Phase II Trial of a DNA Vaccine Encoding Prostatic Acid Phosphatase (pTVG-HP) and Nivolumab in Patients with Non-Metastatic, PSA-Recurrent Prostate Cancer

UW 18008 **Trial Registration Number: NCT 03600350**

Investigational Agent:

BB IND 12109 - pTVG-HP DNA encoding human prostatic acid phosphatase

Study Supporters:
Bristol-Myers Squibb – Nivolumab
Madison Vaccines Inc. (MVI) – pTVG-HP

STUDY SITE INFORMATION

Study Sites: University of Wisconsin Carbone Cancer Center (UWCCC)
1111 Highland Avenue
Madison, WI 53705

Johns Hopkins School of Medicine (JHU)
Baltimore, MD 21287

Sponsor Investigator: Douglas G. McNeel, M.D., Ph.D.
UWCCC
7007 Wisconsin Institutes for Medical Research
1111 Highland Ave.
Madison, WI 53705
Tel: (608) 263-4198
Fax: (608) 265-0614
dm3@medicine.wisc.edu

UWCCC Local Principal Investigator :
Hamid Emamekhoo, MD
UWCCC
7009 Wisconsin Institutes for Medical Research
1111 Highland Ave.
Madison, WI 53705
Tel : (608) 263-3398
Fax : (608) 265-5146
emamekhoo@wisc.edu

Other UWCCC Investigators:

Christos Kyriakopolous, M.D. – Clinical Investigator
Glenn Liu, M.D. – Clinical Investigator
Joshua Lang, M.D. – Clinical Investigator
Robert Jeraj, Ph.D. – Medical Physics, Quantitative Total Bone Imaging
Scott Perlman, M.D. – Nuclear Medicine

JHU Principal Investigator:

Channing Paller, MD
Associate Professor,
Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins
Viragh Floor 9, Box 7
201 N. Broadway, Room 9162
Baltimore, MD 21287
Phone: 410-955-8239
Email: cpaller1@jhmi.edu

Study Coordinator:

Mary Jane Staab, R.N. B.S.N.
UWCCC
600 Highland Ave. K6/5 CSC
Madison, WI 53792
Tel: (608) 263-7107
mjs@medicine.wisc.edu

Study Biostatistician:

Jens Eickhoff, Ph.D.
UWCCC
600 Highland Ave. K6/434 CSC
Madison, WI 53792
Tel: (608) 265-5376
eickhoff@biostat.wisc.edu

Projected Start Date:	May 1, 2018
Projected End of Accrual Date (First Stage):	October 31, 2019
Projected End of Study Date (First Stage):	October 31, 2020
Projected End of Long-Term Follow-Up (First Stage):	October 31, 2022

TABLE OF CONTENTS

SYNOPSIS:

Purpose: To evaluate the safety of an investigational DNA vaccine, pTVG-HP, a plasmid DNA encoding human prostatic acid phosphatase (PAP), in combination with nivolumab, and the efficacy of this combination in decreasing serum PSA in patients with non-metastatic, non-castrate prostate cancer (clinical stage D0/M0).

Primary Objectives:

1. To evaluate the safety and tolerability of pTVG-HP DNA vaccine and nivolumab in patients with clinical stage D0/M0 prostate cancer
2. To determine the PSA complete response rate (PSA < 0.2 ng/mL)

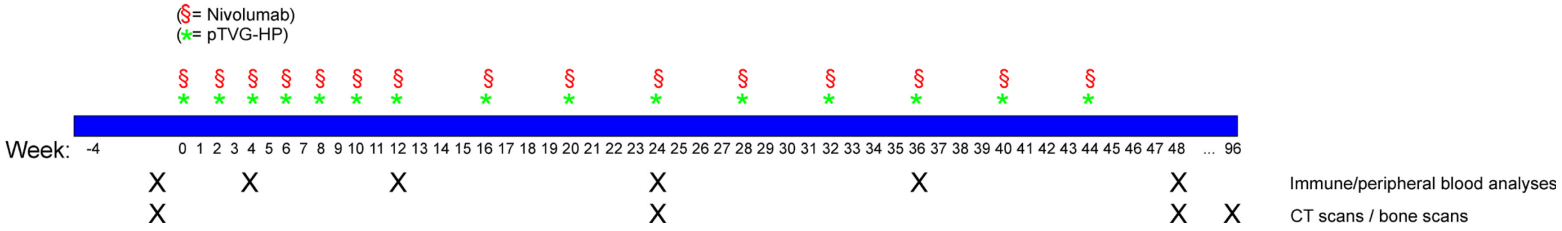
Secondary Objectives:

1. To evaluate 2-year metastasis-free survival rate
2. To evaluate median radiographic progression-free survival
3. To evaluate changes in PSA doubling time or slope
4. To evaluate PSA response rate (≤ 50% of baseline)
5. To determine whether GM-CSF is required as a vaccine adjuvant for pTVG-HP vaccine when used in combination with nivolumab

Laboratory/ Exploratory Biomarker Objectives:

1. To determine if antigen-specific T-cell and/or IgG responses are elicited with treatment
2. To determine if the development of PAP-specific T-cell immune responses are associated with PSA response (decline ≥ 50%)
3. To determine if antigen spread (the development of immune responses to other cancer-associated proteins) is associated with PSA response (decline ≥ 50%)
4. To determine if Quantitative Total Bone Imaging (QTBI) by NaF PET/CT can identify bone lesions in this patient population not detected by standard bone scintigraphy
5. To determine whether the presence of lesions detected by NaF PET/CT at baseline is associated with early disease progression (within 1 year) by standard radiographic imaging methods (CT and bone scintigraphy)
6. To determine if growth rates, and changes in growth rates, determined by NaF PET/CT are associated with PSA doubling time and changes in PSA doubling time

Study Scheme:



Study Calendar:

Vaccine / Treatment Visit:	Pre-Screen (within 4 wks registration)	Screen/Randomization (within 2 weeks of day 1)	Day 1 (wk 0) – V1	Week 2 – V2 (+/- 3 d)[d]	Week 4 – V3 (+/- 3 d)	Week 6 - V4 (+/- 3 d)	Week 8 – V5 (+/- 3 d)	Week 10 - V6 (+/- 3 d)	Week 12 – V7 (+/- 3 d)	Week 16 – V8 (+/- 3 d)	Week 20 – V9 (+/- 3 d)	Week 24 – V10 (+/- 3 d)	Week 28 – V11 (+/- 3 d)	Week 32 – V12 (+/- 3 d)	Week 36 – V13 (+/- 3 d)	Week 40 – V14 (+/- 3 d)	Week 44 – V15 (+/- 3d)	Week 48 – V16 EOT (+/- 3d)	Week 60 – V17 (+/- 14d)[k]	Week 72 – V18 (+/- 14d)[k]	Week 84 – V19 (+/- 14d)[k]	Week 96 – EOS (+/- 14d)[k]	28-day Follow Up (+/- 7d)[hp]	Annual Long-Term F/U
History & Physical Exam																								
Consenting	X																							
History	X																							
Physical Exam[m]		X[n]	X		X		X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
Toxicity assessment[a]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
Lab Tests																								
CBC[b]		X	X[g]		X		X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
Chemistry panel[c]		X	X[g]		X		X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
Serum testosterone		X																						
PSA[l] and PAP		X	X[g]		X[o]		X		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	
Procedures / Treatments																								
CT abdomen/pelvis[f]	X											X[e]						X[e]		X[e]		X[e]		
Bone scan	X											X[e]						X[e]		X[e]		X[e]		
NaF-PET/CT		X[q,r]							X[q,r]			X[q,r]												
Nivolumab			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X							
pTVG-HP immunization			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X							
rhGM-CSF adjuvant[j]					X	X	X	X	X	X	X	X	X	X	X	X	X							
Blood for immune studies		X			X				X			X			X			X					X[i]	

[a] Study coordinator or research nurse review of systems

[b] CBC to include differential and platelets

[c] Chemistry panel includes Chem7 (sodium, potassium, bicarbonate, BUN, creatinine, glucose), ALT, AST, total bilirubin, alkaline phosphatase, amylase, TSH (thyroid stimulating hormone), and LDH

[d] Time interval (+/- 3 days) is from last visit

[e] Can be +/- 7 days of this visit

[f] CT of chest only as clinically indicated

[g] If not performed within 7 days of this visit

[h] 28 days +/- 7d after off-study date, if prior to week 48, otherwise this visit not required

[i] If not already collected within previous 60 days

[j] Only use GM-CSF adjuvant if serum PSA at week 4 (V3) is higher than that obtained at day 1 (V1).

[k] Can be +/- 14 days of this visit

[l] PSA values used for screening/eligibility should all be from same clinical laboratory; for day 1 and subsequent visits, all PSA values should be from same lab, but not necessarily from same lab as used for screening

[m] Physical exam to include vital signs and ECOG status

[n] Physical exam does not need to be repeated on Day 1 if obtained within 14 days prior to the visit

[o] PSA obtained at week 4 and used to determine whether GMCSF will be added can be obtained within 7 days of the visit

[p] Patients will be followed for adverse events for 90 days after the last dose of nivolumab

[q] NaF PET/CT within +/- 7 days of study visit, exceptions may be allowed with prior approval from the study PI. The same PET/CT scanner should be used for each individual subject.

[r] NaF PET/CT should NOT be done within 2 days of GM-CSF injection.

1. Introduction

Prostate cancer is the most common tumor among men, and the second leading cause of male cancer-related death in the United States [1]. Despite advances in screening and early detection, nearly 30,000 U.S. men are estimated to die as a result of prostate cancer in 2017 [1]. Treatment with surgery and radiation remain effective for presumed organ-confined disease, however approximately one third of these patients will have progressive or metastatic disease at 10 years [2]. A retrospective review of patients with prostate cancer treated with prostatectomy demonstrated that with evidence of a rise in serum PSA after definitive therapy, so-called "stage D0" (or M0) disease, patients ultimately developed radiographically apparent metastatic disease within a median of 8 years [3]. Prostate cancer, once it becomes metastatic, is not curable and is generally treated initially with androgen ablation therapy with an average three-year progression-free survival before the disease becomes refractory to hormonal manipulations. Many patients with this stage D0/M0 disease (rising PSA after definitive treatment, and without evidence of radiographically-apparent metastatic disease) are often treated with androgen deprivation. Androgen deprivation in this context could be by orchiectomy or treatment with LH-RH agonists, with or without a nonsteroidal anti-androgen. Observation is also an option, in particular given the potentially long natural history of this stage of disease. In patients with rising serum PSA after definitive therapy, without radiographic evidence of metastases and not on androgen deprivation therapy, the rate of rise of the serum PSA blood test (PSA doubling time, PSA DT, or PSA slope) may be the most important prognostic indicator. Several recent retrospective reports have highlighted that patients with rapid PSA DT in this stage of disease have a markedly shorter time to the development of metastases and death [4-7]. In a prospective analysis, the most important contributors to metastatic disease progression were the PSA DT, the original Gleason score, and the baseline PSA at the start of prospective monitoring [5]. Consequently, this clinical stage D0/M0 disease represents a high-risk population of patients for whom there is not a standard therapy, for whom observation is typically employed, and whose progression-free survival can be estimated based on known factors, including PSA DT [7]. This represents a population for whom new treatments without the side effects associated with androgen deprivation could be evaluated.

Vaccine-based strategies, also known as active immunotherapies, are particularly appealing as potentially safe and less costly treatments that have the potential to eradicate micrometastatic disease and prevent the progression from limited-stage disease to metastatic disease, or at least slow this progression. Once a patient is diagnosed with presumably organ-confined prostate cancer, the gland is usually removed by prostatectomy or destroyed *in vivo* with radiation. Hence, an immune response directed at the prostate following such procedures, to elicit a prostate tissue-specific rejection, might have therapeutic benefit to destroy micrometastatic disease, the goal of active immunotherapies. The target of such therapy would not need to be specific to malignant prostate tissue, but could be to any prostate tissue. We and others have investigated prostatic acid phosphatase (PAP) as a prostate tumor vaccine antigen. PAP is a well-defined protein whose expression is essentially restricted to normal and malignant prostate tissue [8]. It is also one of only a few known prostate-specific proteins for which there is a rodent homologue, which thereby provided an animal model for evaluating vaccine strategies and assessing toxicity [9]. Data from independent labs has demonstrated that, in a rat model, vaccine strategies targeting PAP can result in PAP-specific CD8+ T-cells, the presumed population mediating tumor cell destruction, and anti-tumor responses [10-13]. An autologous antigen-presenting cell vaccine loaded *ex vivo* with a PAP derivative protein (sipuleucel-T, Dendreon Corporation, Seattle, WA) demonstrated

improved overall survival in patients with advanced castration-resistant metastatic prostate cancer in a placebo-controlled randomized phase III trial, leading to the FDA approval of sipuleucel-T in 2010 [14]. Together, these results demonstrate that the PAP protein is a reasonable target antigen for immunotherapy trials of prostate cancer. However, the use of autologous cell-based vaccines is labor-intensive and costly. The development of novel, more feasible, immunization strategies would provide a significant advantage to the development of immunotherapeutic treatments for prostate cancer.

Based on previous studies in rat models using a plasmid DNA vaccine encoding PAP, we conducted a phase I/II trial in patients with high-risk stage D0 prostate cancer using this same DNA vaccine (pTVG-HP). No significant adverse events were observed in 22 subjects treated over a 12-week period of time. Moreover, several patients developed evidence of PAP-specific CD8+ T-cells, and several patients experienced a prolongation in the PSA doubling time, demonstrating immunological efficacy and possible clinical efficacy [15]. IFNγ-secreting immune responses to PAP detectable at multiple times months after immunization were detectable in individuals with evidence of prolonged PSA doubling time [16]. A second trial was conducted in patients with castration-resistant, non-metastatic prostate cancer (clinical stage D0.5/M0) that evaluated treatment over a 2-year period of time and with different schedules of administration. That trial similarly demonstrated safety, and the induction of durable Th1-biased immune responses specific for the PAP target antigen [17]. These findings have justified further evaluation of this vaccine in a randomized phase II clinical trial (NCT01341652), evaluating whether vaccination with pTVG-HP versus placebo increases the 2-year metastasis-free survival of patients with high-risk PSA-recurrent, non-metastatic prostate cancer (stage D0) at high-risk for metastatic progression as defined by a PSA doubling time of less than 12 months. That trial has completed accrual, and is expected to be completed in 2018.

We have further explored means to increase the immunogenicity of DNA vaccines in murine models, and found that DNA vaccination elicited PD-L1 expression in tumors as a result of tumor-specific T cells elicited with vaccination that secrete IFNγ. Combining vaccination with anti-PD-1 or anti-PD-L1 antibody treatment resulted in a greater anti-tumor response and eradication of tumors in some animals [18]. We have recently identified that this also occurs following human immunization using cryopreserved blood samples collected from patients with advanced prostate cancer treated with pTVG-HP. Using *in vitro* and *trans vivo* methods, we found that immune responses to PAP were detected and/or augmented when combined with PD-1 blockade [19]. Moreover, we detected increased expression of PD-L1 on circulating tumor cells following DNA vaccination, and we found that higher expression correlated with the development of persistent antigen-specific IFNγ-secreting T-cell immunity [19]. These data suggested that combining PD-1 blockade with pTVG-HP should elicit superior anti-tumor effects in patients. We have now completed accrual to the first part of a pilot clinical trial using pTVG-HP in combination with pembrolizumab, in patients with metastatic, castration-resistant prostate cancer (mCRPC, NCT02499835). In this trial, patients received both agents, together or in sequence, over a 12-to-24-week course. We identified that treatment elicited PAP-specific Th1-biased immunity in some patients, and anti-tumor responses as measured by declines in serum PSA, and objective radiographic tumor responses as measured by CT imaging, were only detected in individual receiving the combination of agents (not sequentially). These are encouraging findings, notably because PD-1 blockade with either nivolumab or pembrolizumab alone has not demonstrated single-agent clinical activity in patients with advanced prostate cancer in previous phase I trials

[20, 21], and PSA declines and objective radiographic responses are rare following vaccine therapies. The current trial will further evaluate this treatment approach in patients with early recurrent prostate cancer, a stage that we have most studied using vaccines as monotherapy. The ability to drive PSA to undetectable levels, possibly curing or significantly delaying the metastatic recurrence of prostate cancer prior to the need for androgen depriving therapies, could be a substantial and clinically meaningful advance in the therapy of this disease. This will be conducted as a pilot, safety and feasibility multiple-institution study in this population.

2. Background and Rationale

A. PAP is a tumor antigen in prostate cancer and CTL are necessary to destroy prostate tissue

PAP was first identified in 1938 and was initially used as a serum marker for the detection of prostate cancer [8, 22]. PAP expression in normal and malignant prostate cells is well-documented, and is still used in immunohistochemical staining to establish a prostate origin of metastatic carcinoma [23]. The ubiquitous expression of PAP in prostate tissue makes it an appealing antigen as a potential "universal" target for immune-directed therapies of prostate cancer, unlike specific oncogenes that may or may not be expressed by a particular tumor. Moreover, it has been demonstrated that some patients with prostate cancer have preexisting antibody and T-cell responses to PAP, suggesting that tolerance to this "self" protein can be circumvented *in vivo* [24, 25]. In particular, the demonstration of Th1-like immune responses specific for PAP suggest that an immune environment permissive of an anti-tumor response can exist in patients even without immunization.

Results from researchers at Dendreon Corporation have demonstrated that PAP is a human prostate tumor vaccine antigen. They have reported that CTL specific for HLA-A2 peptide epitopes derived from PAP could be expanded from the peripheral blood of HLA-A2-expressing normal donors, and that some peptide-specific CTL lines generated were capable of lysing an HLA-A2-expressing prostate cancer cell line [26]. They then initiated human clinical trials targeting PAP using autologous antigen-presenting cell vaccines pulsed with a protein conjugate of human PAP and GM-CSF. In a phase II follow-on trial with the PAP-GM-CSF treatment approach, one of 21 patients experienced a complete response [27]. These trials formed the basis for a double-blinded phase III randomized clinical trial designed initially to evaluate the time to disease progression in patients with asymptomatic, androgen-independent prostate cancer. In that study, D9901, 127 patients were randomized to three infusions, two weeks apart, of sipuleucel-T (n=82) or placebo (n=45) [28]. The median time to progression was found to be 11.7 weeks on the treatment arm compared with 10.0 weeks on the placebo arm (p=0.052, log-rank test), not meeting its original endpoint [28]. However, that trial demonstrated a significant increase in median survival (25.9 months for patients treated with sipuleucel-T, compared with 21.4 months in patients treated with placebo, p=0.01 log-rank test), with 34% of patients treated with sipuleucel-T alive at 36 months compared with 11% of patients treated on the placebo arm (p=0.005, chi-square analysis). Consequently, a confirmatory phase III trial was conducted with overall survival as the primary endpoint. That trial demonstrated a significant increase in overall survival [14], and was consistent with results from previous trials [29], leading to the FDA approval for this treatment in 2010. Interestingly, few "PSA responses" used in the assessment of cytotoxic treatments for prostate cancer were observed, despite increases in overall survival. The results from the Dendreon trials

underscore the rationale for targeting PAP as a vaccine antigen, and the importance of clinical progression endpoints in the design of anti-tumor vaccine trials. Moreover, the development of less costly and cumbersome immunization approaches to elicit responses to the PAP antigen could be advantageous, as could their application to an earlier stage of disease.

B. DNA vaccines can elicit antigen-specific Th1/CTL immune responses

Over the last decade, there has been considerable interest in the development of plasmid DNA-based vaccines, a strategy that offers several distinct advantages over other methods of antigen delivery. DNA can be rapidly and inexpensively purified, and there are no problems with solubility as there frequently are with peptides and recombinant protein vaccines. In addition, because the DNA in nucleic acid vaccines has been demonstrated to be taken up and expressed by host tissues and presented by host antigen-presenting cells (APC) directly [30-32], antigen presentation occurs through naturally processed epitopes. DNA vaccines can therefore be theoretically employed in an HLA-independent fashion unlike peptide-based vaccines that are necessarily HLA-restricted. This strategy is ideal in the HLA-diverse human situation. In many ways, this method of immunization is similar to the use of viral immunization vectors, however without the additional foreign antigens introduced with a viral vector and consequently less of a risk of an overwhelming immune response to the vector itself [12, 33]. Of importance in tumor immunization models, several groups have demonstrated that immunization with plasmid DNA encoding a target antigen is a potent means of eliciting Th1-biased immune responses [34] and CD8+ T-cells specific for the targeted antigen [30, 35-38]. In animal models, the use of an intradermal route of vaccine administration, in particular, tends to promote this Th1/CTL-biased immune response [34, 39, 40].

C. GM-CSF can function as an immunological adjuvant for DNA vaccines

Granulocyte-macrophage colony-stimulating factor (GM-CSF) has been studied extensively by many groups in animal and human models as a vaccine adjuvant [41]. GM-CSF is a known growth and differentiation factor for human dendritic cells [42, 43]. Dendritic cells are specialized antigen-presenting cells (APC) that are believed to be responsible for stimulating naïve T-cell responses and to be the best cells at augmenting secondary immune responses [44]. They are known to cross present antigens encoded by DNA vaccines and taken up by bystander cells [45]. The dermis is a site for skin dendritic cells (Langerhans' cells) that are important in initiating immune responses by migrating to draining lymph nodes after antigen exposure for presentation of antigen to T-cells [46]. In human studies, soluble GM-CSF has been used as a vaccine adjuvant for a hepatitis B vaccine. In this phase I study, doses of 20-80 μg GM-CSF were administered just prior to and at the same site as the hepatitis B protein vaccination. Of 81 subjects treated with GM-CSF, 15 developed anti-HBs after only 1 injection (10 had protective titers) while only 1 of 27 patients vaccinated without GM-CSF produced a weak and transient antibody response [47]. GM-CSF-expressing autologous cells have also been demonstrated in murine and human studies to be able to elicit immune responses to autologous antigens [48-50]. GM-CSF has also been demonstrated to be a potent adjuvant for plasmid DNA vaccines. Several groups have demonstrated in animal models that plasmid DNA encoding GM-CSF can enhance the immune response generated to a target antigen when co-immunized with plasmid DNA encoding the antigen [39, 51, 52]. We have previously used recombinant human GM-CSF as a vaccine adjuvant for DNA vaccines, however its use has been dispensable in preclinical murine and rat studies,

suggesting it may or may not be required as an adjuvant for human use. This is being more formally evaluated in a separate trial (NCT02411786). In the current trial, GM-CSF will be used as an adjuvant in patients without evidence of PSA decline, to determine whether it required as an adjuvant for the pTVG-HP vaccine when used in combination with nivolumab.

D. PD-1 blockade and cancer treatment

The PD-1 receptor-ligand interaction is a major pathway hijacked by tumors to suppress immune control. The normal function of PD-1, expressed on the cell surface of activated T-cells under healthy conditions, is to down-modulate unwanted or excessive immune responses, including autoimmune reactions. PD-1 (encoded by the gene *Pdcd1*) is an immunoglobulin (Ig) superfamily member related to cluster of differentiation 28 (CD28) and cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) that has been shown to negatively regulate antigen receptor signaling upon engagement of its ligands (PD-L1 and/or PD-L2) [53, 54].

A major mechanism by which tumors can avoid immune detection is by expression of PD-L1 or PD-L2, ligands for a receptor on T cells (PD-1), activation of which can decrease T-cell function and lead to immune tolerance. There is currently great enthusiasm to specifically develop PD/PD-L blockade inhibitors given the relative paucity of adverse events observed with these agents in clinical trials, and long-term disease response observed in some instances in early phase clinical trials. Targeting PD-1, in particular, should be a universal therapy, as it targets the T-cell compartment rather than the tumor directly. However, clinical trial experience to date suggests that patients with some solid tumor types (notably renal cell cancer, melanoma and non-small cell lung cancer) experience more benefit than patients with other histologies, including prostate cancer [20, 21]. This disparity suggests that differences are due to differences in the T-cells of responding and non-responding patients. In particular, higher frequencies of tumor-infiltrating lymphocytes (TIL) are typically observed in patients with renal cell cancer and melanoma than prostate cancer [55]. In addition, early phase clinical trials using PD-1 or PD-L1 inhibitors have identified that the expression of at least one of the ligands for PD-1 (PD-L1) on the target tumor cell by biopsy is associated with clinical response to therapy [21]. This is expected, given that tissue-infiltrating T cells can induce the expression of PD-L1 via the expression of IFNγ, and ligand binding of PD-1 leads to decrease in T-cell effector function. It has been demonstrated that prostate cancers can express PD-L1, and can have infiltrating PD-1-expressing T cells [56]. Taken together, these results suggest that the efficacy of anti-tumor immunotherapy could be increased for prostate cancer by combining agents able to increase the number of tumor-specific T cells, such as through vaccination, and by PD-1/PD-ligand blockade.

The structure of murine PD-1 has been resolved [57]. PD-1 and its family members are type I transmembrane glycoproteins containing an Ig-variable–type (IgV-type) domain responsible for ligand binding and a cytoplasmic tail responsible for the binding of signaling molecules. The cytoplasmic tail of PD-1 contains 2 tyrosine-based signaling motifs, an immunoreceptor tyrosine-based inhibition motif, and an immunoreceptor tyrosine-based switch motif. Following T-cell stimulation, PD-1 recruits the tyrosine phosphatases, SHP-1 and SHP-2, to the immunoreceptor tyrosine-based switch motif within its cytoplasmic tail, leading to the dephosphorylation of effector molecules such as CD3 zeta (CD3ζ), protein kinase C-theta (PKCθ), and zeta-chain-associated protein kinase (ZAP70), which are involved in the CD3 T-cell signaling cascade [54, 58-60]. The mechanism by which PD-1 down-modulates T-cell responses is similar to, but distinct

from, that of CTLA-4, because both molecules regulate an overlapping set of signaling proteins [61, 62]. As a consequence, the PD-1/PD-L1 pathway is an attractive target for therapeutic intervention in multiple types of cancer, including prostate cancer.

Nivolumab is a potent human immunoglobulin G4 (IgG4) monoclonal antibody (mAb) with high specificity of binding to the programmed cell death 1 (PD-1) receptor, thus inhibiting its interaction with programmed cell death ligand 1 (PD-L1) and programmed cell death ligand 2 (PD-L2). Based on preclinical *in vitro* data, nivolumab has high affinity and potent receptor blocking activity for PD-1. Nivolumab has an acceptable safety profile and has been FDA approved for the treatment of several malignancy types, including melanoma, renal cell cancer, non small cell lung cancer, Hodgkin lymphoma, squamous cell head and neck cancer, urothelial cancers, microsatellite instability-high metastatic colorectal cancer, and heptocellular cancer. For more details on specific indications refer to the Investigator brochure.

E. DNA vaccine with PD pathway blockade elicits anti-tumor responses in murine models and a pilot human clinical trial

Given our findings in human trials that some patients did not develop evidence of immune response, and even those that did still showed evidence of disease progression, we have sought to evaluate methods to increase the efficacy of DNA vaccines and evaluate potential mechanisms of tumor escape. As described above, we have identified that immunization elicits CD8+ T cells, which, upon secretion of IFNγ, leads to increased PD-L1 expression in tumors [18]. Moreover, efforts to increase the immunogenicity of vaccination by encoding epitopes with greater affinity for MHC class I led to an inferior anti-tumor response that was mediated by increased and prolonged expression of PD-1 on antigen-specific CD8+ T cells [18, 63]. The anti-tumor efficacy of vaccination could be increased by blocking the PD-1/PD-L1 interaction at the time of immunization [18, 63]. The importance of these findings for human tumor immunotherapy has recently been demonstrated in a pilot clinical trial (NCT02499835, manuscript in preparation). The goal of this trial was to determine if it was better to first elicit a PD-1-regulated T cell response with vaccination, and then block PD-1 (by using vaccine followed by PD-1 blockade), or to block PD-1 at the time T cells are activated with vaccination prior to them being able to be deregulated in the PD-L1-expressing tumor environment (by using vaccine delivered concurrently with PD-1 blockade). 26 patients with castration-resistant, metastatic prostate cancer were randomized to receive the pTVG-HP DNA vaccine, administered every 2 weeks x 6, followed by pembrolizumab administered every 3 weeks x 4 over 24 weeks, or to receive these agents concurrently on the same schedule over 12 weeks. As shown in Figure 1, PSA declines were observed almost exclusively in patients treated with the concurrent schedule. PSA declines were observed in approximately half of individuals treated with the concurrent schedule.

F. NaF PET/CT and Quantitative Total Bone Imaging (QTBI)

In the setting of PSA recurrence after definitive therapy, this stage D0/M0 being evaluated, it is assumed that patients have metastatic disease that is simply below the level of current methods of detection. We and others have demonstrated that NaF PET/CT provides a more sensitive method of detecting bone metastases in patients with more advanced prostate cancer {Morisson, 2013 #4088}, suggesting that this method may be more sensitive in detecting minimal residual disease in the setting of PSA recurrence. Moreover, NaF PET/CT can provide a more

quantitative assessment of total bone involvement, an assessment that cannot be determined from standard bone scintigraphy{Morisson, 2013 #4088}, when combined with advanced image analysis, such as in Quantitative Total Bone Imaging (QTBI), developed at University of Wisconsin Carbone Cancer Center. QTBI can be used to evaluate changes in total prostate cancer bone involvement over time, effectively a measure of tumor growth rate in bone. Our group explored the role of NaF PET/CT in a similar patient cohort in a subset of patients enrolled in a randomized phase II trial of pTVG-HP versus GM-CSF adjuvant in patients with non-metastatic prostate cancer (NCT01341652). Among 34 patients who had NaF PET/CT scans, only 4 of 34 did not have a lesion identified at baseline, and only 1 of 34 patients had no lesions at any time point. Although the median number of lesions detected and change in total activity (SUV_{total}) was not significantly different for the two treatment cohorts, the median SUV_{total} increased 50.4% in the GM-CSF arm and decreased 23.0% in the pTVG-HP arm [manuscript under preparation].

In this study we wish to further explore, whether these methods could be used to identify individuals at particularly high risk for early disease progression and to evaluate whether treatment with a DNA vaccine in combination with nivolumab can affect the tumor growth rate analogous to changes in PSA doubling time. Moreover, we wish to determine whether the pattern of disease progression (continued growth of all lesions, or rapid growth of individual lesions) differs among patients based on their response to treatment.

G. Summary

Given these findings, the current protocol aims to expand on these findings, and determine if the efficacy of anti-tumor vaccination can be improved by combination with PD-1 blockade in patients with early recurrent disease, specifically in a population with minimal residual disease that should theoretically be more amenable to anti-tumor vaccination. This trial will also evaluate whether GM-CSF is required as a vaccine adjuvant when using this combination, and gather safety and PSA response data using nivolumab alone for two cycles in patients with this earlier stage of disease. The hypothesis to be tested is that vaccination with PD-1 blockade will elicit a greater magnitude of tumor-specific CD8+ T cells, and this will result in an objective anti-tumor effect as measured by PSA declines, possibly PSA complete responses that are durable, and absence of metastatic progression at two years in patients who experience substantial ($\geq$ 50%) PSA declines. The ability to drive PSA to undetectable levels, possibly curing or significantly delaying the metastatic recurrence of prostate cancer prior to the need for androgen depriving therapies, would be a "game changing" advance in the therapy of this disease. This will be conducted as a pilot, multiple-institution safety and feasibility trial. Identification of PSA responses (defined as PSA decline $\geq$ 50%), and complete responses (PSA decline to < 0.2 ng/mL), and delays in the time to disease progression will serve as evidence of clinical activity be used for planning future confirmatory clinical trials.

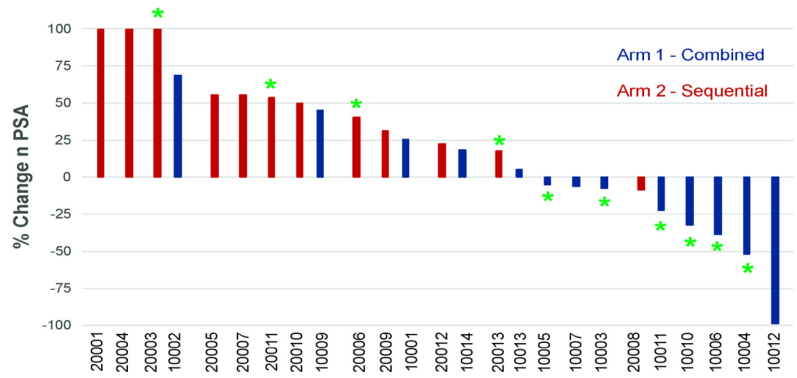


Figure 1: Changes in serum PSA – patients treated with pTVG-HP and pembrolizumab. 26 patients were randomized to treatment with pTVG-HP delivered 6 times over 12 weeks either concurrently with pembrolizumab (blue, delivered 4 times over 12 weeks), or sequentially (red, DNA vaccination over 12 weeks followed by pembrolizumab treatment over subsequent 12 weeks). Shown is the greatest change in serum PSA from baseline (p=0.013, Fisher's exact test, for any PSA decrease with respect to treatment arm). Green asterisks indicate those individuals that developed immune response to the PAP target antigen.

3. Objectives

A. Primary Objectives

1. To evaluate the safety and tolerability of pTVG-HP DNA vaccine and nivolumab in patients with clinical stage D0/M0 prostate cancer

2. To determine the PSA complete response rate (PSA < 0.2 ng/mL)

B. Secondary Objectives

1. To evaluate 2-year metastasis-free survival rate

2. To evaluate median radiographic progression-free survival

3. To evaluate changes in PSA doubling time or slope

4. To evaluate PSA response rate (≤ 50% of baseline)

5. To determine whether GM-CSF is required as a vaccine adjuvant for pTVG-HP vaccine when used in combination with nivolumab

Laboratory/Exploratory Biomarker Objectives:

1. To determine if antigen-specific T-cell and/or IgG responses are elicited with treatment

2. To determine if the development of PAP-specific T-cell immune responses are associated with PSA response (decline ≥ 50%)

3. To determine if antigen spread (the development of immune responses to other cancer-associated proteins) is associated with PSA response (decline ≥ 50%)

4. To determine if Quantitative Total Bone Imaging (QTBI) by NaF PET/CT can identify bone lesions in this patient population not detected by standard bone scintigraphy

5. To determine whether the presence of lesions detected by NaF PET/CT at baseline is associated with early disease progression (within 1 year) by standard radiographic imaging methods (CT and bone scintigraphy)

6. To determine if growth rates, and changes in growth rates, determined by NaF PET/CT are associated with PSA doubling time and changes in PSA doubling time

4. Vaccine and Nivolumab Preparation

A. Anti-PD-1 monoclonal antibody (Nivolumab, Opdivo®)

Nivolumab (Opdivo®, Bristol Myers Squibb) is a human programmed death receptor-1 (PD-1)-blocking antibody indicated for the treatment of patients with multiple different types of cancer. While this agent is commercially available, its use is investigational under this protocol. A fixed dose will be used based on the approved dosing of 240 mg IV every 2 weeks for several malignancies. This dose will be used for all treatments, even during the period of monthly administrations, given that nivolumab is being used in combination rather than as a single-agent therapy. Nivolumab is supplied in 100 mg (10 mL of a 10mg/mL solution in a single-dose vial) quantities. For each administration, three 100-mg vials will be used to achieve the 240 mg fixed dose.

The most common adverse reactions (reported in ≥ 20% of patients) included fatigue, cough, nausea, pruritis, rash, decreased appetite, constipation, arthralgia, and diarrhea. Immune-mediated adverse reactions have also been observed, including immune-mediated pneumonitis, colitis, hepatitis, hypophysitis, nephritis, hyperthyroidism and hypothyroidism. The management of suspected adverse reactions is discussed in Section 7 below.

B. Plasmid DNA vaccine

pTVG-HP pTVG4 vector containing cDNA for human PAP

The sequence of the pTVG-HP plasmid has been confirmed by standard DNA sequencing to confirm its identity. A bacterial strain was transformed with this plasmid and transferred to the National Gene Vector Laboratories (NGVL) manufacturing facility (Center for Biomedicine and Genetics, City of Hope/Beckman Research Institute, Duarte, CA) from which a master cell bank was prepared. This master cell bank was shipped in 2008 to the Waisman Clinical Biomanufacturing Facility (WCBF) at the University of Wisconsin. Several GMP-grade lots of

plasmid DNA have been prepared at the WCBF. The biological activity of each lot has been tested by production of PAP following transfection of reporter cells *in vitro.* Lots were also tested for appearance, plasmid homogeneity, DNA identity by restriction endonuclease evaluation, protein contamination, RNA contamination, genomic DNA contamination, sterility, endotoxin, and pH, and criteria for each of these have been established for future lot release. The vaccine will be supplied in single-use vials containing 0.6 mL 0.2 mg/mL pTVG-HP in phosphate-buffered saline. Vials will be stored at –80°C (or -20°C if further stability testing confirms that this is appropriate) until the day of use.

C. GM-CSF

GM-CSF (Leukine®, Sargramostim), will be obtained from Berlex Laboratories (Montville, NJ), or other commercial pharmaceutical vendors, and may be used as a vaccine adjuvant, provided without cost to subjects participating in this trial. GM-CSF, as described above, is a growth factor that supports the survival, clonal expansion and differentiation of hematopoietic progenitor cells including dendritic antigen presenting cells. In preliminary animal experiments and human clinical trials GM-CSF has been shown to be safe and serve as an effective adjuvant for the induction of antibody and T-cell responses to the immunized antigen [41, 64]. The use of GM-CSF is associated with little toxicity [42, 47, 65]. GM-CSF is a sterile, white, preservative-free, lyophilized powder supplied in 250 µg-dose vials.

Recombinant human GM-CSF (rhGM-CSF), when administered intravenously or subcutaneously is generally well tolerated at doses ranging from 50 to 500 µg/m^2/day. Severe toxic manifestations are extremely rare in patients treated with rhGM-CSF. Diarrhea, asthenia, rash and malaise were the only events observed in more than 5% of the rhGM-CSF group compared with the placebo group in phase III controlled studies of patients undergoing autologous bone marrow transplantation. In uncontrolled phase I/II studies for various indications, the adverse effects reported most frequently were fever, chills, nausea, vomiting, asthenia, headache and pain in the bones, chest, abdomen, joints, or muscles. Most of these systemic events reported with rhGM-CSF administration were mild to moderate in severity and rapidly reversed by the administration of analgesics or antipyretics. Other events reported infrequently were dyspnea, edema, local injection site reactions, and rash. Thrombosis and cardiac arrhythmia have also been reported, and there have been infrequent reports of tissue sloughing, leukemic progression, congestive heart failure, hepatomegaly, and intracranial bleeding; and isolated reports of Guillain-Barré syndrome and increased histiocytes. Neutropenia, although usually a pre-existing condition in the patients receiving rhGM-CSF, has been reported in association with administration. There was an increased frequency of severe thrombocytopenia in patients receiving concurrent chemotherapy and radiotherapy with GM-CSF. Eosinophilia and other blood abnormalities may occur.

There have been rare reports of 1) sequestration of granulocytes in the lungs with respiratory symptoms; 2) a syndrome characterized by respiratory distress, hypoxia, flushing, orthostatic hypotension, and partial loss of consciousness; 3) peripheral edema, pericardial or pleural effusions, and capillary leak of fluid; and 4) serious allergic or anaphylactic reactions.

Administration of rhGM-CSF may aggravate fluid retention in patients with pre-existent edema, capillary leak syndrome, or pleural or pericardial effusions. In some patients with pre-existing renal or hepatic dysfunction, elevation of the serum creatinine or bilirubin and hepatic enzymes

has occurred during the administration of rhGM-CSF. Dose reduction or interruption of rhGM-CSF administration has resulted in a decrease to pretreatment values. Occasional transient and reversible supraventricular arrhythmia has been reported in uncontrolled studies, particularly in patients with a previous history of cardiac arrhythmia.

Stimulation of marrow precursors with rhGM-CSF may result in a rapid rise in white blood cell count. Dosing should be stopped if the ANC exceeds 20,000/cm^3. rhGM-CSF may stimulate the growth of myeloid malignancies; therefore, caution must be exercised in its use in these malignancies or myelodysplastic syndromes.

Because GM-CSF will only be administered at most once every two weeks on the current protocol, most of these side effects are not anticipated. In previous vaccine studies using rhGM-CSF as an adjuvant in an identical fashion, local skin reactions consisting of erythema and induration have occurred in many patients. These resolved without need for treatment over a 2-3 week period for some, and over 2-3 days for most. Transient leukopenia (lasting only a couple of hours) has been observed in some patients, and resolved without treatment and without clinical sequelae [66]. Furthermore, the generation of a transient immune response to rhGM-CSF following its use as a vaccine adjuvant has been observed, however no clinical sequelae were noted, and the significance of this observation is not clear at present [65].

D. Vaccine preparation and administration

Vials will be thawed, and the plasmid DNA will be used by itself or will be used to reconstitute the GM-CSF, on the day of administration. Specifically:

For each of the immunizations: 0.25 mL of 0.2 mg/mL pTVG-HP will be drawn into each of two tuberculin syringes. This effectively provides a 100-µg dose of DNA. If GM-CSF is used as an adjuvant, 0.6 mL of 0.2 mg/mL pTVG-HP will be withdrawn and used to reconstitute 250 µg GM-CSF. 0.25 mL will then be drawn into each of two tuberculin syringes. This effectively provides a 100-µg dose of DNA and 208 µg GM-CSF.

The vaccine with or without GM-CSF will then be administered intradermally on the deltoid area of the lateral aspect of the arm (left arm, preferentially) in two adjacent sites, with the total syringe contents (0.25 mL) administered at each site.

5. Patient Selection

A. Inclusion Criteria

1. Patients must be at least 18 years of age with a histologic diagnosis of adenocarcinoma of the prostate
2. Patients must have undergone radical prostatectomy
3. Patients must have completed local therapy by surgery and any adjuvant/salvage radiation therapy at least 3 months prior to entry, with removal or ablation of all visible disease, including seminal vesical and/or local lymph node involvement.

4. Patients must have biochemically recurrent, non-metastatic (by CT and bone scan) clinical stage D0/M0 disease defined by the following:
   - Patients must have evidence of detectable serum PSA with at least 4 serum PSA measurements available, from the same clinical laboratory, at least two weeks apart up to one year, and the final serum PSA value must be $\geq$ 2.0 ng/mL.
   - PSA doubling time, calculated from most recent 4 serum PSA values (collected up to one year prior to enrollment, at least 2 weeks apart, and all from the same clinical laboratory), must be a positive number (i.e. evidence of PSA rise over time).
   - PSA doubling time will be calculated using the Memorial Sloan-Kettering Cancer Center nomogram: (http://www.mskcc.org/applications/nomograms/prostate/PsaDoublingTime.aspx).
   - Patients must not have definitive evidence of metastases as determined by CT of the abdomen/pelvis and bone scintigraphy (bone scan). Note: patients with lesions detectable by highly sensitive methods (e.g. NaF PET imaging or PSMA PET imaging) will be considered eligible as long as these lesions do not meet size criteria on CT imaging (visceral lesions suspicious for metastases or lymph node > 15 mm in short axis) and/or are not independently observed on bone scan.
5. Patients with a prior history of a second malignancy are eligible provided they have been treated with curative intent and have been free of disease greater than three years. There will be no exclusion for patients with a history of basal cell carcinoma, squamous cell skin cancer, superficial bladder cancer, or other *in situ* carcinoma that has been adequately treated.
6. Patients who are sexually active must use a reliable form of contraception while on study and for 4 weeks after the last immunization.
7. ECOG performance score < 2 and life expectancy of at least 12 months.
8. Patients must have normal hematologic, renal and liver function as defined by: WBC $\geq$ 3000/mm^3, hematocrit $\geq$ 30%, platelet count $\geq$ 100,000/mm^3, serum creatinine $\leq$ 1.5 mg/dl or a calculated creatinine clearance $\geq$ 60 cc/min, AST or ALT < 3.0x ULN, and serum bilirubin $\leq$ 2.0 mg/dl (except participants with Gilbert Syndrome, who can have total bilirubin < 3.0 mg/dL), within 4 weeks prior to first immunization.
9. Patients must be informed of the experimental nature of the study and its potential risks and must sign an IRB-approved written informed consent form indicating such an understanding.
10. Willingness to provide blood samples for immune studies, per study calendar, up to one year after study, even if off study treatment.

B. Exclusion Criteria

1. Small cell or other variant prostate cancer histology
2. Patients cannot have evidence of immunosuppression or have been treated with immunosuppressive therapy, such as chemotherapy or chronic treatment dose corticosteroids (greater than the equivalent of 10 mg prednisone per day), within 3 months of the first vaccination.

3. Seropositive for HIV, hepatitis B (HBV) or hepatitis C (HCV) per patient history due to the immunosuppressive features of these diseases.
4. Prior treatment with an LHRH agonist or nonsteroidal antiandrogen, except in the following circumstances: Neoadjuvant/adjuvant androgen deprivation therapy administered with radiation therapy or at the time of prostatectomy is acceptable, provided that there was no evidence of PSA progression while on treatment. In this situation, patients must not have received more than 24 months of androgen deprivation treatment. Other treatment with androgen deprivation therapy is prohibited.
5. Serum testosterone at screening < 50 ng/dL.
6. Patients must not be concurrently taking other medications or supplements with known hormonal effects, including PC-SPES, megestrol acetate, finasteride, ketoconazole, estradiol, or Saw Palmetto. All other medications with possible anti-cancer effects must be discussed with the PI prior to study entry.
7. Patients previously treated with herbal supplements as described in 5.B.6 or other potential or experimental therapies for prostate cancer must have discontinued these treatments and completed at least a 4 week washout prior to beginning treatment.
8. Patients must not have evidence of bone metastases or lymph node involvement as determined by bone scan or CT scan of the abdomen and pelvis within 4 weeks of study registration. Note: Advanced imaging modalities (such as NaF-PET/CT, choline PET/CT, fluviclovine, or PSMA PET scans) will NOT be used to determine evidence of metastases for eligibility purposes or for defining disease progression.
9. Patients must not have been treated with a prior DNA vaccine therapy for prostate cancer.
10. Patients must not have known psychological or sociological conditions, addictive disorders or family problems, which would preclude compliance with the protocol.
11. Patients must not have known allergic reactions to GM-CSF.
12. Patients with unstable or severe intercurrent medical conditions or laboratory abnormalities that would impart, in the judgment of the PI, excess risk associated with study participation or study agent administration.
13. Patients cannot have concurrent enrollment on other phase I, II, or III investigational therapeutic treatment studies.

6. Experimental Design

This will be a single-arm, multiple-institution, two-stage phase II trial designed to evaluate the safety and effect of serial intradermal vaccinations of a DNA vaccine encoding PAP with or without rhGM-CSF, and co-administered with nivolumab, on serum PSA in patients with PSA-recurrent, non-metastatic, non-castrate prostate cancer. A total of 20-41 patients will be accrued, treated over a maximum of one year each, and followed for up to two years for evidence of radiographic progression. All subjects will be followed an additional 2 years after their last treatment for evidence of delayed adverse events. The treatment will be as follows:

Nivolumab 240 mg IV every two weeks x 6 beginning day 1, then every four weeks x 9 beginning week 12
pTVG-HP (100 µg) administered intradermally (i.d.) every two weeks x 6 beginning day 1, then every four weeks x 9 beginning week 12
rhGM-CSF (208 µg) administered intradermally (i.d.) every two weeks x 4 beginning week 4, then every four weeks x 9 beginning week 12 NOTE: Only administered to patients for whom serum PSA obtained week 4 > serum PSA obtained at day 1.

A. Study Arm Assignment and Toxicity Assessment

Patients with non-metastatic prostate cancer, with evidence of rising PSA, and a calculable PSA doubling time with at least 4 serum PSA values collected over a one-year period of time, will be invited to participate. Given the absence of significant adverse events observed in a previous phase I study, no adverse events > grade 2 are anticipated attributable to vaccine alone. Similarly, given the absence of grade 4 events observed among 26 patients with advanced castration-resistant metastatic prostate cancer treated with this vaccine in combination with pembrolizumab, we do not anticipate toxicity that has not been observed with nivolumab alone. While not common, previous immune-associated toxicities have been observed with nivolumab, including immune-mediated pneumonitis, colitis, hepatitis, hypophysitis, nephritis, hyperthyroidism, and hypothyroidism. Hence these may be anticipated events. All study patients will be evaluated for evidence of toxicity over time, and further accrual to the trial will halt and be reviewed by the UWCCC DSMC if the toxicity rate is deemed to be excessive. A toxicity rate of 30% for Grade 3 events or 10% Grade 4 events given an attribution of at least possibly related to study treatment will be considered excessive. Grade 3 limited site reactions (lasting < 48 hours) and fevers/chills (lasting < 48 hours) will be excluded from this assessment as expected possible adverse events of limited duration.

Continuous toxicity monitoring based on repeated significance testing with a Pocock boundary will be applied after the first 5 patients have been accrued and evaluated for toxicity. A toxicity rate p0 of at most 15% of Grade ≥ 3 toxicity events will be considered as acceptable while a toxicity rate p1=35% or more will be considered as unacceptably high. Assuming a boundary probability of 0.1 under p0, the stopping boundaries of the repeated significance testing procedure within an arm are as follows: 2/5, 3/9, 4/12, 5/17, 6/21, 7/26, 8/31, 9/35, or 10/40. If the number of Grade ≥ 3 toxicities exceeds the boundaries, accrual will be temporarily suspended and the study will be reviewed for safety, dose modification and safety modification, and the study will be reviewed for safety by the UW DSMC (data and safety monitoring committee). Once the study is suspended, it would only be reopened if modifications could be made, and approved by the DSMC and the UW-IRB, to assure that patient safety is reestablished. If not, the study would be closed.

B. Endpoints

The primary endpoints for the trial will be safety, tolerability, and PSA complete response (PSA CR) rate. A PSA CR will be defined as a serum PSA <0.2 ng/mL, with a confirmatory PSA <0.2 ng/mL at least 4 weeks later, as per PCWG2 recommendations [67]. To qualify as a PSA CR, there must be no evidence of radiographic progression. Patients will come off study at the time of appearance of metastatic disease (radiographic progression), at the time of undue toxicity (as defined below), or at the discretion of the patient and treating physician that other therapies for

prostate cancer are warranted. Patients should be discouraged from discontinuing protocol treatment for PSA rise only. Patients will continue to have blood drawn for immune studies, as per study calendar, up to one year after screening, even if off study prior to that time.

All subjects will undergo radiographic imaging (CT of abdomen and pelvis and bone scan) prior to treatment, and at 6-month intervals (or as clinically indicated). The appearance of lesions consistent with metastatic disease will be used to define radiographic progression, and from these the 2-year metastatic progression-free survival will be calculated for each treatment arm, as well as the median progression-free survival. NOTE: Lesions detected by ^{18}F-NaF-PET/CT or other advanced imaging (such as NaF-PET/CT, choline PET/CT, fluviclovine, or PSMA PET scans) only will not be used to define metastatic disease or disease progression. MRI imaging may substitute for CT, but the same type of imaging must be used to determine eligibility and progression. RECIST 1.1 criteria will be used to define metastatic disease (appearance of a visceral lesion > 10 mm by CT or MRI, lymph node > 15 mm in short axis, or appearance of new lesions on bone scan consistent with metastatic disease). The 2-year metastasis-free survival will be determined by investigator review of radiographic studies (CT/MRI and bone scintigraphy) performed 2 years after study initiation in subjects who have not already met criteria for radiographic progression.

PSA doubling times will be calculated from four available serum PSA values obtained up to one year from the same clinical laboratory prior to study enrollment, up to and including the baseline value, and used to determine a pre-treatment PSA doubling time (PSADT). Similarly, a post-treatment doubling time will be calculated from:

(1) All PSA values obtained from the same clinical laboratory using the same PSA assay beginning with the PSA value at day 1 (week 0) and continuing until the end-of study (or month 24) value ($PSADT_{0-24}$).

(2) All PSA values obtained from the same clinical laboratory using the same PSA assay beginning with the PSA value at day 85 (month 3) to the day 253 (month 9) value ($PSADT_{3-9}$).

These time points for PSADT assessment were chosen to correspond to those being obtained in a randomized phase 2 trial using the pTVG-HP vaccine alone (NCT01341652).

7. Definition and Management of Limiting Toxicities and Adverse Events

A treatment-limiting toxicity will be defined as any Grade 3 or greater toxicity (using the NCI Common Terminology Criteria version 4), or a specific Grade 2 event as described below that affects agent dosing, with an attribution of at least possibly related to the study or treatment procedures, and occurring between the pre-study visit and within one month of the final study vaccine treatment or within 90 days of the last treatment with nivolumab (whichever is later). If a patient develops Grade 3 toxicity with an attribution of at least possibly related to nivolumab and unlikely related (or unrelated) to pTVG-HP, the nivolumab treatment will be held until the toxicity resolves to Grade 1 or less, with the vaccine schedule continuing as per protocol. In general, if a patient develops a second Grade 3 event (or any Grade 4 event) believed to be at least possibly

due to nivolumab, but unlikely related (or unrelated) to pTVG-HP vaccine, no further nivolumab treatments will be given. However, Grade 3 or 4 events that would not limit continued treatment or intervention (e.g. lymphopenia or asymptomatic elevations of amylase or lipase) will not require holding the dosing or discontinuation of nivolumab. The specific management for nivolumab-associated adverse events (and the indications for delaying or discontinuing nivolumab treatment) are shown in the table below. In the absence of adverse events attributed to DNA vaccine, the patient will continue with DNA vaccine administration only as per protocol. However, if a patient develops a Grade 3 toxicity with an attribution of at least possibly related to pTVG-HP vaccine, both vaccine and nivolumab treatment will be held until the toxicity resolves to Grade 1 or less. If a patient develops a second Grade 3 event (or any Grade 4 event) believed at least possibly related to pTVG-HP vaccine, no further treatments (nivolumab or vaccine) will be given. The patient will remain on study, however, with collection of radiographic and laboratory data as per protocol. The rationale for these modifications is that patients could continue to benefit in terms of anti-tumor response and this could in fact be related to the development of other autoimmune toxicities, and patients who have toxicity from nivolumab could still potentially benefit from continued DNA vaccination. While rare overall, autoimmune events observed following nivolumab treatment of patients with melanoma typically resolved and did not necessarily lead to discontinuation of treatment.

AEs associated with nivolumab exposure may represent an immunologic etiology. These immune-related AEs (irAEs) may occur shortly after the first dose or several months after the last dose of nivolumab treatment and may affect more than on body system simultaneously. Therefore, early recognition and initiation of treatment is critical to reduce complications. Based on existing clinical study data, most irAEs were reversible and could be managed with interruptions of nivolumab, administration of corticosteroids and/or other supportive care. For suspected irAEs, ensure adequate evaluation to confirm etiology or exclude other causes. Additional procedures or tests such as bronchoscopy, endoscopy, skin biopsy may be included as part of the evaluation. Based on the severity of irAEs, it may be necessary to withhold or permanently discontinue nivolumab and administer corticosteroids. In general, if IV corticosteroids are used, these can be switched to oral dose equivalent (e.g. prednisone), and then tapered, once sustained clinical improvement is observed. Dose modification and toxicity management guidelines for irAEs associated with nivolumab are provided in the following Table:

Dose modification and toxicity management guidelines for immune-related AEs associated with nivolumab

Immune-related AEs	Toxicity grade or conditions (CTCAEv4.0)	Management	Monitor and follow-up
Diarrhea / Colitis	Grade 1 (<4 stools per day over baseline; asymptomatic colitis)	Continue per protocol Symptomatic treatment	Close monitoring for worsening symptoms Educate patient to report worsening immediately If worsens, treat as grade 2 or 3/4
	Grade 2 (4-6 stools per day over baseline; IV fluids required < 24 hours; not interfering with ADL. Colitis:	Delay nivolumab per protocol Symptomatic treatment	If improves to grade 1, resume nivolumab per protocol If persists > 5-7 days or recurs: 0.5-1.0 mg/kg/day methylprednisolone or oral equivalent

	abdominal pain, blood in stool		When resolves to grade 1, taper steroids over at least one month, consider prophylactic antibiotics, and resume nivolumab If worsens or persists >3-5 days with oral steroids, treat as grade 3/4
	Grade 3-4 (≥ 7 stools per day over baseline; IV fluids ≥ 24 hours; interfering with ADL. Colitis: severe abdominal pain, medical intervention required, peritoneal signs. Grade 4: life threatening, or perforation	Discontinue nivolumab 1.0 to 2.0 mg/kg/day methylprednisolone or IV other corticosteroid equivalent Add prophylactic antibiotics for opportunistic infections Consider lower endoscopy	If improves, continue steroids to grade 1, then taper over at least one month If persists > 3-5 days, or recurs after improvement: Add infliximab 5 mg/kg (if no contraindication). Note: infliximab should not be used if perforation or sepsis
Renal	Grade 1 (creatinine > ULN and > baseline, but ≤ 1.5x baseline	Continue as per protocol Monitor creatinine weekly	If returns to baseline, monitor creatinine as per protocol If worsens, treat as grade 2 or ¾
	Grade 2-3 (creatinine >1.5x baseline and ≤ 6x ULN	Delay nivolumab Monitor creatinine daily 0.5 to 1.0 mg/kg/day methylprednisolone or oral equivalent Consider bx or renal consult	If returns to grade 1: Taper steroids over at least 1 month, consider prophylactic antibiotics, resume nivolumab and creatinine monitoring as per protocol If elevation persists > 7 days: Treat as grade 4
	Grade 4 (creatinine > 6x ULN)	Discontinue nivolumab Monitor creatinine daily 1.0 to 2.0 mg/kg/day methylprednisolone or IV other corticosteroid equivalent Consult nephrologist, consider biopsy	If returns to grade 1: Taper steroids over at least 1 month, and add prophylactic antibiotics for opportunistic infections
Pneumonitis	Grade 1 (radiographic changes only)	Consider delay of nivolumab Monitor symptoms every 2-3 days Consider pulmonary and ID consults	Reimage at least every 3 weeks If worsens, treat as grade 2 or 3/4
	Grade 2 (mild to moderate new symptoms)	Delay nivolumab Pulmonary and ID consults	Re-image every 1-3 days If improves:

		Monitor symptoms daily, possible hospitalization 1.0 mg/kg/day methylprednisolone or oral equivalent Consider bronchoscopy, lung biopsy	When symptoms improve to near baseline, taper steroids over at least 1 month, and then resume nivolumab as per protocol. Consider prophylactic antibiotics If not improved after 2 weeks or worsening: Treat as grade 3/4
	Grade 3/4 (severe new symptoms, new/worsening hypoxia; life threatening)	Discontinue nivolumab Hospitalize Pulmonary and ID consults 2-4 mg/kg/day methylprednisolone or IV equivalent Add prophylactic antibiotics for opportunistic infections Consider bronchoscopy, lung biopsy	If improves to baseline: Taper steroids over at least 6 weeks If not improving after 48 hours or worsening: Add additional immunosuppression
Hepatic / Liver Function Test Elevation	Grade 1 (AST or ALT > ULN to 3x ULN and/or total bili > ULN to 1.5x ULN)	Continue nivolumab as per protocol	Continue LFT monitoring as per protocol If worsens, treat as grade 2 or 3/4
	Grade 2 (AST or ALT >3.0 to ≤ 5x ULN and/or total bili > 1.5 to ≤ 3x ULN)	Delay nivolumab Increase monitoring LFTs to every 3 days	If returns to baseline: Resume routine monitoring and resume nivolumab If elevation persist > 5-7 days or worsens: 0.5 – 1 mg/kg/day methylprednisolone or oral equivalent and when LFT returns to grade 1 or baseline, taper steroids over at least 1 month, consider prophylactic antibiotics, and resume nivolumab as per protocol
	Grade 3/4 (AST or ALT > 5x ULN or total bili > 3x ULN	Discontinue nivolumab NOTE: May be delayed rather than discontinued if AST/ALT ≤ 8xULN or total bili ≤ 5x ULN Increase LFT monitoring to every 1-2 days 1.0 to 2.0 mg/kg/day methylprednisolone or IV equivalent NOTE: 2 mg/kg/day recommended for grade 4 Add prophylactic antibiotics for opportunistic infections Consult gastroenterologist	If returns to grade 2: Taper steroids over at least 1 month If does not improve in >3-5 days, worsens, or rebounds: Add mycophenolate mofetil 1 g bid If no response within an additional 3-5 days, consider other immunosuppressants

Endocrino-pathies	Asymptomatic TSH elevation	Continue nivolumab as per protocol	If TSH < 0.5 x LLN or > 2x ULN, or consistently out of range in 2 measurements, include free T4 at subsequent cycles and treat as clinically indicated. Consider endocrinology consult
	Symptomatic endocrinopathy	Evaluate endocrine function Consider pituitary scan (MRI) Grade 3 drug-related endocrinopathies, adequately controlled with only physiologic hormone replacement do not require discontinuation. Adrenal insufficiency requires discontinuation regardless of control with hormone replacement. Grade 4 drug-related endocrinopathy AEs, such as, hyper- or hypothyroidism, or glucose intolerance, that resolve or are adequately controlled with physiologic hormone replacement (corticosteroids, thyroid hormones) or glucose-controlling agents, respectively, may not require discontinuation. Grade 4 drug-related adrenal insufficiency or hypophysitis requires discontinuation regardless of control with hormone replacement.	
	Suspicion of adrenal crisis (e.g. severe dehydration, hypotension, shock out of proportion to current illness)	Discontinue nivolumab for any drug-related grade 3 or 4 adrenal insufficiency Rule out sepsis Stress dose of IV steroids with mineralocorticoid activity IV fluids Consult endocrinologist	
Dermatolo-gic (Skin rash)	Grade 1-2 (covering $\leq$ 30% BSA)	Continue nivolumab Symptomatic therapy (e.g. antihistamines, topical steroids)	If persists >1-2 weeks or recurs: Consider skin biopsy Delay nivolumab Consider 0.5-1.0 mg/kg/day methylprednisolone or oral equivalent. Once improving, taper steroids over at least 1 month, consider prophylactic antibiotics, and resume nivolumab as per protocol If worsens: Treat as grade 3/4
	Grade 3-4 (covering >30% BSA or with life	Delay or discontinue nivolumab Consider skin biopsy Consult dermatology	If improves to grade 1: Taper steroids over at least 1 month and add prophylactic antibiotics

	threatening consequences)	1.0-2.0 mg/kg/day methylprednisolone or IV equivalent	Resume nivolumab as per protocol NOTE: If SJS/TEN is diagnosed, permanently discontinue nivolumab
Neurologic	Grade 1 (asymptomatic or mild symptoms, intervention not indicated)	Continue nivolumab as per protocol	Continue monitoring. If worsens, treat as grade 2 or 3/4
	Grade 2 (moderate symptoms, limiting instrumental ADL)	Delay nivolumab as per protocol Treat symptoms as clinically indicated Consider 0.5 to 1.0 mg/kg/day methylprednisolone or oral equivalent	If improves to baseline: Resume nivolumab as per protocol If worsens: Treat as grade 3/4
	Grade 3/4 (severe symptoms; limiting self-care ADL; life threatening)	Discontinue nivolumab Neurology consult Treat symptoms as clinically indicated 1.0 – 2.0 mg/kg/day methylprednisolone or IV equivalent Add prophylactic antibiotics	If improves to grade 2: Taper steroids over at least 1 month If worsens or atypical presentation: Consider IVIG or other immunosuppressive therapies as clinically indicated
All other immune-related AEs	Grade 3, or intolerable/ persistent Grade 2	Delay nivolumab as per protocol Consider corticosteroid therapy	Ensure adequate evaluation to confirm etiology or exclude other causes; Resume nivolumab as per protocol if symptoms resolve
	Grade 4 or recurrent Grade 3	Discontinue nivolumab 1.0 to 2.0 mg/kg/day methylprednisolone or IV equivalent Add prophylactic antibiotics	Taper steroids over at least 1 month after improvement in symptoms

Any infusional drug, including nivolumab, may cause severe or life threatening infusion-reactions including severe hypersensitivity or anaphylaxis. Signs and symptoms usually develop during or shortly after drug infusion and generally resolve completely within 24 hours of completion of infusion. Dose modification and toxicity management guidelines for infusion reactions are provided in the Table below:

NCI CTCAE Grade	Treatment	Premedication at Subsequent Dosing
Grade 1 Mild reaction; infusion interruption not indicated; intervention not indicated	Increase monitoring of vital signs as medically indicated until the participant is deemed medically stable in the opinion of the investigator.	None
Grade 2 Requires therapy or infusion interruption but responds promptly to symptomatic treatment (e.g., antihistamines, NSAIDs, narcotics, IV fluids); prophylactic medications indicated for ≤24 hrs	**Stop Infusion.** Additional appropriate medical therapy may include but is not limited to: IV fluids Antihistamines NSAIDs Acetaminophen Narcotics Increase monitoring of vital signs as medically indicated until the participant is deemed medically stable in the opinion of the investigator. If symptoms resolve within 1 hour of stopping drug infusion, the infusion may be restarted at 50% of the original infusion rate (e.g. from 100 mL/hr to 50 mL/hr). Otherwise dosing will be held until symptoms resolve and the participant should be premedicated for the next scheduled dose. **Participants who develop Grade 2 toxicity despite adequate premedication should be permanently discontinued from further nivolumab treatment**	Participant may be premedicated 1.5h (± 30 minutes) prior to infusion of nivolumab with: Diphenhydramine 50 mg po (or equivalent dose of antihistamine). Acetaminophen 500-1000 mg po (or equivalent dose of analgesic).
Grades 3 or 4 Grade 3: Prolonged (i.e., not rapidly responsive to symptomatic medication and/or brief interruption of infusion); recurrence of symptoms following initial improvement; hospitalization indicated for other clinical sequelae (e.g., renal impairment, pulmonary infiltrates) Grade 4: Life-threatening; pressor or ventilatory support indicated	**Stop Infusion.** Additional appropriate medical therapy may include but is not limited to: Epinephrine** IV fluids Antihistamines NSAIDs Acetaminophen Narcotics Oxygen Pressors Corticosteroids Increase monitoring of vital signs as medically indicated until the participant is deemed medically stable in the opinion of the investigator. Hospitalization may be indicated. **In cases of anaphylaxis, epinephrine should be used immediately. **Participant is permanently discontinued from further nivolumab treatment.**	No subsequent dosing
Appropriate resuscitation equipment should be available at the bedside and a physician readily available during the period of drug administration. For further information, please refer to the Common Terminology Criteria for Adverse Events v4.0 (CTCAE) at http://ctep.cancer.gov		

Nivolumab may be interrupted for situations other than treatment-related AEs such as medical / surgical events or logistical reasons not related to study therapy. Participants should be placed back on study therapy within 3 weeks of the scheduled interruption, unless otherwise discussed with the Sponsor. The reason for interruption should be documented in the patient's study record.

8. Plan of Treatment

The following section describes the schedule for prescreening, treatment, and clinical and laboratory evaluations. When a chemistry panel is indicated the following tests are performed: Chem 7 (including sodium, potassium, bicarbonate, BUN, creatinine, and glucose), ALT, AST, total bilirubin, alkaline phosphatase, amylase, thyroid stimulating hormone (TSH) and LDH. Whenever a CBC is indicated, this will include differential and platelet count. This plan is summarized in the Schema.

A. Prescreen (performed within 4 weeks of study registration unless otherwise noted)

1. Confirm potential eligibility by history, pathology, diagnosis, and serial serum PSA levels; no exclusions by history
2. CT scan of abdomen and pelvis, bone scan
3. Sign consent form (to be done within 4 months prior to study registration)

B. Screening Evaluation (performed within 2 weeks of study Day 1; can coincide with prescreen evaluation)

1. Physical examination, including vital signs, symptoms assessment, and ECOG performance score
2. Evaluation of blood cell counts (CBC with platelets), chemistry panel, serum prostate specific antigen (PSA), serum PAP, serum testosterone.
3. 20 ml peripheral blood (red-top tubes) for sera to evaluate baseline antibody responses, and 200 ml peripheral blood (green-top heparinized tubes) for T-cell response evaluation.
4. NaF PET/CT (scan within +/- 7 days of study visit, exceptions may be allowed with prior approval from the study PI.). NOTE: This PET scan is for research purposes only, will not be used to determine eligibility, and will not be used for clinical response evaluation or treatment decisions. NaF PET/CT should NOT be obtained within 2 days of GM-CSF injection.

C. First treatment visit (day 1)

1. Physical examination, including vital signs, symptoms assessment, and ECOG performance score (not required if already obtained within 14 days)
2. Blood draw CBC, chemistry panel, PSA and PAP. May be done up to 7 days in advance of Day 1, and could coincide with screening evaluation.
3. pTVG-HP immunization intradermally on the deltoid area of the lateral arm (preferentially left arm) with 0.25 mL administered at each of two adjacent sites
4. Following vaccination, subjects will be observed for 60 min. for adverse events

5. Nivolumab 240 mg, administered intravenously over 30 minutes

D. Second treatment visit (week 2, 14 +/- 3 days after day 1 visit)

1. Symptoms assessment
2. pTVG-HP immunization intradermally on the deltoid area of the lateral arm (preferentially left arm) with 0.25 mL administered at each of two adjacent sites
3. Following vaccination, subjects will be observed for 60 min. for adverse events
4. Nivolumab 240 mg, administered intravenously over 30 minutes

E. Third treatment visit (week 4, 14 +/- 3 days after week 2 visit)

1. Physical examination, including vital signs, symptoms assessment, and ECOG performance score
2. Blood draw CBC, chemistry panel, PSA and PAP. NOTE: for this visit the PSA can be obtained up to 7 days prior to the visit in order to have the results available to determine whether rhGM-CSF will be co-administered.
3. 10 ml peripheral blood (red-top tube) for sera to evaluate baseline antibody responses, and 100 ml peripheral blood (green-top heparinized tubes) for T-cell response evaluation
4. pTVG-HP immunization intradermally on the deltoid area of the lateral arm (preferentially left arm) with 0.25 mL administered at each of two adjacent sites
5. NOTE: If serum PSA obtained at this visit is higher than that obtained at day 1 visit, then rhGM-CSF (208 μg) will be co-administered with the pTVG-HP immunization as adjuvant for this and all subsequent immunizations
6. Following vaccination, subjects will be observed for 60 min. for adverse events
7. Nivolumab 240 mg, administered intravenously over 30 minutes

F. Fourth treatment visit (week 6, 14 +/- 3 days after week 4 visit)

1. Symptoms assessment
2. pTVG-HP immunization intradermally on the deltoid area of the lateral arm (preferentially left arm) with 0.25 mL administered at each of two adjacent sites: NOTE: If serum PSA obtained at week 4 was higher than that obtained at day 1 visit, then rhGM-CSF (208 μg) will be co-administered with the pTVG-HP immunization as adjuvant for this and all subsequent immunizations
3. Following vaccination, subjects will be observed for 60 min. for adverse events
4. Nivolumab 240 mg, administered intravenously over 30 minutes

G. Fifth treatment visit (week 8, 14 +/- 3 days after week 6 visit)

1. Physical examination, including vital signs, symptoms assessment, and ECOG performance score
2. Blood draw CBC, chemistry panel, PSA and PAP.
3. pTVG-HP immunization intradermally on the deltoid area of the lateral arm (preferentially left arm) with 0.25 mL administered at each of two adjacent sites NOTE: If serum PSA obtained at week 4 was higher than that obtained at day 1

visit, then rhGM-CSF (208 μg) will be co-administered with the pTVG-HP immunization as adjuvant for this and all subsequent immunizations
4. Following vaccination, subjects will be observed for 60 min. for adverse events
5. Nivolumab 240 mg, administered intravenously over 30 minutes

H. Sixth treatment visit (week 10, 14 +/- 3 days after week 8 visit)

1. Symptoms assessment
2. pTVG-HP (+/- GM-CSF adjuvant) immunization intradermally on the deltoid area of the lateral arm (preferentially left arm) with 0.25 mL administered at each of two adjacent sites NOTE: If serum PSA obtained at week 4 was higher than that obtained at day 1 visit, then rhGM-CSF (208 μg) will be co-administered with the pTVG-HP immunization as adjuvant for this and all subsequent immunizations
3. Following vaccination, subjects will be observed for 60 min. for adverse events
4. Nivolumab 240 mg, administered intravenously over 30 minutes

I. Seventh treatment visit (week 12, 14 +/- 3 days after week 10 visit)

1. Physical examination, including vital signs, symptoms assessment, and ECOG performance score
2. Blood draw CBC, chemistry panel, PSA and PAP.
3. 10 ml peripheral blood (red-top tube) for sera to evaluate baseline antibody responses, and 100 ml peripheral blood (green-top heparinized tubes) for T-cell response evaluation
4. pTVG-HP (+/- GM-CSF adjuvant) immunization intradermally on the deltoid area of the lateral arm (preferentially left arm) with 0.25 mL administered at each of two adjacent sites NOTE: If serum PSA obtained at week 4 was higher than that obtained at day 1 visit, then rhGM-CSF (208 μg) will be co-administered with the pTVG-HP immunization as adjuvant for this and all subsequent immunizations
5. Following vaccination, subjects will be observed for 60 min. for adverse events
6. Nivolumab 240 mg, administered intravenously over 30 minutes
7. NaF PET/CT (NaF PET/CT within +/- 7 days of week 12 study visit, exceptions may be allowed with prior approval from the study PI). NOTE: This PET scan is for research purposes only and will not be used for clinical response evaluation or treatment decisions. This study will be performed only at designated sites. NaF PET/CT should NOT be obtained within 2 days of GM-CSF injection.

J. Eighth treatment visit (week 16, 28 +/- 3 days after week 12 visit)
Ninth treatment visit (week 20, 28 +/- 3 days after week 16 visit)

1. Physical examination, including vital signs, symptoms assessment, and ECOG performance score
2. Blood draw CBC, chemistry panel, PSA and PAP.
3. pTVG-HP (+/- GM-CSF adjuvant) immunization intradermally on the deltoid area of the lateral arm (preferentially left arm) with 0.25 mL administered at each of two adjacent sites NOTE: If serum PSA obtained at week 4 was higher than that

obtained at day 1 visit, then rhGM-CSF (208 µg) will be co-administered with the pTVG-HP immunization as adjuvant for this and all subsequent immunizations
4. Following vaccination, subjects will be observed for 60 min. for adverse events
5. Nivolumab 240 mg, administered intravenously over 30 minutes

K. Tenth treatment visit (week 24, 28 +/- 3 days after week 20 visit)

1. Physical examination, including vital signs, symptoms assessment, and ECOG performance score
2. Blood draw CBC, chemistry panel, PSA and PAP (can be performed +/- 7 days of this visit)
3. CT scan of abdomen and pelvis, and bone scan (can be performed +/- 7 days of this visit)
4. 10 ml peripheral blood (red-top tube) for sera to evaluate baseline antibody responses, and 100 ml peripheral blood (green-top heparinized tubes) for T-cell response evaluation (can be performed +/- 7 days of this visit)
5. pTVG-HP (+/- GM-CSF adjuvant) immunization intradermally on the deltoid area of the lateral arm (preferentially left arm) with 0.25 mL administered at each of two adjacent sites NOTE: If serum PSA obtained at week 4 was higher than that obtained at day 1 visit, then rhGM-CSF (208 µg) will be co-administered with the pTVG-HP immunization as adjuvant for this and all subsequent immunizations
6. Following vaccination, subjects will be observed for 60 min. for adverse events
7. Nivolumab 240 mg, administered intravenously over 30 minutes
8. NaF PET/CT (NaF PET/CT within +/- 7 days of week 24 study visit, exceptions may be allowed with prior approval from the study PI.). NOTE: This PET scan is for research purposes only and will not be used for clinical response evaluation or treatment decisions. This study will be performed only at designated sites. NaF PET/CT should NOT be obtained within 2 days of GM-CSF injection.

L. Eleventh treatment visit (week 28, 28 +/- 3 days after week 24 visit)
Twelfth treatment visit (week 32, 28 +/- 3 days after week 28 visit)

1. Physical examination, including vital signs, symptoms assessment, and ECOG performance score
2. Blood draw CBC, chemistry panel, PSA and PAP.
3. pTVG-HP (+/- GM-CSF adjuvant) immunization intradermally on the deltoid area of the lateral arm (preferentially left arm) with 0.25 mL administered at each of two adjacent sites NOTE: If serum PSA obtained at week 4 was higher than that obtained at day 1 visit, then rhGM-CSF (208 µg) will be co-administered with the pTVG-HP immunization as adjuvant for this and all subsequent immunizations
4. Following vaccination, subjects will be observed for 60 min. for adverse events
5. Nivolumab 240 mg, administered intravenously over 30 minute

M. Thirteenth treatment visit (week 36, 28 +/- 3 days after week 32 visit)

1. Physical examination, including vital signs, symptoms assessment, and ECOG performance score
2. Blood draw CBC, chemistry panel, PSA and PAP.
3. 10 ml peripheral blood (red-top tube) for sera to evaluate baseline antibody responses, and 100 ml peripheral blood (green-top heparinized tubes) for T-cell response evaluation
4. pTVG-HP (+/- GM-CSF adjuvant) immunization intradermally on the deltoid area of the lateral arm (preferentially left arm) with 0.25 mL administered at each of two adjacent sites NOTE: If serum PSA obtained at week 4 was higher than that obtained at day 1 visit, then rhGM-CSF (208 μg) will be co-administered with the pTVG-HP immunization as adjuvant for this and all subsequent immunizations
5. Following vaccination, subjects will be observed for 60 min. for adverse events
6. Nivolumab 240 mg, administered intravenously over 30 minutes

N. Fourteenth treatment visit (week 40, 28 +/- 3 days after week 36 visit)
Fifteenth treatment visit (week 44, 28 +/- 3 days after week 40 visit)

1. Physical examination, including vital signs, symptoms assessment, and ECOG performance score
2. Blood draw CBC, chemistry panel, PSA and PAP.
3. pTVG-HP (+/- GM-CSF adjuvant) immunization intradermally on the deltoid area of the lateral arm (preferentially left arm) with 0.25 mL administered at each of two adjacent sites NOTE: If serum PSA obtained at week 4 was higher than that obtained at day 1 visit, then rhGM-CSF (208 μg) will be co-administered with the pTVG-HP immunization as adjuvant for this and all subsequent immunizations
4. Following vaccination, subjects will be observed for 60 min. for adverse events
5. Nivolumab 240 mg, administered intravenously over 30 minute

O. End of treatment period visit (week 48, 28 +/- 3 days after week 44 visit)

1. Physical examination, including vital signs, symptoms assessment, and ECOG performance score
2. Blood draw CBC, chemistry panel, PSA and PAP (can be performed +/- 7 days of this visit).
3. CT scan of abdomen and pelvis, and bone scan (can be performed +/- 7 days of this visit)
4. 10 ml peripheral blood (red-top tube) for sera to evaluate baseline antibody responses, and 100 ml peripheral blood (green-top heparinized tubes) for T-cell response evaluation (can be performed +/- 7 days of this visit)

P. Week 60 visit (84 +/- 14 days after week 48 visit)

1. Physical examination, including vital signs, symptoms assessment, and ECOG performance score
2. Blood draw CBC, chemistry panel, PSA and PAP.

Q. Week 72 visit (84 +/- 14 days after week 60 visit)

1. Physical examination, including vital signs, symptoms assessment, and ECOG performance score
2. Blood draw CBC, chemistry panel, PSA and PAP.
3. CT scan of abdomen and pelvis, and bone scan (can be performed +/- 7 days of this visit)

R. Week 84 visit (84 +/- 14 days after week 72 visit)

1. Physical examination, including vital signs, symptoms assessment, and ECOG performance score
2. Blood draw CBC, chemistry panel, PSA and PAP.

S. Week 96 visit (84 +/- 14 days after week 72 visit) – END OF STUDY

1. Physical examination, including vital signs, symptoms assessment, and ECOG performance score
2. Blood draw CBC, chemistry panel, PSA and PAP.
3. CT scan of abdomen and pelvis, and bone scan (can be performed +/- 7 days of this visit)

T. 28-Day Follow Up Visit (28 +/- 7 days after Off-study date, if prior to week 48)

Patients will come off study at the time of disease progression, undue toxicity, or at the discretion of the patient and/or treating physician that it is not in the interest of the patient to continue treatment. A separate follow-up visit is not required for patients completing the week 48 evaluation, however for patients coming off study prior to week 48, 28-day follow up visit will include the following:

1. Physical examination, symptoms assessment, and ECOG performance score.
2. Blood draw for CBC, chemistry panel, serum PSA and PAP
3. IF NOT ALREADY COLLECTED WITHIN PREVIOUS 60 DAYS: 10 ml peripheral blood (red-top tubes) for sera to evaluate antibody responses, and 100 ml peripheral blood (green-top heparinized tubes) for T-cell response evaluation

U. Long-term Follow-Up

Subjects will be contacted by telephone (if not already being seen in clinic) annually for 2 years following the last DNA vaccination to collect clinical information to identify any potential long-term risks. This information has been requested by FDA for all gene delivery trials to assess potential long-term risks. The specific information to be collected annually will include:

- Date of contact
- Current medications
- Hospitalizations (dates and reasons for hospital admission)

- Stage of prostate cancer, treatments for prostate cancer, and recent serum PSA level
- New cancer diagnoses
- New autoimmune disorders
- New hematologic or neurologic disorders
- Other new medical diagnoses
- Date of death if patient deceased

9. Response Monitoring

A. Radiographic Monitoring – Primary Endpoint

CT scans of the abdomen and pelvis and bone scans will be obtained at 6-month intervals, or at times clinically indicated at the discretion of the treating physician. Progression is defined as the appearance of metastases by radiographic imaging using the same criteria as for defining study eligibility – RECIST 1.1 criteria (appearance of a visceral lesion > 10 mm by CT or MRI, lymph node > 15 mm in short axis, or appearance of new lesions on bone scan consistent with metastatic disease).

PSA rise will not be used to define disease progression, however PSA progression will be determined, as per Section 13 below.

B. Immunological Monitoring

Blood (up to 220 mL) will be collected pre-immunization, and at weeks 4,12, 24, 36, and 48 after study start for immunological monitoring. From the heparinized blood peripheral blood mononuclear cells (PBMC) will be prepared by density centrifugation over Ficoll-Paque using standard techniques (included in separate laboratory manual). PBMC will be cryopreserved in liquid nitrogen using 90% autologous serum collected at the time of blood draw, or 90% fetal calf serum, and 10% DMSO. Sera will be prepared from the red-top tubes and stored in aliquots at –80°C for antibody analyses.

(1) Quantitative assessment of PAP-specific CD8+ T-cell effector immunity

PAP-specific IFNγ- and granzyme B-secreting T-cell precursor frequency quantification by ELISPOT: ELISPOT will be used as the preferred methodology, as it permits analysis of low-frequency events (LOD < 1:10,000 cells) and also permits simultaneous analysis of cryopreserved batched specimens. Specifically, cryopreserved PBMC from subjects at the various time points will be rapidly thawed, and then transferred to 96-well nitrocellulose microtiter (ELISPOT) plates previously coated with monoclonal capture antibodies specific for IFNγ or granzyme B. 10^5 cells per well will be cultured in the presence of media (RPMI 1640 supplemented with L-glutamine, penicillin/streptomycin, ß-mercaptoethanol and 10% human AB serum) only (no antigen), 2 µg/ml PAP protein, 2 µg/ml PSA protein (negative control), 250 ng/ml tetanus toxoid, or 2.5 µg/ml PHA (positive mitogenic control) for 24-48 hours. Plates will then be washed with PBS containing 0.05% Tween-20 and incubated for 2.5 hours at room temperature with 50 µl/well PBS containing 5 µg/ml biotinylated detection antibodies for either IFNγ or granzyme B. After incubation, wells

will be washed with PBS, and further incubated with 100 µl/well streptavidin-labeled alkaline phosphatase (BioRad, Hercules, CA) and then developed with 100 µl/well BCIP/NBT colorimetric substrate (BioRad). The colorimetric reaction will be stopped by rinsing the plates under cool tap water, and wells will be allowed to dry completely before spots are enumerated with an ELISPOT automatic plate reader. Fluorometric assays of detection may be substituted for those described above, and include the evaluation of other cytokines.

REPORTING AND RESPONSE DEFINITION: Results will be presented as previously reported as the mean (+/- standard deviation) number of spot-forming-units (sfu) per 10^6 cells (frequency), calculated by subtracting the mean number of spots obtained from the no antigen control wells from the mean number obtained in the experimental wells, normalized to 10^6 starting PBMC, from 3- to 8-well replicate assays [17]. Comparison of experimental wells with control, no antigen, wells will be performed using a two-sample t-test, with $p < 0.05$ (two-sided) defined as a significant antigen-specific T-cell response. A significant antigen-specific response resulting from immunization will then be defined as a PAP-specific response detectable at least twice post treatment that is significantly higher than to media only (as above), at least 3-fold higher than the mean baseline value, and with a frequency > 10 per 10^6 PBMC.

(2) Quantitative assessment of antigen-specific antibody immunity

Enzyme-linked immunosorbent assay (ELISA) for the detection of antibodies responses to PAP: The presence of a coexisting humoral immune response to PAP will be evaluated by ELISA using an indirect method similar to that described previously [24]. Specifically, Immulon-4 ELISA plates (Dynex Technologies Inc.) will be coated with 2 µg/ml purified PAP protein (Research Diagnostics, Inc.) in 0.1 M $NaHCO_3/Na_2CO_3$ buffer (pH 9.6) overnight at 4ºC. After blocking with PBS/1% BSA for 1 hour at room temperature, wells will be washed with PBS + 0.05% Tween-20 (PBS-Tween) and then incubated for 1 hour with human sera diluted 1:25, 1:50, 1:100 and 1:200. After washing, plates will then be sequentially incubated with a peroxidase-conjugated anti-human IgG detection antibody (Amersham), followed by peroxidase enzyme TMB substrate (Kierkegaard and Perry Laboratories). The color reaction will be stopped with 1N H_2SO_4 and the optical density measured at 450 nm. Antibody titers for PAP-specific IgG antibodies will be determined as previously described [24].

REPORTING AND RESPONSE DEFINITION: These are not strictly quantitative assays. IgG response will be reported graphically demonstrating sera dilution curves, and by titer – defined as the highest sera dilution at which IgG responses are detectable above the mean + 3 standard deviations of the negative control. A positive IgG response resulting from immunization will be defined as an antigen-specific IgG titer at least 4-fold higher than the baseline titer detectable at the 6-month post-treatment time point (or other post-treatment time point evaluated).

(3) Assessment of antigen-spread to other prostate-associated antigens

High-throughput immunoblot (HTI): An exploratory objective of the study will be to determine if patients treated develop "off-target" prostate cancer antigen-specific immune responses as evidence of antigen spread. Future studies may evaluate T-cell responses to non-targeted antigens. However, the primary evaluation will be to evaluate IgG responses to a panel of prostate-associated antigens as we have previously reported in patients treated with vaccines or other immune-

modulating agents [68-70]. IgG specific for 126 antigens, including 29 cancer-testis antigens [71, 72] and 97 prostate antigens frequently immunologically recognized [73-76] will be identified by screening a high-density phage array expressing these individual antigens, as we have previously reported [68, 70, 71]. An alternative to this approach may include the evaluation of responses to panels of protein or peptide antigens fixed to support membranes provided by commercial vendors. The primary analysis will be conducted using sera obtained at 6 months, and compared with IgG responses identified at baseline, to determine whether IgG specific for individual antigens are elicited over time. Other time points will be assessed to determine the durability and kinetics of immune response development, and confirmatory ELISA studies will be performed where feasible.

REPORTING AND RESPONSE DEFINITION: A positive IgG response will be defined as an immunoreactive spot to a defined antigen, scored by at least 3 of 4 independent reviewers, and detectable to at least 2 of 3 replicates per immunoblot membrane as previously described [68, 77], or by statistically defined criteria using commercial antigen sources. An IgG response resulting from immunization will be defined as an immunoreactive antigen identified at the 6-month (or other post-treatment time for subsequent analyses) time point that was not identified at the pre-treatment time point.

C. Serum PSA Doubling Time Evaluation

See Section 11 for further details. Serum PSA doubling-time (or PSA slope) response will be considered a secondary endpoint. The pre-treatment serum PSA doubling time will be calculated from four serum PSA values available from the same clinical laboratory using the same assay for the period up to one year prior to study treatment, not on other treatments for prostate cancer, up to and including the PSA value obtained at screening, using the Memorial Sloan-Kettering Cancer Center nomogram (http://www.mskcc.org/applications/nomograms/prostate/PsaDoublingTime.aspx). The post-treatment PSA doubling time will be calculated using the same nomogram from:

(1) All PSA values obtained from the same clinical laboratory using the same PSA assay beginning with the PSA value at day 1 (week 0) and continuing until the end-of study (or month 24) value ($PSADT_{0-24}$).

(2) All PSA values obtained from the same clinical laboratory using the same PSA assay beginning with the PSA value at week 12 (month 3) to the week 36 (month 9) value ($PSADT_{3-9}$).

An increase in the PSA doubling time to at least double the baseline value, and at least 15 months, will be considered a PSA doubling time "response" [7].

D. Quantitative Total Bone Imaging (QTBI) using NaF PET/CT

An assessment of small volume bone metastatic disease (potentially not detected by standard bone scintigraphy) and tumor growth rates will be conducted using QTBI. Patients selected to participate in the NaF PET/CT part of the study will be assessed at baseline, week 12, and week 24 by NaF PET/CT. Metastatic prostate cancer lesions in bone will first be localized and identified based on functional NaF PET uptake, assisted with the anatomical information

provided by CT scans. Segmentation will be performed using an automatic segmentation method (e.g. a fixed SUV threshold), and adjusted with physician guidance. Scans from different time points will be registered to one another using an articulated registration technique employing a rigid registration of skeletal elements (bones) from CT followed by registration optimization by combining with deformable registration of bones and lesions from NaF PET/CT. The lesions between pre-treatment and follow-up scans will be matched to establish longitudinal correspondence of the lesions. For each patient, comprehensive treatment response metrics will be calculated, consisting of SUV_{total} (total disease burden), SUV_{max} (maximum intensity lesion), SUV_{mean} (average intensity), the number of lesions, and total volume of bone lesions. In addition, imaging response metrics will be calculated for each individual lesion. This methodology will specifically be used to assess the growth rate of bone metastatic disease by evaluating changes from baseline to month 3 and from month 3 to month 6. In terms of logistics, each of the clinical centers has the capacity and expertise to perform NaF PET imaging.

NaF-PET/CT IMAGING PROCEDURES: To ensure uniformity as well as optimal data interpretation, a standardized image acquisition protocol will be utilized and the overall imaging analyses will be conducted centrally under the supervision of Dr. Robert Jeraj, PhD (co-investigator) at the University of Wisconsin Image Analysis Core (IMAC) facility, with all analysis blinded to study treatment arm. Please refer to the Standard Operating Procedures (SOP) document for NaF-PET/CT procedures, which will be adhered to at both sites, for details of image acquisition, image analysis, image interpretation, image submission for central review, and other imaging-related details.

10. Statistical Considerations

A. Overview

This will be a single-arm, multiple-institution, two-stage phase II trial designed to evaluate the safety and effect of serial intradermal vaccinations of a DNA vaccine encoding PAP with or without rhGM-CSF, and co-administered with nivolumab, on serum PSA in patients with PSA-recurrent, non-metastatic, non-castrate prostate cancer.

B. Objectives

Primary Objectives:

1. To evaluate the safety and tolerability of pTVG-HP DNA vaccine and nivolumab in patients with clinical stage D0/M0 prostate cancer
2. To determine the PSA complete response rate (PSA < 0.2 ng/mL)

Secondary Objectives

1. To evaluate 2-year metastasis-free survival rate
2. To evaluate median radiographic progression-free survival
3. To evaluate changes in PSA doubling time or slope
4. To evaluate PSA response rate (≤ 50% of baseline)
5. To determine whether GM-CSF is required as a vaccine adjuvant for pTVG-HP vaccine when used in combination with nivolumab

Laboratory/Exploratory Biomarker Objectives:

1. To determine if antigen-specific T-cell and/or IgG responses are elicited with treatment
2. To determine if the development of PAP-specific T-cell immune responses are associated with PSA response (decline ≥ 50%) To determine if antigen spread (the development of immune responses to other cancer-associated proteins) is associated with PSA response (decline ≥ 50%)
3. To determine if antigen spread (the development of immune responses to other cancer-associated proteins) is associated with PSA response (decline ≥ 50%)
4. To determine if Quantitative Total Bone Imaging (QTBI) by NaF PET/CT can identify bone lesions in this patient population not detected by standard bone scintigraphy
5. To determine whether the presence of lesions detected by NaF PET/CT at baseline is associated with early disease progression (within 1 year) by standard radiographic imaging methods (CT and bone scintigraphy)
6. To determine if growth rates, and changes in growth rates, determined by NaF PET/CT are associated with PSA doubling time and changes in PSA doubling time

C. Study Design and Sample Size Calculation

The primary efficacy endpoint is the PSA complete response (PSA < 0.2 ng/mL) rate. A confirmed PSA CR will be defined as one in which serum PSA at least 4 weeks later is < 0.2 ng/mL, as per PCWG2 recommendations [67]. In this patient population, the PSA CR rate is expected to be less than 5% with standard care. Hence, the null hypothesis that the PSA complete response rate is at most 5% will be tested against the alternative hypothesis that the rate is greater than 5%. A PSA complete response rate of 20% or more, on the other hand, will be considered sufficient evidence to consider further clinical trial investigation. In order to ensure sufficient accuracy in the estimation of PSA complete response, an adequate number of patients should be enrolled in this study. However, in order to avoid treating patients with a potentially ineffective therapy, a Simon optimal two-stage design with one interim analysis for efficacy will be utilized.

In the first stage, 21 patients will be enrolled and evaluated for PSA response. If a PSA complete response is observed in at most one out of the 21 patients, then study will be terminated after the first stage. Otherwise, an additional 20 patients will be enrolled in the second stage for a total of 41 patients.

Assuming that the number of subjects with a PSA complete response is binomially distributed and using a Simon-optimal two-stage design, this design has a significance of 5% and 90% power for detecting a true success (PSA complete response) probability of 20%. If the true PSA complete response rate is only 5%, the study will be terminated after the first stage with 66% probability. The expected sample size is 27 under this scenario. If, however, the true efficacy response rate is 20%, the study will be terminated early with only 6% probability with an expected sample size of 40 patients. The two-stage procedure has 98% power to detect an efficacy response rate of 25% and 71% power to detect an efficacy response rate of only 15%. The operating characteristic of the proposed balanced two-stage design are summarized in the following table:

Table 1 *Operating characteristics of the Simon-optimal two-stage design*

True PSA complete response rate	Probability of	Power	Expected

	early stop after stage 1		sample size
5%	66%	6%	27
10%	36%	35%	34
15%	16%	71%	38
20%	6%	90%	40
25%	2%	98%	41

A sample size of 21 patients for the first stage and 41 patients in total will be also adequate for estimating toxicity rates with sufficient accuracy. Specifically, toxicity rates will be estimated with a standard error of less than 10% with a sample size of 21 for the first stage and the corresponding 95% confidence intervals will be no wider than 40%. Analogously, with a total sample size of up to 41 patients, toxicity rates will be estimated with a standard error of less than 8% and the corresponding 95% confidence intervals will be no wider than 32%.

D. Analysis

(1) General

This section outlines the statistical analysis strategy and procedures for the study. Descriptive statistics will primarily be generated to summarize the data. For continuous variables, descriptive statistics may include the number of subjects reflected in the calculation (n), mean, standard deviation, median, minimum, and maximum; frequencies and percentages may be displayed for categorical data. A detailed Statistical Analysis Plan (SAP) will be generated before the data analysis will be conducted.

(2) Analysis of Primary Endpoints

PSA complete response: PSA complete responses will be analyzed by descriptive statistics and summarized in tabular format (frequency tables). The PSA complete response rate will be estimated using the Whitehead's bias adjustment method which takes into account the sequential testing procedure of the proposed two-stage design. The table below shows the estimated objective response rates, the p-values and 90% confidence intervals for various scenarios:

Table 2: *Estimated PSA complete response rates, p-values and confidence intervals*

Number of PSA complete responses	Estimated PSA complete response rate[†]	p-value	Lower 95% CI bound	Upper 95% CI bound
0	0.0%	1.0000	0.0%	0.0%
1	5.7%	0.6590	0.2%	13.3%
2	5.9%	0.2830	1.7%	20.7%
3	8.5%	0.2120	2.6%	20.8%
4	10.9%	0.1140	3.7%	21.3%
5	13.2%	0.0460	5.1%	22.7%
6	15.4%	0.0140	6.7%	24.8%

7	17.7%	0.0040	8.4%	27.2%
8	20.0%	0.0010	10.1%	29.8%
9	22.3%	0.0000	12.0%	32.5%
10	24.6%	0.0000	13.9%	35.2%

† Estimated using Whitehead's bias-adjustment approach

For example, if at the end of the study, PSA complete response efficacy responses are observed in 5 out of the 41 patients, then the estimated (bias adjusted) response rate is 13.2% with a 95% confidence intervals ranging form 5.1% to 22.7%.

A minimum of 5 complete PSA responses are required to reject the null hypothesis which warrants further clinical investigation.

Toxicity and adverse events: Subjects will be evaluated at each visit by a review of systems based on the most recent version of the NCI common toxicity criteria. Toxicities will be summarized by type and severity in tabular format. Toxicity rates (grade 2, grade 3, grade 4, grade ≥ 2, grade ≥ 3, etc.) will be calculated and reported along the corresponding 95% confidence intervals. The 95% confidence intervals will be constructed using the Wilson score method.

(3) Analysis of Secondary Endpoints

2-year metastasis-free Survival Rate: The 2-year metastasis-free survival rate will be estimated using the Kaplan-Meier method and reported along with the corresponding two-sided 95% confidence interval.

Median radiographic progression-free survival: Radiographic progression-free survival will be estimated using the Kaplan-Meier method. The Brookmeyer-Crowley method will be used to construct the 95% confidence interval of the median radiographic progression-free survival.

PSA doubling time: PSA doubling time will be estimated for each patient using linear regression analysis. Specifically, PSA doubling time will be calculated as log(2) divided by the slope parameter of the regression of log(PSA) on time. PSA doubling times and slopes will be summarized in terms of means, medians, standard deviation, and 95% confidence interval. Changes in PSA doubling time and slopes will be evaluate using a paired t-test or nonparametric Wilcoxon Signed Rank test.

(4) Exploratory Objectives

The number of antigen-specific T-cell and/or IgG responses will be summarized in terms of means, standard deviations, medians and ranges for each assessment time point. Changes from baseline will be evaluated using a nonparametric Wilcoxon Signed Rank test. A negative binomial or overdispersed Poisson model (depending on the model fit) will be utilized to evaluate whether antigen-specific T-cell and/or IgG responses are elicited with treatment. Univariate and multivariate logistic regression analysis will be conducted to examine whether the development of PAP-specific T-cell immune responses and antigen spread are associated with PSA response.

Results from the QTBI analysis will be used to determine (1) whether QTBI by NaF PET/CT can identify bone lesions in this patient population not detected by standard bone scintigraphy, (2) whether the presence of lesions detected by NaF PET/CT at baseline is associated with early disease progression (within 1 year) by standard radiographic imaging methods (CT and bone scintigraphy), and (3) whether growth rates, and changes in growth rates, determined by NaF PET/CT are associated with PSA doubling time and changes in PSA doubling time. Sensitivity of QTBI by NaF PET/CT to identify bone lesions will be summarized in tabular format and compared to sensitivity of standard bone scintigraphy using a paired McNemar's test. Univariate and multivariate Cox proportional hazard regression analyses will be conducted to evaluate the associations between presence of lesions detected by NaF PET/CT at baseline and time to disease progression. Analogously, univariate and multivariate logistic regression analyses will be performed to evaluate the associations between presence of lesions detected by NaF PET/CT at baseline and 1-year PFS rate. The associations between changes in QTBI parameters (SUVmax, SUVmean, SUVtotal) and changes in PSA doubling time will be evaluated using linear mixed effects modeling with subject specific random effects.

11. Administrative Considerations

A. Specimen Handling

Upon entry into the study, each subject will be assigned a unique identification number. All materials collected on that subject will be labeled with that number only, for reasons of confidentiality. Lymphocytes collected will be stored in liquid nitrogen, and sera will be aliquoted and stored at –80°C in research laboratories at the investigator sites, and shipped in bulk to the central UWCCC laboratory for immune analysis.

B. Future Use of Samples

Not all of the blood components obtained during this study may be required for the tests that are part of the clinical trial. Following the conclusion of the study, the samples may be used for future cancer research at University of Wisconsin or other sites. This research will help to understand disease subtypes, drug response and toxicity, and possibly identify new drug targets or biomarkers that predict subject response to treatment. Patients will be given the option to opt out of the sample banking process.

Samples will be coded with the subject's unique study identification number and date sample was drawn, which can be linked back to individually identifiable information. The link between the unique subject identification number and the identifiable information will be stored in a secure location in a password protected computer, with only appropriately trained staff having access to this link. Samples will be stored in the research laboratory of the University of Wisconsin Carbone Cancer Center in Madison, Wisconsin. This is a secure facility and only appropriate people will have access to the blood samples.

C. Institutional Review Board

In accordance with federal regulations (21 CFR 312.66), an Institutional Review Board (IRB) that complies with the regulations in 21 CFR 56 must review and approve this protocol and the informed consent form prior to the initiation of the study at each investigator site. In addition, the study cannot be instituted without FDA approval of the vaccine formulations. Finally, the trial will be conducted with adherence to this protocol, following good clinical practice (GCP) guidelines, and in compliance with other applicable regulatory requirements. Any modifications to the protocol must follow the procedure as outlined in Section 17 below.

As the Coordinating Center for a trial, it is UWCCC's responsibility for oversight of regulatory documentation for each participating site including verifying that the participating has an IRB and a Federal wide assurance to cover its activities. After UW Health Sciences Institutional Review Board (HS-IRB) grants initial approval, the UWCCC GU Oncology Group staff will provide the approved protocol, and consent form to the participating site. The participating site will use the UW HS-IRB approved consent document as a template. No substantial changes, including changes to the risk language will be allowed without first receiving approval from the UWCCC GU Oncology Group.

Each participating site must receive local IRB approval of both the current protocol and consent prior to activation of the study at their site. The local IRB approval notice, approved HIPAA and consent will be forwarded to the UWCCC GU Oncology Group at the contact listed below either by mail, fax or email. Once all of the UWCCC administrative requirements are completed, the UWCCC GU Oncology Group will issue an activation notice for the site and subjects can be enrolled into the study:

In addition, the participating institution must provide the UWCCC GU Oncology Group with a copy of the institution's approved continuing review. Registration will be halted at the participating institution if a current continuing review approval is not on file at UWCCC.

All local IRB approvals and informed consent documents should be forwarded to the UWCCC GU Oncology Group by email at uwcccgu@medicine.wisc.edu or fax at 608-265-5146 or regular mail at:
UWCCC GU Oncology Group
University of Wisconsin Carbone Cancer Center
600 Highland Avenue, CSC K4/6
Madison, WI 53792

D. Consent

The Principal Investigator or their associates must explain verbally and in writing the nature, duration, and purpose of the study and possible consequences of the treatment. Patients must also be informed that they may withdraw from the study at any time and for any reason without jeopardizing their future treatment. In accordance with Federal regulations (21 CFR 312), all patients must sign the IRB-approved consent form.

Documentation of both the informed consent process and that the process occurred prior to a subject's entry into the study should be recorded in the subject's source documents. The original

consent form, signed and dated by the subject and by the person consenting the subject prior to the subject's entry into the study, must be maintained in the investigator's study files at each site.

12. Data and Safety Monitoring Plan

A. Definitions

Adverse events (AE) are defined as any unfavorable and unintended sign (including abnormal laboratory finding), symptom or disease temporally associated with a medical treatment or procedure, regardless of whether it is considered related to the treatment or procedure. Adverse events are categorized as definite, probable, possible, unlikely or unrelated in relation to the medical treatment or procedure performed.

AEs include the following:
1. An exacerbation, or an unexpected increase in frequency or intensity of a pre-existing condition (other than condition under investigation), including intermittent or episodic conditions.
2. Significant or unexpected worsening or exacerbation of the condition/indication under investigation.
3. A suspected drug interaction.
4. An intercurrent illness.
5. Any clinically significant laboratory abnormality.

An AE does not include:
1. Anticipated day-to-day fluctuations of any pre-existing conditions, including the disease under study.
2. Signs and symptoms of the disease under study that do not represent a significant worsening or exacerbation.
3. Expected progression of the disease under investigation.
4. Abnormal laboratory findings that are grade 1 or 2 and are considered clinically insignificant.

Serious adverse events (SAE) are any events occurring that result in any of the following outcomes:

1. Subject death
2. Life-threatening adverse event
3. Inpatient hospitalization or prolongation of existing hospitalization
4. Persistent or significant disability/incapacity
5. Congenital anomaly or birth defect

A life-threatening event is defined as any adverse event that places the subject, in the view of the investigator, at immediate risk of death from the reaction.

Note: In addition to the above criteria, adverse events meeting any of the below criteria, although not serious per ICH definition, are reportable to the BMS in the same timeframe as SAEs to meet

certain local requirements. Therefore, these events are considered serious by BMS for collection purposes.

- Is another important medical event
- Is a new cancer (that is not a condition of the study)
- Is associated with an overdose.

Severity rating/grading scale

Adverse events are classified by organ system and graded by severity according to the current NIH Common Terminology Criteria V. 5, as described in Appendix A. The defined grades use the following general guidelines:

0 No adverse event or within normal limits
1 Mild adverse event
2 Moderate adverse event
3 Severe adverse event
4 Life-threatening or disabling adverse event
5 Fatal adverse event

All adverse events will be recorded from time of treatment initiation through 30 days after the final dose of study treatment, as well as any time after these 30 days for events that are believed to be at least possibly related to study treatment. Please note: SAEs, regardless of relationship, occurring from the time of consent until 100 days after the final dose of nivolumab will be collected and reported as per section E.1.

Appropriate clinical, diagnostic, and laboratory measures to attempt to delineate the cause of the adverse reaction in question must be performed and the results reported. All tests that reveal an abnormality considered to be drug- or vaccine-related will be repeated at appropriate intervals until the course is determined or a return to normal (or pre-treatment) values occurs. Information will be recorded as noted above.

B. Disease Oriented Team Meetings

UWCCC site: This study undergoes review of subject safety at regularly scheduled Disease Oriented Team (DOT) meetings where the following are discussed as applicable: number of subjects enrolled, subject treatments given, dose holds/modifications, significant toxicities, response to treatment, and the subjects' overall status. These discussions are documented in the DOT meeting minutes.

C. UWCCC Data and Safety Monitoring Committee (DSMC) Study Progress Review and Oversight

The review of Protocol Summary Reports (PSRs) enables the UWCCC DSMC to assess whether the study should continue, continue with modifications, be suspended, or be closed. Following their review, the UWCCC DSMC will notify the sponsor-investigator of their recommendation and the authority for continuing, modifying, suspending, or closing the protocol is the responsibility of the applicable sponsor-investigator, Principal Investigator, IRB, FDA or other regulatory authority associated with the protocol.

Based on the risk level of this study, as determined by the UWCCC Protocol Review and Monitoring Committee, PSRs must be submitted to the UWCCC DSMC by the UWCCC site on a quarterly basis.

The UWCCC site is responsible for ensuring participating sites enter data used to populate the PSRs in a timely manner into the UWCCC instance of OnCore. This data includes: accrual information, Serious Adverse Events (SAEs), response to treatment, events reportable to IRB (e.g., non-compliance, unanticipated problems).

a. UWCCC DSMC Review of Auditing and/or Monitoring Reports

Reports created through the auditing and/or monitoring activities at all sites are submitted in real-time by the UWCCC site to the UWCCC DSMC. Summary data and/or query reports are submitted in lieu of detailed reports. Following the review of these reports, the committee may issue a request for corrective and/or preventive action(s), protocol suspension, or for-cause audit(s).

b. UWCCC DSMC Review of Non-compliance, Unanticipated Problems, and Other IRB Reportable Events

UWCCC site: Reports of non-compliance, unanticipated problems, and other IRB reportable events are submitted to the IRB of record for the study and to the UWCCC DSMC, via an email to DSMC@carbone.wisc.edu, simultaneously.

Non-UWCCC participating site(s): Reports of non-compliance, unanticipated problems, and other IRB reportable events are submitted to the IRB of record for the site at which the event occurred and to the UWCCC site, via an email to uwcccgu@medicine.wisc.edu, simultaneously. The UWCCC site forwards the event information to the UWCCC DSMC within 1 business day of receipt.

c. Real-time UWCCC DSMC Review of Serious Adverse Events (SAEs)

The UWCCC DSMC Chair, or designee, reviews all SAEs occurring on the study, regardless of site, to determine if immediate action is required.

UWCCC site: The Principal Investigator (PI), or designee, notifies the following individuals/entities of SAEs as applicable:

- Other investigators involved with the study at the UWCCC
- IRB of record for the study conducted at the UWCCC, per the IRB's reporting requirements
- UWCCC DSMC (Refer to section D)
- Sponsor-Investigator
- UW Institutional Biosafety Officer [Andrea Ladd: AndreaLadd@wisc.edu]

Non-UWCCC participating sites: The site PI, or designee, notifies the following individuals/entities of SAEs as applicable:

- Other investigators involved with the study at the site
- IRB of record for the study conducted at the site, per the IRB's reporting requirements Institutional Biosafety Committee of record for the participating site
- UWCCC site via an email to uwcccgu@medicine.wisc.edu

UWCCC site (Sponsor-Investigator or designee): responsibilities are noted in section E

D. Serious Adverse Event Reporting

Serious Adverse Events Requiring 24-Hour Reporting

UWCCC site: All Serious Adverse Events must be reported within 24 hours to the UWCCC DSMC Chair via an email to saenotify@uwcarbone.wisc.edu within one business day. The OnCore SAE Details Report must be submitted along with other report materials as appropriate (FDA Medwatch Form #3500, UWCCC routing form, UWCCC Sponsor-Investigator Determination Form for FDA Reporting of Safety Events, de-identified supporting documentation available at that time of initial reporting). The UWCCC DSMC Chair reviews the information and determines if immediate action is required. Within 5 calendar days, all available subsequent SAE documentation is submitted electronically, along with a 24 hour follow-up SAE Details Report, to saenotify@uwcarbone.wisc.edu. This information is entered and tracked in the UWCCC secure, password protected computer network. Refer to the current UWCCC SAE Standard Operating Procedure (SOP) and related documents to facilitate reporting.

UWCCC site (Sponsor-Investigator): Refer to section E for sponsor-investigator responsibilities.

Non-UWCCC participating site(s): All Serious Adverse Events must be reported within 24 hours to the UWCCC site via an email to uwcccgu@medicine.wisc.edu and a phone call (608-263-7107) within one business day of the site being aware of the event. The OnCore SAE Details Report must be submitted along with other report materials as appropriate (FDA Medwatch Form #3500 and de-identified supporting documentation available at that time of initial reporting). The UWCCC site sends this information, along with the completed UWCCC Sponsor-Investigator Determination Form for FDA Reporting of Safety Events, to the UWCCC DSMC Chair via an email to saenotify@uwcarbone.wisc.edu within one business day of receipt. The DSMC Chair reviews the information and determines if immediate action is required. If immediate action is required, it is communicated by the UWCCC DSMC Chair to the UWCCC site, who is then responsible for contacting the non-UWCCC participating site. Within 5 calendar days, all available subsequent SAE documentation is submitted electronically, along with a 24 hour follow-up SAE Details Report, to the UWCCC site via an email to uwcccgu@medicine.wisc.edu. The UWCCC site sends this information to the UWCCC DSMC Chair via an email to saenotify@uwcarbone.wisc.edu within one business day of receipt. This information is entered and tracked in the UWCCC secure, password protected computer network.

E. Sponsor-Investigator Responsibilities for SAE Review

The sponsor-investigator (i.e., IND holder), assumes the responsibilities of the study sponsor in accordance with FDA 21 CFR 312.32. In this capacity, the sponsor-investigator reviews all reports of serious adverse events occurring on the study at the UWCCC and non-UWCCC participating sites and makes a documented determination of 1) suspectedness (i.e., whether there is a reasonable possibility that the drug caused the AE); and 2) unexpectedness (the event is not listed in the Investigator's Brochure or is not listed at the specificity or severity that has been observed) in the context of this study. SAE with suspected causality to study drug and deemed unexpected are reported as IND Safety Reports by the sponsor-investigator, or designee, to the following within 15 calendar days unless otherwise designated below:

- FDA
- All participating investigators on the study, and the external global sponsor (if applicable).
- Other oversight committees (NIH OSP)
- Study supporters within 1 day (For additional details, see section E.1):
  Madison Vaccines, Inc.
  Attn: Safety Manager
  Phone: (608) 467-5269

  Bristol Myers Squibb (BMS)
  SAE Email Address: Worldwide.Safety@BMS.com
  SAE Facsimile Number: +1 609-818-3804

All fatal or life-threatening SAE that are unexpected and have suspected causality to the study drug will be reported by the sponsor-investigator, or designee, to the following within 7 calendar days unless otherwise designated below:

- FDA
- All participating investigators on the study, and the external global sponsor (if applicable)
- Other oversight committees (NIH OSP)
- Study supporters within 1 day (For additional details, see section E.1):
  Madison Vaccines, Inc.
  Attn: Safety Manager
  Phone: (608) 467-5269

  Bristol Myers Squibb (BMS)
  SAE Email Address: Worldwide.Safety@BMS.com
  SAE Facsimile Number: +1 609-818-3804

a. UWCCC Sponsor-Investigator Reporting to the Industry Supporters:

Madison Vaccines, Inc.
All SAEs that occur from the signing of the study specific consent through the duration of the post- therapy adverse event collection period (30 days after the last treatment administration) of the study vaccine must be reported to Madison Vaccines Inc. within 24 hours of being made aware of the SAE. Notifications can be made via phone a copy of the FDA Med Watch form to:

Madison Vaccines Inc. Attn: Safety Manager Phone: (608) 467-5269

Significant new information regarding an ongoing SAE and the resolution must be sent to Madison Vaccines Inc. within 3 business days of awareness of the new information via the same reporting mechanism.

Bristol Myers Squibb (BMS):

- All Serious Adverse Events (SAEs) that occur following the subject's written consent to participate in the study until [redacted] days following discontinuation of dosing must be reported to BMS Worldwide Safety, whether related or not related to study drug. If applicable, SAEs

must be collected that relate to any later protocol-specified procedure (eg, a follow-up skin biopsy).

- Following the subject's written consent to participate in the study, all SAEs, whether related or not related to study drug, are collected, including those thought to be associated with protocol-specified procedures. The investigator should report any SAE occurring after these aforementioned time periods, which is believed to be related to study drug or protocol-specified procedure.
- An SAE report should be completed for any event where doubt exists regarding its seriousness;
- If the investigator believes that an SAE is not related to study drug, but is potentially related to the conditions of the study (such as withdrawal of previous therapy or a complication of a study procedure), the relationship should be specified in the narrative section of the SAE Report Form.
- If the BMS safety address is not included in the protocol document (e.g. multicenter studies where events are reported centrally), the procedure for safety reporting must be reviewed/approved by the BMS Protocol Manager. Procedures for such reporting must be reviewed and approved by BMS prior to study activation.
- An appropriate SAE form (e.g. ex-US = CIOMS form or USA = Medwatch form) should be used to report SAEs to BMS. The BMS protocol ID number must be included on whatever form is submitted by the Sponsor/Investigator.
    - The CIOMS form is available at: http://www.cioms.ch/index.php/cioms-form-i
    - The Med Watch form is available at:

      https://www.fda.gov/downloads/AboutFDA/ReportsManualsForms/Forms/UCM048334.pdf

- In accordance with local regulations, BMS will notify investigators of all reported SAEs that are suspected (related to the investigational product) and unexpected (ie, not previously described in the IB). An event meeting these criteria is termed a Suspected, Unexpected Serious Adverse Reaction (SUSAR). Investigator notification of these events will be in the form of a SUSAR Report.
    - Other important findings which may be reported by BMS as an Expedited Safety Report (ESR) include: increased frequency of a clinically significant expected SAE, an SAE considered associated with study procedures that could modify the conduct of the study, lack of efficacy that poses significant hazard to study subjects, clinically significant safety finding from a nonclinical (e.g. animal) study, important safety recommendations from a study data monitoring committee, or sponsor decision to end or temporarily halt a clinical study for safety reasons.
    - Upon receiving an ESR from BMS, the investigator must review and retain the ESR with the IB. Where required by local regulations or when there is a central IRB/IEC for the study, the sponsor will submit the ESR to the appropriate IRB/IEC. The investigator and IRB/IEC will determine if the informed consent requires revision. The investigator

should also comply with the IRB/IEC procedures for reporting any other safety information.

- In addition to the Sponsor Investigator's responsibility to report events to their local HA, suspected serious adverse reactions (whether expected or unexpected) shall be reported by BMS to the relevant competent health authorities in all concerned countries according to local regulations (either as expedited and/or in aggregate reports).

SAEs, whether related or not related to study drug, and pregnancies in partners of study participants must be reported to BMS within 24 hours. SAEs must be recorded on either CIOMS or MedWatch form & pregnancies must be reported on a Pregnancy Surveillance Form or can be submitted on the aforementioned SAE form to BMS.

Overdoses (either accidental or intentional dose that is considered both excessive and medically important) must be reported as an SAE.

SAE Email Address: Worldwide.Safety@BMS.com

SAE Facsimile Number: +1 609-818-3804

If only limited information is initially available, follow-up reports are required. (Note: Follow-up SAE reports should include the same investigator term(s) initially reported.)

If an ongoing SAE changes in its intensity or relationship to study drug or if new information becomes available, a follow-up SAE report should be sent within 24 hours to BMS (or designee) using the same procedure used for transmitting the initial SAE report.

All SAEs should be followed to resolution or stabilization.

13. Potential Risks and Benefits, and Procedures to Minimize Risk

A. Potential Risks

(1) From immunization with DNA plasmid encoding PAP

Two potential toxicities might be predicted to occur from DNA-based vaccines. The first would be immediate toxicity due to the vaccination itself, and a second would be due to immunological consequences of the vaccination targeting other unrelated tissues. An intradermal route of administration will be used, and is preferred given the presence of Langerhans' antigen-presenting cells in the dermis. Intradermal administrations, however, carry a risk of immediate allergic reactions. For that reason, subjects will be remain for 60 minutes following each treatment to evaluate the skin site of treatment and for any immediate allergic events. Subjects will also be asked to keep a record of unusual site or other reactions for two days after immunization. To date, in studies using pTVG-HP only without anti-PD-1, allergic reactions have been rare (grade 3 angioedema observed in 1 of over 50 treated subjects, <5% individuals).

With respect to eliciting unwanted immunological reactions, PAP, a protein whose expression is essentially restricted to the prostate, does share homology with other tissue phosphatases, notably lysosomal acid phosphatase (LAP), most prevalent in pancreatic tissue. No such toxicity has been reported in clinical trials targeting PAP by means of a dendritic cell vaccine, and the generation of PAP-specific CTL in rat models similarly did not elicit detectable evidence of autoimmune disease in non-prostate tissues. Moreover, this was not seen in previous clinical trials with this DNA vaccine with over 100 subjects. This could theoretically be potentiated, however, with the anti-PD-1 therapy. Consequently, in order to further evaluate this potential toxicity in humans, subjects will be evaluated at each visit by a review of systems based on the NCI common toxicity criteria. In addition, subjects will be examined and serum chemistries, including renal function tests, blood counts, liver function tests, and serum amylase, will be evaluated at 6-12 week intervals, as outlined above. The serum amylase will be used as a serum marker to monitor for evidence of subclinical pancreatic inflammation. Again, while no autoimmune treatment-limiting adverse events have been observed to date in other trials, it is conceivable that by using a checkpoint inhibitor such as anti-PD-1 with an immunization approach targeting the PAP antigen that more autoimmune events could be observed than with either agent alone. For this reason, these blood tests will be continued to be monitored in this trial.

While there has been a suggestion that the plasmid DNA could insert into the host chromosomal DNA, this has not been documented in any other study, and several laboratory investigations have suggested the possibility of this occurring is less than the spontaneous mutation rate, and therefore not a real risk [78]. Even should this occur, there is little reason to suspect that this would put subjects at any increased risk, and in this patient population with treated prostate cancer, there is little risk of transfer to offspring. There are no known risks to blood donation during or after immunization, however subjects will be requested to not donate blood from study entry until completion of all study procedures.

The DNA itself is not hazardous (in that it encodes no viral proteins, is not radioactive, and is not itself a carcinogen) and does not pose additional risk to subjects or study personnel. However, standard precautions to reduce the risk of needle sticks to study personnel will be performed.

(2) <u>From intradermal treatment with rhGM-CSF</u>

GM-CSF, administered intradermally with the vaccine, is being used as a vaccine adjuvant, and has demonstrated safety from multiple other vaccine trials in humans. Common side effects include erythema and induration at the site of immunization (lasting for several days), and a transient decrease in peripheral white blood cell counts (lasting for several hours), mild flu-like symptoms (lasting for several hours). It has also been observed and reported that T-cell and antibody responses can occur to GM-CSF, but without any known clinical sequelae [65]. Uncommon and rare side effects, which have been less frequently observed with daily administration of higher doses of GM-CSF, include: vomiting; diarrhea; fatigue; weakness; headache; decreased appetite; feeling of faintness; facial flushing; pain in the bones, muscles, chest, abdomen, or joints; blood clots or unusual bleeding symptoms; rapid or irregular heartbeat or other heart problems; kidney and liver dysfunction; fluid accumulation or worsening of pre-existing fluid accumulation in arms and legs, in the lungs, and around the heart that may result in breathing problems and heart failure; allergic reactions; sloughing of skin; liver enlargement; Guillain-Barré syndrome; hypotension; loss of consciousness; dyspnea. In a previous phase I trial

with this DNA vaccine and GM-CSF, two patients experienced chest and back pain, attributed to the GM-CSF, which lasted less than 10 minutes and occurred within one hour of receiving GM-CSF. For this reason, all subjects will be monitored for one hour after receiving each DNA immunization treatment.

(3) From treatment with nivolumab

Nivolumab (Opdivo®, Bristol Myers Squibb) is a human programmed death receptor-1 (PD-1)-blocking antibody. Given that PD-1 is expressed on T cells, and that ligation of PD-1 by one of its ligands causes inactivation of T-cell function, blocking PD-1 by means of an antibody such as nivolumab can release the function of T cells that may have reactivity to normal cells. In fact, the goal of this therapy is to "unleash" T cells, and cytolytic CD8+ T cells in particular, that recognize tumor cells. In this trial, the goal is to elicit a specific population of CD8+ T cells that recognize prostate tumors, and allow them to lyse prostate tumor by not permitting expression of the ligand PD-L1 on prostate tumors to inhibit their function. But because PD-1 blockade is itself not specific, the release of T cells with specificity to other normal tissues can result, leading to autoimmune adverse events. To date, while autoimmune adverse events have been relatively uncommon (occurring in <10% of patients with melanoma treated), multiple different immune adverse events have been observed. These have included immune-mediated pneumonitis, colitis, hepatitis, hypophysitis, nephritis, hyperthyroidism and hypothyroidism. Other clinically significant, immune-mediated adverse reactions have been observed in <1% of patients treated with nivolumab, including exfoliative dermatitis, uveitis, arthritis, myositis, pancreatitis, hemolytic anemia, partial seizures, adrenal insufficiency, myasthenic syndrome, optic neuritis, and rhabdomyolysis. Most of these events resolved following discontinuation of nivolumab, or in some cases following treatment with systemic corticosteroids. But because of these rare, but potentially serious immune adverse events, patients will be followed closely for unusual new symptoms.

In addition to these immune-mediated adverse events, other common adverse reactions (reported in $\geq$ 20% of patients) included fatigue, cough, nausea, pruritis, rash, decreased appetite, constipation, arthralgia, and diarrhea.

(4) From blood tests

Drawing blood may cause temporary discomfort and bruising at the site of venipuncture. Skin infections, while possible, are extremely rare as a result of blood draws

(5) From NaF PET/CT imaging

The main potential risk to subjects is during and immediately after the tracer injection, not because of the tracer, but because of the saline, which represents greater than 99% of the injection volume, so risks associated with any injection apply. The NaF PET/CT scan will be performed for at least 60 minutes after the injection, so there is a built-in 60 minute observation period for adverse events. The 60-minute time period for collection of AE's will also cover the risk of radiation, which is very low, and no different than a standard PET procedure. For this study, subjects will be assessed for adverse events (AE) starting from immediately after the injection of the Sodium Fluoride F-18 tracer for each of the NaF PET/CT scans until 60 minutes

after the injection. All events assessed as at least possibly related to the study procedure (NaF tracer or NaF PET/CT scan) will be collected.

(6) Confidentiality

The research staff will make their best effort to keep samples and data confidential at all times. However, absolute confidentiality cannot be guaranteed. There is a slight risk of breach of confidentiality which could be embarrassing or stigmatizing.

B. Potential Benefits

No benefits are guaranteed. It is hoped that individual patients treated with the DNA vaccine and nivolumab will derive a clinical response indicated by a decrease in PSA and prolonged time to development of metastatic disease. While no direct benefit is guaranteed, the results from this trial will guide the direction of future vaccine trials targeting PAP or potentially other antigens for the treatment of prostate cancer, and could suggest means by which anti-PD-1 therapies could be used in prostate cancer, as these agents have demonstrated substantial benefit for patients with other types of malignancies other than prostate cancer. Thus, it is hoped that study participants and future patients will benefit from research participation in the current study.

14. Study Data Management and Procedural Issues

A. Study Enrollment Procedures – Recruitment and Informed Consent Process:

(1) For Subjects enrolled at UWCCC

Potentially eligible subjects at the UWCCC will be patients regularly followed or referred to the University of Wisconsin Hospital and Clinics, and seen in the Medical Oncology, Urology, or Radiation Oncology outpatient clinics at the University of Wisconsin. No specific advertisement or recruiting tools will be used. Subjects will be identified by their primary radiation, surgical, or medical oncologist, and informed about this study, alternatives to this study, and the possible risks and benefits. Potentially eligible subjects will be informed that their decision to participate or not participate will in no way affect their ongoing medical care. Subjects who are interested at that point in obtaining more information will then be introduced to one of the GU research nurses who will then present the study (review rationale, describe time commitment, discuss again possible risks and benefits, and answer procedural questions) to the subject and provide them with a consent form. In order to allow research subjects time to review the consent form thoroughly with their family and referring or primary physician, the subject will be instructed to take the consent form home without signing. If the patient wishes to proceed with enrollment, they are then asked to contact the GU research office (608) 263-7107. To avoid the possibility of unintended coercion, incarcerated subjects and subjects unable to provide their own informed consent will not be considered eligible. In addition, the research staff will call the patient to further discuss the study and its requirements. Any questions the subject has will be addressed by the research staff or investigators, and the time commitment and alternatives to treatment will again be reviewed. If the subject agrees to participate in the study, they will be instructed to return to clinic to meet with the research staff and to sign and date the consent form. Our research staff will sign and date the

consent form as the person obtaining consent. No screening procedures done solely for purposes of the study will be obtained prior to the subject signing the consent form.

(2) Enrollment Procedures For Subjects enrolled at Participating Institution:

Eligible patients will be entered on study centrally at the University of Wisconsin by the Study Coordinator. All sites should call the UWCCC GU Oncology Program at 608-263-7107 to verify a slot is available.

To register a patient, the following documents should be completed by the research nurse or data manager and faxed 608-265-5146 or e-mailed to uwcccgu@medicine.wisc.edu to the Study Coordinator:

- Copy of required laboratory tests and other source documents verifying eligibility
- Signed patient consent form
- HIPAA authorization form
- Eligibility checklist

The research nurse or data manager at the participating site will then call 608-263-7107 or e-mail uwcccgu@medicine.wisc.edu to verify eligibility. To complete the registration process, the UWCCC will:

- assign a patient study number
- register the patient on study via UWCCC's On-line Clinical Oncology Research Environment (ONCORE)
- fax or e-mail the patient study number to the participating site

B. Data Collection Procedures

Electronic case report forms (e-CRFs) will be submitted to the UWCCC GU Oncology Office via UWCCC's On-line Clinical Oncology Research Environment (ONCORE). Completion of the e-CRFs will be done in accordance with the instructions provided by the UWCCC GU office in a study-specific data capture plan. The e-CRFs are found in the study specific calendar that has been created in ONCORE. The system will prompt the user to the forms that are required based upon the patient's enrollment and treatment dates.

Research personnel entering data into the ONCORE data base at both UWCCC and at the participating institution must have completed human subjects training and Health Insurance Portability and Accountability Act (HIPAA) training. The UWCCC GU Oncology Office will verify that the required training has occurred.

The Principal Investigator will be responsible for assuring that all the required data is entered onto the e-CRFs accurately and within 2 weeks of the date in which the previous cycle was completed.

Periodically, monitoring and/or auditing visits will be conducted by staff from the UWCCC GU Oncology Office. The Principal Investigator at each participating center will provide access to his/her original records to permit verification of data entry.

All clinical safety and clinical response data will be collected by the study coordinators on electronic case report forms as noted in section 14.B. and maintained by the individual sites.

The UWCCC will serve as the Coordinating Center responsible for initial study configuration and database setup and any future changes.

Research data evaluating immune responses will be maintained in individual laboratory research charts. All laboratory analysis conducted at the UWCCC will be maintained in research charts in the laboratory of Dr. McNeel and stored a minimum of 7 years.

Adverse events, clinical responses, and issues related to disease progression are reviewed each week by the Genitourinary Malignancy clinical research Disease Oriented Working Group (DOWG), attended by GU medical oncologists, GU malignancy research nurses, data coordinators, and the GU malignancy program manager. Minutes from these meetings are recorded, and bi-annual reports from this group are submitted to and reviewed by the UWCCC Data Safety and Monitoring Committee. All clinical safety, immunological response, and clinical response data will be analyzed by the study statistician, with the University of Wisconsin Department of Biostatistics and Medical Informatics.

All subject research charts will be maintained at individual treatment sites for a minimum of seven years after study completion. All clinical information maintained in the UWCCC clinical trials database will be stored indefinitely. All research samples and data derived from these specimens will be maintained indefinitely in the laboratory of Dr. McNeel. All information with patient identifiers or medical history information will be kept in locked cabinets or secured databases available only to the study personnel to maintain patient confidentiality.

C. Description of Procedures to Maintain Confidentiality of Research Specimens

All specimens obtained for the immunological evaluation of this trial (blood specimens) will be delivered to the laboratory of Dr. McNeel. Specimens received by Dr. McNeel's lab will only be handled by laboratory personnel who have undergone HIPAA training and annual UWHC-mandated blood-borne pathogen safety training. Receipt of specimens will be entered into a database that will provide a unique code for each specimen. All stored specimens (sera, peripheral blood mononuclear cells) will be labeled with this unique code and the date of preparation. Thus, all patient identifiers will be removed from the final stored samples, and any data generated will contain only the unique code as identifier. A database will be maintained to link individual coded specimens with an individual subject (name, hospital medical record number, date/time point) in order to be able to compare information from samples obtained at different time points from a specific individual, and ultimately for transfer of research data to the clinical trials database, if necessary. This research database will only be available to the study investigators, not other laboratory personnel, to maintain confidentiality. Subject sera will be maintained in Dr. McNeel's laboratory, stored in aliquots at –20°C to –80°C. Peripheral blood mononuclear cells will be stored in Dr. McNeel's laboratory, stored in aliquots in liquid nitrogen. If warranted, samples may be sent to an external institution or a contract research organization for the purpose of collaboration and /or analysis.

Subjects will have the option to consent to future research using both their samples and data which may be used independently. Samples remaining after the primary analysis has been completed and available for future research will be "banked" and stored as they were for this study unless they are selected to be sent for research elsewhere, which may be within or outside of the University of Wisconsin. Subjects wishing to withdraw their banked samples will be required to contact the study team with their request , otherwise their samples may be used indefinitely for cancer research. All research samples and data derived from these specimens will be maintained indefinitely in the laboratory of Dr. McNeel. All information with patient identifiers or medical history information will be kept in locked cabinets or secured databases available only to the study personnel to maintain patient confidentiality.

D. Modifications of Protocol and Deviations from Protocol

Any changes to the preceding protocol after approval by the University of Wisconsin IRB must be submitted as an amendment to this IRB with a description of specific changes. All changes must be approved by the IRB before implementation. If these changes were related to possible adverse events that could potentially affect subject safety, the consent form will also be modified, and submitted with the protocol to the IRB. After approval, all enrolled subjects will be asked to review and sign the new consent form to proceed with treatment. If these changes were related to a severe adverse event, or could affect the scientific integrity of the study, the events would also be reviewed internally by the DSMC, as described above, and the study could be placed on hold pending a protocol and consent form amendment, as described above, or study closure. In addition, any information that might affect the immediate safety of currently enrolled subjects will be communicated with them directly as well.

The UWCCC GU Oncology Group will forward a copy of all UW HS-IRB approved protocol and consent changes and new study information to the participating site. The participating institution must provide the UWCCC GU Oncology Group with a copy of the institution's approvals. The UWCCC GU Oncology Group will maintain a copy of all amendments, consent forms and approvals from each participating institution.

Note that any changes to the protocol will require approval from the UWCCC Study PI, Dr. Hamid Emamekhoo, and from the Sponsor Investigator, Dr. Douglas McNeel.

Deviations from the study protocol, for reasons other than patient safety, are not permitted. Any possible deviations, intended or not, will be documented in the subjects' research chart. Deviations that are unlikely to affect subject safety, such as missing a specific study lab draw, will be documented in the research chart and discussed with the study PI. Deviations that could potentially affect subject safety, such as missing safety labs, will be documented, performed as soon as possible, and reviewed at the weekly GU clinical research (DOWG) meetings, with prompt reporting as soon as any deviation is identified to the IRB if there are concerns for patient safety or scientific integrity of the study.

E. Withdrawal from Study Protocol

Subjects are informed during the consenting process, and in the consent form, that their participation is voluntary and they may withdraw consent at any time and for any reason. If

subjects wish to withdraw from the study, they will be asked, but not required, to perform the off-study procedures/blood draws. They will be again informed that their decision to participate or not participate at any time will not affect their routine medical care or any other benefits to which they were otherwise entitled. In addition, subjects will be removed from study participation if there is a concern for patient safety, as described above, if they are unable to comply with study procedures, or if the study is terminated by local or national regulatory agencies.

15. Roles and Responsibilities of Study Personnel at UWCCC

Protocol sponsor:

Dr. Douglas McNeel MD PhD, Professor of Medicine, is a genitourinary medical oncologist, with a clinical research and laboratory interest in immune-based therapies for prostate cancer. He has served as the principal investigator for multiple other clinical trials, and has had formalized training in the ethics and conduct of clinical trials and human subjects protection. He is overall responsible for the design of the trial, interpretation of the protocol as study sponsor, and its laboratory analysis, and holds physician INDs for the pTVG-HP DNA vaccines. Because of his ownership interest in these vaccines that have been licensed by the WARF (Wisconsin Alumni Research Foundation, intellectual property manager for the University of Wisconsin) to a company that is seeking to commercialize them (Madison Vaccines, Inc), however, he will not be involved in any direct patient care aspects of this trial (recruitment, treatment of subjects on trial, review of imaging studies, or review of clinical laboratories as they pertain to the treatment of individual subjects).

UWCCC Principal Investigator:

Dr. Hamid Emamekhoo MD, Assistant Professor of Medicine, is a genitourinary medical oncologist with a primary interest in genitourinary cancer clinical trials research. He will be the UWCCC Principal Investigator for this study. As UWCCC PI he will be responsible for activities involving direct patient care and interaction, including recruitment, consent, clinical care and adverse event reporting. The UWCCC local PI will also assume the role of principal investigator on the protocol and UW HS-IRB submission, working with the UW HS-IRB as the responsible investigator to obtain and maintain UW HS-IRB approval for the study. The UWCCC local PI will also be responsible for reviewing significant adverse events that occur at UWCCC and will oversee the care provided by the UWCCC co-investigators on this study.

Biostatistician:

Dr. Jens Eickhoff, PhD is a biostatistician with a primary interest in cancer clinical trials, and significant experience with immunotherapy clinical trials. He has been actively involved in the study design and he, or designee, will be primarily responsible for the statistical analysis of the study as proposed.

16. References

1. Siegel, R.L., K.D. Miller, and A. Jemal. (2017). "Cancer Statistics, 2017." *CA Cancer J Clin*.**67**:7-30.
2. Oefelein, M.G., N.D. Smith, J.T. Grayhack, A.J. Schaeffer, and K.T. McVary. (1997). "Long-term results of radical retropubic prostatectomy in men with high grade carcinoma of the prostate." *J Urol*.**158**:1460-1465.
3. Pound, C.R., A.W. Partin, M.A. Eisenberger, D.W. Chan, J.D. Pearson, and P.C. Walsh. (1999). "Natural history of progression after PSA elevation following radical prostatectomy." *Jama*.**281**:1591-7.
4. Freedland, S.J., E.B. Humphreys, L.A. Mangold, M. Eisenberger, F.J. Dorey, P.C. Walsh, and A.W. Partin. (2005). "Risk of prostate cancer-specific mortality following biochemical recurrence after radical prostatectomy." *Jama*.**294**:433-9.
5. Slovin, S.F., A.S. Wilton, G. Heller, and H.I. Scher. (2005). "Time to detectable metastatic disease in patients with rising prostate-specific antigen values following surgery or radiation therapy." *Clin Cancer Res*.**11**:8669-73.
6. Lee, A.K., L.B. Levy, R. Cheung, and D. Kuban. (2005). "Prostate-specific antigen doubling time predicts clinical outcome and survival in prostate cancer patients treated with combined radiation and hormone therapy." *Int J Radiat Oncol Biol Phys*.**63**:456-62.
7. Freedland, S.J., E.B. Humphreys, L.A. Mangold, M. Eisenberger, F.J. Dorey, P.C. Walsh, and A.W. Partin. (2007). "Death in patients with recurrent prostate cancer after radical prostatectomy: prostate-specific antigen doubling time subgroups and their associated contributions to all-cause mortality." *J Clin Oncol*.**25**:1765-71.
8. Gutman, A.B. and E.B. Gutman. (1938). "An acid phosphatase in the serum of patients with metastasizing carcinoma of the prostate gland." *J. Clin. Invest.***17**:473-479.
9. Terracio, L., A. Rule, J. Salvato, and W.H. Douglas. (1985). "Immunofluorescent localization of an androgen-dependent isoenzyme of prostatic acid phosphatase in rat ventral prostate." *Anat Rec*.**213**:131-9.
10. Fong, L., C.L. Ruegg, D. Brockstedt, E.G. Engleman, and R. Laus. (1997). "Induction of tissue-specific autoimmune prostatitis with prostatic acid phosphatase immunization; implications for immunotherapy of prostate cancer." *J. Immunol.***159**:3113-3117.
11. Johnson, L.E., T.P. Frye, A.R. Arnot, C. Marquette, L.A. Couture, A. Gendron-Fitzpatrick, and D.G. McNeel. (2006). "Safety and immunological efficacy of a prostate cancer plasmid DNA vaccine encoding prostatic acid phosphatase (PAP)." *Vaccine*.**24**:293-303.
12. Johnson, L.E., T.P. Frye, N. Chinnasamy, D. Chinnasamy, and D.G. McNeel. (2007). "Plasmid DNA vaccine encoding prostatic acid phosphatase is effective in eliciting autologous antigen-specific CD8+ T cells." *Cancer Immunol Immunother*.**56**:885-95.
13. Laus, R., D.M. Yang, C.L. Ruegg, M.H. Shapero, P.H. Slagle, E. Small, P. Burch, and F.H. Valone. (2001). "Dendritic cell immunotherapy of prostate cancer: preclinical models and early clinical experience." *Canc Res Ther Control*.**11**:1-10.
14. Kantoff, P.W., C.S. Higano, N.D. Shore, E.R. Berger, E.J. Small, D.F. Penson, C.H. Redfern, A.C. Ferrari, R. Dreicer, R.B. Sims, Y. Xu, M.W. Frohlich, and P.F. Schellhammer. (2010). "Sipuleucel-T immunotherapy for castration-resistant prostate cancer." *N Engl J Med*.**363**:411-22.
15. McNeel, D.G., E.J. Dunphy, J.G. Davies, T.P. Frye, L.E. Johnson, M.J. Staab, D.L. Horvath, J. Straus, D. Alberti, R. Marnocha, G. Liu, J.C. Eickhoff, and G. Wilding. (2009).

"Safety and immunological efficacy of a DNA vaccine encoding prostatic acid phosphatase in patients with stage D0 prostate cancer." *J Clin Oncol*.**27**:4047-54.
16. Becker, J.T., B.M. Olson, L.E. Johnson, J.G. Davies, E.J. Dunphy, and D.G. McNeel. (2010). "DNA vaccine encoding prostatic acid phosphatase (PAP) elicits long-term T-cell responses in patients with recurrent prostate cancer." *J Immunother*:(in press).
17. McNeel, D.G., J.T. Becker, J.C. Eickhoff, L.E. Johnson, E. Bradley, I. Pohlkamp, M.J. Staab, G. Liu, G. Wilding, and B.M. Olson. (2014). "Real-time immune monitoring to guide plasmid DNA vaccination schedule targeting prostatic acid phosphatase in patients with castration-resistant prostate cancer." *Clin Cancer Res*.**20**:3692-704.
18. Rekoske, B.T., H.A. Smith, B.M. Olson, B.B. Maricque, and D.G. McNeel. (2015). "PD-1 or PD-L1 Blockade Restores Antitumor Efficacy Following SSX2 Epitope-Modified DNA Vaccine Immunization." *Cancer Immunol Res*.**3**:946-55.
19. Rekoske, B.T., B.M. Olson, and D.G. Mcneel. (2016). "Anti-tumor vaccination of prostate cancer patients elicits PD-1/PD-L1 regulated antigen-specific immune responses." *Oncoimmunology*:(in press).
20. Brahmer, J.R., C.G. Drake, I. Wollner, J.D. Powderly, J. Picus, W.H. Sharfman, E. Stankevich, A. Pons, T.M. Salay, T.L. McMiller, M.M. Gilson, C. Wang, M. Selby, J.M. Taube, R. Anders, L. Chen, A.J. Korman, D.M. Pardoll, I. Lowy, and S.L. Topalian. (2010). "Phase I study of single-agent anti-programmed death-1 (MDX-1106) in refractory solid tumors: safety, clinical activity, pharmacodynamics, and immunologic correlates." *J Clin Oncol*.**28**:3167-75.
21. Topalian, S.L., F.S. Hodi, J.R. Brahmer, S.N. Gettinger, D.C. Smith, D.F. McDermott, J.D. Powderly, R.D. Carvajal, J.A. Sosman, M.B. Atkins, P.D. Leming, D.R. Spigel, S.J. Antonia, L. Horn, C.G. Drake, D.M. Pardoll, L. Chen, W.H. Sharfman, R.A. Anders, J.M. Taube, T.L. McMiller, H. Xu, A.J. Korman, M. Jure-Kunkel, S. Agrawal, D. McDonald, G.D. Kollia, A. Gupta, J.M. Wigginton, and M. Sznol. (2012). "Safety, activity, and immune correlates of anti-PD-1 antibody in cancer." *N Engl J Med*.**366**:2443-54.
22. Griffiths, J. (1980). "Prostate-specific acid phosphatase: re-evaluation of radioimmunoassay in diagnosing prostatic disease." *Clin. Chem.***26**:433-436.
23. Jobsis, A.C., G.P. De Vries, R.R. Anholt, and G.T. Sanders. (1978). "Demonstration of the prostatic origin of metastases: an immunohistochemical method for formalin-fixed embedded tissue." *Cancer*.**41**:1788-1793.
24. McNeel, D.G., L.D. Nguyen, B.E. Storer, R. Vessella, P.H. Lange, and M.L. Disis. (2000). "Antibody immunity to prostate cancer-associated antigens can be detected in the serum of patients with prostate cancer." *J. Urol.***164**:1825-1829.
25. McNeel, D.G., L.D. Nguyen, W.J. Ellis, C.S. Higano, P.H. Lange, and M.L. Disis. (2001). "Naturally occurring prostate cancer antigen-specific T cell responses of a Th1 phenotype can be detected in patients with prostate cancer." *Prostate*.**47**:222-229.
26. Peshwa, M.V., J.D. Shi, C. Ruegg, R. Laus, and W.C. van Schooten. (1998). "Induction of prostate tumor-specific CD8+ cytotoxic T-lymphocytes in vitro using antigen-presenting cells pulsed with prostatic acid phosphatase peptide." *Prostate*.**36**:129-138.
27. Burch, P.A., G.A. Croghan, D.A. Gastineau, L.A. Jones, J.S. Kaur, J.W. Kylstra, R.L. Richardson, F.H. Valone, and S. Vuk-Pavlovic. (2004). "Immunotherapy (APC8015, Provenge) targeting prostatic acid phosphatase can induce durable remission of metastatic androgen-independent prostate cancer: a Phase 2 trial." *Prostate*.**60**:197-204.
28. Small, E.J., P.F. Schellhammer, C.S. Higano, C.H. Redfern, J.J. Nemunaitis, F.H. Valone, S.S. Verjee, L.A. Jones, and R.M. Hershberg. (2006). "Placebo-controlled phase III trial of

immunologic therapy with sipuleucel-T (APC8015) in patients with metastatic, asymptomatic hormone refractory prostate cancer." *J Clin Oncol*.**24**:3089-94.
29. Higano, C.S., P.F. Schellhammer, E.J. Small, P.A. Burch, J. Nemunaitis, L. Yuh, N. Provost, and M.W. Frohlich. (2009). "Integrated data from 2 randomized, double-blind, placebo-controlled, phase 3 trials of active cellular immunotherapy with sipuleucel-T in advanced prostate cancer." *Cancer*.**115**:3670-9.
30. Iwasaki, A., C.A. Torres, P.S. Ohashi, H.L. Robinson, and B.H. Barber. (1997). "The dominant role of bone marrow-derived cells in CTL induction following plasmid DNA immunization at different sites." *J Immunol*.**159**:11-14.
31. La Cava, A., R. Billetta, G. Gaietta, D.B. Bonnin, S.M. Baird, and S. Albani. (2000). "Cell-mediated DNA transport between distant inflammatory sites following intradermal DNA immunization in the presence of adjuvant." *J Immunol*.**164**:1340-5.
32. Corr, M., A. von Damm, D.J. Lee, and H. Tighe. (1999). "In vivo priming by DNA injection occurs predominantly by antigen transfer." *J Immunol*.**163**:4721-4727.
33. Irvine, K.R. and N.P. Restifo. (1995). "The next wave of recombinant and synthetic anticancer vaccines." *Seminars in Canc Biol*.**6**:337-347.
34. Raz, E., H. Tighe, Y. Sato, M. Corr, J.A. Dudler, M. Roman, S.L. Swain, H.L. Spiegelberg, and D.A. Carson. (1996). "Preferential induction of a Th1 immune response and inhibition of specific IgE antibody formation by plasmid DNA immunization." *Proc Natl Acad Sci U S A*.**93**:5141-5145.
35. Chen, Y., R.G. Webster, and D.L. Woodland. (1998). "Induction of CD8+ T cell responses to dominant and subdominant epitopes and protective immunity to Sendai virus infection by DNA vaccination." *J. Immunol.***160**:2425-2432.
36. Thomson, S.A., M.A. Sherritt, J. Medveczky, S.L. Elliott, D.J. Moss, G.J. Fernando, L.E. Brown, and A. Suhrbier. (1998). "Delivery of multiple CD8 cytotoxic T cell epitopes by DNA vaccination." *J. Immunol.***160**:1717-1723.
37. Cho, H.J., K. Takabayashi, P.M. Cheng, M.D. Nguyen, M. Corr, S. Tuck, and E. Raz. (2000). "Immunostimulatory DNA-based vaccines induce cytotoxic lymphocyte activity by a T-helper cell-independent mechanism." *Nat Biotechnol*.**18**:509-514.
38. Corr, M., D.J. Lee, D.A. Carson, and H. Tighe. (1996). "Gene vaccination with naked plasmid DNA: mechanism of CTL priming." *J Exp Med*.**184**:1555-60.
39. Svanholm, C., L. Bandholtz, A. Lobell, and H. Wigzell. (1999). "Enhancement of antibody responses by DNA immunization using expression vectors mediating efficient antigen secretion." *J Immunol Methods*.**228**:121-30.
40. Rahman, F., A. Dahmen, S. Herzog-Hauff, W.O. Bocher, P.R. Galle, and H.F. Lohr. (2000). "Cellular and humoral immune responses induced by intradermal or intramuscular vaccination with the major hepatitis B surface antigen." *Hepatology*.**31**:521-527.
41. Disis, M.L., H. Bernhard, F.M. Shiota, S.L. Hand, J.R. Gralow, E.S. Huseby, S. Gillis, and M.A. Cheever. (1996). "Granulocyte-macrophage colony-stimulating factor: an effective adjuvant for protein and peptide-based vaccines." *Blood*.**88**:202-210.
42. Bernhard, H., M.L. Disis, S. Heimfeld, S. Hand, J.R. Gralow, and M.A. Cheever. (1995). "Generation of immunostimulatory dendritic cells from human CD34+ hematopoietic progenitor cells of the bone marrow and peripheral blood." *Cancer Res.***55**:1099-104.
43. Caux, C., C. Dezutter-Dambuyant, D. Schmitt, and J. Banchereau. (1992). "GM-CSF and TNF-alpha cooperate in the generation of dendritic Langerhans cells." *Nature*.**360**:258-61.
44. Mellman, I., S.J. Turley, and R.M. Steinman. (1998). "Antigen processing for amateurs and professionals." *Trends Cell Biol*.**8**:231-237.

45. Mincheff, M., I. Altankova, S. Zoubak, S. Tchakarov, C. Botev, S. Petrov, E. Krusteva, G. Kurteva, P. Kurtev, V. Dimitrov, M. Ilieva, G. Georgiev, T. Lissitchkov, I. Chernozemski, and H.T. Meryman. (2001). "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." *Crit Rev Oncol Hematol*.**39**:125-32.
46. Hu, H.M., H. Winter, W.J. Urba, and B.A. Fox. (2000). "Divergent roles for CD4(+) T cells in the priming and effector/memory phases of adoptive immunotherapy." *J Immunol*.**165**:4246-4253.
47. Tarr, P.E., R. Lin, E.A. Mueller, J.M. Kovarik, M. Guillaume, and T.C. Jones. (1996). "Evaluation of tolerability and antibody response after recombinant human granulocyte-macrophage colony-stimulating factor (rhGM-CSF) and a single dose of recombinant hepatitis B vaccine." *Vaccine*.**14**:1199-204.
48. Dranoff, G., E. Jaffee, A. Lazenby, P. Golumbek, H. Levitsky, K. Brose, V. Jackson, H. Hamada, D. Pardoll, and R.C. Mulligan. (1993). "Vaccination with irradiated tumor cells engineered to secrete murine granulocyte-macrophage colony-stimulating factor stimulates potent, specific, and long-lasting anti-tumor immunity." *Proc. Natl. Acad. Sci. U. S. A.***90**:3539-3543.
49. Simons, J.W., B. Mikhak, J.F. Chang, A.M. DeMarzo, M.A. Carducci, M. Lim, C.E. Weber, A.A. Baccala, M.A. Goemann, S.M. Clift, D.G. Ando, H.I. Levitsky, L.K. Cohen, M.G. Sanda, R.C. Mulligan, A.W. Partin, H.B. Carter, S. Piantadosi, F.F. Marshall, and W.G. Nelson. (1999). "Induction of immunity to prostate cancer antigens: results of a clinical trial of vaccination with irradiated autologous prostate tumor cells engineered to secrete granulocyte-macrophage colony-stimulating factor using ex vivo gene transfer." *Cancer Res.***59**:5160-5168.
50. Jaffee, E.M., R. Abrams, J. Cameron, R. Donehower, M. Duerr, J. Gossett, T.F. Greten, L. Grochow, R. Hruban, S. Kern, K.D. Lillemoe, S. O'Reilly, D. Pardoll, H.A. Pitt, P. Sauter, C. Weber, and C. Yeo. (1998). "A phase I clinical trial of lethally irradiated allogeneic pancreatic tumor cells transfected with the GM-CSF gene for the treatment of pancreatic adenocarcinoma." *Hum Gene Ther*.**9**:1951-71.
51. Kanellos, T.S., I.D. Sylvester, V.L. Butler, A.G. Ambali, C.D. Partidos, A.S. Hamblin, and P.H. Russell. (1999). "Mammalian granulocyte-macrophage colony-stimulating factor and some CpG motifs have an effect on the immunogenicity of DNA and subunit vaccines in fish." *Immunology*.**96**:507-10.
52. Disis, M.L., F.M. Shiota, D.G. McNeel, and K.L. Knutson. (2003). "Soluble cytokines can act as effective adjuvants in plasmid DNA vaccines targeting self tumor antigens." *Immunobiology*.**207**:179-86.
53. Greenwald, R.J., G.J. Freeman, and A.H. Sharpe. (2005). "The B7 family revisited." *Annu Rev Immunol*.**23**:515-48.
54. Okazaki, T., A. Maeda, H. Nishimura, T. Kurosaki, and T. Honjo. (2001). "PD-1 immunoreceptor inhibits B cell receptor-mediated signaling by recruiting src homology 2-domain-containing tyrosine phosphatase 2 to phosphotyrosine." *Proc Natl Acad Sci U S A*.**98**:13866-71.
55. Mercader, M., B.K. Bodner, M.T. Moser, P.S. Kwon, E.S. Park, R.G. Manecke, T.M. Ellis, E.M. Wojcik, D. Yang, R.C. Flanigan, W.B. Waters, W.M. Kast, and E.D. Kwon. (2001). "T cell infiltration of the prostate induced by androgen withdrawal in patients with prostate cancer." *Proc Natl Acad Sci U S A*.**98**:14565-14570.

56. Sfanos, K.S., T.C. Bruno, A.K. Meeker, A.M. De Marzo, W.B. Isaacs, and C.G. Drake. (2009). "Human prostate-infiltrating CD8+ T lymphocytes are oligoclonal and PD-1+." *Prostate*.**69**:1694-703.
57. Zhang, X., J.C. Schwartz, X. Guo, S. Bhatia, E. Cao, M. Lorenz, M. Cammer, L. Chen, Z.Y. Zhang, M.A. Edidin, S.G. Nathenson, and S.C. Almo. (2004). "Structural and functional analysis of the costimulatory receptor programmed death-1." *Immunity*.**20**:337-47.
58. Chemnitz, J.M., R.V. Parry, K.E. Nichols, C.H. June, and J.L. Riley. (2004). "SHP-1 and SHP-2 associate with immunoreceptor tyrosine-based switch motif of programmed death 1 upon primary human T cell stimulation, but only receptor ligation prevents T cell activation." *J Immunol*.**173**:945-54.
59. Sheppard, K.A., L.J. Fitz, J.M. Lee, C. Benander, J.A. George, J. Wooters, Y. Qiu, J.M. Jussif, L.L. Carter, C.R. Wood, and D. Chaudhary. (2004). "PD-1 inhibits T-cell receptor induced phosphorylation of the ZAP70/CD3zeta signalosome and downstream signaling to PKCtheta." *FEBS Lett*.**574**:37-41.
60. Riley, J.L. (2009). "PD-1 signaling in primary T cells." *Immunol Rev*.**229**:114-25.
61. Parry, R.V., J.M. Chemnitz, K.A. Frauwirth, A.R. Lanfranco, I. Braunstein, S.V. Kobayashi, P.S. Linsley, C.B. Thompson, and J.L. Riley. (2005). "CTLA-4 and PD-1 receptors inhibit T-cell activation by distinct mechanisms." *Mol Cell Biol*.**25**:9543-53.
62. Francisco, L.M., P.T. Sage, and A.H. Sharpe. (2010). "The PD-1 pathway in tolerance and autoimmunity." *Immunol Rev*.**236**:219-42.
63. Zahm, C.D., V.T. Colluru, and D.G. McNeel. (2017). "Vaccination with High-Affinity Epitopes Impairs Antitumor Efficacy by Increasing PD-1 Expression on CD8+ T Cells." *Cancer Immunol Res*.**5**:630-641.
64. Disis, M.L., K.H. Grabstein, P.R. Sleath, and M.A. Cheever. (1999). "Generation of immunity to the HER-2/neu oncogenic protein in patients with breast and ovarian cancer using a peptide-based vaccine." *Clin. Cancer Res.***5**:1289-1297.
65. McNeel, D.G., K. Schiffman, and M.L. Disis. (1999). "Immunization with recombinant human granulocyte-macrophage colony-stimulating factor as a vaccine adjuvant elicits both a cellular and humoral response to recombinant human granulocyte-macrophage colony-stimulating factor." *Blood*.**93**:2653-2659.
66. Devereux, S., H.A. Bull, D. Campos-Costa, R. Saib, and D.C. Linch. (1989). "Granulocyte macrophage colony stimulating factor induced changes in cellular adhesion molecule expression and adhesion to endothelium: in-vitro and in-vivo studies in man." *Br J Haematol*.**71**:323-30.
67. Scher, H.I., S. Halabi, I. Tannock, M. Morris, C.N. Sternberg, M.A. Carducci, M.A. Eisenberger, C. Higano, G.J. Bubley, R. Dreicer, D. Petrylak, P. Kantoff, E. Basch, W.K. Kelly, W.D. Figg, E.J. Small, T.M. Beer, G. Wilding, A. Martin, and M. Hussain. (2008). "Design and end points of clinical trials for patients with progressive prostate cancer and castrate levels of testosterone: recommendations of the Prostate Cancer Clinical Trials Working Group." *J Clin Oncol*.**26**:1148-59.
68. Smith, H.A., B.B. Maricque, J. Eberhardt, B. Petersen, J.L. Gulley, J. Schlom, and D.G. McNeel. (2011). "IgG responses to tissue-associated antigens as biomarkers of immunological treatment efficacy." *J Biomed Biotech*.**2011:454861**.
69. Fong, L., S.S. Kwek, S. O'Brien, B. Kavanagh, D.G. McNeel, V. Weinberg, A.M. Lin, J. Rosenberg, C.J. Ryan, B.I. Rini, and E.J. Small. (2009). "Potentiating endogenous

antitumor immunity to prostate cancer through combination immunotherapy with CTLA4 blockade and GM-CSF." *Cancer Res*.**69**:609-15.

70. Zabransky, D.J., H.A. Smith, C.J. Thoburn, M. Zahurak, D. Keizman, M. Carducci, M.A. Eisenberger, D.G. McNeel, C.G. Drake, and E.S. Antonarakis. (2011). "Lenalidomide modulates IL-8 and anti-prostate antibody levels in men with biochemically recurrent prostate cancer." *Prostate*:(in press).
71. Dubovsky, J.A., M.R. Albertini, and D.G. McNeel. (2007). "MAD-CT-2 identified as a novel melanoma cancer-testis antigen using phage immunoblot analysis." *J Immunother*.**30**:675-683.
72. Hoeppner, L.H., J.A. Dubovsky, E.J. Dunphy, and D.G. McNeel. (2006). "Humoral immune responses to testis antigens in sera from patients with prostate cancer." *Cancer Immun*.**6**:1-7.
73. Dunphy, E.J., J.C. Eickhoff, C.H. Muller, R.E. Berger, and D.G. McNeel. (2004). "Identification of antigen-specific IgG in sera from patients with chronic prostatitis." *J. Clin. Immunol.***24**:492-501.
74. Dunphy, E.J. and D.G. McNeel. (2005). "Antigen-specific IgG elicited in subjects with prostate cancer treated with flt3 ligand." *J Immunother*.**28**:268-75.
75. Mooney, C.J., E.J. Dunphy, B. Stone, and D.G. McNeel. (2006). "Identification of autoantibodies elicited in a patient with prostate cancer presenting as dermatomyositis." *Int J Urol*.**13**:211-7.
76. Morse, M.D. and D.G. McNeel. (2010). "Prostate Cancer Patients Treated with Androgen Deprivation Therapy Develop Persistent Changes in Adaptive Immune Responses." *Hum Immunol*.**71**:496-504.
77. Maricque, B.B., J.C. Eickhoff, and D.G. McNeel. (2011). "Antibody responses to prostate-associated antigens in patients with prostatitis and prostate cancer." *Prostate*.**71**:134-146.
78. Martin, T., S.E. Parker, R. Hedstrom, T. Le, S.L. Hoffman, J. Norman, P. Hobart, and D. Lew. (1999). "Plasmid DNA malaria vaccine: the potential for genomic integration after intramuscular injection." *Hum Gene Ther*.**10**:759-68.