotoxic immunotherapy (GMCI) involves the intratumoral delivery of an adenovirus encoding the Herpes simplex virus enzyme thymidine kinase (AdV-tk). Transduced tumor cells become susceptible to systemically administered prodrugs such as valcyclovir (VCV) or ganciclovir (GCV), which are selectively converted by thymidine kinase to cytotoxic nucleotide analogs.[68,69] These activated drugs can then affect neighboring cells (in particular highly proliferating cells) through multiple processes, collectively known as the local bystander effect.[70] GMCI has also been shown to elicit an immunological, systemic bystander effect that attacks metastases and protects against tumor rechallenge.[71] A Phase I/II clinical trial on 23 prostate cancer patients with locally advanced disease prior to prostatectomy was initiated to test the safety and preliminary therapeutic potential of AdV-tk.[34] This study involved (1–4) intraprostatic injections of AdV-tk followed by 2 weeks of GCV-based therapy and (2–4 weeks later) prostatectomy. The analysis of resected prostates revealed a highly significant influx of $CD8^+$ T lymphocytes into the tumors of patients who had received AdV-tk compared with those of control patients. The levels of activated $CD8^+$ T lymphocytes were also significantly increased in the blood. This was in contrast to changes in the abundance of $CD4^+$ T lymphocytes, natural killer cells and B lymphocytes, which either remained unaffected or decreased. GMCI was shown to preferentially cause cytopathological effects to malignant (rather than on benign) tissues. However, there was no significant amelioration in the prognosis of AdV-tk-treated patients, as measured in terms of biochemical recurrence (PSA levels). This lack of clinical efficacy prompted the investigation of combinatorial treatments involving chemotherapy or radiotherapy and GMCI. Although the combination of chemotherapy

and AdV-tk-based GMCI has not yet been investigated in clinical trials, a Phase I/II study testing AdV-tk-based GMCI combined with radiotherapy in patients bearing prostate cancer at various stages has been completed.[72] In this setting, patients were grouped into three categories/arms which included 29 low-risk patients (stage T1-T2a disease, Gleason score < 7), 26 high-risk patients (stage T2b-T3 disease, Gleason score > 6) and 4 patients with stage D1 disease. Promising observations were made on bioptic data, demonstrating a good locoregional control achieved by AdV-tk-based GMCI in all low-risk and high-risk patients. With median follow-ups > 13 mo, a control of PSA levels was also noted for all low-risk and high-risk patients. However, 3 out of 4 patients with stage D1 disease manifested biochemical failure at 3, 3 and 7 mo, respectively. A double-blind, randomized, placebo-controlled Phase III trial of ProstAtak™ (AdV-tk plus VCV) in combination with standard external beam radiation therapy, with or without androgen deprivation therapy, is currently recruiting patients with intermediate-high risk localized prostate cancer (NCT01436968).

Concluding Remarks

Results from the clinical trials presented here indicate that prostate cancer vaccines are generally safe and, encouragingly, capable of generating tumor-specific T-lymphocyte responses. It is becoming evident that prostate cancer patients with early-stage disease may be those who obtain the main benefits from vaccines.[22] This was particularly apparent in patients receiving ProstVac VF, but a similar trend was also suggested by the results of studies involving Ad5-PSA and GMCI.[22,34,47,63] A Phase IIIB clinical trial for sipuleucel-T (NCT00779402) is ongoing to test the effectiveness of this vaccine on early-stage (non-metastatic) hormone-sensitive prostate cancer patients. In patients with advanced tumors, effective immune responses are possibly being suppressed by either tumor cells themselves and/or by regulatory T lymphocytes and myeloid-derived suppressor cells, both of which accumulate in the tumor microenvironment as disease progresses. In an attempt to (at least partially) address this issue, both GVAX-PCa and ProstVac VF have been combined with antibodies (ipilimumab or nivolumab) that limit the activity of regulatory T lymphocytes in Phase I clinical trials, yielding encouraging results that warrant further investigation.[49,54] Aside from immunosuppression, patients with advanced prostate cancer also bear bulky lesions, which the immune system cannot effectively eradicate. Chemotherapeutic agents that efficiently reduce disease burden, in particular docetaxel, are currently being tested in combination with ProstVac VF (NCT01145508) for the treatment of patients with metastatic CRPC. Hopefully, these combinations and others that are currently being trialed (**Table 1**) will ultimately lead to the development of vaccination strategies that not only improve the survival of prostate cancer patients but also their quality of life. Vaccines are under intensive investigation for the therapy of a wide range of cancers, yielding promising results that have been recently summarized elsewhere.[73]

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

References

1. Siegel R, Naishadham D, Jemal A. Cancer statistics, 2012. CA Cancer J Clin 2012; 62:10-29; PMID:22237781; http://dx.doi.org/10.3322/caac.20138
2. Ferlay J, Parkin DM, Steliarova-Foucher E. Estimates of cancer incidence and mortality in Europe in 2008. Eur J Cancer 2010; 46:765-81; PMID:20116997; http://dx.doi.org/10.1016/j.ejca.2009.12.014
3. Kirby M, Hirst C, Crawford ED. Characterising the castration-resistant prostate cancer population: a systematic review. Int J Clin Pract 2011; 65:1180-92; PMID:21995694; http://dx.doi.org/10.1111/j.1742-1241.2011.02799.x
4. Inoue T, Segawa T, Kamba T, Yoshimura K, Nakamura E, Nishiyama H, et al. Prevalence of skeletal complications and their impact on survival of hormone refractory prostate cancer patients in Japan. Urology 2009; 73:1104-9; PMID:19394511; http://dx.doi.org/10.1016/j.urology.2008.07.062
5. Smith MR, Kabbinavar F, Saad F, Hussain A, Gittelman MC, Bilhartz DL, et al. Natural history of rising serum prostate-specific antigen in men with castrate nonmetastatic prostate cancer. J Clin Oncol 2005; 23:2918-25; PMID:15860850; http://dx.doi.org/10.1200/JCO.2005.01.529
6. Beltran H, Beer TM, Carducci MA, de Bono J, Gleave M, Hussain M, et al. New therapies for castration-resistant prostate cancer: efficacy and safety. Eur Urol 2011; 60:279-90; PMID:21592649; http://dx.doi.org/10.1016/j.eururo.2011.04.038
7. Coffey DS, Isaacs JT. Prostate tumor biology and cell kinetics--theory. Urology 1981; 17(Suppl 3):40-53; PMID:7010755
8. Kiessling A, Füssel S, Wehner R, Bachmann M, Wirth MP, Rieber EP, et al. Advances in specific immunotherapy for prostate cancer. Eur Urol 2008; 53:694-708; PMID:18061335; http://dx.doi.org/10.1016/j.eururo.2007.11.043
9. 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; PMID:10873066
10. 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; PMID:11099318
11. 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; PMID:19536890; http://dx.doi.org/10.1002/cncr.24429
12. Kantoff PW, Higano CS, Shore ND, Berger ER, Small EJ, Penson DF, et al.; IMPACT Study Investigators. Sipuleucel-T immunotherapy for castration-resistant prostate cancer. N Engl J Med 2010; 363:411-22; PMID:20818862; http://dx.doi.org/10.1056/NEJMoa1001294
13. Sonpavde G, Di Lorenzo G, Higano CS, Kantoff PW, Madan R, Shore ND. The role of sipuleucel-T in therapy for castration-resistant prostate cancer: a critical analysis of the literature. Eur Urol 2012; 61:639-47; PMID:22036643; http://dx.doi.org/10.1016/j.eururo.2011.10.027
14. Drake CG. Prostate cancer as a model for tumour immunotherapy. Nat Rev Immunol 2010; 10:580-93; PMID:20651745; http://dx.doi.org/10.1038/nri2817
15. Geary SM, Lemke CD, Lubaroff DM, Salem AK. Proposed mechanisms of action for prostate cancer vaccines. Nat Rev Urol 2013; 10:149-60; PMID:23399727; http://dx.doi.org/10.1038/nrurol.2013.8
16. Huber ML, Haynes L, Parker C, Iversen P. Interdisciplinary critique of sipuleucel-T as immunotherapy in castration-resistant prostate cancer. J Natl Cancer Inst 2012; 104:273-9; PMID:22232132; http://dx.doi.org/10.1093/jnci/djr514
17. Drake CG. Re: interdisciplinary critique of sipuleucel-T as immunotherapy in castration-resistant prostate cancer. J Natl Cancer Inst 2012; 104:1422, author reply 1422-3; PMID:22911668; http://dx.doi.org/10.1093/jnci/djs340
18. Gulley JL, Leitman SF, Dahut W, Schlom J. Re: interdisciplinary critique of sipuleucel-T as immunotherapy in castration-resistant prostate cancer. J Natl Cancer Inst 2012; 104:1106, author reply 1109-12; PMID:22825555; http://dx.doi.org/10.1093/jnci/djs280
19. 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; PMID:17707059; http://dx.doi.org/10.1016/j.juro.2007.05.117
20. DiPaola RS, Plante M, Kaufman H, Petrylak DP, Israeli R, Lattime E, et al. A phase I trial of pox PSA vaccines (PROSTVAC-VF) with B7-1, ICAM-1, and LFA-3 co-stimulatory molecules (TRICOM) in patients with prostate cancer. J Transl Med 2006; 4:1; PMID:16390546; http://dx.doi.org/10.1186/1479-5876-4-1

21. 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; PMID:20100959; http://dx.doi.org/10.1200/JCO.2009.25.0597
22. 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; PMID:19890632; http://dx.doi.org/10.1007/s00262-009-0782-8
23. Higano C, Saad F, Somer B, Curti B, Petrylak DP, Drake CG, et al. A phase III trial of GVAX immunotherapy for prostate cancer vs. docetaxel plus prednisone in asymptomatic, castration-resistant prostate cancer (CRPC). Genitourinary Cancer Symposium: Proc Am Soc Clin Oncol, 2009:Abstract # LBA150
24. 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; PMID:16489082; http://dx.doi.org/10.1158/1078-0432.CCR-05-2059
25. Lubaroff DM, Konety BR, Link B, Gerstbrein J, Madsen T, Shannon M, et al. Phase I clinical trial of an adenovirus/prostate-specific antigen vaccine for prostate cancer: safety and immunologic results. Clin Cancer Res 2009; 15:7375-80; PMID:19920098; http://dx.doi.org/10.1158/1078-0432.CCR-09-1910
26. Small EJ, Sacks N, Nemunaitis J, Urba WJ, Dula E, Centeno AS, et al. Granulocyte macrophage colony-stimulating factor--secreting allogeneic cellular immunotherapy for hormone-refractory prostate cancer. Clin Cancer Res 2007; 13:3883-91; PMID:17606721; http://dx.doi.org/10.1158/1078-0432.CCR-06-2937
27. 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; PMID:18646045; http://dx.doi.org/10.1002/cncr.23669
28. Small EJDT. Gerritsen WR, 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). 2009 Genitourinary Cancers Symposium (ASCO Annual Meeting Abstracts) 2009:LBA150
29. van den Eertwegh AJ, Versluis J, van den Berg HP, Santegoets SJ, van Moorselaar RJ, van der Sluis TM, et al. Combined immunotherapy with granulocyte-macrophage colony-stimulating factor-transduced allogeneic prostate cancer cells and ipilimumab in patients with metastatic castration-resistant prostate cancer: a phase 1 dose-escalation trial. Lancet Oncol 2012; 13:509-17; PMID:22326922; http://dx.doi.org/10.1016/S1470-2045(12)70007-4
30. 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; PMID:19636017; http://dx.doi.org/10.1200/JCO.2008.19.9968
31. Pavlenko M, Roos AK, Lundqvist A, Palmborg A, Miller AM, Ozenci V, et al. A phase I trial of DNA vaccination with a plasmid expressing prostate-specific antigen in patients with hormone-refractory prostate cancer. Br J Cancer 2004; 91:688-94; PMID:15280930
32. Herman JR, Adler HL, Aguilar-Cordova E, Rojas-Martinez A, Woo S, Timme TL, et al. In situ gene therapy for adenocarcinoma of the prostate: a phase I clinical trial. Hum Gene Ther 1999; 10:1239-49; PMID:10340555; http://dx.doi.org/10.1089/10430349950018229
33. Miles BJ, Shalev M, Aguilar-Cordova E, Timme TL, Lee HM, Yang G, et al. Prostate-specific antigen response and systemic T cell activation after in situ gene therapy in prostate cancer patients failing radiotherapy. Hum Gene Ther 2001; 12:1955-67; PMID:11686937; http://dx.doi.org/10.1089/104303401753204535
34. Ayala G, Satoh T, Li R, Shalev M, Gdor Y, Aguilar-Cordova E, et al. Biological response determinants in HSV-tk + ganciclovir gene therapy for prostate cancer. Mol Ther 2006; 13:716-28; PMID:16480930; http://dx.doi.org/10.1016/j.ymthe.2005.11.022
35. Fong L, Weinberg VK, Corman JM, Amling CL, Stephenson RA, Formaker C, et al. Immune responses in prostate tumor tissue following neoadjuvant sipuleucel-T in patients with localized prostate cancer. 2012 Genitiurinary Cancers Symposium. J Clin Oncol 2012
36. Le DT, Pardoll DM, Jaffee EM. Cellular vaccine approaches. Cancer J 2010; 16:304-10; PMID:20693840; http://dx.doi.org/10.1097/PPO.0b013e3181eb33d7
37. van den Eertwegh AJ, Versluis J, van den Berg HP, Santegoets SJ, van Moorselaar RJ, van der Sluis TM, et al. Combined immunotherapy with granulocyte-macrophage colony-stimulating factor-transduced allogeneic prostate cancer cells and ipilimumab in patients with metastatic castration-resistant prostate cancer: a phase 1 dose-escalation trial. Lancet Oncol 2012; 13:509-17; PMID:22326922; http://dx.doi.org/10.1016/S1470-2045(12)70007-4
38. van Elsas A, Hurwitz AA, Allison JP. Combination immunotherapy of B16 melanoma using anti-cytotoxic T lymphocyte-associated antigen 4 (CTLA-4) and granulocyte/macrophage colony-stimulating factor (GM-CSF)-producing vaccines induces rejection of subcutaneous and metastatic tumors accompanied by autoimmune depigmentation. J Exp Med 1999; 190:355-66; PMID:10430624; http://dx.doi.org/10.1084/jem.190.3.355
39. Hurwitz AA, Yu TF, Leach DR, Allison JP. CTLA-4 blockade synergizes with tumor-derived granulocyte-macrophage colony-stimulating factor for treatment of an experimental mammary carcinoma. Proc Natl Acad Sci U S A 1998; 95:10067-71; PMID:9707601; http://dx.doi.org/10.1073/pnas.95.17.10067
40. 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; PMID:20525992; http://dx.doi.org/10.1056/NEJMoa1003466
41. Robert C, Thomas L, Bondarenko I, O'Day S, M D JW, Garbe C, et al. Ipilimumab plus dacarbazine for previously untreated metastatic melanoma. N Engl J Med 2011; 364:2517-26; PMID:21639810; http://dx.doi.org/10.1056/NEJMoa1104621
42. 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 vs. docetaxel plus prednisone in symptomatic, castration-resistant prostate cancer (CRPC). Genitourinary Cancer Symposium: Proc Am Soc Clin Oncol 2009:Abstract #7
43. Drake CG. Immunotherapy for prostate cancer: walk, don't run. J Clin Oncol 2009; 27:4035-7; PMID:19635998; http://dx.doi.org/10.1200/JCO.2009.22.2299
44. 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; PMID:15169798; http://dx.doi.org/10.1200/JCO.2004.08.083
45. 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; PMID:12242725; http://dx.doi.org/10.1002/pros.10130
46. 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; PMID:12663709; http://dx.doi.org/10.1200/JCO.2003.06.100
47. Gulley JL, Madan RA, Schlom J. Impact of tumour volume on the potential efficacy of therapeutic vaccines. Curr Oncol 2011; 18:e150-7; PMID:21655153; http://dx.doi.org/10.3747/co.v18i3.783
48. Gulley JL, Madan RA, Stein WD, Wilkerson J, Dahut WL, Heery CR, et al. Effect of PSA-tricom, a pox-viral vaccine in prostate cancer (PCa), on tumor growth rates within 80 days after initiation in nonmetastatic PCa. Genitourinary Cancer Symposium: J Clin Oncol 2013:suppl 6:abstract #57
49. Liakou CI, Kamat A, Tang DN, Chen H, Sun J, Troncoso P, et al. CTLA-4 blockade increases IFNgamma-producing CD4+ICOShi cells to shift the ratio of effector to regulatory T cells in cancer patients. Proc Natl Acad Sci U S A 2008; 105:14987-92; PMID:18818309; http://dx.doi.org/10.1073/pnas.0806075105
50. Madan RA, Mohebtash M, Arlen PM, Vergati M, Rauckhorst M, Steinberg SM, et al. Ipilimumab and a poxviral vaccine targeting prostate-specific antigen in metastatic castration-resistant prostate cancer: a phase 1 dose-escalation trial. Lancet Oncol 2012; 13:501-8; PMID:22326924; http://dx.doi.org/10.1016/S1470-2045(12)70006-2
51. Sanderson K, Scotland R, Lee P, Liu D, Groshen S, Snively J, et al. Autoimmunity in a phase I trial of a fully human anti-cytotoxic T-lymphocyte antigen-4 monoclonal antibody with multiple melanoma peptides and Montanide ISA 51 for patients with resected stages III and IV melanoma. J Clin Oncol 2005; 23:741-50; PMID:15613700; http://dx.doi.org/10.1200/JCO.2005.01.128
52. Phan GQ, Yang JC, Sherry RM, Hwu P, Topalian SL, Schwartzentruber DJ, et al. Cancer regression and autoimmunity induced by cytotoxic T lymphocyte-associated antigen 4 blockade in patients with metastatic melanoma. Proc Natl Acad Sci U S A 2003; 100:8372-7; PMID:12826605; http://dx.doi.org/10.1073/pnas.1533209100
53. Attia P, Phan GQ, Maker AV, Robinson MR, Quezado MM, Yang JC, et al. Autoimmunity correlates with tumor regression in patients with metastatic melanoma treated with anti-cytotoxic T-lymphocyte antigen-4. J Clin Oncol 2005; 23:6043-53; PMID:16087944; http://dx.doi.org/10.1200/JCO.2005.06.205
54. Brahmer JR, Drake CG, Wollner I, Powderly JD, Picus J, Sharfman WH, et al. Phase I study of single-agent anti-programmed death-1 (MDX-1106) in refractory solid tumors: safety, clinical activity, pharmacodynamics, and immunologic correlates. J Clin Oncol 2010; 28:3167-75; PMID:20516446; http://dx.doi.org/10.1200/JCO.2009.26.7609
55. Andersen MH, Junker N, Ellebaek E, Svane IM, Thor Straten P. Therapeutic cancer vaccines in combination with conventional therapy. J Biomed Biotechnol 2010; 2010:237623; PMID:20617155; http://dx.doi.org/10.1155/2010/237623
56. Schlom J. Recent advances in therapeutic cancer vaccines. Cancer Biother Radiopharm 2012; 27:2-5; PMID:22251077; http://dx.doi.org/10.1089/cbr.2012.1200
57. Liniger M, Zuniga A, Naim HY. Use of viral vectors for the development of vaccines. Expert Rev Vaccines 2007; 6:255-66; PMID:17408374; http://dx.doi.org/10.1586/14760584.6.2.255
58. Cheng C, Gall JG, Kong WP, Sheets RL, Gomez PL, King CR, et al. Mechanism of ad5 vaccine immunity and toxicity: fiber shaft targeting of dendritic cells. PLoS Pathog 2007; 3:e25; PMID:17319743; http://dx.doi.org/10.1371/journal.ppat.0030025

59. Miller G, Lahrs S, Pillarisetty VG, Shah AB, DeMatteo RP. Adenovirus infection enhances dendritic cell immunostimulatory properties and induces natural killer and T-cell-mediated tumor protection. Cancer Res 2002; 62:5260-6; PMID:12234994
60. Siemens DR, Elzey BD, Lubaroff DM, Bohlken C, Jensen RJ, Swanson AK, et al. Cutting edge: restoration of the ability to generate CTL in mice immune to adenovirus by delivery of virus in a collagen-based matrix. J Immunol 2001; 166:731-5; PMID:11145643
61. Lubaroff DM, Konety B, Link BK, Ratliff TL, Madsen T, Shannon M, et al. Clinical protocol: phase I study of an adenovirus/prostate-specific antigen vaccine in men with metastatic prostate cancer. Hum Gene Ther 2006; 17:220-9; PMID:16454655; http://dx.doi.org/10.1089/hum.2006.17.220
62. Lubaroff DM. Prostate cancer vaccines in clinical trials. Expert Rev Vaccines 2012; 11:857-68; PMID:22913261; http://dx.doi.org/10.1586/erv.12.54
63. Lubaroff DM, Williams RD, Vaena D, Joudi F, Brown J, Smith M, et al. An ongoing Phase II trial of an adenovirus/PSA vaccine for prostate cancer. 103rd Annual Meeting of the American Association for Cancer Research. Chicago IL: Cancer Res, 2012
64. Ahmad S, Sweeney P, Sullivan GC, Tangney M. DNA vaccination for prostate cancer, from preclinical to clinical trials - where we stand? Genet Vaccines Ther 2012; 10:9; PMID:23046944; http://dx.doi.org/10.1186/1479-0556-10-9
65. 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; PMID:20551832; http://dx.doi.org/10.1097/CJI.0b013e3181dda23e
66. Roos AK, Pavlenko M, Charo J, Egevad L, Pisa P. Induction of PSA-specific CTLs and anti-tumor immunity by a genetic prostate cancer vaccine. Prostate 2005; 62:217-23; PMID:15389792; http://dx.doi.org/10.1002/pros.20135
67. Roos AK, Moreno S, Leder C, Pavlenko M, King A, Pisa P. Enhancement of cellular immune response to a prostate cancer DNA vaccine by intradermal electroporation. Mol Ther 2006; 13:320-7; PMID:16185933; http://dx.doi.org/10.1016/j.ymthe.2005.08.005
68. Fyfe JA, Keller PM, Furman PA, Miller RL, Elion GB. Thymidine kinase from herpes simplex virus phosphorylates the new antiviral compound, 9-(2-hydroxyethoxymethyl)guanine. J Biol Chem 1978; 253:8721-7; PMID:214430
69. Eastham JA, Chen SH, Sehgal I, Yang G, Timme TL, Hall SJ, et al. Prostate cancer gene therapy: herpes simplex virus thymidine kinase gene transduction followed by ganciclovir in mouse and human prostate cancer models. Hum Gene Ther 1996; 7:515-23; PMID:8800746; http://dx.doi.org/10.1089/hum.1996.7.4-515
70. Freeman SM, Abboud CN, Whartenby KA, Packman CH, Koeplin DS, Moolten FL, et al. The "bystander effect": tumor regression when a fraction of the tumor mass is genetically modified. Cancer Res 1993; 53:5274-83; PMID:8221662
71. Aguilar LK, Guzik BW, Aguilar-Cordova E. Cytotoxic immunotherapy strategies for cancer: mechanisms and clinical development. J Cell Biochem 2011; 112:1969-77; PMID:21465529; http://dx.doi.org/10.1002/jcb.23126
72. Teh BS, Ayala G, Aguilar L, Mai WY, Timme TL, Vlachaki MT, et al. Phase I-II trial evaluating combined intensity-modulated radiotherapy and in situ gene therapy with or without hormonal therapy in treatment of prostate cancer-interim report on PSA response and biopsy data. Int J Radiat Oncol Biol Phys 2004; 58:1520-9; PMID:15050332; http://dx.doi.org/10.1016/j.ijrobp.2003.09.083
73. Senovilla L, Garcia P, Eggermont A, Fridman WH, Galon J, Zitvogel L, et al. Trial watch: DNA vaccines for cancer therapy. OncoImmunology 2013; 2:e23803; http://dx.doi.org/10.4161/onci.23803
74. Teh BS, Aguilar-Cordova E, Kernen K, Chou CC, Shalev M, Vlachaki MT, et al. Phase I/II trial evaluating combined radiotherapy and in situ gene therapy with or without hormonal therapy in the treatment of prostate cancer--a preliminary report. Int J Radiat Oncol Biol Phys 2001; 51:605-13; PMID:11597799; http://dx.doi.org/10.1016/S0360-3016(01)01692-3