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Preclinical and Clinical Development of DNA Vaccines for Prostate Cancer

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Abstract

Prostate cancer is the most commonly diagnosed cancer in the United States. It is also the second leading cause of cancer-related death in men, making it one of the largest public health concerns today. Prostate cancer is an ideal disease for immunotherapies because of the generally slow progression, the dispensability of the target organ in the patient population, and the availability of several tissue-specific antigens. As such, several therapeutic vaccines have entered clinical trials, with one autologous cellular vaccine (sipuleucel-T) recently gaining FDA approval after demonstrating overall survival benefit in randomized phase III clinical trials. DNA-based vaccines are safe, economical, alternative “off-the-shelf” approaches that have undergone extensive evaluation in pre-clinical models. In fact, the first vaccine approved in the United States for the treatment of cancer was a DNA vaccine for canine melanoma. Several prostate cancer-specific DNA vaccines have been developed in the last decade, and have shown promising results in early phase clinical trials. This review summarizes anti-cancer human DNA vaccine trials, with a focus on those conducted for prostate cancer. We conclude with an outline of special considerations important for the development and successful translation of DNA vaccines from the laboratory to the clinic.

Keywords

DNA vaccines; tumor vaccine; prostate cancer; clinical trials

Introduction and Background

The primary goal of vaccination is to elicit a host immune response, cellular and/or humoral, to a defined antigen or set of antigens. In the case of infectious disease vaccines, this is usually with the goal of establishing protective immunity. In the case of anti-tumor vaccines, the goal is typically to elicit and/or augment an immune response with anti-tumor activity in subjects with existing disease. DNA vaccines represent one type of this “active”

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Conflicts of Interest:
DGM has an ownership interest in Madison Vaccine, Inc which has licensed technology reported in this publication. The other authors declare no conflict of interest.

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immunotherapy. In their simplest application, DNA vaccines are bacterial plasmids containing the coding nucleic acid sequence of a target antigen under the control of a eukaryotic promoter. Immunization with DNA vaccines has been shown to elicit both humoral and T cell-mediated immune responses with anti-tumor activity in multiple preclinical models and in early human clinical trials. In this article, we review DNA vaccines with respect to anti-tumor immunization approaches, review the clinical development of DNA vaccines specifically in the context of human prostate cancer, and discuss specific considerations for "next generation" DNA vaccines.

DNA Vaccines – Comparison with Other Methods of Immunization

DNA vaccines have several advantages relative to other antigen-specific vaccine approaches, as summarized in Table 1. First, plasmid DNA is relatively stable, and easy and inexpensive to manufacture. Similar to peptide and protein-based vaccines, DNA vaccines also represent an "off-the-shelf" approach, but are not MHC restricted as are most peptide-based approaches. In addition, plasmid DNA is more temperature-stable than peptides and proteins, as well as bacterial and viral vectors, making DNA vaccines easier to transport and store, likely with a longer shelf life. Given these particular advantages, there is interest in the development of DNA vaccines as global immunization strategies. The ability to easily construct and manipulate the backbone of plasmid DNA offers another particular advantage. For example, DNA vaccines can be simply constructed to encode multiple antigens, portions of proteins, or other agents used to aid or enhance the immune response elicited. Such agents might include adjuvants and cytokines, or even siRNA to decrease the expression of particular genes.

An additional advantage of DNA vaccines is afforded by the adjuvant property of the bacterial plasmid DNA itself. The bacterial backbone of DNA vaccine intrinsically has been shown to elicit innate immunostimulatory properties through the recognition of unmethlyated CpG-rich regions present in non-eukaryotic DNA via toll-like receptor 9, or by the recognition of double stranded DNA through other intracellular DNA sensors such as AIM2 and/or sensors involved in the STING/TBK cascade [1–3]. Thus, administration of bacterial DNA can engage immune cells and inflammatory cytokines at the vaccination site, effectively acting as a vaccine adjuvant.

Finally, like other genetic vaccine approaches such as viral and bacterial vaccine approaches, the encoded antigen can enter the endogenous antigen-presentation pathway, leading to a cellular CD8+ T cell response. However, unlike viral or bacterial vaccines, there has been no evidence of immune responses being elicited to the vector itself. Moreover, the multitude of foreign immunogenic proteins encoded by bacterial or viral delivery methods could potentially compromise the immune response elicited to the antigen of interest [4].

Anti-Tumor DNA Vaccines – Clinical Trials

DNA vaccines have been demonstrated to elicit antigen-specific cellular and antibody immune responses in anti-microbial and anti-tumor preclinical models. Recently, DNA vaccines have been approved by the USDA for the treatment of West Nile virus in horses, and infectious hematopoietic necrosis factor disease in salmon [5, 6]. In 2010, the first anti-

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tumor DNA vaccine was approved in the U.S. by the USDA for the treatment of canine melanoma based on results from non-randomized clinical trials demonstrating safety and likely benefit [7]. With the demonstration that immune responses, and cytolytic T-cells in particular, can be elicited in larger mammals, DNA vaccines as a therapeutic treatment for cancer have entered human clinical trials. As demonstrated in Table 2, plasmid DNA vaccines have been evaluated in phase I and II clinical trials for numerous types of cancer, including melanoma, colorectal, breast, head/neck, bladder, and prostate. Overall, results from the studies reported in Table 2 have shown DNA vaccines to be safe; the most common adverse events reported being fever and pain, redness, and swelling at the injection sites. Most trials have been early phase, and hence little clinical efficacy has been demonstrated to date, however most have demonstrated immunological activity. Among the furthest in development are vaccines specifically for the treatment of prostate cancer, highlighted in Table 2, and which we review here.

Among the first clinical trials for prostate cancer, a phase I clinical trial using a DNA vaccine targeting PSA (pVAX/PSA), was investigated in patients with castration-resistant prostate cancer [8]. To determine the biologically active dose of the vaccine, patients were administered one of three doses, 100, 300, or 900 μg, five times at 4-week intervals in combination with the cytokines granulocyte-macrophage colony-stimulating factor (GM-CSF) and IL-2 as vaccine adjuvants. The vaccine was found to be safe with no adverse effects. No PSA-specific immune responses, as assessed by IFNγ production, were detected in patients before immunization or in patients who received the lowest doses of the vaccine, while two of the three patients administered 900 μg of the vaccine developed PSA-specific IFNγ production and anti-PSA antibodies [8, 9]. Further analysis showed that five out of six patients analyzed showed an increase in PSA peptide-specific immune responses after vaccination with the highest responses observed in patients who received the highest dose of vaccine [10]. A decrease in PSA slope was observed in two patients exhibiting PSA-specific IFNγ release.

Prostate-specific membrane antigen (PSMA) is another antigen that has been targeted using DNA vaccine-based approaches. In a highly complicated, phase I/II clinical trial, prostate cancer patients received two immunizations at weekly intervals with a DNA vaccine encoding the extracellular domain of PSMA or CD86 in separate plasmids or combined in a single plasmid, PSMA/CD86, along with GM-CSF as an adjuvant [11]. Two weeks after the initial immunization, patients were vaccinated with a recombinant adenovirus-PSMA (Ad5-PSMA) followed by two additional immunizations (ranging from 100 μg to 800 μg) of PSMA/CD86 plasmid along with GM-CSF. All patients developed a positive delayed-type hypersensitivity (DTH) skin response. Due to the wide variation of disease status of the patient population and prior or concurrent treatment, clinical outcome to the vaccine could not be determined. A xenogeneic approach using a PSMA DNA vaccine has also been evaluated in patients with metastatic prostate cancer [12]. Patients were vaccinated three times at three-week intervals with either a DNA vaccine encoding human PSMA followed by three immunizations with a DNA vaccine encoding mouse PSMA, or first with mouse PSMA followed by human PSMA. This approach was investigated at three different doses: 100 μg, 1500 μg, and 4000 μg. In selected patients, T-cell responses to 3T3 fibroblast cells expressing PSMA were observed. An increase in the PSA doubling time (PSA-DT) was

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seen in patients that were immunized with the 4000 μg dose [12]. Further analyses of T-cell responsiveness are being conducted. In another phase I/II dose escalation trial for HLA-A2+ patients with biochemically, recurrent prostate cancer, Ottensmeier *et. al.* investigated a PSMA DNA vaccine in which a strong immunogeneic helper domain (DOM) from fragment C of tetanus toxin was linked to a PSMA-specific, HLA-A2-binding epitope, $PSMA_{27-35}$ [13]. The DNA vaccine was administered intramuscularly five times at 0, 4, 8, 24 and 48 weeks. The dose of plasmid varied depending whether vaccine was administered with (400, 800, or 1600 μg) or without (800, 1600, or 3200 μg) electroporation. The immunogenicity to the $PSMA_{27-35}$ peptide was independent of the DNA vaccine dose, however delivery of the vaccine by electroporation resulted in an increase of the antibody response to the DOM region. Only a trend towards induction of higher frequencies of antigen-specific CD4+ and CD8+ T cells were seen. The vaccine induced CD8+ T cell immunity to $PSMA_{27-35}$ in 16/30 patients and lead to a significant increase in PSA-DT from 11.97 months pre-treatment to 16.82 months over the 72-week study period.

Plasmid DNA vaccines encoding prostatic acid phosphatase (PAP) have also been investigated. In a phase I/IIa clinical trial, patients with biochemically recurrent prostate cancer (clinical stage M0) were treated in a dose-escalation fashion with 100 μg, 500 μg, or 1500 μg plasmid DNA encoding PAP (pTVG-HP) along with 200 μg of granulocyte-macrophage colony-stimulating factor (GM-CSF) protein as an adjuvant [14]. Patients received six immunizations intradermally at 14-day intervals. Three out of 22 patients developed PAP-specific, IFNγ-secreting CD8+ T cells and nine out 22 patients developed PAP-specific, proliferating CD4+ or CD8+ T cells after the 12-week immunization period. No PAP-specific antibody responses were detected. No significant adverse events were detected, and an increase in PSA-DT from 6.5 months pre-treatment to 8.5 months on-treatment was observed [14]. In a longitudinal immune analysis (one year post treatment), of 8 patients who experienced at least a doubling of the PSA-DT in the year follow-up, six had detectable long-term PAP-specific, IFNγ-secreting T-cell responses [15]. Currently, a randomized phase II trial (NCT01341652) in the same patient population (clinical stage M0) is underway investigating the two year metastasis-free rate in patients receiving DNA vaccine encoding PAP plus GM-CSF compared to GM-CSF alone. A separate clinical trial (NCT00849121) is evaluating whether long-term, repetitive vaccination with this plasmid DNA may be required to circumvent tolerance for some individuals, or whether for this specific approach of using plasmid DNA alone some individuals are simply not able to be immunized, or not able to be immunized to this specific target antigen [16]. This trial is being conducted in patients with PSA-recurrent, non-radiographically metastatic, castration-resistant prostate cancer.

The cancer-testis antigen NY-ESO-1 has also been targeted using a DNA vaccine in a trial that included patients with prostate cancer [17]. This trial, open to multiple solid tumor types, included patients with non-small cell lung cancer (n=5) and esophageal carcinoma (n=1) as well as prostate cancer (n=10). Patients were immunized with plasmid DNA by a particle-mediated epidermal delivery method. The prostate cancer patients were divided into two cohorts. The first cohort (3 patients) received 8 μg vaccine administered monthly at weeks 1, 5, and 9 and the second cohort (7 patients) received 8 μg vaccine administered as

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clustered dosing with 2 μg doses on days 1, 3, 5, and 8 of each week and repeated monthly at weeks 1, 5, and 9. NY-ESO-1-specific CD4+ T-cell responses and some CD8+ T-cell responses were observed after vaccination. However, these responses were transient, potentially a result of suppression by regulatory T cells observed after immunization. In some patients *in vitro* depletion of regulatory T cells restored detectable levels of antigen-specific effector T cells. An increase in PSA DT was similarly observed during the vaccination period, however all patients were deemed to have progressed as evidenced by PSA increase at the time of study completion. The authors concluded that combining the NY-ESO-1 DNA vaccine with therapies to overcome regulatory T-cell mechanisms, such as depletion of regulatory T cells or by the use of other immunostimulatory adjuvants, would be needed for the development of a clinically effective therapy.

Specific Considerations in the Translation of DNA Vaccine to Clinical Practice and Evaluation of Next Generation Vaccines

As described above, the ease of preparation and storage, low cost, and simple administration of plasmid DNA has led to great interest in its use as an antigen delivery method to specifically elicit antigen-specific T cells with cytolytic activity as an approach for treating tumors. To date, however, despite multiple trials demonstrating safety in human subjects, DNA vaccines have been criticized as being poorly immunogenic in humans, and many trials have demonstrated infrequent immune response rates (Table 2). Much of this has been attributed to low transfection rates of antigen-presenting cells following administration, hence most efforts to improve the immunogenicity of DNA vaccines have focused on methods to increase plasmid DNA transfer, including the use of electroporation, or the use of alternative routes and methods of delivery. This has been supported by preclinical data demonstrating efficacy of approaches such as electroporation [18], as well as some preclinical data demonstrating that there may be a relationship between the magnitude of the immune response generated and the dose of plasmid DNA [19]. We describe here several priorities and considerations, specifically applicable to the development of anti-tumor DNA vaccines for prostate cancer, based on preclinical and clinical experience from our group and others.

1. Can DNA vaccines elicit immune responses to human autologous tumor antigens?

This question is fundamental to whether or not DNA vaccines might serve as a foundation for immune therapies, either alone or in combination with other treatments. It has been previously suggested that, in fact, DNA vaccines encoding autologous tumor antigens cannot overcome immunological tolerance [20]. In preclinical studies in Lewis rats we observed that a plasmid DNA vaccine encoding the human PAP gene elicited robust Th1-biased immune responses with as few as two immunizations when delivered intradermally [19]. Responses were found to be directed to human-specific epitopes, despite the high similarity between human and rat PAP homologues [21]. We were not able to immunize rats to the rat homologue using the same plasmid DNA vector unless multiple booster immunizations were used [4]. This result was not unsurprising, given tolerance to the PAP "self" antigen, and suggested that, while DNA delivery could certainly be improved, the primary barrier is tolerance and not delivery of the vaccine to the appropriate antigen-

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presenting cell, or limitations in DNA uptake. Further, the observation that multiple immunizations could circumvent tolerance in rats confirmed that DNA encoding an autologous antigen can elicit immune response, and suggested that a similar approach using increased doses or prolonged schedules of immunization might be necessary in human studies. This was the basis for testing the immune efficacy in a phase I/II trial with this same DNA vaccine, in which patients were immunized six times at 2-week intervals, as we had performed in rat studies. We found that PAP-specific T cells could be elicited in some patients, irrespective of dose, and these immune responses were similarly only detectable after several immunizations and could persist for many months [14, 15]. We observed that ~40% of patients developed PAP-specific T-cell responses, a frequency similar to what has been reported using the FDA-approved sipuleucel-T vaccine which targets this same PAP antigen [22]. At this point it is unclear whether the T-cell repertoire may simply not exist, or be completely tolerant, to this particular antigen in some individuals. And whether DNA vaccines are superior or inferior to other immunization approaches may be answered in the future by using DNA vaccines targeting multiple antigens, together or sequentially, or using different vaccine approaches targeting the same antigen to determine whether other immunization approaches can elicit responses in patients unable to be immunized with DNA alone. To date this remains unanswered in human trials. In any case, as evidenced by the results summarized in Table 2, it is clear that DNA vaccines encoding autologous antigens can, in fact, elicit antigen-specific T-cells in prostate cancer patients. Therefore this approach may serve as a simple framework on which one might build effective immunization approaches in combination with other agents.

2. What is the optimal target antigen?

As described above, DNA vaccines targeting different antigens have entered clinical trials for patients with prostate cancer. At present it remains unknown whether one antigen is superior to another in terms of frequency of immune response or clinical effect [23]. The FDA approval of a cellular vaccine targeting PAP, based on prolonged survival in randomized clinical trials, suggests that this is a relevant antigen [22]. PSA may similarly be a relevant antigen, based on preliminary results in a randomized phase II clinical trial using poxviral vaccines encoding PSA [24]. To date, no studies have evaluated the same plasmid DNA construct encoding different antigens to determine whether one antigen is preferred, or can be more effectively targeted than another. This is a future direction of research, as are studies targeting multiple antigens simultaneously. Ultimately studies demonstrating clinical benefit in randomized trials will be necessary to determine whether one particular antigen truly is preferred over another, or whether this is entirely related to an individual subject's pre-existing T-cell repertoire.

3. Is there a need for heterologous prime-boost immunization approaches?

It has been demonstrated using viral vaccines that there is an advantage to heterologous prime-boost immunization approaches, primarily to focus the immune response on a target antigen rather than augment responses to other immunogenic viral proteins encoded by the priming vector [25]. This was the presumed basis for the trial of Mincheff and colleagues targeting PSMA by means of an adenovirus and plasmid DNA approach [11]. We and others have demonstrated that this is not necessary using plasmid DNA vaccines [4]. However,

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prime-boost strategies may be advantageous to use immunologically potent strategies and potentially minimize the number of immunizations required. Multiple preclinical studies demonstrate that DNA vaccines can be used in various prime-boost sequences to elicit antigen-specific responses [26]. We are currently evaluating in a pilot clinical trial (NCT01706458) whether a plasmid DNA vaccine encoding PAP can augment responses primed with sipuleucel-T, an antigen-presenting cell vaccine targeting the same antigen.

4. Are there optimal routes of delivery, preferred schedules, or superior adjuvants?

These all remain relevant variables that have not been entirely answered in human trials. The optimal route of administration may depend on several factors, including the nature of antigen (extracellular or intracellular, whole protein or epitope), amount of plasmid, type of adjuvant employed, and mechanism of administration (gene gun, particle-mediated delivery, electroporation, etc). These variables may significantly influence the mechanisms involved in induction of antigen-specific immunity by DNA vaccines, including innate immunity, antigen processing by bystander cells, and presentation by regional professional APCs. In the case of antibody responses to the hepatitis B surface antigen (HBsAg), comparison of 8 injected and 6 non-injected routes revealed that highest titers of antigen-specific antibodies were obtained after intramuscular and intravenous administration of the DNA vaccine, although intradermal and sublingual injections yielded significant titers as well [27]. In a tumor model, gene gun mediated administration of plasmid was superior to intramuscular injection [28]. Another study employing plasmid DNA encoding HBsAg found that intramuscular injection elicited both antibody and CTL responses in mice, whereas intradermal injection was able to elicit only detectable antibody responses [29], in contrast to observations by our laboratory, which has reported robust elicitation of CTL responses to three different antigens upon intradermal vaccination [21, 30, 31]. In human clinical trials (Table 2), several routes have been evaluated for safety, though intramuscular (either by direct injection or electroporation) administration appears to be the most popular modality. As demonstrated in Table I, there is no clear superiority of any one method, with immune responses observed using multiple routes of delivery. In one study directly comparing intradermal and intramuscular immunization using a needle-free injection device, the authors concluded that a low-dose intradermal administration was preferred [32]. Each clinical or preclinical study cited used slightly different methodologies of schedule or dosage, preventing direct comparison. However, a recurrent theme is that distinct immune responses (in terms of kinetics, quantum, polarity and nature) are induced by different vaccination strategies, suggesting the importance of preclinical studies to determine an optimal route of administration for each specific vaccine.

5. Are there preferred stages of disease for clinical evaluation of plasmid DNA vaccines?

This remains a further unanswered question. Preclinical data would suggest that earlier stages of disease, with minimal tumor burden, are preferred times for immunization to minimize peripheral tolerance and the immunosuppressive mechanisms evoked by the tumor [33, 34]. Notwithstanding, sipuleucel-T has demonstrated a survival benefit in patients with more advanced metastatic, castrate-resistant prostate cancer, although subset analyses suggested that the magnitude of benefit was highest in patients with lower tumor burdens [22]. The poxviral vaccine approach, Prostvac-VF, has similarly demonstrated a possible

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survival benefit in this same population [24]. Consequently, while metastatic tumor burden may not preclude the possibility of benefit from anti-tumor immunization, this is still a population with a relatively short life expectancy. Modeling would still suggest that generating an adaptive anti-tumor immune response with memory should be more even effective in earlier stages of disease [35]. Moreover, multiple immunizations using plasmid DNA over a prolonged period of time would require that patients have relatively stable disease for the period of immunization, or at least not requiring intervention with other therapies that might have counterproductive effects. For these reasons, we have elected to pursue clinical studies using DNA vaccines in patients with minimal residual disease, detectable by PSA only, without obvious evidence of radiographically apparent metastases. This is also a population for which there is not a current standard-of-care treatment, yet for which the rate of PSA rise is predictive of metastatic progression and death, providing a means to stratify individuals at greatest risk for metastatic progression [36].

In the case of prostate cancer, a further consideration is whether there is an advantage or disadvantage of using androgen deprivation, the cornerstone of therapy for advanced prostate cancer, in combination with vaccines [37]. Most preclinical studies have suggested that androgen deprivation can mitigate peripheral tolerance to prostate tumor-expressed proteins, potentially by regrowth of the thymus, and the production of naïve T cells [38, 39]. It has also been observed that a Th1-biased systemic immune response occurs shortly after androgen deprivation, suggesting this may be an optimal time to immunize [40]. This consideration, however, must be weighed against the side effects of androgen deprivation, a therapy that most patients are keen to avoid. Preliminary results from a trial with sipuleucel-T, delivered before or after androgen deprivation, suggested that immune response changes were greater when delivered after androgen deprivation, suggesting this may be an optimal time for immunization [41]. Notwithstanding, the absolute benefit of vaccination in the context of androgen deprivation remains to be demonstrated in clinical trials.

6. Do these vaccines have anti-tumor effect, and how do we best measure this in human trials?

Ultimately these are the most important questions, and the ones most important to answer over the next ten years as trials progress towards randomized phase III trials. It is clear from preclinical studies that DNA vaccines can elicit anti-tumor responses, and from human clinical trials that DNA vaccines can elicit antigen-specific T cells with cytolytic activity. However, relevant clinical measures of anti-tumor efficacy are needed. As has been extensively reviewed elsewhere, patterns of anti-tumor response seen following immune-based therapies are different, both in terms of the often-delayed kinetics of radiographic response and durability off treatment, from what are typically observed following traditional cytotoxic therapies [34, 42]. This is a challenge for the treating oncologist to know whether a vaccine is "working," and when it is not, and also a challenge for the clinical trialist to measure these responses and build on these therapies. The use of longer-term endpoints in stages of disease with a defined natural history (e.g. progression-free survival over several years) in randomized clinical trials may be necessary to identify these benefits, as may be the development of quantitative measures to identify early changes in tumor growth rates (or regression) following DNA vaccines. In addition, as has been demonstrated in multiple

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vaccine approaches, the anti-tumor efficacy of immune cells augmented with vaccination can certainly be outweighed by immunosuppressive mechanisms of the tumor itself, including expression of regulatory ligands, recruitment of regulatory cell types, or by secretion of immunosuppressive factors. Thus it is clear that studies of these immune regulatory mechanisms are critical to design specific combination strategies to block or circumvent these regulatory mechanisms in combination with vaccines, including DNA vaccines. Many of these agents, including T-cell checkpoint inhibitors, are also being evaluated as single agents in clinical trials [43]. Trials combining these therapies with DNA vaccines are eagerly anticipated over the next several years.

Conclusions

In summary, over the last decade DNA vaccines targeting tumor-associated antigens have progressed from the laboratory to early phase clinical trials. Phase I clinical trials have been conducted targeting most major tumor types, and have generally demonstrated safety and measurable immune activity to the target antigen. Ongoing and future studies are exploring the clinical benefit of these vaccines, specifically addressing the choice of the particular target antigen, the route and schedule of administration, the optimal stages of disease for treatment, and the requirement for adjuvants and other complementary therapies. Over the next decade we anticipate multiple phase II clinical trials, and well-designed randomized phase II trials in particular, to clarify the future role of DNA vaccines in the treatment of prostate cancer as well as other cancer types.

Acknowledgments

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Table 1

Advantages of DNA vaccines

1. Adaptive immunity – Can induce robust CTL responses, helper T cells and antibodies.
2. No MHC restriction – Epitopes applicable to any MHC haplotype can be presented from a DNA plasmid encoding an entire antigen.
3. Safety – DNA vaccines have demonstrated safety in multiple early clinical trials. Moreover, they preclude handling of virulent pathogens or pathogenic proteins from other vaccine approaches that could potentially subdue immune responses, mask critical epitopes, or cause infection or transformation. No immune response against the vector is induced.
4. Adjuvant effect – Double stranded DNA and hypomethylated CpG motifs of plasmid DNA can stimulate innate immune receptors to cause cytokine release.
5. Adaptability – Can encode altered proteins or epitopes to enhance immune responses. Can be coupled with various adjuvants in protein or DNA form.
6. Stability – Plasmid DNA is a stable moiety and does not require unusual storage and transport conditions.
7. Economy – Can be easily and cost-effectively manufactured.

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Table 2

Clinical trials evaluating DNA vaccines for the treatment of cancer

Disease, number of patients	Target Antigen	Amount, Mechanism, and Route of Delivery	Phase Trial	Immunological Responses	Clinical Responses	Ref./Trial No.
Anal (n=12)	HPV-16 E7 fragment	i.m. (50–400μg) Up to four total immunizations, delivered every four weeks	I	10/12 patients developed antigen-specific immune responses	3/12 patients had PR	[44]
B-cell lymphoma (n=12)	Idiotype	i.m. (200, 600, 1800μg) Up to three immunizations delivered monthly, followed 17 weeks later by booster immunizations (1800μg delivered i.m. and i.d. three times monthly via Biojector). Booster immunizations could be repeated again 14 months later.	I/II	1/12 patients developed T-cell response to autologous Id following initial immunizaiton course 6/12 patients developed anti-Id responses following booster immunizations	At median follow-up (44 months) four patients had NED	[45]
B-cell lymphoma	Extraceullular domain of mouse CD20	i.m. injection, 5 doses administered every 3 weeks	I	TBD	TBD	NCT00561756
Breast (n=8)	HER2 (signaling-deficient)	i.m. (270μg) and i.c. (30μg) Up to three total vaccinations, delivered every four weeks	I	3/8 individuals had enhanced CD4+ T cell responses 3/5 patients have enhanced HER2 Ab responses	No clinical responses	[46]
Breast/Ovarian	ICD of HER2	i.d., 3 monthly doses along with admixed GM-CSF	I	TBD	TBD	NCT00436254
Breast	Mammoglobin-A	i.m. using jet delivery, 3 doses at weeks 1,4,8	I	TBD	TBD	NCT00807781
Cervical	HPV16 E6/E7, HPV18 E6/E7 fusion proteins	i.m. electroporation (0.6, 2 or 6mg) 3 doses at 1 month intervals	I	TBD	TBD	NCT00685412
Cervical	HPV16 E6/E7, HPV18 E6/E7 fusion proteins	1 ml of VGX-3100 delivered i.m. electroporation at Day 0, Week 4 and Week 12.	I	TBD	TBD	NCT01304524
Cervical	Calreticulin (CRT) linked to a detox form of human papillomavirus (HPV) type 16 E7 antigen	i.d. gene gun, i.m, or i.t., 3 doses at weeks 0,4,8	I	TBD	TBD	NCT00988559
Cervical	HPV16 E6/E7, HPV18 E6/E7 fusion proteins along with Fms-like tyrosine kinase-3 ligand (FLT3L)	i.m. electroporation	I	TBD	TBD	NCT01634503
Cervical	HPV E7/Hsp70	i.m, 2 doses on days 1 and 29, followed by TA-HPV on day 57	I	TBD	TBD	NCT00788164

Disease, number of patients	Target Antigen	Amount, Mechanism, and Route of Delivery	Phase Trial	Immunological Responses	Clinical Responses	Ref./Trial No.
Colorectal (n=10)	CEA fused to T-helper epitope	i.d. (2mg) or i.m. (8mg) Up to three total immunizations, delivered every two weeks	I	Erythema at injection site increased over time	8/10 patients showed no evidence of disease at follow-up	[32]
Colorectal (n=17)	CEA (along with HBV surface antigen)	i.m. (100, 400, 1000, 2000μg) Up to three total immunizations, delivered every three weeks	I	4/17 patients developed CEA-specific lymphoproliferative responses	No clinical responses observed	[47]
HNSCC (n=21)	Bacterial Hsp65	i.t. (150, 400, or 600μg) Up to three total immunizations, delivered every three weeks	I	4/13 patients developed Hsp65-specific IL-10 responses No responses to Hsp60 (human homologue)	5/14 patients had disease stabilization or regression	[48]
Head and Neck	Calreticulin (CRT) linked to a detox form of human papillomavirus (HPV) type 16 E7 antigen	i.m. electroporation (0.5,1.0,2.0 or 4.0 mg/dose) 3 doses on days 1, 22 and 43, preceded by cyclophosphamide injections	I	TBD	TBD	NCT01493154
Kidney	PSMA (human and mouse)	i.m, up to 6 doses, once every 3 weeks)	I	TBD	TBD	NCT00096629
Lymphoma	Autologous Ig derived ScFV-Chemokine fusion	i.m, 3 doses at weeks 0,4, 8	I	TBD	TBD	NCT01209871
Melanoma (n=34)	gp100 (mouse)	i.m. (2000 μg per injection) or PMED (4 μg per injection) Up to eight total vaccinations, delivered every two weeks	I	4/27 patients developed gp100 tetramer+ CD8+ T cells (all effector-memory phenotype) 5/27 developed IFNγ+ CD8+ responses, one of which was tetramer+	Median PFS = 17mo.	[49]
Melanoma (n=19)	gp100 (mouse and human)	i.m. (100, 500, or 1500μg) Up to six total immunizations, delivered every three weeks. First three immunizations using vaccine encoding one species, and next three with other species.	I	6/18 patients developed gp100-specific T cell responses	Median follow-up time of 30 months Median PFS of 44 months Median survival not reached	[50]
Melanoma (n=18)	Tyrosinase (human and mouse)	i.m. (100, 500, or 1500μg) Up to six total immunizations, delivered every three weeks. First three immunizations using vaccine encoding one species, and next three with other species.	I	7/17 patients developed antigen-specific T-cell responses	Median follow-up time of 42 months	[51]
Melanoma (n=12)	MART-1	i.m. (100, 300, 1000μg) Up to four total immunizations, delivered on days 1, 43, 85, 127	I	No enhancement in antigen-specific immune responses	Not determined	[52]
Melanoma (n=19)	MART-1 and tyrosinase T-cell epitopes	Intra-lymph node injections (500, 1000, 1500μg) Up to four total immunizations, delivered every two weeks	I	4/19 patients developed immune responses to MART-1	No clinical responses observed	[53]

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Disease, number of patients	Target Antigen	Amount, Mechanism, and Route of Delivery	Phase Trial	Immunological Responses	Clinical Responses	Ref./Trial No.
Melanoma (n=41)	Epitopes from five melanoma antigens	i.m. (2000 or 4000μg) followed by viral boosters Up to four immunizations, delivered every three weeks, followed by up to four viral injections, delivered every three weeks	I	22/31 patients developed antigen-specific T-cell responses	8/41 patients showed clinical benefit (PR, SD, and mixed responses)	[54]
Melanoma (n=12)	gp100	PMED (0.5 or 1μg) Vaccinations delivered every three weeks	I	No antigen-specific immune responses detected	1/12 patient had SD, and 4/12 maintained NED	[55]
Melanoma (n=10)	HLA-B7	DNA-containing liposomes injected i.t. (2, 9, 90mg) Up to three total vaccinations, delivered every two weeks	I	2/2 patients developed enhanced TIL cytotoxicity	2/10 patients had inhibition of growth of targeted lesion 1/10 patient had a PR, and following transfer of autologous TIL, a CR	[56]
Melanoma (n=26)	Tyrosinase epitopes	Intra-lymph node (200, 400, 800μg) Up to four total immunizations, delivered every two weeks	I	11/26 patients had antigen-specific T-cell responses	No clinical responses	[57]
Melanoma (n=22)	gp100	i.m. or i.d. (1000μg) Up to four total immunizations, delivered monthly	I	0/13 patients developed detectable antigen-specific immune responses	1/22 patients had a PR	[58]
Melanoma	Mouse TYRP2	i.m injection (0.5,2.0,4.0 or 8.0mg) 6 doses every 3 weeks	I	TBD	TBD	NCT00680589
Melanoma	gp75	i.m (0.1,0.5,2.0,4.0 or 8.0mg), 5 doses every 3 weeks	I	TBD	TBD	NCT00034554
Melanoma	Mouse gp100	PMED, 17 doses on days 1, 3, 5, 8, 22, 24, 26, 29, 43, 45, 47, 50, 64, 66, 68, and 71 i.m. jet injection, 16 doses on days 1, 3, 5, 8, 22, 24, 26, 29, 43, 45, 47, 50, 64, 66, 68, and 71.	Pilot	TBD	TBD	NCT00398073
Melanoma	Mouse tyrosinase	i.m. electroporation	Ia/b	TBD	TBD	NCT00471133
Melanoma	TRP2 epitope plus modified mAb	i.m. electroporation, 5 doses at weeks 0, 3, 6, 12 and 24	I/II	TBD	TBD	NCT01138410
NSCLC (n=13)	L523S	DNA injected i.m. via Biojector (8mg) on weeks 1 and 3, followed by viral boosters (1–400×10^9 viral particles) on weeks 4 and 8)	I	1/10 patient developed antigen-specific antibody response	Median overall survival of 290 days	[59]

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Disease, number of patients	Target Antigen	Amount, Mechanism, and Route of Delivery	Phase Trial	Immunological Responses	Clinical Responses	Ref./Trial No.
Ovarian	Amino Acids 1–163 of Insulin-Like Growth Factor Binding Protein-2 (IGFBP-2)	i.d., 3 monthly doses	I	TBD	TBD	NCT01322802
Prostate (n=9)	PSA	i.m. and i.d. (100, 300, 900μg) Up to five total immunizations, delivered monthly	I	2/8 patients developed antigen-specific immune responses In long-term follow-up, 4/8 patients had antigen-specific responses	Patients with PSA-specific immune responses had decrease in serum PSA	[8, 9]
Prostate (n=22)	PAP	i.d. (100, 500, or 1500μg) Up to six total immunizations, delivered every two weeks	I	10/22 patients had PAP-specific immune responses two weeks following final immunization 7/22 patients had PAP-specific immune responses 3–12 months following final immunization	10/22 patients had greater than 200% increase in PSA doubling time	[14, 15]
Prostate (n=30)	PSMA fused to T-helper epitope	i.m. (800, 1600, 3200μg) or e.p. (400, 800, 1600μg) Up to five immunizations, delivered at week 0, 4, 8, 24, and 48	I/II	21/30 patients developed antibodies to T-helped epitope up to 72 weeks following immunization 29/30 patients developed a measurable CD4+ T-cell response 16/30 patients developed epitope-specific CD8+ T cells	14/24 patients had an increase in PSA doubling-time at any point during the study 4/24 patients had an increase in PSA doubling-time at 72 weeks	[13]
Prostate (n=26)	PSMA (extra-cellular domain)	i.d. (100–800μg) Up to two immunizations, delivered every two weeks. Followed by up to 15 viral booster immunizations 5×10^8 PFU of adenovirus encoding PSMA delivered every 12 weeks	I/II	36/42 patients developed antigen-specific antibody responses	4/26 patients had decreases in serum PSA	[11]
Prostate	PSMA (human and mouse)	i.m, up to 6 doses, once every 3 weeks) First three immunizations using vaccine encoding one species, and next three with other species.	I	TBD	TBD	[20]
Prostate	PAP	i.d., 6 biweekly doses for first 12 weeks, then every 12 weeks till disease progression i.d., 6 biweekly doses for first 12 weeks followed by vaccination as per results of immune monitoring	II	TBD	TBD	NCT00849121
Prostate	PAP	i.d., 4 biweekly doses along with rhGMCSF at weeks 6,8, 10, 12 after sipuleucel T administration – 3 biweekly doses at weeks 0,2,4	II	TBD	TBD	NCT01706458

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Disease, number of patients	Target Antigen	Amount, Mechanism, and Route of Delivery	Phase Trial	Immunological Responses	Clinical Responses	Ref./Trial No.
Prostate	PAP	i.d., 6 biweekly doses, followed by one dose every 3 months to complete a 2 year period	II	TBD	TBD	NCT01341652
Multiple Solid Tumors (n=26)	PRAME and PSMA fragments	Intra-lymph node injections (2400μg, with 1200μg delivered on day 1 and 1200μg on day 4 each cycle) delivered along with PRAME and PSMA peptides Up to four total immunizations, delivered every two weeks	I	15/24 patients developed antigen-specific T-cell responses	10/26 patients developed stable disease	[60]
Multiple Solid Tumors NSCLC (n=5), **esophageal** (n=1), **prostate** (n=11)	NY-ESO-1	PMED (4μg or 8μg per injection at week 1, 5, and 9) or clustered PMED dosing (2μg at day 1, 3, 5, and 8 at weeks 1, 5, and 9)	I	1/17 patients developed Ab response 16/17 patients developed CD4+ T cell response 5/17 patients developed CD8+ T cell response	2 NSCLC patients with SD 2 NSCLC and 1 esophageal patient with NED	[17]
Multiple Solid Tumors	Inactivated form of the carcinogen activator cytochrome P450 1B1	400 μg DNA/total dose every two weeks for a maximum of six doses (6 cycles), preceded by i.v injections of cyclophosphamide	I	TBD	TBD	NCT00381173

Abbreviations

Ab – antibody

CEA – carcinoembryonic antigen

CR – complete response

EP – electroporation

Hsp – heat-shock protein

IC – intracutaneous

ID – intradermal

IFNγ – Interferon gamma

IM – intramuscular

IT – intratumoral

NED – no evidence of disease

ND – not determined

NSCLC – non-small cell lung cancer

PAP – prostatic acid phosphatase

PFS – progression-free survival

PFU – plaque-forming units

PMED – particle-mediated epidermal delivery

PSMA – prostate specific membrane antigen

PSA – prostate specific antigen

PR – partial response

SD – stable disease

TIL – tumor infiltrating lymphocytes