Published in final edited form as:

Curr Cancer Ther Rev. 2012 November 1; 8(4): 254–263. doi:10.2174/157339412804143113.

DNA Vaccines for Prostate Cancer

Douglas G. McNeel*, Jordan T. Becker, Laura E. Johnson, and Brian M. Olson

Department of Medicine, University of Wisconsin Carbone Cancer Center, 1111 Highland Avenue, Madison, WI 53705, USA

Abstract

Delivery of plasmid DNA encoding an antigen of interest has been demonstrated to be an effective means of immunization, capable of eliciting antigen-specific T cells. Plasmid DNA vaccines offer advantages over other anti-tumor vaccine approaches in terms of simplicity, manufacturing, and possibly safety. The primary disadvantage is their poor transfection efficiency and subsequent lower immunogenicity relative to other genetic vaccine approaches. However, multiple preclinical models demonstrate anti-tumor efficacy, and many efforts are underway to improve the immunogenicity and anti-tumor effect of these vaccines. Clinical trials using DNA vaccines as treatments for prostate cancer have begun, and to date have demonstrated safety and immunological effect. This review will focus on DNA vaccines as a specific means of antigen delivery, advantages and disadvantages of this type of immunization, previous experience in preclinical models and human trials specifically conducted for the treatment of prostate cancer, and future directions for the application of DNA vaccines to prostate cancer immunotherapy.

Keywords

DNA vaccines; prostate cancer; tumor antigens; clinical trials

INTRODUCTION: CANCER IMMUNOTHERAPY AND ANTI-TUMOR VACCINES

The goal of tumor immunotherapy is to harness components of the immune system to treat existing cancer. Cancer immunotherapies are broadly divided into two subtypes: passive and active therapies. Passive immunotherapy involves the transfer of immunological mediators (such as tumor-specific antibodies, autologous T-cells, or immune-modulating agents) into tumor-bearing hosts, where the transferred immune agents are able to directly mediate or modulate an anti-tumor response. Alternatively, active immunotherapies (or anti-tumor vaccines) are designed to elicit and/or augment the host's own intrinsic anti-tumor immune responses. In the last year, both approaches have demonstrated success in clinical trials leading to FDA approval of ipilimumab (a passive approach, using a monoclonal antibody targeting CTLA-4) for the treatment of metastatic melanoma [1], and FDA approval of sipuleucel-T (an active approach, using autologous antigen-presenting cells loaded *ex vivo* with a prostate-specific antigen) for the treatment of metastatic prostate cancer [2]. The FDA approval of sipuleucel-T, in particular, underscores the potential for active immunotherapies,

© 2012 Bentham Science Publishers

*Address correspondence to this author at the 7007 Wisconsin Institutes for Medical Research, 1111 Highland Avenue, Madison, WI 53705, USA; Tel: (608) 265-8131; Fax: (608) 265-0614; dm3@medicine.wisc.edu.

CONFLICT OF INTEREST

The author(s) confirm that this article content has no conflicts of interest.

and specifically anti-tumor vaccines, to be further developed and improved for the treatment of prostate cancer.

Active immunotherapies can be further subdivided into either antigen-specific or antigen-non-specific vaccines. Antigen non-specific vaccines are designed to elicit an anti-tumor immune response to any or all antigens displayed by tumor cells. A common example of these vaccines includes irradiated tumor cell vaccines, including gene-modified cellular vaccines such as the GVAX vaccine approach, which has been extensively evaluated in prostate cancer [3, 4]. Antigen-specific vaccines are designed to elicit an anti-tumor immune response against one or more specific antigenic target(s) made and displayed by tumor cells. These vaccines differ in the means of delivering the particular antigenic target, using purified protein or peptide, using genetic carriers (bacteria, virus, naked DNA) encoding the antigen, or using antigen-presenting cells loaded *ex vivo* with the target antigen, such as the sipuleucel-T vaccine. Many of these approaches are described elsewhere in this journal issue. This review will focus on DNA vaccines as a specific means of antigen delivery, advantages and disadvantages of this means of immunization, previous experience in preclinical models and human trials specifically conducted for the treatment of prostate cancer, and future directions for the application of DNA vaccines to prostate cancer immunotherapy.

DNA Vaccines – History

DNA vaccines are typically bacterial DNA plasmids that encode the cDNA of an antigen of interest. The use of bacterial plasmid DNA as a means of delivering a vaccine antigen was initially discovered serendipitously by Wolff and colleagues, who found that when naked DNA was injected intramuscularly into rodents, host muscle cells could take up this DNA and express the encoded antigen [5]. Moreover, plasmid DNA could persist in cells long-term as an extrachromosomal plasmid [6]. It was subsequently demonstrated that proteins encoded and expressed by the plasmid DNA, under a eukaryotic promoter, could elicit immune responses, notably cytotoxic T-cell type responses [7]. The generation of immune responses has been demonstrated to be due to transfection of MHC class 1-expressing bystander cells as well as professional antigen-presenting cells, by direct transfection and via cross-presentation [8]. As a result, plasmid DNA vaccines are able to elicit both CD4+ and CD8+ cellular antigen-specific immunity, a characteristic that sets DNA vaccines apart from many other types of active immunotherapy [9, 10]. In addition, the presence of unmethylated CpG motifs present in the bacterial DNA may further serve as an immune stimulant by TLR9 activation [11]. Moreover, recent studies suggest that the presence of specific intracellular DNA sensors, including DAI (DNA-dependent activator of IFN-regulatory factors [12]), and members of the interferon-inducible gene family (IFI16, AIM2 [13]) can recognize double stranded cytosolic DNA and elicit inflammatory type immune responses via activation of STING (stimulator of interferon genes) independent of TLR9 [14–16]. By one or both of these mechanisms, TLR activation or intracellular DNA sensors, the bacterial DNA itself may act as a vaccine adjuvant [17]. DNA vaccines have been widely studied in infectious disease models and as anti-tumor vaccines, both in preclinical models and human clinical trials. In fact, prior to the FDA approval of sipuleucel-T, the only anti-cancer vaccine approved as an anti-tumor therapy in the U.S. was a DNA vaccine encoding human tyrosinase, Oncept, approved by USDA in 2010 for the treatment of canine melanoma, based on studies demonstrating a marked increase in survival of treated dogs [18].

DNA Vaccines – Advantages and Disadvantages

Like peptide and protein vaccines, DNA vaccines have an advantage in being highly-purified “off-the-shelf” reagents that are relatively inexpensive to manufacture. This is in

contrast to antigen-presenting cell vaccines, such as sipulcucel-T, which require preparation from autologous cells, and thus add significant cost and labor. Plasmid DNA is also stable, more stable than protein-based vaccines, and hence of particular interest as a means of vaccination for underdeveloped regions where loss of drug stability due to storage conditions remains problematic.

Another major advantage to DNA vaccines relates to their mechanism of action. Like viral vaccines, DNA vaccines enter host cells and guide the expression of antigens that are then expressed and presented by the host's antigen-presentation machinery. This means that post-translational modifications can take place, and the antigen is appropriately MHC-restricted and presented, leading to a CD8-biased T cell immune response. This is in contrast to protein vaccines, which may lead to more Th2-biased immune responses via cross-presentation by antigen-presenting cells, and to peptide vaccines in which the specific MHC type must be necessarily restricted, thus limiting their application only to individuals of specific MHC types. Furthermore, as the DNA plasmid can persist long-term in transfected cells, this continued antigen expression might help maintain antigen-specific T-cell activation. This is in contrast to peptide-based vaccines which bind to MHC complexes, but once recycled back into cells likely lose any ability to further prime antigen-specific immune responses.

Another advantage to DNA vaccines, in particular compared with viral or bacterial vaccines, is that the number of antigens expressed can be tightly controlled. Viral vaccines, while able in many cases to elicit robust immune responses, suffer from immune responses being generated against expressed foreign viral antigens (which may be prioritized over the targeted antigen), thus potentially compromising the response against the encoded tumor antigens and negating the effect of booster immunizations for some viruses [19]. DNA vaccines, on the other hand, are comprised solely of a DNA plasmid backbone, do not require expression of any foreign antigens, and consequently can be used to prime and boost immunizations without generating immune responses to the vector itself. Moreover, DNA vaccines can also be easily engineered to encode multiple antigens, portions of antigens, vaccine adjuvants, or subcellular localization signals to guide the expression or processing of encoded antigens.

The primary disadvantage of DNA vaccines has been that direct injection of plasmid DNA is inefficient in transfecting host cells, notably less efficient in primates compared with rodents [20]. Consequently, DNA vaccines are less potently immunogenic than viral vaccines. For this reason many of the approaches in translating DNA vaccines to human application have investigated means to increase the immunogenicity of these vaccines by using multiple or heterologous vaccination approaches, using different vaccine adjuvants, co-expressing molecules involved in antigen-presentation or T-cell activation, using carriers or electroporation to increase cell transfection, using different routes of DNA administration, or making modifications to the antigen coding sequence itself. These approaches are reviewed in the following sections, specifically highlighting strategies in preclinical studies that are being incorporated into human trials for prostate cancer aimed at improving the efficacy of DNA vaccines.

DNA Vaccines for Prostate Cancer – Preclinical Studies

The development of DNA vaccines for prostate cancer have largely focused on targeting antigens with prostate-specific expression, including prostate specific antigen (PSA), prostate specific membrane antigen (PSMA), and prostate acid phosphatase (PAP), which have been extensively studied in both preclinical models and clinical trials. Other prostate-associated antigens, including prostate stem cell antigen (PSCA), six transmembrane epithelial antigen of the prostate (STEAP), tumor protein D52 (TPD52), and the cancer-

testis antigen synovial sarcoma X breakpoint-2 (SSX-2), have also been investigated in preclinical models. These approaches are summarized in Table 1.

One of the first antigens to be targeted using DNA vaccines for prostate cancer was PSA. PSA, a member of the kallikrein family of serine proteases, is a prostate tissue-specific protein secreted by healthy prostate cells and frequently overexpressed by prostate cancer cells. PSA has become the standard clinical biomarker for diagnosis and progression of prostate cancer, and due to its high tissue specificity has been a rational target for antigen-specific immunotherapy. First studied in rodent models, several groups found that immune responses elicited to PSA by DNA vaccination could induce immune responses that could recognize and lyse prostate cancer cells [21–23]. Kim *et al* found that female BALB/c mice immunized with a DNA plasmid encoding PSA under a CMV promoter could elicit persistent PSA-specific antibodies as well as IFN γ -secreting cytotoxic T lymphocytes (CTLs) capable of lysing PSA-expressing tumor cells [22]. Because there is not a prostate-specific murine homologue of PSA, this vaccine was further investigated in nonhuman primates. The PSA of rhesus in nonhuman primates. The PSA of rhesus macaques is 94% homologous to human PSA. Rhesus macaques or cynomolgus monkeys immunized with PSA-expressing DNA vaccines encoding the human orthologue developed PSA-specific antibodies and Th1-biased T-cell responses [23, 24]. T helper cell proliferation responses were further augmented when the PSA DNA vaccine was combined with plasmids encoding the cytokines interleukin-2 (IL-2), interleukin-12 (IL-12), and interleukin-18 (IL-18) [24]. Further studies in rhesus macaques indicated that frequent immunizations with high dosages of the PSA DNA vaccine were well tolerated and enhanced the PSA-specific proliferative responses. Together, these studies suggested that dose of plasmid DNA, and co-administration with inflammatory cytokines, may be important for maximizing immunological efficacy.

PSMA is a transmembrane protein with a large extracellular domain and thus has been explored as a target for cell-surface directed antibodies as well as T-cell therapies [25]. Moreover, while PSMA is expressed in some other tissues, it becomes increasingly overexpressed on prostate cancer cells with disease progression and in regions of high intratumoral angiogenesis. Consequently, it serves as a rational target for more aggressive prostate cancer [26]. Several groups have investigated DNA vaccines encoding PSMA, predominantly using xenogeneic immunization approaches. McGregor *et al* demonstrated that mice vaccinated with a DNA plasmid encoding human PSMA (hPSMA) developed CD8 $^{+}$ T cells specific for hPSMA and the production of autoantibodies to mouse PSMA (mPSMA) while no immune responses were detected in mice vaccinated with a DNA vaccine encoding mPSMA [27, 28]. The mPSMA-specific antibodies elicited with DNA vaccination also recognized the hPSMA indicating that they arose from a response to epitopes shared between mPSMA and hPSMA. Mincheff and colleagues explored a similar approach using a prime-boost strategy in Copenhagen rats. Rats were first primed with a xenogeneic DNA vaccine encoding the extracellular domain of human PSMA (hPSMA $_{\text{t}}$) followed by a booster immunization with a DNA plasmid encoding the extracellular domain of rat PSMA (rPSMA $_{\text{t}}$) [29]. Rats immunized with the prime-boost strategy developed the strongest cytotoxic response to hPSMA-transfected prostate cancer cell lines and were more protected from implanted hPSMA-expressing tumor cells than rats immunized with hPSMA $_{\text{t}}$ or rPSMA $_{\text{t}}$ alone. Together, these authors concluded that DNA vaccine approaches using highly homologous xenoantigens as priming immunizations would be preferred in clinical trials in order to elicit immunity to the autologous PSMA antigen.

In a different approach to targeting the PSMA antigen by means of DNA vaccines, Stevenson and colleagues have investigated DNA fusion gene approaches in which the DNA vaccine encodes a target MHC class I-restricted epitope fused to a protein (protein domain

of tetanus toxin, DOM) that provides a strong CD4⁺ T-cell helper response [30]. Using DNA gene fusion constructs encoding different HLA-A2-restricted epitopes derived from PSMA, HLA-A2 transgenic mice were immunized intramuscularly with plasmid DNA alone and evaluated for peptide-specific T cells. The investigators found that immunization with DNA/DOM fusion DNA vaccines encoding the PSMA epitopes PSMA₂₇ and PSMA₆₆₃ elicited CTL that could lyse PSMA-expressing target cells, and the magnitude of the immune response could be further augmented with subsequent electroporation [30].

Prostate stem cell antigen (PSCA) and six-transmembrane epithelial antigen of the prostate (STEAP) are other prostate-associated antigens that have been evaluated as target antigens by Kast *et al* using DNA vaccines with heterologous vector prime-boost approaches. PSCA is a glycosylphosphatidylinositol (GPI)-anchored cell surface protein expressed in the epithelial tissue of the urinary bladder, kidney, stomach, skin, esophagus, and prostate. As of yet, the physiological function of this protein is unknown [31]. Human PSCA is overexpressed in ~90% of primary or metastatic prostate cancers with higher levels associated with higher tumor grade and stage [32]. A murine PSCA homologue exists, 65% homologous to the human protein, and is known to be expressed in prostate tumor cells in the transgenic adenocarcinoma of the mouse prostate (TRAMP) cancer model, thus providing a relevant preclinical model to investigate vaccines encoding PSCA [33, 34]. Similarly, STEAP is also expressed in normal prostate tissues but is overexpressed in human prostate cancer tissue. Its expression has also been detected in colon, bladder, ovarian, and pancreatic cancer cell lines. A murine STEAP homologue (80% homology with human STEAP) exists, and was detected at high levels in malignant prostate tissue in TRAMP mice and the TRAMP-C2 derivative prostate cancer cell line [35]. Using a heterologous prime/boost strategy, Garcia-Hernandez *et al.* vaccinated TRAMP mice with a DNA vaccine encoding PSCA, using a gene gun delivery, followed by a booster immunization with Venezuelan equine encephalitis virus replicons (VRP) encoding PSCA (mPSCA-VRP) [33]. Mice were subsequently challenged with TRAMP-C2 murine prostate tumor cells. Immunized mice, relative to controls receiving vector DNA and/or VRP, had slower growth of tumors 52 days after challenge and had an increase in overall survival. Native tumors of the PSCA-vaccinated mice were infiltrated with macrophages, dendritic cells, CD4⁺ and CD8⁺ T cells, demonstrating the ability of this approach to circumvent tolerance to the autologous antigen [33]. Similarly, mice immunized with either a DNA vaccine encoding mSTEAP, VRP encoding mSTEAP (mSTEAP-VRP), or a combination of both, were then challenged with TRAMP-C2 cells [36]. Tumor growth was inhibited in mice immunized with either the mSTEAP DNA vaccine or mSTEAP-VRP, however DNA vaccination followed with a boost with VRP was the most effective in both protecting against tumor establishment following vaccination and in treating existing tumors [36].

Other groups have also evaluated PSCA as a target using DNA vaccines using approaches to increase antigen presentation at the time of immunization [34, 37]. In particular, Zhang and colleagues have evaluated DNA vaccines encoding PSCA with or without HSP70. Expression of HSP70 family proteins have been demonstrated to increase MHC class I expression and antigen presentation, thus facilitating anti-tumor immune responses [38, 39]. Consequently, the goal of these studies was to determine if co-expression of HSP70 could facilitate antigen presentation and increase the immunogenicity of this DNA vaccine. Mice were immunized with four different plasmids, encoding PSCA alone, encoding HSP70 alone, encoding 5'-PSCA-HSP70-3', and encoding 5'-HSP70-PSCA-3'. They found that immunization with the PSCA-HSP70, HSP70-PSCA, or a combination of the two antigen/HSP70 fusion plasmids, could enhance PSCA-specific CD8⁺ T-cell immune responses but did not substantially augment PSCA-specific CD4⁺ T_H-cell responses or antibody responses [37].

Similar results have been found using a plasmid DNA vaccine encoding the tumor-associated antigen TPD52 [40–42]. TPD52 is involved in cellular transformation, proliferation, and metastasis and it is overexpressed in breast, prostate, and ovarian cancer. The mouse orthologue shares 86% protein identity with the human orthologue. Lewis and colleagues reported that a DNA vaccine encoding murine TPD52, administered with GM-CSF, was superior to protein-based immunization in eliciting an antigen-specific Th1-type immune response and protection from challenge with the TRAMP-C1 murine prostate cancer cell line that naturally expresses the TPD52 antigen [40, 41]. In addition, a memory immune response was elicited by the TPD 52 DNA vaccine since the tumor-free mice re-challenged with TRAMP-C1 cell line 150 days later were still protected.

Our group has evaluated DNA vaccines using repetitive intradermal immunization approaches. These vaccines have included as targets PAP, a prostate-specific protein, and the cancer-testis antigen synovial sarcoma X breakpoint-2 (SSX-2), a target expressed by metastatic prostate cancers and not expressed by normal prostate cells. Like PSA, PAP is expressed in normal and malignant prostate tissue, and was initially used as a serum biomarker for prostate cancer detection and monitoring prior to the discovery of PSA [43]. PAP is the most abundant phosphatase in human prostate tissue, expressed primarily by epithelial cells, although its expression may decrease with disease progression [44]. While its exact physiological function is still unknown, PAP has been shown to play a role in tumor proliferation, fertility, and sperm motility, and based on its antinociceptive properties in neurons is being explored as a treatment for chronic pain [43, 45]. Although PAP is expressed at very low levels in some other tissues, including placenta, kidney, testis, and pancreas, the expression in the prostate is one to two orders of magnitude higher as determined by a variety of methods [46]. Unlike PSA, where no native rodent homologue exists, a highly homologous rat prostate-specific PAP (rPAP) exists, making the rat an appropriate preclinical model [47]. Using a plasmid DNA construct encoding rat (rPAP), we found that Th1-biased rPAP-specific T-cell responses could be elicited following multiple immunizations when co-administered with GM-CSF as a vaccine adjuvant, without the need for heterologous immunization approaches, in contrast to viral vaccines in which repetitive immunization elicited exclusively viral antigen-specific immunity [19]. Similarly, HLA-A2 transgenic mice immunized repetitively intradermally with a DNA vaccine encoding SSX-2, and even without the GM-CSF cytokine adjuvant, elicited Th1-biased immune responses to SSX-2-specific immunodominant epitopes; CTL specific for SSX-2 were able to lyse HLA-A2+ prostate cancer cell lines [48].

Other groups have evaluated methods of DNA delivery to increase the transfection efficiency in preclinical rodent models. Roos *et al*, specifically evaluated electroporation in the context of a DNA vaccine encoding PSA [49]. Specifically, C57B1/6 mice were immunized intramuscularly or intradermally, followed by electroporation. These studies identified that intradermal DNA electroporation resulted in rapid and stable transgene expression, and led to the generation of PSA-specific CD8⁺ T cells. In another study described earlier, a DNA vaccine encoding mPSCA was delivered intramuscularly by electroporation to C57/B16 mice and then mice were challenged with TRAMP-C1 cells [34]. mPSCA vaccinated mice demonstrated inhibition of tumor growth or and greater survival relative to control vaccinated mice, however the specific enhancement provided by electroporation was not investigated [34].

DNA Vaccines for Prostate Cancer – Clinical Studies

Table 2 summarizes the clinical trials for prostate cancer using DNA vaccines that have been conducted to date. Among the first clinical trials was a vaccine encoding PSA in patients with hormone-refractory prostate cancer led by Pisa and colleagues [50]. Patients

were immunized in a dose-escalation study with 100, 300, or 900 μ g plasmid DNA, five times every four weeks. The vaccine was given on day 0 of each cycle, with 40 μ g GM-CSF given daily prior to immunization (days -2 through 0), and 75 μ g IL-2 given daily after each immunization (days 1 through 7). This vaccine approach was found to be safe; no patients experienced adverse events greater than WHO grade 2. Patients that were immunized with the highest vaccine dose developed PSA-specific immune responses as measured by PSA-specific IFN γ secretion or antibodies, and a decrease in serum PSA levels was observed in some patients [50, 51]. The authors concluded that DNA vaccines could elicit immune responses in patients with advanced prostate cancer, and that higher dose of plasmid DNA was associated with detectable immune responses. This group has gone on to evaluate a modified DNA vaccine, encoding rhesus PSA, and delivered by intradermal electroporation, and a phase 1 dose-escalation trial with this approach is currently underway in patients with early recurrent prostate cancer (NCT00859729).

Three groups have evaluated DNA vaccines targeting PSMA in patients with prostate cancer. Mincheff *et al.* conducted a phase I/II trial using a prime-boost strategy with adenovirus and plasmid DNA encoding the extracellular portion of PSMA [52]. The PSMA DNA vaccine was evaluated alone, in combination with a separate vector encoding the costimulatory molecule CD86, or a plasmid encoding both PSMA and CD86. Some patients also received soluble GM-CSF as a vaccine adjuvant. Given the complicated trial design, heterogeneous subject population, and primary immunological endpoint (DTH responses at the injection site), interpretation of the trial's clinical and immunological results remains difficult. Nevertheless, no significant adverse events were observed and DTH responses were detected in more patients when GM-CSF was included as a vaccine adjuvant and in individuals receiving a DNA vaccine encoding CD86 [52, 53]. In separate studies, Slovin, Wolchok and colleagues have investigated DNA vaccines targeting PSMA using a xenoantigen prime-boost approach [54]. Specifically, in a phase 1 dose-escalation trial, 36 patients with metastatic prostate cancer received three immunizations using a high pressure intramuscular delivery system with DNA encoding either mPSMA or hPSMA followed by three immunizations with DNA encoding the other orthologue (hPSMA or mPSMA). Final results from this trial have not yet been reported, however no antibody responses to PSMA were detected in doses up to 4000 μ g plasmid DNA [55]. Finally, Low and colleagues have reported the results of a phase I/II trial in which 30 HLA-A2-expressing patients with early recurrent prostate cancer (non-radiographically metastatic) were immunized in a dose-escalation fashion with a fusion gene construct encoding a PSMA HLA-A2-restricted epitope with a tetanus domain protein, as described above [56]. In this trial, the DNA was administered intramuscularly by direct injection, or intramuscularly by electroporation, three times at monthly intervals. Antibody responses to the tetanus domain were reported, but no responses to the PSMA epitope were reported, and no clinical efficacy data was reported [56].

Our group has investigated a DNA vaccine encoding PAP. In a phase I/IIa clinical trial, patients with non-castrate, non-metastatic, PSA-recurrent (clinical stage D0) prostate cancer were immunized with a DNA vaccine targeting PAP (pTVG-HP) in a dose escalation fashion. Patients received 100, 500, or 1500 μ g plasmid intradermally six times at 14-day intervals, with 200 μ g GM-CSF co-administered as a vaccine adjuvant, analogous to studies performed in rats. No significant adverse events were detected, and ten of 22 patients generated PAP-specific T-cell responses immediately after immunization [57]. Responses observed were Th1 type, and cytolytic T-cell responses were specifically elicited in HLA-A2-expressing subjects [58]. Approximately 1/3 of individuals were observed to have at least a 100% increase in PSA doubling time, and this was associated with the detection of long-term PAP-specific IFN γ -secreting immune responses detectable at multiple times during the follow up period (up to 1 year after immunization). PAP-specific CD8 $^{+}$ T-cell

immune responses were detected at each dose level, and one patient treated at the lowest (100 μ g) dose was subsequently re-immunized at this dose with evidence of immune response, making the 100 μ g dose the dose chosen for further trials [58]. Given the safety, immunological efficacy, and prolongation of PSA doubling time observed in some individuals, a randomized phase II clinical trial is currently underway to evaluate the 2-year metastasis-free rate in high-risk individuals (PSA doubling time less than 12 months) in patients treated with this vaccine or GM-CSF adjuvant alone (NCT01341652). In addition, a separate pilot clinical trial using this DNA vaccine is currently accruing patients with castrate-resistant, non-metastatic prostate cancer using different schedules of vaccine administration (NCT00849121). The goal of this trial is to determine if immune responses can be elicited in some patients with prolonged immunization, and if optimal booster immunization schedules can thus be defined.

DNA Vaccines for Prostate Cancer – Future Directions

As discussed above, DNA vaccines have demonstrated immunological efficacy and anti-tumor responses in animal models, and multiple strategies are being employed to increase the immunogenicity and anti-tumor effect of these vaccines. DNA vaccines targeting three antigens have already entered early phase clinical trials specifically for patients with prostate cancer, and to date have demonstrated safety and immunological effects. Sufficiently powered trials evaluating anti-tumor efficacy have not yet been completed, and the furthest in development is a randomized phase II trial using a DNA vaccine encoding PAP that is currently underway. In addition to trials evaluating clinical efficacy, we predict that over the next five to ten years the primary areas of focus for future clinical trials using DNA vaccines will be efforts to: 1) increase the immunogenicity of these vaccines; 2) evaluate new antigens and combinations of antigens; 3) explore other stages of disease, notably earlier stages of disease; 4) explore combination approaches with other conventional anti-tumor therapies; and 5) explore strategies combining vaccines with methods to deplete regulatory/tolerant immune responses, augment long-term memory immune responses, and alter the tumor microenvironment to make tumors more amenable to T-cell mediated lysis.

As described earlier, there are many efforts being evaluated in preclinical models to improve the immunological efficacy of DNA vaccines. These have included heterologous prime-boost strategies, and the use of homologous xenoantigens. These are logical directions for future clinical trials, and as noted above, the use of xenoantigens as targets has already been explored in the case of PSMA vaccines. With the development of viral and bacterial genetic vaccines targeting the same antigens, heterologous prime-boost approaches such as have been evaluated for PSCA and STEAP will likely enter clinical trials. Similarly, given the approval of sipuleucel-T, a logical direction will be combination trials with this vaccine and other vaccines, including a DNA vaccine encoding the same PAP antigen targeted in the sipuleucel-T vaccine, as heterologous prime-boost approaches. Moreover, to date there remains little information in human trials about the optimal doses, adjuvants, and schedules of DNA vaccines able to elicit long-term immunity. These will certainly be goals of future clinical trials.

We also predict that future trials will explore new antigen targets and earlier stages of disease. At present it remains unknown whether one particular antigen is superior to another, and hence the evaluation of different antigens remains important. Similarly, given preclinical models suggesting that immune targeting can lead to antigen-loss escape variants, simultaneous targeting of multiple antigens will likely be important to reduce this possibility. Qin and colleagues have reported the delivery of a DNA vaccine encoding multiple epitopes from several prostate-associated antigens in a murine model and human *in vitro* model, hence this is a logical and feasible approach for future trials [59]. Efforts to

rank particular antigens based on preclinical data and biological importance have been proposed, and will likely factor into the choice of high priority antigens [60]. In addition, we believe that future trials will be evaluated in earlier stages of disease. Kast and colleagues have reported that DNA immunization in early stages of prostate tumor development in the murine TRAMP system had markedly superior results in terms of animal survival than if immunization was initiated in later stages of tumor development [61]. This is particularly relevant in the case of prostate cancer, where it has been documented that late-stage tumors frequently lose expression of MHC class I or have defects in antigen presentation or T-cell signaling [62, 63]. Consequently, trials conducted in the adjuvant or early recurrent disease settings, and ultimately in the prophylactic setting to prevent disease, or prevent the outgrowth of tumors expressing specific targets that might be associated with a worse phenotype (e.g. SSX-2), are all logical directions for future clinical trials.

Finally, it is clear from the failures of many anti-tumor vaccines to demonstrate clinical responses when used as monotherapies, despite having evidence of biological/immunological effects, that combination approaches will be necessary to more successfully treat established tumors. Such treatments may include other standard therapies for prostate cancer, such as chemotherapy, radiation therapy, or androgen deprivation, all of which may have effects on tumor antigen presentation and/or can produce an inflammatory tumor micro environment [64–70]. In addition, agents capable of reversing or inhibiting the generation of tolerant/regulatory immune responses that counterbalance the inflammatory immune response elicited by vaccination will be critical. Such agents may include T-cell checkpoint inhibitors, such as anti-CTLA-4 or anti-PD1 blockade [71], or T-cell activating agents such as anti-OX40 [72, 73]. Anti-tumor vaccine trials using non-DNA vaccine approaches have entered clinical trials with these agents, however the best use and sequence of these therapies remains unknown.

In summary, plasmid DNA vaccines offer advantages over other anti-tumor vaccine approaches in terms of simplicity, manufacturing, and potentially safety. Multiple preclinical models demonstrate anti-tumor efficacy, and many efforts are underway to improve the immunogenicity and anti-tumor effect of these vaccines. Clinical trials using DNA vaccines as treatments for prostate cancer have begun, and to date have demonstrated safety and immunological effect. We expect that future clinical trials will continue to explore DNA vaccines alone, in combination with other vaccine approaches, and in combination with other immunomodulatory therapies, to further augment the anti-tumor efficacy of these vaccines.

Acknowledgments

This work is supported by NIH R01 CA142608 and by the US Department of Defense Prostate Cancer Research Program (W81XWH-11-1-0196).

ABBREVIATIONS

PSA	prostate-specific antigen
PAP	prostatic acid phosphatase
PSMA	prostate-specific membrane antigen
STEAP	six transmembrane epithelial antigen of the prostate
PSCA	prostate stem cell antigen
TPD52	tumor protein D52

SSX-2 synovial sarcoma X breakpoint-2

References

1. Hodi FS, O'Day SJ, McDermott DF, et al. Improved survival with ipilimumab in patients with metastatic melanoma. *N Engl J Med*. 2010; 363:711–23.
2. Kantoff PW, Higano CS, Shore ND, Berger ER, Small EJ, Peuson DF, et al. Sipuleucel-T immunotherapy for castration-resistant prostate cancer. *N Engl J Med*. 2010; 363:411–22.
3. Higano CS, Corman JM, Smith DC. 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.
4. Emens LA. GM-CSF-secreting vaccines for solid tumors. *Curr Opin Investig Drugs*. 2009; 10:1315–24.
5. Wolff JA, Malone RW, Williams P, Chong, et al. Direct gene transfer into mouse muscle *in vivo*. *Science*. 1990; 247:1465–8. [PubMed: 1690918]
6. Danko I, Wolff JA. Direct gene transfer into muscle. *Vaccine*. 1994; 12:1499–502.
7. Chen Y, Webster RG, Woodland DL. Induction of CD8+ T cell responses to dominant and subdominant epitopes and protective immunity to Sendai virus infection by DNA vaccination. *Immunol*. 1998; 160:2425–32.
8. Iwasaki A, Torres CA, Ohashi PS, Robinson HL, Barber BH. The dominant role of bone marrow-derived cells in CTL induction following plasmid DNA immunization at different sites. *J Immunol*. 1997; 159:11–4.
9. Rice J, Ottensmeier CH, Stevenson FK. DNA vaccines: precision tools for activating effective immunity against cancer. *Nat Rev Cancer*. 2008; 8:108–20.
10. Anderson RJ, Schneider J. Plasmid DNA and viral vector-based vaccines for the treatment of cancer. *Vaccine*. 2007; 25 (Suppl 2):B24–34.
11. Krieg AM. CpG DNA: trigger of sepsis, mediator of protection, or both? *Scand J Infect Dis*. 2003; 35:653–9.
12. Takaoka A, Wang Z, Choi MK, et al. DAI (DLM-1/ZBP1) is a cytosolic DNA sensor and an activator of innate immune response. *Nature*. 2007; 448:501–5. [PubMed: 17618271]
13. Choubey D, Duan X, Dickerson E, Ponomareva L, Panchanathan R, Shen H, et al. Interferon-inducible p200-family proteins as novel sensors of cytoplasmic DNA: role in inflammation and autoimmunity. *J Interferon Cytokine Res*. 2010; 30:371–80.
14. Suzuki K, Mori A, Ishii KJ, et al. Activation of target-tissue immune-recognition molecules by double-stranded polynucleotides. *Proc Natl Acad Sci U S A*. 1999; 96:2285–90.
15. Ishii KJ, Coban C, Kato H, et al. A Toll-like receptor-independent antiviral response induced by double-stranded B-form DNA. *Nat Immunol*. 2006; 7:40–8.
16. Ishikawa H, Ma Z, Barber GN. STING regulates intracellular DNA-mediated, type I interferon-dependent innate immunity. *Nature*. 2009; 461:788–92.
17. Klinman DM. Adjuvant activity of CpG oligodeoxynucleotides. *Int Rev Immunol*. 2006; 25:135–54.
18. Bergman PJ, McKnight J, Novosad A, et al. Long-term survival of dogs with advanced malignant melanoma after DNA vaccination with xenogeneic human tyrosinase: a phase I trial. *Clin Cancer Res*. 2003; 9:1284–90.
19. Johnson LE, Frye TP, Chinnasamy N, Chinnasamy D, McNeel DG. Plasmid DNA vaccine encoding prostatic acid phosphatase is effective in eliciting autologous antigen-specific CD8+ T cells. *Cancer Immunol Immunother*. 2007; 56:885–95. [PubMed: 17102977]
20. Jiao S, Williams P, Berg RK, et al. Direct gene transfer into nonhuman primate myofibers *in vivo*. *Hum Gene Ther*. 1992; 3:21–33.
21. 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. [PubMed: 15389792]

22. Kim JJ, Trivedi NN, Wilson DM, et al. Molecular and immunological analysis of genetic prostate specific antigen (PSA) vaccine. *Oncogene*. 1998; 17:3125–35.
23. Marshall DJ, San Mateo LR, Rudnick KA, et al. Induction of Th1-type immunity and tumor protection with a prostate-specific antigen DNA vaccine. *Cancer Immunol Immunother*. 2005; 54:1082–94.
24. Kim JJ, Yang JS, Dang K, Manson KH, Weiner DB. Engineering enhancement of immune responses to DNA-based vaccines in a prostate cancer model in rhesus macaques through the use of cytokine gene adjuvants. *Clin Cancer Res*. 2001; 7:882s–9s.
25. Saffran DC, Reiter RE, Jakobovits A, Witte ON. Target antigens for prostate cancer immunotherapy. *Cancer Metastasis Rev*. 1999; 18:437–49.
26. Ben Jemaa A, Bouraoui Y, Sallami S, Banasr A, Ben Rais N, Ouertani L, et al. Co-expression and impact of prostate specific membrane antigen and prostate specific antigen in prostatic pathologies. *J Exp Clin Cancer Res*. 2010; 29:171. [PubMed: 21189143]
27. Gregor PD, Wolchok JD, Ferrone CR, et al. CTLA-4 blockade in combination with xenogeneic DNA vaccines enhances T-cell responses, tumor immunity and autoimmunity to self antigens in animal and cellular model systems. *Vaccine*. 2004; 22:1700–8.
28. Gregor PD, Wolchok JD, Turaga V, et al. Induction of autoantibodies to syngeneic prostate-specific membrane antigen by xenogeneic vaccination. *Int J Cancer*. 2005; 116:415–21.
29. Mincheff M, Zoubak S, Makogonenko Y. Immune responses against PSMA after gene-based vaccination for immunotherapy-A: results from immunizations in animals. *Cancer Gene Ther*. 2006; 13:436–44.
30. Vittes GE, Harden EL, Ottensmeier CH, Rice J, Stevenson FK. DNA fusion gene vaccines induce cytotoxic T-cell attack on naturally processed peptides of human prostate-specific membrane antigen. *Eur J Immunol*. 2011; 41:2447–56.
31. Saeki N, Gu J, Yoshida T, Wu X. Prostate stem cell antigen: a Jekyll and Hyde molecule? *Clin Cancer Res*. 2010; 16:3533–8.
32. Reiter RE, Gu Z, Watabe T, et al. Prostate stem cell antigen: a cell surface marker overexpressed in prostate cancer. *Proc Natl Acad Sci U S A*. 1998; 95:1735–40.
33. de Garcia-Hernandez ML, Gray A, Hubby B, Klinger OJ, Kast WM. Prostate stem cell antigen vaccination induces a long-term protective immune response against prostate cancer in the absence of autoimmunity. *Cancer Res*. 2008; 68:861–9.
34. Ahmad S, Casey G, Sweeney P, Tangney M, O'Sullivan GC. Prostate stem cell antigen DNA vaccination breaks tolerance to self-antigen and inhibits prostate cancer growth. *Mol Ther*. 2009; 17:1101–8.
35. Yang D, Holt GE, Velders MP, Kwon ED, Kast WM. Murine six-transmembrane epithelial antigen of the prostate, prostate stem cell antigen, and prostate-specific membrane antigen: prostate-specific cell-surface antigens highly expressed in prostate cancer of transgenic adenocarcinoma mouse prostate mice. *Cancer Res*. 2001; 61:5857–60. [PubMed: 11479226]
36. de Garcia-Hernandez ML, Gray A, Hubby B, Kast WM. *In vivo* effects of vaccination with six-transmembrane epithelial antigen of the prostate: a candidate antigen for treating prostate cancer. *Cancer Res*. 2007; 67:1344–51.
37. Zhang X, Yu C, Zhao J, et al. Vaccination with a DNA vaccine based on human PSCA and HSP70 adjuvant enhances the antigen-specific CD8+ T-cell response and inhibits the PSCA+ tumors growth in mice. *The journal of gene medicine*. 2007; 9:715–26.
38. Wells AD, Rai SK, Salvato MS, Band H, Malkovsky M. Hsp72-mediated augmentation of MHC class I surface expression and endogenous antigen presentation. *Int Immunol*. 1998; 10:609–17. [PubMed: 9645609]
39. Wells AD, Malkovsky M. Heat shock proteins, tumor immunogenicity and antigen presentation: an integrated view. *Immunol Today*. 2000; 21:129–32.
40. Lewis JD, Sullivan LA, Byrne JA, de Riese W, Bright RK. Memory and cellular immunity induced by a DNA vaccine encoding self antigen TPD52 administered with soluble GM-CSF. *Cancer Immunol Immunother*. 2009; 58:1337–49.

41. Payton LA, Lewis JD, Byrne JA, Bright RK. Vaccination with metastasis-related tumor associated antigen TPD52 and CpG/ODN induces protective tumor immunity. *Cancer Immunol Immunother.* 2008; 57:799–811.
42. Boutros R, Fanayan S, Shehata M, Byrne JA. The tumor protein D52 family: many pieces, many puzzles. *Biochemical and biophysical research communications.* 2004; 325:1115–21.
43. Hassan MI, Aijaz A, Ahmad F. Structural and functional analysis of human prostatic acid phosphatase. *Expert Rev Anticancer Ther.* 2010; 10:1055–68.
44. Hakalahti L, Vihko P, Henttu P, Autio-Harminen H, Soini Y, Vihko R. Evaluation of PAP and PSA gene expression in prostatic hyperplasia and prostatic carcinoma using northern-blot analyses, in situ hybridization and immunohistochemical stainings with monoclonal and bispecific antibodies. *Int J Cancer.* 1993; 55:590–7.
45. Zylka MJ, Sowa NA, Taylor-Blake B, et al. Prostatic acid phosphatase is an ectonucleotidase and suppresses pain by generating adenosine. *Neuron.* 2008; 60:111–22. [PubMed: 18940592]
46. Graddis TJ, McMahan CJ, Tamman J, Page KJ, Trager JB. Prostatic acid phosphatase expression in human tissues. *International journal of clinical and experimental pathology.* 2011; 4:295–306.
47. Roiko K, Janne OA, Vihko P. Primary structure of rat secretory acid phosphatase and comparison to other acid phosphatases. *Gene.* 1990; 89:223–9.
48. Smith HA, McNeel DG. Vaccines targeting the cancer-testis antigen SSX-2 elicit HLA-A2 epitope-specific cytolytic T cells. *J Immunother.* 2011; 34:569–80.
49. 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. [PubMed: 16185933]
50. Pavlenko M, Roos AK, Lundqvist A, et al. A phase 1 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.
51. Miller AM, Ozenci V, Kiessling R, Pisa P. Immune monitoring in a phase 1 trial of a PSA DNA vaccine in patients with hormone-refractory prostate cancer. *J Immunother.* 2005; 28:389–95. [PubMed: 16000958]
52. Mincheff M, Tchakarov S, Zoubak S, et al. Naked DNA and Adenoviral Immunizations for Immunotherapy of Prostate Cancer: A Phase I/II Clinical Trial. *Eur Urol.* 2000; 38:208–17.
53. Mincheff M, Altankova I, Zoubak S, et al. *In vivo* transfection and/or cross-priming of dendritic cells following DNA and adenoviral immunizations for immunotherapy of cancer--changes in peripheral mononuclear subsets and intracellular IL-4 and IFN-gamma lymphokine profile. *Critical reviews in oncology/hematology.* 2001; 39:125–32.
54. Wolchok JD, Gregor PD, Nordquist LT, Slovin SF, Scher HI. DNA vaccines: an active immunization strategy for prostate cancer. *Seminars in oncology.* 2003; 30:659–66.
55. Slovin S, Gregor P, Wolchok J, et al. A xenogeneic PSMA DNA vaccine for patients with non-castrate metastatic (NCMP) and castrate metastatic prostate cancer (CMPC) - A phase I trial of proof of principle. *J Clin Oncology.* 2007; 25:3073S.
56. Low L, Mander A, McCann K, et al. DNA vaccination with electroporation induces increased antibody responses in patients with prostate cancer. *Hum Gene Ther.* 2009; 20:1269–78.
57. McNeel DG, Dunphy EJ, Davies JG, 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.
58. 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.
59. Qin H, Zhou C, Wang D, et al. Specific antitumor immune response induced by a novel DNA vaccine composed of multiple CTL and T helper cell epitopes of prostate cancer associated antigens. *Immunology letters.* 2005; 99:85–93. [PubMed: 15894116]
60. Cheever MA, Allison JP, Ferris AS, et al. The prioritization of cancer antigens: a national cancer institute pilot project for the acceleration of translational research. *Clin Cancer Res.* 2009; 15:5323–37.

61. Gray A, de la Luz Gareia-Hernandez M, van West M, Kanodia S, Hubby B, Kast WM. Prostate cancer immunotherapy yields superior long-term survival in TRAMP mice when administered at an early stage of carcinogenesis prior to the establishment of tumor-associated immunosuppression at later stages. *Vaccine*. 2009; 27 (Suppl 6):G52–9. [PubMed: 20006141]
62. Blades RA, Keating PJ, McWilliam LJ, George NJ, Stern PL. Loss of HLA class I expression in prostate cancer: implications for immunotherapy. *Urology*. 1995; 46:681–6.
63. Healy CG, Simons JW, Carducci MA, et al. Impaired expression and function of signal-transducing zeta chains in peripheral T cells and natural killer cells in patients with prostate cancer. *Cytometry*. 1998; 32:109–19. [PubMed: 9627224]
64. Arlen PM, Gulley JL, Parker C, et al. A randomized phase II study of concurrent docetaxel plus vaccine versus vaccine alone in metastatic androgen-independent prostate cancer. *Clin Cancer Res*. 2006; 12:1260–9. [PubMed: 16489082]
65. Garnett CT, Schlom J, Hodge JW. Combination of docetaxel and recombinant vaccine enhances T-cell responses and antitumor activity: effects of docetaxel on immune enhancement. *Clin Cancer Res*. 2008; 14:3536–44. [PubMed: 18519787]
66. Friedman EJ. Immune modulation by ionizing radiation and its implications for cancer immunotherapy. *Current pharmaceutical design*. 2002; 8:1765–80.
67. Reits EA, Hodge JW, Herberts CA, et al. Radiation modulates the peptide repertoire, enhances MHC class I expression, and induces successful antitumor immunotherapy. *J Exp Med*. 2006; 203:1259–71.
68. Cameron RB, Spiess PJ, Rosenberg SA. Synergistic antitumor activity of tumor-infiltrating lymphocytes, interleukin 2, and local tumor irradiation. Studies on the mechanism of action. *J Exp Med*. 1990; 171:249–63. [PubMed: 2295878]
69. Harris TJ, Hipkiss EL, Borzillary S, et al. Radiotherapy augments the immune response to prostate cancer in a time-dependent manner. *Prostate*. 2008; 68:1319–29.
70. Morse MD, McNeel DG. Prostate Cancer Patients Treated with Androgen Deprivation Therapy Develop Persistent Changes in Adaptive Immune Responses. *Human immunology*. 2010; 71:496–504.
71. Korman AJ, Peggs KS, Allison JP. Checkpoint blockade in cancer immunotherapy. *Adv Immunol*. 2006; 90:297–339.
72. Weinberg AD, Rivera MM, Prell R, et al. Engagement of the OX-40 receptor *in vivo* enhances antitumor immunity. *J Immunol*. 2000; 164:2160–9.
73. Wolchok JD, Yang AS, Weber JS. Immune regulatory antibodies: are they the next advance? *Cancer journal Sudbury, Mass*. 2010; 16:311–7.
74. Roos AK, Eriksson F, Timmons JA, et al. Skin electroporation: effects on transgene expression, DNA persistence and local tissue environment. *PloS one*. 2009; 4:e7226.

Table 1

Prostate Cancer DNA Vaccines – Preclinical Studies

Antigen	Model	Method to Increase Efficacy/Immunogenicity	Reference
PSA	Mouse	None	[22]
		Co-administered with protein cytokines or plasmids encoding GM-CSF and IL-2	[24]
		Intramuscular delivery with electroporation	[74]
	Cynomolgus monkeys	Administered human PSA gene	[23]
	Rhesus macaques	Co-administered with plasmids encoding IL-2, IL-12, IL-18	[24]
PSMA	Mouse	Immunization with homologous human xenoantigen	[28]
	Rat (Copenhagen)	Prime-boost with xenoantigen and native antigen	[29]
	Mouse (HHD, HLA-A2 transgenic)	HLA-A2-restricted epitope fused with tetanus DOM protein, +/- electroporation	[30]
PSCA	Mouse (TRAMP)	Prime-boost with DNA followed by Venezuelan equine encephalitis virus replicons (VRP) encoding PSCA	[33]
		Intramuscular delivery with electroporation	[34]
	Mouse	Co-expression with HSP70	[37]
STEAP	Mouse (TRAMP)	Prime-boost with DNA followed by VRP encoding STEAP	[36]
PAP	Rat (Lewis)	Prime-boost with vaccinia, and multiple repetitive immunization with GM-CSF adjuvant	[19]
SSX-2	Mouse (HLA-A2/HLA-DR1, transgenic)	Immunization with a cancer testis antigen	[48]
TPD52	Mouse (TRAMP)	DNA administered with GM-CSF	[40]

Table 2

Prostate Cancer DNA Vaccines – Clinical Trials

Antigen	Phase of Trial	Disease Stage	Method to Increase Efficacy/Immunogenicity	Results	Reference
PSA	I	Hormone-refractory	Administered with GM-CSF and IL-2	900 mcg dose elicited PSA-specific IFN γ secretion and PSA-specific antibodies	[50]
	I	PSA-recurrent	Intradermal delivery with electroporation	(trial results pending)	
PSMA	I/II	Mixed	Delivered with adenoviral vector +/- CD86 co-expression, and GM-CSF protein adjuvant	DTH responses observed	[52]
	I	Metastatic	Prime-boost with DNA encoding xenotigen (mouse) alone or native protein followed by xenotigen	No PSMA-specific antibody responses up to 4000 μ g dose	[55]
	I	Non-metastatic	DNA fusion encoding HLA-A2 epitope and tetanus DOM protein	Antibody responses to DOM protein	[56]
PAP	I/IIa	D0 (rising PSA, non-castrate, non-metastatic)	Multiple immunizations and co-administered with GM-CSF	CD8+ immune responses elicited, IFN γ -secreting responses associated with increased PSA doubling time	[57, 58]
	II	D0.5 (rising PSA, castrate, non-metastatic)	Multiple immunizations and boosters, evaluation of different schedules, co-administered with GM-CSF	2-arm pilot trial – results pending	
	II	D0 (rising PSA, non-castrate, non-metastatic)	Multiple immunizations and co-administered with GM-CSF	Randomized to vaccine + GM-CSF or GM-CSF only – results pending	