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Safety and Immunological Efficacy of a DNA Vaccine Encoding the Androgen Receptor Ligand-Binding Domain (AR-LBD)

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Abstract

Background—The androgen receptor (AR) is a key oncogenic driver of prostate cancer, and has been the primary focus of prostate cancer treatment for several decades. We have previously demonstrated that the AR is also an immunological target antigen, recognized in patients with prostate cancer, and targetable by means of vaccines in rodent models with delays in prostate tumor growth. The current study was performed to determine the safety and immunological efficacy of a GMP-grade plasmid DNA vaccine encoding the ligand-binding domain (LBD) of the AR, pTVG-AR.

Methods—Groups of male mice (n=6-10 per group) were evaluated after four or seven immunizations, using different schedules and inclusion of GM-CSF as a vaccine adjuvant. Animals were assessed for toxicity using gross observations, pathological analysis, and analysis of serum chemistries. Animals were analyzed for evidence of vaccine-augmented immunity by tetramer analysis. Survival studies using different immunization schedules and inclusion of GM-CSF were conducted in an autochthonous genetically-engineered mouse model.

Results—No significant toxicities were observed in terms of animal weights, histopathology, hematological changes, or changes in serum chemistries, although there was a trend to lower serum glucose in animals treated with the vaccine. There was specifically no evidence of toxicity in other tissues that express AR, including liver, muscle, hematopoietic and brain. Vaccination was found to elicit AR LBD-specific CD8+ T cells. In a subsequent study of tumor-bearing animals, animals treated with vaccine had prolonged survival compared with control-immunized mice.

Conclusions—These studies demonstrate that, in immunocompetent mice expressing the target antigen, immunization with the pTVG-AR vaccine was both safe and effective in eliciting AR-specific cellular immune responses, and prolonged the survival of prostate tumor-bearing mice. These findings support the clinical evaluation of pTVG-AR in patients with recurrent prostate cancer.

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Keywords

androgen receptor; DNA vaccine; prostate cancer; mouse; toxicology

Introduction

Despite decreases in the number of new diagnoses per year, prostate cancer remains a major health concern, being the second leading cause of cancer-related death in men (1). While patients who develop recurrent disease following treatment for localized prostate cancer have a high initial response rate to hormonal therapy, prostate cancer nearly always becomes refractory to androgen ablation, and metastatic prostate cancer that is castrate-resistant (mCRPC) is the lethal form of the disease for which effective, well-tolerated treatments are urgently needed. Among the agents recently approved for the treatment of mCRPC, sipuleucel-T, a vaccine targeting the prostate tumor antigen prostatic acid phosphatase (PAP), was approved on the basis an improvement in survival, and Prostavac® (rilimogene galvacirepvec/rilimogene glafovec), a viral-based vaccine targeting prostate-specific antigen (PSA), is currently being evaluated in a phase III trial based on encouraging survival results observed in a phase II trial (2,3). Despite these modest effects on overall survival, an outstanding question in the field of prostate vaccine development remains whether the PAP and PSA antigens are ideal targets for vaccine development, or whether more effective immune responses can be safely elicited targeting more functionally-relevant tumor associated antigens.

The androgen receptor is a steroid hormone receptor that plays a crucial role in the development of the prostate and is also important to the progression of prostate cancer. For many decades the primary treatment for recurrent prostate cancer has been androgen deprivation by surgical or pharmacological castration. However, even in the setting of castration-resistant disease the AR remains active and often overexpressed by means of gene regulation and amplification (4,5). Given the role and increased expression of AR in the progression of prostate cancer, we have evaluated it as a potential immunotherapy target of CD8+ T cells. Our concept has been that the AR is a critical molecule in prostate cancer development, and hence loss of AR expression as a means of avoiding immune detection should be uncommon. Moreover, overexpression of the AR following androgen deprivation should lead to greater presentation of AR-derived MHC-presented epitopes, permitting these tumor cells to be more readily detected by AR-specific cytolytic CD8+ T cells. Finally, while AR splice variants such as AR-V7 that lose expression of the LBD have emerged as major means of resistance to androgen depriving pharmacological therapies, expression of these variants is typically lower than continued expression of the full-length AR protein (6). Consequently, expression of AR-V7 or other LBD-loss splice variants may be less of a concern as a means of immunological resistance following LBD-targeted vaccination. In previous work we have demonstrated that patients with prostate cancer can have spontaneous immunity to the AR, and in particular CD8+ T cells specific for AR-derived epitopes can be identified in patients, and these CD8+ T cells have cytolytic activity to prostate cancer cells (7,8). To evaluate it as an immunotherapy target we have focused on the ligand-binding domain (LBD) of the AR, as the protein sequence of this domain is identical

among multiple species, permitting evaluation in immunocompetent murine models. Using a DNA vaccine encoding the AR LBD, we have found that immunization of HLA-A2-expressing male mice elicits AR LBD-specific CD8+ T cells with cytolytic activity against human prostate tumor cells (9). Moreover, immunization delayed the development of prostate tumors in, and prolonged the overall survival of, transgenic TRAMP mice (9).

The current study was prospectively designed in consultation with FDA reviewers, and conducted in male mice with or without prostate tumors, to comprehensively evaluate the safety of this vaccine using two different schedules of immunization, and with or without GM-CSF as a vaccine adjuvant, similar to what might be considered for future human clinical testing. The vaccine used for all studies was prepared using Good Manufacturing Practice (GMP) conditions. We report that with the immunization schedules used in mice, this vaccine was safe, elicited AR LBD-specific CD8+ T cells, and prolonged the survival of prostate tumor-bearing mice.

Materials and Methods

Animals

Male C57BL/6 mice at 40-65 days of age were purchased from Harlan Laboratories (Madison, WI). Male A2/TRAMP mice, also 40-65 days of age, were the F1 progeny of TRAMP mice and HHDII-DR1 mice, as previously described (9). Animals were housed in an American Association for the Accreditation of Laboratory Animal Care-accredited facility. The mice were housed in a 12 hour light:12 hour dark cycle and fed a chow diet (8604 Harlan Teklab, Wilmington, DE) and consumed distilled water *ad libitum*. All experimental protocols were received and approved by the University of Wisconsin-Madison Institutional Animal Care and Use Committee (IACUC).

Protocol and Regulatory Oversight

A specific prospective animal protocol describing all aspects of animal husbandry, *in vivo* observations, treatments, procedures, and laboratory analysis was reviewed and approved by the study investigators in compliance with Good Laboratory Practice requirements. Records of study deviations and amendments were kept, and the entire study (including review of the protocol, deviations, test article preparation, dose administration, necropsy, and final report) was reviewed by an independent study monitor.

Plasmids

The plasmids pTVG4 and pTVG-AR were previously described (9,10). The pTVG-AR DNA used for the current study was prepared from a master cell bank under GMP conditions by the Waisman Clinical Biomanufacturing Facility (WCBF) at the University of Wisconsin. Prior to release the plasmid lot was found to be >80% supercoiled; have low levels of endotoxin (2 EU/mg DNA), protein (0.17%), RNA (none detectable), and *E. coli* DNA (1%); and tested negative for bacterial or fungal contamination. The pTVG4 DNA used for the current study was purified using a Qiagen endonuclease-free kit, according to the manufacturer's recommendations (Qiagen, Valencia, CA).

Vaccinations

Groups of six to ten mice were immunized with 100 µg pTVG4 or pTVG-AR, and with or without 5 µg recombinant murine GM-CSF (Peprotech Inc., Rocky Hill, NJ), on two different schedules as illustrated in Figure 1. Vaccinations were performed intradermally in the ear pinna.

Tissue Collection

At the time of necropsy, blood was collected for measurements of serum chemistries and hematology parameters and Wright stained blood smears. In addition, tissue samples were collected from the following tissues: spleen, liver, kidney, seminal vesicle, prostate, urinary bladder, heart, lung, testis, skeletal muscle, bone, stomach, colon, ear injection site, brain, and cervical and/or inguinal and/or epitrochlear lymph nodes. Tissue samples for histopathological analysis were placed in 10% buffered formalin.

Toxicity Assessment

Cage-side observations were performed every day, and weights were recorded at least twice weekly. Blood collected into EDTA-containing tubes at the time of necropsy was evaluated for complete blood counts by the UW Veterinary School Clinical Pathology laboratory. Blood smears were also independently reviewed by a certified hematopathologist. Plasma prepared from blood collected was evaluated for changes in serum chemistries, including blood urea nitrogen (BUN), creatinine, calcium, sodium, potassium, glucose, phosphate, albumin, globulin, total protein, amylase, total bilirubin, alkaline phosphatase, alanine aminotransferase (ALT), creatine kinase (CK), and testosterone. Analysis for all parameters except CK and testosterone was performed by the UW Veterinary School Clinical Pathology laboratory using the Comprehensive Diagnostic Profile rotors in combination with the VetScan VS2 instrument (Abaxis, Union City, CA). ELISA kits were used for CK (XpressBio Life Science Products, Frederick, MD) and testosterone (Monobind Inc., Lake Forest, CA) measurements, according to the manufacturers' instructions. Tissues collected at the time of necropsy from experimental animals were fixed in 10% buffered formalin, and embedded in paraffin. Hematoxylin and eosin stained sections were reviewed by a single veterinary pathologist, in blinded fashion with respect to treatment group, with comparisons made to tissues obtained from age-matched, untreated control animals.

Tetramer Staining

Splenocytes from A2/TRAMP animals were evaluated for the frequency of HLA-A2-restricted AR LBD-specific CD8⁺ T cells by tetramer staining for HLA-A2 specific epitopes previously identified (8). Statistical comparison between treatment groups was performed using a Student's t test of log-transformed data, with $p < 0.05$ defined as a significant T-cell response.

Survival Analysis

In a study separate from the GLP study described above, A2/TRAMP animals, beginning at 6 weeks of age, were immunized as above with the same lot of pTVG-AR plasmid, or pTVG4 control, and with or without 5 µg GM-CSF administered as a vaccine adjuvant.

Immunizations were given either biweekly or on a cyclical schedule (immunizations at day 1 and 15 of an 84-day cycle, see Figure 1) and continued until the time of natural death or veterinarian-directed compassionate euthanasia. Additional survival studies were conducted using A2/TRAMP animals beginning at 6, 12, 18, 24, or 30 weeks of age, in which animals were immunized biweekly with either pTVG4 or pTVG-AR and continued until the time of natural death or veterinarian-directed compassionate euthanasia. Survival curves were generated using the Kaplan-Meier method and comparisons between experimental conditions were conducted using the log-rank test.

Results

Study Design

The current study was conducted to evaluate the safety and potential immunological efficacy of a plasmid DNA vaccine encoding the AR LBD that was prepared under GMP conditions. The study was designed to evaluate two schedules of administration using a fixed dose, based on our prior laboratory and clinical studies demonstrating immunological effect at an equivalent 100 µg dose (9,11,12). The evaluation of schedule and use of GM-CSF as an adjuvant were chosen, in consultation with FDA reviewers, to be similar to endpoints of a proposed human clinical trial using this plasmid DNA vaccine in patients with recurrent prostate cancer. Male mice were chosen as a model given that the expression of the androgen receptor among normal tissues is similar between mice and humans (13). Male mice were immunized at defined times, with collection of tissues for histopathological analysis, according to the schema in Figure 1. Spleens were collected under aseptic conditions for immunological analysis. Blood was collected at the time of euthanasia for the evaluation of complete blood counts and serum chemistries to assess for other possible toxicities.

Safety and Toxicity Assessment

Mice were examined multiple days per week for general changes in health and weighed at least twice weekly. As shown in Figure 2, there were no significant differences observed in growth rates among the groups. Apart from excoriations due to fighting, no other physical evidence of toxicity was observed. Five animals, in different groups, died due to wounds attributed to animal fighting. One animal died within one hour of anaesthesia used during vaccination. One animal, treated with pTVG-AR, was compassionately euthanized due to behavioral changes, and was found to have a high serum BUN, but no histological abnormalities on necropsy. Given the absence of similar findings in any other animal, attribution to the test article seemed unlikely. Another animal, in the vector DNA control group, was found at scheduled necropsy to have widespread lymphoma.

Blood drawn at the time of euthanasia was evaluated for abnormalities in complete blood counts, and peripheral blood smears were evaluated for white blood cell counts, hemoglobin concentration, hematocrit, and platelet count. No specific treatment-related abnormalities were observed, or out of range of normal controls, on standard blood counts. Specifically, the final off-study white blood cell counts for C57Bl/6 mice treated with pTVG-AR (n=17 available) were $6.77 \pm 2.25 \times 10^3$ cells/µL. Final off-study hemoglobin levels were 13.2 ± 1.1 g/dL, and platelet counts were $1219 \pm 246 \times 10^3$ /µL. All of these were within the

normal ranges for untreated animals. Evaluation of peripheral blood smears noted decreases in platelet counts not specific to treatment with the test agent, and likely due to microclots in the specimens collected prior to preparation of the smears. Some tumor-bearing animals were found to have anemia and pancytopenia, but no significant abnormalities were observed in wild type mice receiving the pTVG-AR. Sera were also evaluated from all animals for abnormalities in blood urea nitrogen (BUN), creatinine, calcium, sodium, potassium, phosphate, albumin, globulin, total protein, amylase, total bilirubin, alkaline phosphatase, alanine aminotransferase (ALT), creatine kinase (CK), and testosterone. No significant treatment-related abnormalities were observed in any group (Table I), however serum glucose was slightly lower in animals receiving pTVG-AR (274 +/- 135 mg/dL) compared with untreated control animals (344 +/- 94 mg/dL, $p=0.057$).

Tissues obtained at necropsy were evaluated for histological changes. All tissues were reviewed by a single pathologist in blinded fashion with respect to treatment group. No lesions or significant pathology were observed in tissues obtained from spleen, liver, kidney, seminal vesicle, prostate, urinary bladder, heart, lung, testis, skeletal muscle, bone, stomach, colon, brain, and lymph nodes of wild type mice treated with pTVG-AR. Areas of prostatic intraepithelial neoplasia and prostate tumor were identified in the prostates of A2/TRAMP mice, as expected. Dermal edema was observed in most mice at the site of injection.

Immunological Analysis

Tumor specimens were insufficient for direct analysis of tumor-infiltrating AR-specific CD8⁺ T cells. Consequently, systemic immune responses were evaluated using splenocytes collected from the tumor-bearing HLA-A2-expressing animals at the time of necropsy. As shown in Figure 3, AR-specific CD8⁺ T cells could be detected in nearly all A2/TRAMP animals. The median frequency of AR761-specific CD8 T cells was higher in animals treated on the cyclical schedule (0.44% among CD8⁺ T cells) compared with controls (0.14% among CD8⁺ T cells, $p=0.0045$), and overall the median frequency of any HLA-A2-restricted CD8 T cells was higher in animals treated on the cyclical schedule (1.8%) compared with the control vaccine (0.97%, $p=0.036$). However, the median frequency of peripheral AR-specific CD8⁺ T cells was non statistically higher in animals receiving pTVG-AR administered on the continuous biweekly schedule compared to those receiving control vector.

Anti-Tumor Effect: We have previously demonstrated that A2/TRAMP mice, immunized with pTVG-AR, have a decrease in the development of prostate tumors and an increase in overall survival (9). To test the potential anti-tumor efficacy of the plasmid prepared for clinical evaluation, and potentially whether there might be a difference in effect with different schedules of administration, and with or without GM-CSF adjuvant, 6-week old male A2/TRAMP mice were immunized biweekly with 100 μ g pTVG-AR ($n=9$), or on a cyclical schedule with vaccination at days 1 and 15 of an 84-day cycle ($n=9$). An additional group received vaccination on the cyclical schedule and with 5 μ g mGM-CSF co-administered as an adjuvant ($n=8$). A control group received the pTVG4 control vector administered on the same cyclical schedule ($n=9$). Overall, animals receiving the pTVG-AR vaccine ($n=26$) had a median survival significantly longer (338 days) than control-treated

animals (223 days, $n=9$, $p=0.003$). While the median survival was not significantly different between animals receiving pTVG-AR on a continuous biweekly or cyclical schedule (332 days versus 303 days, $p=0.49$), or with the addition of GM-CSF to animals receiving vaccine on a cyclical schedule (303 days versus 367 days, $p=0.26$), nonetheless the highest median survival was observed in the group of animals that received GM-CSF as an adjuvant (Figure 4). As we have previously reported (9), animals immunized with pTVG-AR on a cyclical schedule without GM-CSF had a higher median survival than animals immunized on the same schedule with control vector ($p=0.04$). However, the survival of animals immunized with pTVG-AR continuously every two weeks was not statistically different from animals immunized with the control vector ($p=0.10$). To investigate this biweekly schedule further, older A2/TRAMP mice, with increasing burdens of prostate tumors, were similarly immunized with pTVG-AR or control vector continuously every two weeks. Animals at ages of 12 weeks, 18 weeks, 24 weeks, or 30 weeks of age, ages when tumor growth is apparent (Figure 5A), were randomized to treatment. While there was a similar trend to increased survival in animals receiving the pTVG-AR vaccine (median 337 days versus 291 days), this was not statistically significant (Figure 5B, $p=0.069$).

Discussion

To date the primary antigens that have been investigated as targets for prostate tumor vaccines have been tissue-restricted antigens such as PSA (14-17), PAP (2,12), and prostate-specific membrane antigen (PSMA) (18,19). Curiously there has been little exploration of the AR as an immunological target antigen, despite the fact that it has been the primary pharmacological target in the treatment of prostate cancer for decades. While the AR is not a prostate tissue-specific protein (as is the case with PSA, PAP and PSMA), the fact that it is a key oncogenic protein involved in prostate cancer progression, and overexpressed in late-stage, castration-resistant tumors, suggests that it could also be a rational “next generation” immunotherapy target antigen for prostate cancer. In fact, the AR was highlighted as a key target antigen for tumor vaccines based on these criteria and other factors used in the prioritization of antigenic targets for anti-tumor vaccines (20). The LBD of the AR serves as a preferred portion of this protein for this evaluation, given that the single domain itself is not oncogenic. Moreover, there is large sequence variability in the amino terminal domain of the protein among humans. The LBD, however, has little to no sequence variability among humans, and is also identical in amino acid sequence among many species, permitting evaluation of the LBD as a “tolerant” self-antigen in rodent models without concerns for xenogeneic differences. We have previously identified that immune responses to the AR LBD can be elicited in mice and rats by means of a DNA vaccine, and that immunization delayed tumor progression and increased overall survival of tumor bearing mice (9). Anticipating the evaluation of this DNA vaccine in human studies, the current study was primarily performed to assess the safety of a GMP-grade plasmid in mice. We demonstrate that the pTVG-AR vaccine was safe (as determined by serum chemistries, blood counts, and histological review). In addition, administration was effective in eliciting AR LBD-specific CD8 T cells and prolonging overall survival of tumor-bearing mice, depending on immunization schedule, and with or without GM-CSF as a vaccine adjuvant.

Findings from this study demonstrate that immunization of mice with the pTVG-AR vaccine was not associated with significant toxicity, including multiple tissues (such as prostate, bladder, muscle, liver and brain) which are known to express the AR. Multiple serum chemistries were also evaluated. While not statistically significant, there was a trend to lower serum glucose in animals treated. Animals were evaluated 24 hours and 2 weeks after the final immunization, with no evidence of toxicities at either time point. While the current studies did not assess any possible toxicities beyond two weeks after the final immunization, the survival studies demonstrating improved overall survival of pTVG-AR treated animals suggest that significant toxicities developing at later time points with repetitive immunization were unlikely.

The primary toxicity measurements were made in wild type animals rather than tumor-bearing animals to distinguish effects due to treatment from those potentially caused by tumors. Nonetheless, A2/TRAMP animals were included in this study for the purpose of evaluating anti-tumor effect and whether immunization elicited detectable HLA-A2-restricted CD8+ T cells. As demonstrated, AR LBD-specific CD8+ T cells could be detected in all A2/TRAMP animals, but were significantly more frequent in animals treated with vaccine delivered on a cyclical schedule. Optimal vaccine schedules remain unknown and have been largely selected in human trials on the basis of inducing serum antibody responses to the target antigen (21,22). While optimal vaccine schedules able to elicit anti-tumor efficacy (which should be more dependent on Th1-biased CD8+ T cells than antibody responses) remains unknown, studies by others have suggested that more protracted schedules, with greater intervals between immunization, may elicit better quality CD8+ T cell responses, potentially by permitting the establishment of T cell memory prior to re-boosting (23,24). We have previously reported that an intermittent schedule of immunization using a DNA vaccine could elicit persistent Th1-biased immunity to the prostatic acid phosphatase (PAP) tumor antigen in patients with advanced prostate cancer (11). While the number of animals treated in our current studies was small, our studies similarly suggest that there may be an advantage to a cyclical schedule with intervals between immunization, and not continuously immunizing every 2 weeks, in terms of eliciting an immune response and prolonging survival in tumor-bearing animals. This will be investigated in future studies, to identify if there is a change in the magnitude or quality of antigen-specific effector and memory T cells with different immunization schedules.

GM-CSF has been studied by many groups as a vaccine adjuvant. This interest initially derived from studies performed over 20 years ago in which it was demonstrated that tumor cell lines engineered to secrete GM-CSF, compared with other cytokines, served as better vaccines and led to improved protection from subsequent tumor challenge (25). GM-CSF was subsequently evaluated in human and murine studies for protein and peptide vaccines, and demonstrated to augment antigen-specific CD4+ and CD8+ T-cell frequencies (26). Similarly, human studies with the hepatitis B vaccine demonstrated that GM-CSF was an effective adjuvant to increase antibody titers in patients not responding to vaccination in the absence of GM-CSF (27). However, the role of GM-CSF has been questioned as an adjuvant for peptide vaccines on the basis of a multicenter phase II trial that demonstrated inferior CD4+ and CD8+ T-cell responses if GM-CSF was administered with vaccine in Freund's adjuvant (28). Alternatively, preclinical studies using DNA vaccines by us and others have

suggested that GM-CSF administered as either a plasmid vector or soluble cytokine can increase antigen-specific T cell responses (29,30). These differences could be due to the fact that our studies have not also used oil-based adjuvants such as Freund's adjuvant, which can lead to sequestration of antigen-specific T cells at the site of administration (31). The studies in this report suggest that GM-CSF, administered as a soluble cytokine adjuvant, can act as an adjuvant to increase the frequency of antigen-specific CD8⁺ T cells with immunization. And while not statistically significant, median survival of animals receiving vaccine with GM-CSF was longer than animals not receiving adjuvant. Thus, there is no evidence from our studies that GM-CSF is disadvantageous to vaccination. Additionally, the use of alternative adjuvant strategies aimed at priming T-cell responses at the site of immunization (using cytokines such as IL-15 or the co-expression of costimulatory molecules such as LIGHT to promote T-cell proliferation) are the subject of ongoing preclinical and clinical research (32,33).

In summary, the results from this study demonstrate that the pTVG-AR vaccine did not elicit detectable significant toxicity in mice at equivalent doses (and higher dose-to-body mass ratios) that would be evaluated in clinical trials. Certainly, differences in AR expression in human tissues compared with mice could lead to unanticipated toxicities, and hence safety will be a primary objective of phase I clinical trial evaluation. Moreover, the vaccine was effective in eliciting antigen-specific CD8 T cells. An optimal schedule remains to be determined from the results of ongoing pre-clinical and clinical studies. However, these data suggest that a less frequent administration schedule using a cyclical approach may be preferred, or at least does not appear inferior to a continuous regular immunization schedule. In addition, our data suggest that GM-CSF is an appropriate adjuvant for this DNA vaccine. Together these data support the clinical evaluation of this vaccine in patients with recurrent prostate cancer.

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Figure 1. Schema of trial design

18 male A2/TRAMP mice (Groups 1, 2, and 3) and 75 male wild type C57Bl/6 mice (Groups 4-11) were immunized according to the schedule shown. Groups 1, 5, 6, and 10 received 100 μ g pTVG-AR administered every 14 days for 7 immunizations, with group 10 euthanized 24 hours after the last dose, and the other groups euthanized 2 weeks after the last immunization. Of these groups, groups 1, 6, and 10 also received 5 μ g GM-CSF co-administered with the plasmid DNA as a vaccine adjuvant. Group 4 was immunized on the same schedule with pTVG4 control DNA and with 5 μ g GM-CSF co-administered. Groups 2, 7, and 8 were immunized with pTVG-AR on a cyclical schedule, and received 4 immunizations total. Of these groups, groups 2 and 8 also received 5 μ g GM-CSF as a vaccine adjuvant. Groups 3, 9, and 11 were untreated age-matched controls and underwent necropsy at the same time points as the vaccinated animals as indicated. The grey arrows indicate the times of immunization.

Figure 2. Animal weights

Experimental animals were weighed three times per week. Shown is the mean and standard deviation of the weights (g) of each C57Bl/6 wild type animal per treatment dose group.

Figure 3. Immunization of A2/TRAMP animals with pTVG-AR elicits AR LBD-specific tetramer + CD8+ T cells

Splenocytes from A2/TRAMP animals (groups 1-3) were evaluated for the frequency of AR761, AR805, and AR811 tetramer+ CD8+ T cells among CD3+CD8+ T cells by flow cytometry. The frequency of each population, and sum of these three populations per animal, is shown. Line indicates median and * = $p < 0.05$ by T test (2-tailed) of log-transformed values.

Figure 4. Immunization with pTVG-AR leads to prolonged survival of A2/TRAMP mice
A2/TRAMP mice were immunized beginning at 6 weeks of age with either 100 μ g pTVG4 (vector control) on a cyclical basis (week 6, 8, 18, 20, 30, 32, etc), 100 μ g pTVG-AR administered on the same schedule, and with or without 5 μ g GM-CSF as a vaccine adjuvant, or with 100 μ g pTVG-AR every 2 weeks beginning at week 6. Animals were treated until death or until compassionate euthanasia as directed by veterinary staff. Shown are survival curves by individual groups. P values evaluate comparisons to control group by log-rank test.

Figure 5. Immunization with pTVG-AR leads to prolonged survival of A2/TRAMP mice with established tumors

Panel A: Genitourinary complexes/tumors were collected and massed from naïve A2/TRAMP mice at 6, 12, 18, 24, and 30 weeks of age. Lines indicate median mass. Panel B: Studies were conducted in which animals were immunized biweekly beginning at 12-, 18-, 24-, or 30-weeks of age, and were treated until death or until compassionate euthanasia as directed by veterinary staff. Specifically, 35 mice were treated with biweekly with pTVG4 (n=8 age 12 weeks, n=9 age 18 weeks, n=8 age 24 weeks, n=10 age 30 weeks) and 38 animals were treated biweekly with pTVG-AR (n=9 age 12 weeks, n=9 age 18 weeks, n=8 age 24 weeks, n=12 age 30 weeks). Shown is the Kaplan-Meier survival curve, and p-value indicates comparison to control group by log-rank test.

Serum chemistry evaluation

Table 1

Sera were prepared from blood collected at the time of necropsy, 24 hours or 2 weeks after the last immunization, from the number of animals indicated per group. Serum chemistries were determined by routine clinical laboratory tests at the University of Wisconsin School of Veterinary Medicine Clinical Pathology Laboratory. Shown are the mean $\pm$ standard deviation (SD) of the BUN, creatinine, sodium, potassium, glucose, calcium, phosphorus, albumin, total protein, globulin, alkaline phosphatase, alanine aminotransferase (ALT), total bilirubin, and amylase. Creatine kinase and testosterone evaluations were performed separately by ELISA.

	Group 4	Group 5	Group 6	Group 7	Group 8	Group 10	Group 9	Group 11
	pTVG4 + GM (n=9)	pTVG-AR biweekly (n=9)	pTVG-AR biweekly + GM wk 16 (n=9)	pTVG-AR cyclical (n=7)	pTVG-AR cyclical + GM (n=7)	pTVG-AR biweekly + GM wk 14 (n=7)	Untreated controls - wk 16 (n=9)	Untreated controls - wk 14 (n=4)
BUN (mg/dL)	31.3 $\pm$ 4.8	31.4 $\pm$ 5.4	47.8 $\pm$ 48.6	29.4 $\pm$ 4.8	30.0 $\pm$ 5.3	34.9 $\pm$ 6.7	31.3 $\pm$ 3.9	32.2 $\pm$ 2.6
Creatinine (mg/dL)	0.0 $\pm$ 0.0	0.02 $\pm$ 0.07	0.06 $\pm$ 0.11	0.06 $\pm$ 0.10	0.01 $\pm$ 0.04	0.04 $\pm$ 0.08	0.06 $\pm$ 0.09	0.0 $\pm$ 0.0
Sodium (mmol/L)	101 $\pm$ 76	52 $\pm$ 78	90 $\pm$ 85	136 $\pm$ 61	67 $\pm$ 84	93 $\pm$ 87	84 $\pm$ 79	78 $\pm$ 90
Potassium (mmol/L)	9.0 $\pm$ 3.6	8.2 $\pm$ 2.6	8.1 $\pm$ 1.9	9.3 $\pm$ 2.4	9.3 $\pm$ 1.5	9.2 $\pm$ 2.0	9.3 $\pm$ 2.2	10.1 $\pm$ 2.4
Glucose (mg/dL)	308 $\pm$ 107	313 $\pm$ 162	291 $\pm$ 176	173 $\pm$ 96	266 $\pm$ 96	323 $\pm$ 114	344 $\pm$ 55	382 $\pm$ 33
Calcium (mg/dL)	5.7 $\pm$ 3.5	4.4 $\pm$ 4.4	5.5 $\pm$ 3.4	7.5 $\pm$ 3.5	4.5 $\pm$ 4.0	7.0 $\pm$ 3.6	4.1 $\pm$ 4.0	6.9 $\pm$ 1.0
Phosphorus (mg/dL)	15.9 $\pm$ 4.1	15.2 $\pm$ 5.0	15.5 $\pm$ 6.0	12.6 $\pm$ 5.9	14.9 $\pm$ 3.7	14.9 $\pm$ 2.9	10.9 $\pm$ 3.9	11.4 $\pm$ 4.1
Albumin (g/dL)	4.0 $\pm$ 0.4	3.9 $\pm$ 0.4	3.9 $\pm$ 0.4	3.9 $\pm$ 0.3	4.1 $\pm$ 0.2	4.0 $\pm$ 0.3	3.9 $\pm$ 0.3	4.1 $\pm$ 0.2
Total protein (g/dL)	6.7 $\pm$ 0.6	6.7 $\pm$ 0.8	6.4 $\pm$ 1.0	6.2 $\pm$ 0.7	6.8 $\pm$ 0.2	6.7 $\pm$ 0.8	6.2 $\pm$ 0.5	6.3 $\pm$ 0.4
Globulin (g/dL)	0.0 $\pm$ 0.0	0.5 $\pm$ 1.1	0.3 $\pm$ 0.7	0.8 $\pm$ 1.0	0.0 $\pm$ 0.0	0.2 $\pm$ 0.5	0.4 $\pm$ 0.9	0.5 $\pm$ 1.0
Alkaline phosphatase (U/L)	163 $\pm$ 332	119 $\pm$ 184	258 $\pm$ 319	83 $\pm$ 107	160 $\pm$ 115	181 $\pm$ 254	61 $\pm$ 101	55 $\pm$ 106
ALT (U/L)	161 $\pm$ 430	70 $\pm$ 156	83 $\pm$ 206	286 $\pm$ 427	208 $\pm$ 368	12 $\pm$ 15	258 $\pm$ 437	658 $\pm$ 763
Total bilirubin (mg/dL)	0.08 $\pm$ 0.08	0.09 $\pm$ 0.14	0.08 $\pm$ 0.14	0.20 $\pm$ 0.12	0.06 $\pm$ 0.05	0.20 $\pm$ 0.32	0.18 $\pm$ 0.09	0.18 $\pm$ 0.05
Amylase (U/L)	113 $\pm$ 339	243 $\pm$ 483	489 $\pm$ 764	1002 $\pm$ 1087	183 $\pm$ 485	515 $\pm$ 1362	666 $\pm$ 1232	870 $\pm$ 1006
Creatine kinase (U/L)	228 $\pm$ 64	196 $\pm$ 134	241 $\pm$ 74	173 $\pm$ 117	260 $\pm$ 127	220 $\pm$ 103	135 $\pm$ 73	174 $\pm$ 67
Testosterone (ng/mL)	1.9 $\pm$ 4.5	1.0 $\pm$ 1.2	0.7 $\pm$ 1.0	3.7 $\pm$ 4.6	2.3 $\pm$ 2.6	1.6 $\pm$ 2.1	2.1 $\pm$ 2.1	1.6 $\pm$ 2.3