Version 10/7/2015

# Randomized Phase II Trial of a DNA Vaccine Encoding Prostatic Acid Phosphatase (pTVG-HP) versus GM-CSF Adjuvant in Patients with Non-Metastatic Prostate Cancer

**CO 08801**

Investigational Agent:

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

Study Sponsors:

56 patients – treated at UWCCC and UCSF

Department of Defense Prostate Cancer Research Program federal grant

50 patients in biomarker cohort – treated at UWCCC, UCSF and JHU

Madison Vaccines Inc. (MVI) corporate sponsor

*STUDY SITE INFORMATION*

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

University of California San Francisco (UCSF)

Johns Hopkins School of Medicine (JHU)

Study Principal 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 : Glenn Liu, MD
UWCCC
7051 Wisconsin Institutes for Medical Research
1111 Highland Ave.
Madison, WI 53705
Tel : (608) 265-8689
Fax : (608) 265-5146
gxl@medicine.wisc.edu

Medical Monitor: Mark Albertini, M.D.
UWCCC
600 Highland Ave. K6/5 CSC
Madison, WI 53792
Tel: (608) 265-8131
Fax: (608) 265-8133
mra@medicine.wisc.edu

Other UWCCC Investigators:

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

UCSF Principal Investigator:
Lawrence Fong, M.D.

JHU Principal Investigator:
Emmanuel Antonarakis, MD

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. K4/4 CSC
Madison, WI 53792
Tel: (608) 265-6380
eickhoff@biostat.wisc.edu

Projected Start Date: December 1, 2010

Projected End of Accrual Date: March 31, 2016

Projected End of Study Date: March 31, 2018

## TABLE OF CONTENTS

## SYNOPSIS:

Purpose: To evaluate the efficacy of an investigational vaccine, pTVG-HP, a plasmid DNA encoding human prostatic acid phosphatase (PAP), in delaying disease progression in patients with non-metastatic prostate cancer (clinical stage D0).

Primary Objectives:

1. To evaluate the 2-year metastasis-free survival of patients with non-metastatic prostate cancer (clinical stage D0) treated with a DNA vaccine encoding PAP, with GM-CSF as an adjuvant, versus patients treated with GM-CSF only.

Secondary Objectives:

1. To evaluate if immunization with a DNA vaccine encoding PAP prolongs PSA doubling time in patients with non-metastatic prostate cancer
2. To evaluate the safety and tolerability of the pTVG-HP DNA vaccine administered to patients with clinical stage D0 prostate cancer
3. To evaluate the median progression-free survival, and PSA progression-free survival, of patients with non-metastatic prostate cancer (clinical stage D0) treated with a DNA vaccine encoding PAP, with GM-CSF as an adjuvant, versus patients treated with GM-CSF only.

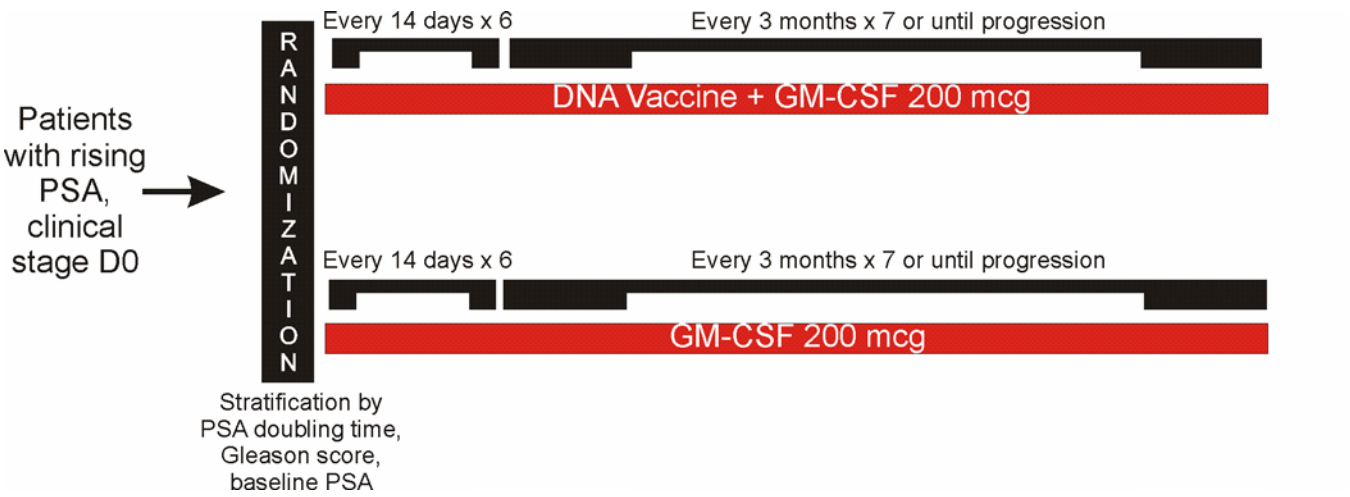
Laboratory Objectives:

1. To determine if immunization with a DNA vaccine encoding PAP elicits antigen-specific T-cell and/or IgG responses
2. To determine if immune monitoring of T-cell responses can be accurately and reproducibly conducted in a multi-center vaccine trial
3. To determine if the development of PAP-specific T-cell immune responses are associated with clinical responses.

Exploratory Biomarker Objectives – Expanded Subject Cohort:

1. To determine if baseline immune responses specific for PAP are predictive (or not) for the development of PAP-specific T-cells and/or are associated with 2-year metastasis-free survival in the cohort of patients who receive a DNA vaccine encoding PAP
2. To determine if antigen spread (the development of immune responses to other cancer-associated proteins) is associated with 2-year metastasis-free survival
3. 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
4. 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)
5. 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:



Plan of Treatment (DOD 56-Patient Cohort):

| **Vaccine / Treatment Visit:** | Pre-Screen (within 4 weeks of registration) | Screen/Randomization (within 2 weeks of day 1) | Day 1 (wk 0) – bV1[a] | Day 15 (wk 2) – bV2 | Day 29 (wk 4) – bV3 | Day 43 (wk 6) – bV4 | Day 57 (wk 8) – bV5 | Day 71 (wk 10) – bV6 | Day 85 (Mo 3 ) – bV7[a] | Day 169 (Mo 6) – qV1 | Day 253 (Mo 9) – qV2 | Day 337 (Mo 12) – qV3 | Day 421 (Mo 15) – qV4 | Day 505 (Mo 18) – qV5 | Day 589 (Mo 21) – qV6 | Day 672 (Mo 24, Year 2) and/or End of study visit[ef] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **History & Physical Exam** | | | | | | | | | | | | | | | | |
| History | X | | | | | | | | | | | | | | | |
| Consenting | X[g] | | | | | | | | | | | | | | | |
| Physical Exam | | X | X[h] | | | X | | | X | X | X | X | X | X | X | X |
| Toxicity assessment[b] | | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X |
| Conmed review | | X | X | X | X | X | X | X | X | X | X | X | X | X | X | X |
| Injection site inspection | | | X | X | X | X | X | X | X | X | X | X | X | X | X | X |
| **Lab Tests** | | | | | | | | | | | | | | | | |
| CBC and platelets | | X | | | | X | | | X | X | X | X | X | X | X | X |
| Creatinine, alk phos, SGOT, glucose, total bilirubin, amylase, LDH | | X | | | | X | | | X | X | X | X | X | X | X | X |
| Serum testosterone | | X | | | | | | | | | | | | | | |
| PSA and PAP | | X | X[c] | | | X | | | X | X | X | X | X | X | X | X |
| **Procedures** | | | | | | | | | | | | | | | | |
| CT abdomen/pelvis | X | | | | | | | | | X | | X | | X | | X[d] |
| Bone scan | X | | | | | | | | | X | | X | | X | | X[d] |
| Tetanus immunization | | X | | | | | | | | | | | | | | |
| DNA immunization vs. GM-CSF only | | | X | X | X | X | X | X | X | X | X | X | X | X | X | |
| Blood for immune studies | | X | | | | | | | X | X | X | X | | | | X[e] |

[a] bV = biweekly vaccination/treatment (+/- 2 days), qV = quarterly vaccination/treatment (+/- 1 week)

[b] Study coordinator or research nurse review of systems

[c] Baseline PSA used for calculating PSA time-to-progression endpoint

[d] Radiographic studies not required at off-study time point if subject already met time-to-progression endpoint and/or studies already performed within 1 month; scans otherwise performed every 6 months

[e] This blood draw not required if patient off-study prior to 12 months. However, all subjects should have blood drawn for immune monitoring up to 12 months, at the quarterly intervals (+/- 1 week) even if off-study prior to that time.

[f]. After the End of Study Visit, subjects will be contacted by telephone annually for up to 2 years 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. See section 10.J.1 for additional details.

[g] Consenting to be performed within 4 months (112 days) of study registration

[h] Not required if already performed within 14 days

Plan of Treatment (Expansion Biomarker 50-Patient Cohort):

| **Vaccine / Treatment Visit:** | Pre-Screen (within 4 weeks of registration) | Screen/Randomization (within 2 weeks of day 1) | Day 1 (wk 0) – bV1[a] | Day 15 (wk 2) – bV2 | Day 29 (wk 4) – bV3 | Day 43 (wk 6) – bV4 | Day 57 (wk 8) – bV5 | Day 71 (wk 10) – bV6 | Day 85 (Mo 3 ) – bV7[a] | Day 127 (Mo 4.5)[g] | Day 169 (Mo 6) – qV1 | Day 253 (Mo 9) – qV2 | Day 337 (Mo 12) – qV3 | Day 421 (Mo 15) – qV4 | Day 505 (Mo 18) – qV5 | Day 589 (Mo 21) – qV6 | Day 672 (Mo 24, Year 2) and/or End of study visit[ef] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **History & Physical Exam** | | | | | | | | | | | | | | | | | |
| Consenting | X[i] | | | | | | | | | | | | | | | | |
| History | X | | | | | | | | | | | | | | | | |
| Physical Exam | | X | X[j] | | | X | | | X | | X | X | X | X | X | X | X |
| Toxicity assessment[b] | | X | X | X | X | X | X | X | X | | X | X | X | X | X | X | X |
| Conmed review | | X | X | X | X | X | X | X | X | | X | X | X | X | X | X | X |
| Injection site inspection | | | X | X | X | X | X | X | X | | X | X | X | X | X | X | X |
| **Lab Tests** | | | | | | | | | | | | | | | | | |
| CBC and platelets | | X | | | | X | | | X | | X | X | X | X | X | X | X |
| Creatinine, alk phos, SGOT, total bilirubin, amylase, LDH | | X | | | | X | | | X | | X | X | X | X | X | X | X |
| Serum testosterone | | X | | | | | | | | | | | | | | | |
| PSA and PAP | | X | X[c] | | | X | | | X | X | X | X | X | X | X | X | X |
| **Procedures** | | | | | | | | | | | | | | | | | |
| CT abdomen/pelvis | X | | | | | | | | | | X | | X | | X | | X[d] |
| Bone scan | X | | | | | | | | | | X | | X | | X | | X[d] |
| NaF-PET/CT[k] | | X | | | | | | | X[h] | | X[h] | | | | | | |
| Tetanus immunization | | X[m] | | | | | | | | | | | | | | | |
| Leukapheresis[l] | | X[lm] | | | | | | | | | X[l] | | | | | | |
| DNA immunization vs. GM-CSF only | | | X | X | X | X | X | X | X | | X | X | X | X | X | X | |
| Blood for immune studies | | X[m] | | | | | | | X | | X | X | X | | | | X[e] |

[a] bV = biweekly vaccination/treatment (+/- 2 days), qV = quarterly vaccination/treatment (+/- 1 week)

[b] Study coordinator or research nurse review of systems

[c] Baseline PSA used for calculating PSA time-to-progression endpoint. May be done up to 3 days in advance of Day 1.

[d] Radiographic studies not required at off-study time point occurring before month 24 if subject already met time-to-progression endpoint and/or studies already performed within 1 month

[e] This blood draw not required if patient off-study prior to 12 months. However, all subjects should have blood drawn for immune monitoring up to 12 months, at the quarterly intervals (+/- 1 week) even if off-study prior to that time.

[f] After the End of Study Visit, subjects will be contacted by telephone annually for up to 2 years 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. See section 10.J.1 for additional details.

[g] Day 127 (Month 4.5) can be +/- 10 days – blood draw visit only

[h] NaF PET/CT within 14 days of study visit

[i] Consenting to be performed within 4 months (112 days) of study registration

[j] Not required if already performed within 14 days

[k] These NaF-PET/CT studies will be performed at UWCCC and JHU clinical sites only

[l] Baseline leukapheresis required at UWCCC site only. 200-cc blood draw (heparinized green-top tubes) permitted as an alternative to leukapheresis at JHU and UCSF trial sites, and at 6-month time point for UWCCC.

[m]Blood and leukapheresis samples at screening must be collected before tetanus immunization. DNA immunization/GM-CSF must be at least 24 hours after the tetanus immunization.

## 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 have died as a result of prostate cancer in 2007 [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" 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 initially treated 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 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) 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, 5, 6, 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 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. Many vaccines for prostate cancer are in various stages of clinical testing, as have been previously reviewed [8, 9, 10], and several vaccines have entered phase III clinical trials for patients with metastatic, castrate-resistant prostate cancer [11].

Prostatic acid phosphatase (PAP) is a model antigen for vaccine-based treatment strategies targeting prostate cancer. PAP is a well-defined protein whose expression is essentially restricted to normal and malignant prostate tissue [12]. It is also one of only a few known

prostate-specific proteins for which there is a rodent homologue, thereby providing an animal model for evaluating vaccine strategies and assessing toxicity [13]. 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 [14, 15, 16, 17]. Finally, an autologous antigen-presenting cell vaccine loaded *ex vivo* with a PAP derivative protein (Provenge, Dendreon Corporation, Seattle, WA) has been demonstrated in a placebo-controlled randomized phase III human clinical trial in patients with metastatic castration-resistant prostate cancer to elicit T-cell immunity, with an observed increase in overall survival in vaccine-treated subjects [18]. A confirmatory nationwide double-blinded placebo-controlled Phase III trial using this vaccination approach was completed and similarly demonstrated findings of increased overall survival (2009 American Urological Association Annual Meeting). That vaccine has been FDA approved for the treatment of patients with castrate-resistant, metastatic prostate cancer. These results suggest 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, a phase I/II trial was conducted in patients with high-risk stage D0 prostate cancer using this same DNA vaccine. 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 [19]. IFNγ-secreting immune responses to PAP detectable at multiple times months after immunization were detectable in individuals with evidence of prolonged PSA doubling time [20]. These findings have justified further evaluation of this vaccine in a randomized phase II clinical trial, and have further suggested that immune biomarkers might be useful as predictors of clinical response.

## 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 [12, 21]. 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 [22]. 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* [23, 24]. 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 [25]. They have then gone on to initiate human clinical trials targeting PAP using autologous antigen-presenting cell vaccines pulsed with a protein conjugate of human PAP and GM-CSF. Preliminary results suggest that immunized patients develop CD4+ T-cell responses against both PAP and GM-CSF [26], and 3/12 patients had a decrease in serum PSA levels [27]. Thus, not only can tolerance to PAP be circumvented *in vivo* by means of vaccination, but this may be associated with a clinical response. A similar approach has been reported by Fong *et al.* using autologous dendritic cells pulsed with a murine homologue of PAP [28]. In a phase II follow-on trial with the PAP-GM-CSF treatment approach, one of 21 patients experienced a complete response [29]. As described above, 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) [18]. 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 [18]. 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. Preliminarily, that trial has been reported to demonstrate a similar increase in median survival in patients receiving the sipuleucel-T (2009 national meeting of the American Urological Association), and Dendreon Corporation was granted 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 more feasible immunization approaches to elicit responses to the PAP antigen could be advantageous, as could their application in 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, 31, 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 [16, 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, 36, 37, 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].

Because of the potential of plasmid DNA to be used as a general means of immunization, the National Institutes of Health-sponsored National Gene Vector Laboratory has maintained a repository of plasmid DNA vectors designed for potential human clinical use (http://www.ngvl.org). The plasmid vector pNGVL3 was obtained from the National Gene Vector Laboratory (courtesy, Dr. Robert Gerard). This vector drives eukaryotic transcription from the CMV promoter, but with the addition of the CMV intron A sequence to enhance protein expression [41]. The vector also contains a multi-cloning site, and does not express a eukaryotic antibiotic resistance gene, such that the only protein expression expected in a eukaryotic system is the one driven from the CMV promoter. Given the additional properties of CG-rich DNA to augment inflammatory immune responses, we have modified the pNGVL3 immunization vector obtained from the National Gene Vector Laboratories described above to contain two copies of a 36-bp CG-rich immunostimulatory (ISS) oligonucleotide containing a 5'-GTCGTT-3' motif [42]. This resulting construct, called pTVG4 (Figure 1), is the parent vector into which the cDNA encoding human PAP was cloned. In the studies presented below and in published studies, it has been demonstrated that the resulting plasmid DNA vaccine encoding PAP (pTVG-HP, Figure 1) can elicit PAP-specific CD8+ T-cells in rodent models and in patients with prostate cancer, and that in rats this approach is superior to immunization with a vaccinia viral vector encoding PAP [16].

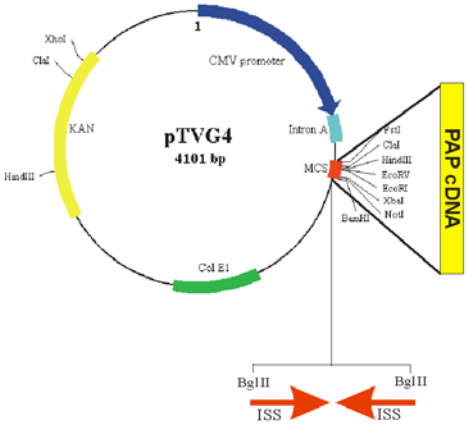



**Figure 1**: pTVG-HP construct for immunization. Shown is a plasmid map for the pTVG4 vector as constructed, into which was cloned the cDNA encoding human PAP to create the immunization construct pTVG-HP.

## 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 [43]. GM-CSF is a known growth and differentiation factor for human dendritic cells [44, 45]. 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 [46]. 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 [47]. 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 [48]. GM-CSF-expressing autologous cells have also been demonstrated in murine and human studies to be able to elicit immune responses to autologous antigens [49, 50, 51]. 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, 52, 53]. The use of plasmid DNA encoding GM-CSF, however, carries a theoretical risk of eliciting an immune response to GM-CSF [54] or to other self proteins. In addition, GM-CSF alone, given at higher doses in more frequent schedules, has been investigated as a treatment for early stage and castrate-resistant prostate cancer [55, 56, 57]. Treatment with GM-CSF alone has been associated with increases in circulating monocytes and lymphocytes, and has been associated with long-term disease control in patients with clinical stage D0 prostate cancer [58]. For all of these reasons, the current trial will investigate the use of soluble GM-CSF as an adjuvant for the DNA vaccine, compared with GM-CSF only as a control group.

D. 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 [59], 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 [59], 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. In the current proposal we wish to determine, in an exploratory fashion, 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 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 with respect to the treatment (vaccine versus control).

E. Summary

The current protocol will examine the use of a plasmid DNA vaccine encoding PAP to induce and/or augment CD8+ T-cell immune responses to PAP in patients with clinical stage D0 prostate cancer. Based on results from a prior trial suggesting an increase in PSA DT in patients treated with this vaccine, the primary endpoint will be to evaluate the time to radiographic disease progression, assuming that higher risk patients (PSA doubling time less than 12 months) have a median time to progression of about 2 years. Secondary endpoints will include

evaluations of changes in PSA doubling time, median time to radiographic disease progression, and a further assessment of safety. The trial will be conducted in a prospectively randomized fashion, with one group receiving vaccine, and the other group receiving the GM-CSF adjuvant only. Given that a CD8+/CTL response to PAP would be predicted to be the most effective immune response to lyse PAP-expressing tissue, the induction of T-cell immune responses specific for PAP will also be a secondary objective of the study, with assays being conducted at individual sites and centrally, to evaluate the reproducibility and predictive value of these assays in a multi-center trial. An additional cohort of subjects, treated in the same randomized fashion, will also be evaluated for additional measures of immune response, present at baseline or following treatment, that might predict for favorable disease outcome (two-year metastasis-free survival) and by QTBI for early detection of bone metastatic lesions and determination of tumor growth rates and patterns of disease progression.

## 3. Preclinical Safety and Toxicity Studies

A. Study Design

75 age-matched male Lewis rats were immunized at two-week intervals according to the study design shown in Table I [15]. Animals were housed in a single room without other experimental animals, and weighed three times per week. Groups of 3-6 animals were euthanized at four-week intervals, two weeks after 2, 4 or 6 immunizations. Groups 5-13 were designed to test the safety of the pTVG-HP vaccine at the same doses to be used in a dose-escalation human trial. Groups 14-16 were designed to test the immunological efficacy in an autologous system, using an identical vaccine but encoding the rat PAP gene in place of the human PAP gene. Groups 1-4 were control groups. Three additional age-matched untreated animals were housed with the treated animals, and one animal from this group was euthanized at the same time points for histopathological tissue controls at these three time points. At the time of euthanization blood was collected for serum chemistries and evaluation for hematologic abnormalities. In addition, tissue samples were taken from multiple sites (blood, cervical lymph nodes, spleen, liver, pancreas, stomach and duodenum, ileum, cecum, colon, bladder, prostate, testis, kidney, heart, lung, brain, skin site of injection, non-immunized skin site, and muscle) for histopathologic analysis and for biodistribution studies. Finally, blood and spleen samples were removed under aseptic conditions for immunological analysis.

Table I: Rat Toxicity Study

| Group | # Animals | Immunizing DNA* | Dose | Adjuvant† | Dose | # Immunizations |
|---|---|---|---|---|---|---|
| 1 | 3 | pTVG4 | 1500 µg | GM-CSF | 5 µg | 2 |
| 2 | 3 | pTVG4 | 1500 µg | GM-CSF | 5 µg | 4 |
| 3 | 3 | pTVG4 | 1500 µg | GM-CSF | 5 µg | 6 |
| 4 | 3 | -- | -- | GM-CSF | 20 µg | 6 |
| 5 | 6 | pTVG-HP | 100 µg | GM-CSF | 5 µg | 2 |
| 6 | 6 | pTVG-HP | 100 µg | GM-CSF | 5 µg | 4 |
| 7 | 6 | pTVG-HP | 100 µg | GM-CSF | 5 µg | 6 |
| 8 | 6 | pTVG-HP | 500 µg | GM-CSF | 5 µg | 2 |
| 9 | 6 | pTVG-HP | 500 µg | GM-CSF | 5 µg | 4 |
| 10 | 6 | pTVG-HP | 500 µg | GM-CSF | 5 µg | 6 |
| 11 | 6 | pTVG-HP | 1500 µg | GM-CSF | 5 µg | 2 |

| | | | | | | |
|---|---|---|---|---|---|---|
| 12 | 6 | pTVG-HP | 1500 µg | GM-CSF | 5 µg | 4 |
| 13 | 6 | pTVG-HP | 1500 µg | GM-CSF | 5 µg | 6 |
| 14 | 3 | pTVG-RP | 100 µg | GM-CSF | 5 µg | 2 |
| 15 | 3 | pTVG-RP | 100 µg | GM-CSF | 5 µg | 4 |
| 16 | 3 | pTVG-RP | 100 µg | GM-CSF | 5 µg | 6 |

[*] pTVG4 (control vector DNA not encoding hPAP) and pTVG-RP (identical to pTVG-HP but encoding the rat homologue of PAP rather than the human homologue) were prepared separately, not under GMP conditions. The pTVG-HP DNA was prepared under GMP conditions, from the same lot (#0553-13-001) to be used for the clinical trial.
[†] The adjuvant used was recombinant rat GM-CSF (Peprotech, Rocky Hill, NJ). Animals received plasmid DNA admixed with GM-CSF and administered intradermally in the ear pinna with a 25-gauge needle and syringe.

## B. Safety Assessment:

Animals were weighed three times per week for evidence of toxicity. As shown in Figure 2, no differences were noted among groups and control animals in weights over the time period of immunization. At the time of euthanization blood was removed for evaluation of hematological parameters (including WBC, hematocrit, and platelet count) and serum chemistries (including creatinine, total bilirubin, alkaline phosphatase, ALT, AST, and amylase). No specific abnormalities were noted in any group with respect to any of the hematological values or serum chemistries. Similarly, biopsy sections of the multiple tissues obtained at the times of euthanization were reviewed by a veterinary pathologist. No significant abnormalities were noted with the exception of mild proximal tubular or inner medullary vacuolation observed in some animals (10/54) treated with the pTVG-HP vaccine. No changes were observed in serum creatinine in these animals. Prostate tissue inflammation and prostate epithelial cell sloughing into the prostatic ducts were observed in some animals, but this was not significantly different in the treated animals (11/54) from controls (1/12) (data not shown).

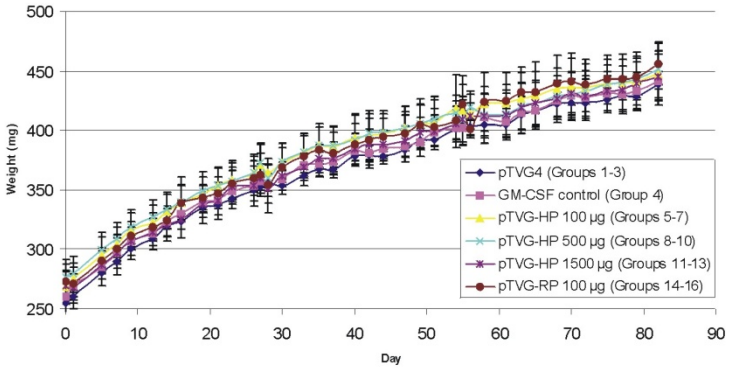



**Figure 2**: Safety assessment: Weights. Animals were weighed three times per week. Shown are the mean and standard deviations of the weights of the animals shown by treatment group per dose level.

## C. Biodistribution studies

Tissue samples obtained at the time of euthanasia were quick frozen and subsequently digested with purification of DNA. Quantitative real time PCR, using oligonucleotide primers specific for the pTVG-HP plasmid, was then used to evaluate the presence of the pTVG-HP plasmid DNA among the tissue specimens. This test was determined to have a sensitivity of detecting 55 copies of plasmid DNA in 1 µg of tissue DNA, or approximately 1 copy in 3,000 cells. Plasmid

DNA could be detected at the site of immunization and regional lymph nodes after two immunizations, and in multiple sites after 4-6 immunizations. No pathologic lesions were noted in association with the detection of plasmid DNA [15].

D. Immunological studies

Blood and spleen tissue were removed at the time of euthanization for immunological studies to evaluate for PAP-specific T-cells, IFNγ secretion, and antibodies. As shown in Figure 3, PAP-specific proliferating CD4+ and CD8+ T-cells were detectable at each dose and after 2, 4, or 6 immunizations, without a specific dose-response relationship. Similarly, PAP-specific secretion of IFNγ could be detected, again without a specific dose-response relationship (Figure 4). PAP-specific IgG antibody responses were detectable, and a dose response was noted with higher doses of plasmid DNA, and with repeated immunizations (not shown).

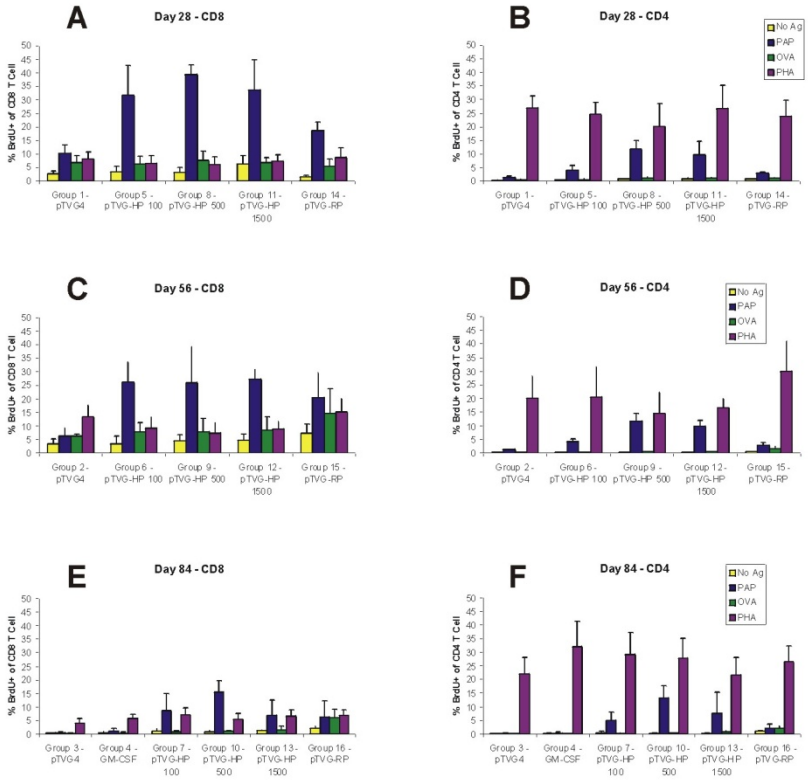



**Figure 3**: CD4+ and CD8+ T-cell proliferative responses. Splenocytes were removed from animals immunized after 2 immunizations (panels A and B), 4 immunizations (panels C and D), or 6 immunizations (panels E and F). Splenocytes were cultured in the presence of 2 µg/ml PAP protein, 2 µg/ml ovalbumin protein, 10 µg/ml PHA, or media only. After 72 hours, cells were pulsed for 8 hours with 10 µM BrdU and then analyzed for BrdU uptake by flow cytometry (BrdU Proliferation kit, Pharmingen). Shown are the % of CD4 T cells (panels A, C and E) or CD8 T cells (panels B, D, and F) that demonstrated BrdU staining. Results show the mean and standard deviation for all animals in the treatment group.

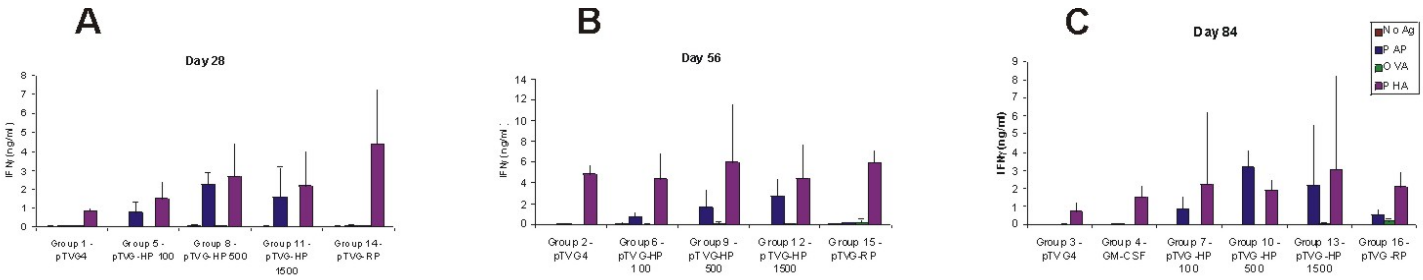



**Figure 4**: IFNγ responses. Splenocytes were removed from animals immunized after 2 immunizations (panel A), 4 immunizations (panel B), or 6 immunizations (panel C). Splenocytes were cultured in the presence of 2 µg/ml PAP protein, 2 µg/ml ovalbumin protein, 10 µg/ml PHA, or media only, as above. After 72 hours, the cell media was removed and assayed for the presence of IFNγ by quantitative ELISA. Shown are the mean and standard deviation of the IFNγ concentrations for all animals in the treatment group.

E. Animal efficacy studies

Male Copenhagen rats were immunized twice intradermally with 100 µg pTVG-HP or a control plasmid not encoding PAP (pTVG4). Fourteen days after the second immunization, rats were challenged subcutaneously with 5 x $10^5$ syngeneic cells from the aggressive Mat-Lu prostate cancer cell line. As shown in Figure 5, rats that received the DNA vaccine encoding either the human PAP antigen had a significant delay in the establishment of tumor compared with control animals.

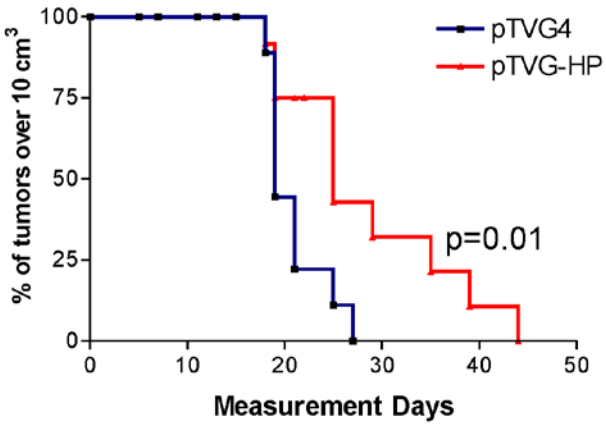



**Figure 5:** Immunization with pTVG-HP elicits protective anti-tumor responses. Male Copenhagen rats were immunized four times with pTVG4 (n=10) or pTVG-HP (n=10). Two weeks after the last immunization, animals were challenged with 5 x $10^5$ Mat-Lu cells administered subcutaneously. Shown are the times at which tumor sizes reached 10 $cm^3$ in size for each treatment group. Statistical comparison of groups was made using a log-rank (Mantel-Haenszel) test.

## 4. Prior Clinical Experience

A phase I/II clinical trial has completed subject accrual in subjects with non-castrate, non-metastatic prostate cancer. The primary objectives of that trial were to evaluate the safety and immunological efficacy of the same pTVG-HP DNA vaccine encoding hPAP when administered to patients with clinical stage D0 prostate cancer [60]. This was a dose-escalation study, with an expanded cohort of subjects treated at the maximum tolerated dose. In the dose-escalation portion, nine subjects were treated in three dose cohorts of 100 µg, 500 µg, or 1500 µg DNA, administered intradermally every two weeks for six total immunizations. 200 µg GM-CSF was co-administered as a vaccine adjuvant with each immunization. 13 additional subjects were treated in an expanded cohort at the 1500 µg DNA dose. Safety evaluation included monthly blood tests (CBC, creatinine, urinalysis, anti-nuclear antibody tests, liver function tests, and serum amylase) and physical examination and review of systems. Subjects were also monitored for one hour after each vaccination for any immediate allergic reactions. No serious adverse events were observed, and no significant laboratory anomalies were observed apart from a single grade 3 amylase that was assessed to not be treatment-related. No other events > grade 2 were observed in any of the 22 subjects treated. Common events observed have been grade 1/2 fevers, chills, and local site reactions lasting typically less than 24 hours. 2/22 subjects experienced grade 2 back pain occurring ~ 45 minutes after treatment and lasting < 10 minutes.

The primary immunological endpoint of this trial was the induction of PAP-specific IFNγ-secreting effector CD8+ T cells detectable two weeks following the final immunization. For this evaluation all study subjects underwent leukapheresis within two weeks before beginning the 12-

week immunization series and again two weeks after the final immunization. With the leukapheresis products, autologous dendritic cells were prepared by culture of flask-adherent monocytes for six days in serum-free medium supplemented with GM-CSF and IL-4. CD8+ T cells, purified from PBMC by negative magnetic bead selection, were cultured with autologous dendritic cells and protein antigens for 96 hours, without *in vitro* restimulation, and ELISPOT was used as a quantitative direct assessment of effector T-cell response. As shown in Figure 6, three patients had a significant increase in the number of PAP-specific IFNγ-secreting CD8+ T cells after immunization compared with pre-immunization, one patient from each dose cohort. In addition, several subjects, including the subject with the greatest magnitude ELISPOT response, were also found to have PAP-specific memory T-cell recall responses as identified by proliferating CD4+ and CD8+ T cells in a 96-hour proliferation assay following antigen-stimulation (Figure 7). Finally, several individuals experienced a prolongation in PSA doubling time over one year following treatment compared with pre-treatment. Overall the median PSA doubling time was 6.5 months in the four months pre-treatment and 8.5 months in the 4-month on-treatment period ($p = 0.033$). Long-term PAP-specific IFNγ-secreting T-cell responses were observed in several patients up to one year after immunization. In addition, one patient went on to receive booster immunizations with 100 µg pTVG-HP at monthly intervals. As shown in Figure 8, booster immunizations were able to augment PAP-specific CD4+ and CD8+ proliferative T cells, as well as PAP epitope-specific CD8+ T cells (Figure 9) months after the initial immunization course. Taken together, these results demonstrate that pTVG-HP can elicit PAP-specific T-cell immune responses, CD8+ T-cell responses in particular, and these responses could be elicited at even the lowest dose (100 µg) of plasmid DNA vaccine tested. In subsequent studies, we have further found that PAP-specific IFNγ responses were detectable in several individuals at multiple time points after immunization, and the presence of this persistent immunity was associated with favorable changes in PSA doubling time. These results, demonstrating immunological efficacy with the 100 µg dose, and safety in at least one individual with ongoing immunization at that dose, form the basis of the current trial design using a similar vaccination induction schedule, but continuing with periodic (quarterly) booster immunizations.

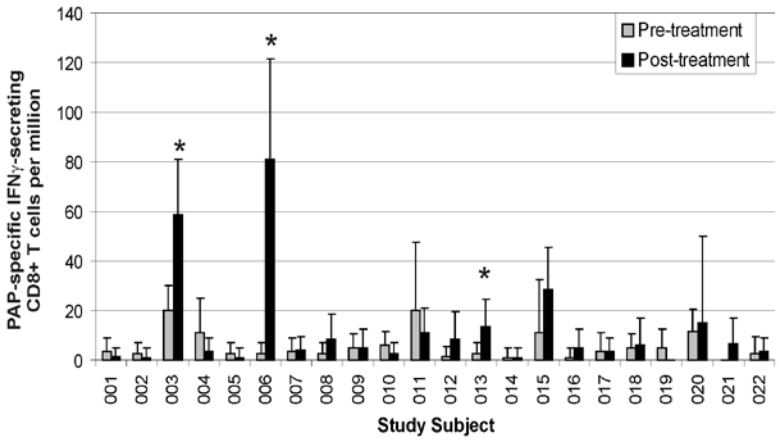



**Figure 6:** Patients with prostate cancer immunized with pTVG-HP develop PAP-specific IFNγ-secreting CD8+ T-cell responses. CD8+ T-cells were isolated from peripheral blood mononuclear cells from patients pre- and 2 weeks post six immunizations with pTVG-HP. These were cultured with autologous dendritic cells and antigen (2 µg/ml PAP protein, 2 µg/ml PSA protein, 1 lfu/ml tetanus toxoid, or 10 µg/ml PHA) for 96 hours without *in vitro* restimulation, and were directly assessed for numbers of CD8+ T cells secreting IFNγ by ELISPOT. The bars indicate the means and standard deviations of the number of spots per million CD8+ T-cells in 8-well replicates for pre-treatment (grey) and post-treatment (black) samples. The asterisks represent statistically significant responses compared with the media-alone control ($p<0.05$, Student's t test).

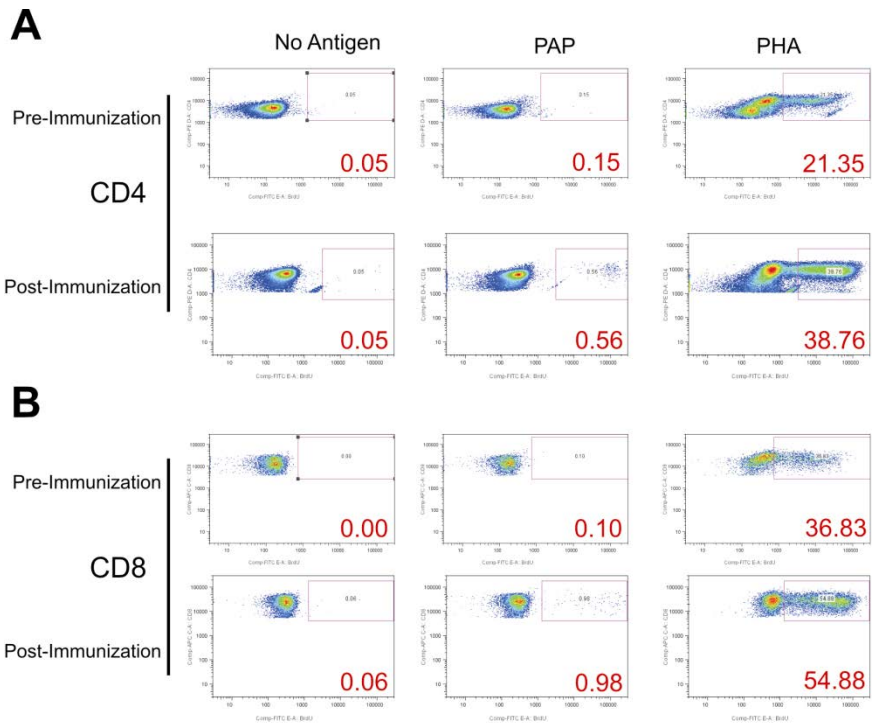



**Figure 7:** Patients with prostate cancer immunized with pTVG-HP develop PAP-specific CD4+ and CD8+ T-cell proliferative recall responses. Peripheral blood mononuclear cells obtained from subject #6 before immunization (top panels) and after six immunizations (bottom panels) were incubated 96 hours in the presence of 2 µg/ml PAP protein (middle panels), 10 µg/ml PHA (right panels), or media only (left panels). T-cell proliferation was assessed by BrdU uptake as described earlier for the rat studies (BrdU Proliferation kit, BD Pharmingen). Shown are the flow cytometry plots, gated on lymphocyte scatter, and by CD4+ (panel A) or CD8+ (panel B) T-cell events. The numbers represent the % of BrdU+ events among CD4+ or CD8+ T-cells.

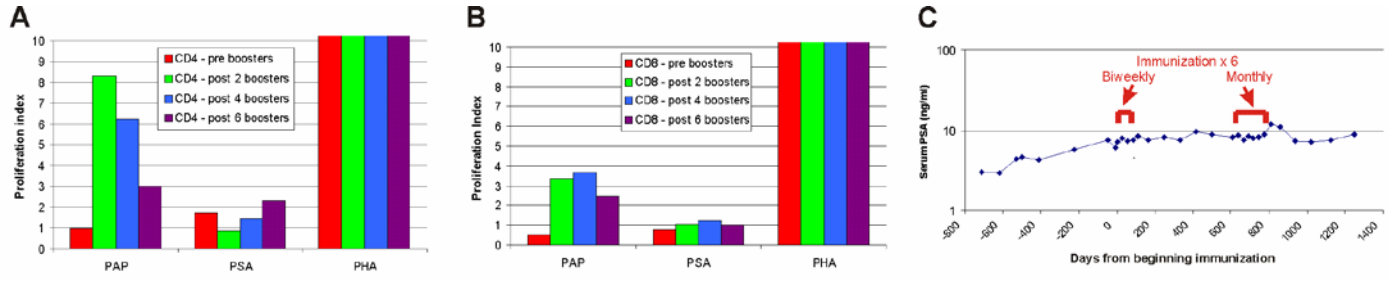



**Figure 8:** PAP-specific cellular immune responses can be boosted months after initial immunization**.** Peripheral blood mononuclear cells were obtained from patient 3 18 months after the initial immunization series (pre boosters), and after 2, 4, or 6 monthly booster immunizations with 100 µg pTVG-HP. Antigen-specific T cells were identified by antigen-specific T-cell proliferation by BrdU incorporation and flow cytometric analysis (BrdU proliferation kit, BD Biosciences). Shown is the proliferation index relative to a media only control of CD4+ T cells (panel A) and CD8+ T cells (panel B). Panel C shows patient's serum PSA levels prior to and after treatment, indicating stable disease.

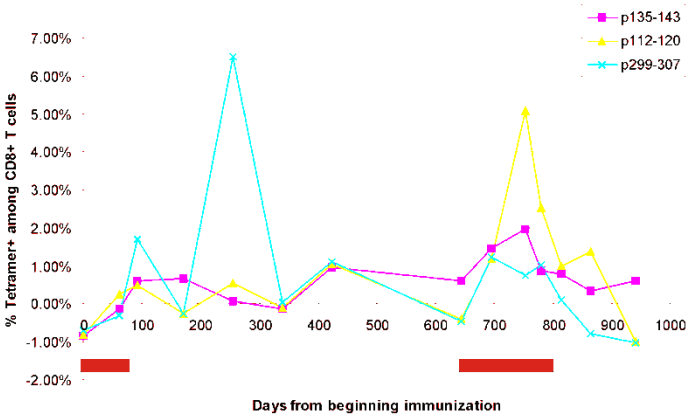



**Figure 9:** PAP-derived epitope-specific cellular immune responses can be detected long-term after initial immunization**.** Peripheral blood mononuclear cells were obtained from an HLA-A2-expressing patient at multiple intervals following an initial 6 biweekly immunization series and 6 monthly booster immunizations 18 months later (red bars indicate timing of these immunizations). Cells were stimulated *in vitro* for 1 week with peptides and assayed for the frequency of epitope-specific T cells among CD3+CD8+ cells by HLA-A2-specific PAP peptide-specific pentamers (ProImmune, Inc.).

## 5. Objectives

A. Primary Objectives

1. To evaluate the 2-year metastasis-free survival of patients with non-metastatic prostate cancer (clinical stage D0) treated with a DNA vaccine encoding PAP, with GM-CSF as an adjuvant, versus patients treated with GM-CSF only.

B. Secondary Objectives:

1. To evaluate if immunization with a DNA vaccine encoding PAP prolongs PSA doubling time in patients with non-metastatic prostate cancer

2. To evaluate the safety and tolerability of the pTVG-HP DNA vaccine administered to patients with clinical stage D0 prostate cancer

3. To evaluate the median progression-free survival, and PSA progression-free survival, of patients with non-metastatic prostate cancer (clinical stage D0) treated with a DNA vaccine encoding PAP, with GM-CSF as an adjuvant, versus patients treated with GM-CSF only.

C. Laboratory Objectives:

1. To determine if immunization with a DNA vaccine encoding PAP elicits antigen-specific T-cell and/or IgG responses

2. To determine if immune monitoring of T-cell responses can be accurately and reproducibly conducted in a multi-center vaccine trial

3. To determine if the development of PAP-specific T-cell immune responses are associated with clinical responses.

D. Exploratory Biomarker Objectives – Expanded Subject Cohort:

1. To determine if baseline immune responses specific for PAP are predictive (or not) for the development of PAP-specific T-cells and/or are associated with 2-year metastasis-free survival in the cohort of patients who receive a DNA vaccine encoding PAP

2. To determine if antigen spread (the development of immune responses to other cancer-associated proteins) is associated with 2-year metastasis-free survival

3. 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

4. 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)

5. 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

## 6. Vaccine Preparation

A. 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. Bacteria from this bank was grown and amplified in culture under kanamycin selection, and two separate GMP-grade lots of plasmid DNA have been prepared (one at the Beckman Research Institute for the initial phase I trial, and one lot at the WCBF). The biological activity of each lot has been tested in rodent studies demonstrating that T-cell immune responses specific for PAP can be elicited *in vivo* following immunization. Lots were 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 containing phosphate-buffered saline only will be used for preparing the placebo/GM-CSF. Vials will be stored at –80°C until the day of use.

B. GM-CSF

GM-CSF (Leukine®, Sargramostim), will be obtained from Berlex Laboratories (Montville, NJ), or other commercial pharmaceutical vendors, and used as a vaccine adjuvant and will be provided free of charge 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 [43, 61]. The use of GM-CSF is associated with little toxicity [44, 48, 54]. 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 $\mu g/m^2$/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 [62]. 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 [54].

C. Vaccine preparation and administration

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

For each of the immunizations/treatments: 0.6 mL of 0.2 mg/mL pTVG-HP (or 0.6 mL phosphate-buffered saline (PBS) from placebo vial) 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/GM-CSF (or PBS/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.

## 7. 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 completed local therapy by surgery and/or ablative 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.
3. Patients must have high-risk clinical stage D0 disease defined by the following:
   - In patients previously treated by prostatectomy, must have evidence of rising PSA with measurements at least two weeks apart, and final serum PSA value must be $\geq$ 2 ng/mL.
   - In patients previously treated with ablative radiation therapy, an absolute increase in serum PSA by at least 2 ng/mL over nadir PSA value after radiation therapy.
   - All patients must have at least four serum PSA values determined over a 12-week-to-six-month period of time prior to study entry from the same clinical laboratory. All available PSA values during this period (up to 6 months) will be used to calculate a PSA doubling time, according to the Memorial Sloan-Kettering Cancer Center nomogram
   - (http://www.mskcc.org/applications/nomograms/prostate/PsaDoublingTime.aspx).
   - The PSA doubling time calculated from this nomogram, up to and including the value obtained at screening, must be < 12 months
4. 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, or *in situ* carcinoma that has been adequately treated.
5. Patients who are sexually active must use a reliable form of contraception while on study and for 4 weeks after the last immunization.
6. ECOG performance score < 2 and life expectancy of at least 4 months.
7. 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, and serum bilirubin $\leq$ 2.0 mg/dl, within 4 weeks prior to first immunization.

8. 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.
9. Willingness to provide blood samples for immune studies, per study calendar, up to one year after study, even if off study.

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 6 months of the first vaccination. Treatment or salvage radiation therapy must have been completed at least 4 weeks prior to 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, and must not have been treated within 12 months prior to screening. 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 7.B.6 or other potential or experimental therapies for prostate cancer must have discontinued these treatments and completed at least a one-month washout prior to beginning treatment.
8. FOR FIRST COHORT ONLY, NOT REQUIRED FOR BIOMARKER COHORT: Patients will be allowed to eat pomegranates and drink pomegranate juice provided that the patient has been taking these for 4 weeks or more with evidence of progressive disease as outlined above (Inclusion Criteria 3.0). These patients should be instructed to continue to take pomegranates and/or pomegranate juice as per the same schedule while on study. Documentation of amount and duration will be captured for those patients taking pomegranates or pomegranate juice. Those patients who have not been taking pomegranates or pomegranate juice prior to study entry will not be allowed to begin these while on study.
9. 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: NaF-PET/CT scan information will NOT be used to determine evidence of metastases for eligibility purposes or for defining disease progression.

10. Patients must not have been treated with a prior vaccine therapy for prostate cancer.
11. Patients must not have known psychological or sociological conditions, addictive disorders or family problems, which would preclude compliance with the protocol.
12. Patients must not have known allergic reactions to GM-CSF.
13. Patients with unstable or severe intercurrent medical conditions or laboratory abnormalities that would impart, in the judgment of the Medical Monitor, excess risk associated with study participation or study agent administration.
14. Patients cannot have concurrent enrollment on other phase I, II, or III investigational treatment studies.

## 8. Experimental Design

This will be a randomized, double-blinded, multi-institutional phase II trial designed to evaluate the effect of serial intradermal vaccinations of a DNA vaccine encoding PAP with rhGM-CSF on time to disease progression as compared to rhGM-CSF alone. 56 patients are to be accrued as part of a DOD sponsored trial. An additional 50 patients (BIOMARKER COHORT), funded by a separate sponsor, will be accrued using the same treatment protocol (same study population, treatment schedule, inclusion/exclusion criteria etc.) Additional biomarker endpoints will be measured in the BIOMARKER COHORT. The treatment, assessment and analysis of these 50 patients and associated data are NOT part of the DOD sponsored trial. There will be no interim analysis conducted after the initial 56 patients have been accrued and the study will remain blinded until the completion of the proposed total number of 106 patients has been completed and all patients have been followed for the primary efficacy endpoint assessment. Study arms will be defined as follows:

Arm 1: pTVG-HP (100 µg) with rhGM-CSF (208 µg) administered intradermally (i.d.) biweekly for 6 total doses, then every 3 months to complete a 2-year treatment period

Arm 2: rhGM-CSF (208 µg) administered intradermally (i.d.) biweekly for 6 total doses, then every 3 months to complete a 2-year treatment period

### 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 3-6 month period of time, will be invited to participate. Subjects will be randomly assigned to a treatment group, stratified by original Gleason score ($\leq 7$ or $> 7$), PSA doubling time ($< 3$ months, 3-6 months, or 6-12 months), and baseline PSA at the time of screening ($\leq 10$ ng/mL or $> 10$ ng/mL). Given the absence of significant adverse events observed in a previous phase I study, no adverse events > grade 2 are anticipated. However, all study patients will be evaluated for evidence of toxicity, and further accrual to either treatment arm will be discontinued if the toxicity rate is deemed to be excessive. A toxicity rate of 20% for Grade 3 events (or Grade > 1 autoimmune 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.

If at any point during the study, the observed toxicity rate within a study arm (after the first 4 patients have been accrued) exceeds one of these thresholds, accrual will be temporarily suspended and the study will be reviewed for safety by the UW DSMC (data and safety monitoring committee) with respect to treatment arm. Operationally, this will occur if any of the following events occurs: Grade ≥ 3 events (or Grade >1 autoimmune events) are present in at least 1 out of 4, 1 out of 5 , 2 out of 10 (or less), 3 out of 15 (or less), 4 out of 20 (or less), 5 out of 25 (or less), 6 out of 30 (or less), 7 out of 35 (or less), 8 out of 40 (or less), 9 out of 45 (or less), 10 out of 50 (or less), 11 out of 53 (or less), of the subjects accrued (within a study arm). Furthermore, accrual will be temporarily suspended if any of the following events occur: Grade 4 events are present in at least 1 out of 4, 1 out of 5, 1 out of 6, 1 out of 7, 1 out of 8, 1 out of 9, 1 out of 10, 2 out of 20 (or less), 3 out of 30 (or less), 4 out of 40 (or less), 5 out of 50 (or less), or 5 out of 53 (or less) of the subjects accrued (within a study arm).

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

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 only will not be used to define metastatic disease or disease progression. The evaluation of these lesions by this method remains investigational, and analysis of changes in these lesions over time by this method will be performed in blinded fashion. 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, blinded to treatment arm, 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 all available values obtained within 6 months 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 ($PSADT_{3-9}$).

## 9. 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, Appendix C) and occurring between the pre-study visit and within one month of the final immunization. For purposes of this study, a Grade 2 autoimmune toxicity will be considered equivalent to a Grade 3 toxicity. If a patient develops Grade 3 toxicity with an attribution of at least possibly related to vaccine, the vaccination schedule will be held until the toxicity resolves to Grade 2 or less (Grade 1 for autoimmune toxicity). There will be no dose reductions due to adverse events. If a patient develops a second Grade 3 event or any Grade 4 event, no further vaccinations will be given and the patient will be removed from study. If the adverse event occurs during the six initial vaccinations, a treatment delay of up to one week will be permitted, with the expectation that the event resolve to $\leq$ grade 1 for retreatment, and that subsequent immunizations will follow at 2-week intervals. A delay > 1 week will result in a skipped dose. If the adverse event occurs during the booster phase, vaccination will be held in the event of toxicity attributed to vaccination. If the toxicity observed is believed to be a result of the immune response generated, a regimen of corticosteroids may be administered, as clinically indicated, and after discussion with the Medical Monitor. The following dose schedule may be used:

Day 1: Intravenous Solu-Medrol at 1 mg/kg IV q12 hr
Day 2: Intravenous Solu-Medrol at 1 mg/kg IV x 1
Day 3-4: Prednisone at 30 mg p.o. bid
Day 5-6: Prednisone at 15 mg p.o. bid
Day 7-8: Prednisone at 10 mg p.o. bid
Day 9-10: Prednisone at 10 mg p.o. qd
Day 11-12: Prednisone at 5 mg p.o. qd

Mild, low-grade fevers and chills, and local skin site reactions, are expected events following immunization and/or treatment with GM-CSF. Grade 1 events will not be treated with anti-inflammatory agents unless they persist 48 hours after an immunization. Constitutional inflammatory events > grade 1, or persisting beyond 48 hours after immunization, will be managed with ibuprofen, if needed, up to 600 mg three times daily.

## 10. Plan of Treatment

The following section describes the schedule for prescreening, vaccine/GM-CSF inoculations, clinical and laboratory evaluations. When a chemistry panel is indicated the following tests are performed: creatinine, glucose, SGOT, bilirubin, alkaline phosphatase, amylase, and LDH. (Note: serum glucose not required in expanded biomarker cohort). 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. Randomization - treatment arm assignment
4. Tetanus immunization: must be at least 24 hours before initial DNA immunization/GM-CSF treatment, and not to be completed until after blood and leukapheresis samples are collected for baseline immune studies.
5. 20 ml peripheral blood (red-top tubes) for sera to evaluate baseline antibody responses, and 100 ml peripheral blood (green-top heparinized tubes) for T-cell response evaluation. Must be collected prior to tetanus immunization.
6. BIOMARKER COHORT ONLY: Leukapheresis (~1.0 blood volume, 100 mL collection) for baseline T-cell response evaluation (can be performed +/- 7 days of this study visit, and replaces 100-ml peripheral blood requirement in 10.B.5 above). NOTE: This is required at UWCCC site only. As an alternative to leukapheresis, 200 ml peripheral blood (green-top heparinized tubes) can be collected at other clinical sites. This is for pre-treatment T-cell response evaluation and immune biomarker studies and must be collected prior to tetanus immunization.
7. BIOMARKER COHORT ONLY: NaF PET/CT (can be performed +/- 14 days of this screening time point). NOTE: This study is for research purposes only, will not be used to determine eligibility, and will not be used for clinical response evaluation or treatment decisions. This study will only be performed at designated sites (UWCCC and JHU).

C. First immunization/GM-CSF 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 for serum PSA and PAP (to be obtained prior to administration of immunization/GM-CSF). May be done up to 3 days in advance of Day 1.
3. Immunization as described in sections 6 and 8 above. Specifically, subjects will receive immunizations intradermally on the deltoid area of the lateral arm (preferentially left arm) with 0.25 cc administered at each of two adjacent sites.
4. Following vaccinations, the subjects will be observed for 60 minutes for unanticipated adverse events.

D. Second, third, fifth and sixth biweekly treatment visits (days 15, 29, 57 and 71, each +/- 2 days)

1. Symptoms assessment
2. Immunization/GM-CSF and post-treatment monitoring as described above

E. Fourth immunization/GM-CSF treatment visit (day 43 +/- 2 days)

1. Physical examination, including vital signs, symptoms assessment, and ECOG performance score
2. Blood draw for CBC, chemistry panel, serum PSA and PAP (to be obtained prior to administration of immunization/GM-CSF)
3. Immunization/GM-CSF and post-treatment monitoring as described above

F. End of initial vaccination series evaluation (2 weeks after sixth vaccination, i.e. month 3 (day 85 +/- 2 days)

1. Physical examination, including vital signs, symptoms assessment, and ECOG performance score.
2. Blood draw for CBC, chemistry panel, serum PSA and PAP (to be obtained prior to administration of immunization/GM-CSF)
3. 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
4. Immunization/GM-CSF and post-treatment monitoring as described above
5. BIOMARKER COHORT ONLY: NaF PET/CT (can be performed +/- 14 days of this 3-month immunization/GM-CSF visit). NOTE: This study 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 (UWCCC and JHU).

G. Day 127 (+/- 10 days) – BIOMARKER COHORT ONLY

1. Blood draw for serum PSA and PAP (does not require a clinic visit; the purpose of this draw is to provide PSA values to determine a PSA doubling time

corresponding with the 3-6 month period over which NaF PET/CT scans are obtained).

H. Quarterly booster immunizations (every 3 months (days 169, 253, 337, 421, 505, 589, each +/- 7 days) – beginning at month 6 immunization – up to 6 total (to complete 2-year treatment course)

1. Physical examination, including vital signs, symptoms assessment, and ECOG performance score
2. Blood draw for CBC, chemistry panel, serum PSA and PAP (to be obtained prior to administration of immunization/GM-CSF)
3. Immunization/GM-CSF and post-treatment monitoring as described above
4. CT scan abdomen/pelvis and bone scan – performed prior to second quarterly booster/treatment (i.e. 6 months after first immunization/treatment), and every 6 months (every other visit) thereafter. Can be performed +/- 7 days of study visit.
5. 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 (Quarterly for 1st year following initial series, 3 time points, only)
6. BIOMARKER COHORT ONLY: Leukapheresis (~1.0 blood volume, 100 mL collection) at month 6 evaluation for T-cell response evaluation (can be performed +/- 14 days of this study visit). This leukapheresis replaces 100-ml peripheral blood requirement in 10.G.5 above for the month 6 time point. NOTE: As an alternative to leukapheresis, 200 ml peripheral blood (green-top heparinized tubes) can be collected. This is for T-cell response evaluation and immune biomarker studies.
7. BIOMARKER COHORT ONLY: NaF PET/CT (to be performed +/- 7 days of the 6-month immunization/GM-CSF visit). NOTE: This study is for research purposes only and will not be used for clinical response evaluation or treatment decisions. This study will also be performed only at designated sites (UWCCC and JHU).

I. End of Study Visit or 2-year (Day 672 +/- 7 days) Off-Study Visit

1. Physical examination, including vital signs, symptoms assessment, and ECOG performance score
2. Blood for CBC, chemistry panel, serum PSA, serum PAP
3. 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. [Note: these specimens not required if subject off study prior to 12 month visit. However, these will continue to be drawn at quarterly intervals up to 12 months after screening/randomization in all subjects, even if off-study prior to 12 months.]

J. Follow up for Subjects Who Come Off Study Prior to 12-Month Visit

1. 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 – to take place quarterly (days 85, 169, 253, 337) for first year following initial series up to 12 months after screening/randomization.

NOTE: The study will be complete at the time the last off-study visit is complete for the last individual on treatment.

K. Long-term Follow-Up

1. Subjects will be contacted by telephone annually for up to 2 years 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

## 11. 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 (120 mL) will be collected pre-immunization (or by leukapheresis in the biomarker cohort at this time point and at 6 months), after the initial biweekly immunization/treatment series, at quarterly intervals up to one year, and then at the final study visit, for immunological monitoring at the clinical trial sites. From the heparinized blood or leukapheresis procedure, 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. Samples drawn at non-UWCCC sites will be prepared as above, may be used immediately for immunological analysis, and will be stored at the research sites to be subsequently shipped in batch to the University of Wisconsin for central analysis. All of the immunological analysis will be conducted by laboratory personnel blinded to the study treatment arm of individual subjects. IFNγ and granzyme B ELISPOT analysis, and PAP-specific T-cell proliferation, may be conducted at the individual centers for subjects treated at those sites. Similar studies will be performed for tetanus-specific immune responses, as a control for immunization. Additional samples from those subjects, and from all samples from all other clinical trial sites, will be shipped to the UWCCC site for duplicate analysis. In this way, the reproducibility of these specialized assays can be determined. Other methods of T-cell response to PAP and other human tissue antigens may be used.

B.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. Results will be determined as either the number of spots per well or by T-cell frequencies, 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 $10^6$ PBMC. Comparison of experimental wells with control, no antigen, wells, and with pre-treatment experimental wells, will be performed using a Student's t test, with $p < 0.05$ defined as a significant T-cell response.

B.2. Assessment of PAP-specific memory T-cell immunity

Ability of T-cell lines to proliferate in response to antigenic stimulation: T-cell proliferation in response to antigen stimulation as a measure of memory T-cell responses will be assessed by a standard 5-day BrdU incorporation assay or by a PKH26 dye dilution assay. As an example, T-cell cultures with and without antigens will be established in replicates using $2x10^5$ peripheral blood mononuclear cells (PBMC)/well, plated in 96-well round bottom microtiter plates (Corning, Cambridge, MA), in media consisting of RPMI 1640 (Gibco) and supplemented with L-glutamine, penicillin/streptomycin, ß-mercaptoethanol and 10% human AB serum (ICN Flow,

Costa Mesa, CA). Antigens may include 1 μg/ml of the PAP peptide library, 2 μg/ml PAP (Research Diagnostics Inc., Flanders, NJ), 2 μg/ml PSA (Research Diagnostics Inc.), 2 μg/ml ovalbumin, 2 μg/ml streptokinase, 2 μg/ml human thyroglobulin, 250 ng/ml tetanus toxoid, and 2.5 μg/ml phytohemaglutinin (PHA). After 5 days of culture at 37°C/5% $CO_2$, wells will be pulsed with 10 μM BrdU for 8-10 hours, and then the % of CD4+ or CD8+ T-cells proliferating in response to antigen will be determined by flow cytometry (BrdU Flow kit, BD Pharmingen). Ovalbumin and human thyroglobulin may be included as negative controls, and streptokine and PHA (a non-specific T-cell mitogen) may be included as positive controls. Other cell surface markers will be stained to characterize the memory phenotype of proliferating cells (CD45R0, CCR7). A proliferation index will be determined for each antigen as the ratio of the % of CD4+ or CD8+ T-cells with BrdU uptake following antigen-stimulation to the % of CD4+ or CD8+ T-cells with BrdU uptake in media only. Alternatively, T-cell precursor frequency will be determined by analysis of PKH26 dye dilution (ModFit software), and used as a quantitative measure of antigen-specific cellular proliferation.

B.3. 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 [23]. 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 4°C. 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 [23].

B.4. Assessment of antigen-specific regulatory immune responses

*Trans-vivo* delayed-type hypersensitivity (tvDTH) evaluation: 7.5-10 x $10^6$ PBMC obtained from patients prior to and after (6 months) immunization will be co-injected into the footpads of 6-to-8-week old SCID mice with 1 μg of PAP protein (Fitzgerald Industries, Acton, MA), or tetanus toxoid (TT/D; Aventis Pasteur, Bridgewater, NJ) as a recall antigen, versus phosphate-buffered saline (PBS) alone as a negative control. Antigen–driven swelling will be determined as previously described [63]. DTH reactivity after 24 hours will be shown as the change in footpad thickness in multiples of $10^{-4}$ inches, measured using a dial thickness gauge (Mitutoyo, Japan), and net swelling will be defined as the antigen-specific swelling subtracted for the contribution obtained with PBMC plus PBS. To determine the effect of neutralizing antibodies, PBMC will be mixed with 1 μg of PAP antigen and 25 μg of either control IgG or rabbit anti-human TGF-β, goat anti-human IL-10 (R&D Systems, Minneapolis, MN), or 1 μg of mouse anti-human CTLA-4 monoclonal Ab (clone AS32, Ab Solutions, Mountain View, CA) and injected into the footpads of SCID mice as above. The extent of bystander suppression, defining an antigen-specific regulatory response, will be measured as inhibition of recall antigen (tetanus) response in the presence of PAP antigen (or prostate-specific antigen (PSA) as a negative

control) and calculated as previously described [64]. Given the nature of the testing and the requirement for SCID mice, data will be shown derived from single measurements, but with key experiments repeated 2-3 times for confirmation.
REPORTING AND RESPONSE DEFINITION: These are not strictly quantitative assays. An antigen-specific (PAP-specific) regulatory response for these purposes will be defined as an inhibition of a recall antigen (tetanus toxoid) DTH response by $\geq$ 50% when performed in the presence of PAP; i.e. a DTH response in the presence of PAP+TT that is $\leq$ 50% of that obtained with TT only.

B.5. 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 [65, 66, 67]. IgG specific for 126 antigens, including 29 cancer-testis antigens [68, 69] and 97 prostate antigens frequently immunologically recognized [70, 71, 72, 73] will be identified by screening a high-density phage array expressing these individual antigens, as we have previously reported [65, 67, 68]. An alternative to this approach may include the evaluation of responses to panels of protein 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, 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 Response

See Section 13 for further details. Serum PSA doubling-time response will be considered a secondary endpoint. The pre-treatment serum PSA doubling time will be calculated from all serum PSA values available from the same clinical laboratory using the same assay for the three-six month period 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 day 85 (month 3) to the day 253 (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 accrued to the Biomarker Cohort at designated clinical trial sites will be assessed at baseline, 3 months and 6 months 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.

## 12. Reporting Adverse Events

Please see section 15 below, which contains in detail the policy and procedures for patient safety monitoring and adverse event reporting.

A. Definition

An adverse event is defined as any unfavorable and unintended sign (including abnormal laboratory finding unless they are grade 1 and deemed clinically insignificant), symptom or disease temporally associated with a medical treatment or procedure, regardless of whether it is considered related to the treatment or procedure. Note that for this study grade 1 laboratory values deemed clinically insignificant will not be reported as adverse events. Adverse events are categorized as definite, probable, possible, unlikely or unrelated in relation to the medical treatment or procedure performed. 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. All nonserious and serious adverse events will be collected from time of informed consent through 30 days after the final dose of study drug or any time after the 30 days if felt to be at least possibly related. Serious adverse events are any events occurring that result in any of the following outcomes:

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

Adverse events are classified by organ system and graded by severity according to the current NIH Common Terminology Criteria, 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

B. Procedure for Reporting Adverse Events

Regulations defining the responsibilities for reporting adverse events are defined by the National Cancer Institute Cancer Therapy Evaluation Program (NCI CTEP, http://ctep.info.nih.gov). Please see the details of adverse event reporting in Section 15.D

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 treatment-related will be repeated at appropriate intervals until the course is determined or a return to normal values occurs. Information will be recorded as noted above.

## 13. Statistical Considerations

**Experimental Design:**
In this multi-center, randomized, controlled phase II study, the primary endpoint is the 2-year metastasis-free survival probability. Participants will be randomly assigned in 1:1 fashion to either a PAP-encoding DNA vaccine with rhGM-CSF as an adjuvant or placebo with rhGM-CSF. Time to metastasis is measured from randomization date to the metastasis confirmation date (via radiographic imaging). Metastasis-free patients at 24 months of follow-up are considered censored at the date of most recent evaluation. For those participants who have not already met criteria for radiographic imaging progression, metastasis assessment will be made by reviewer assessment of the CT images and bone scans obtained at the 24-month time point.

**Power Calculation:**
The primary efficacy endpoint is the 2-year metastasis-free survival probability. Slovin et al reported a median MFS of 19 months in patients with rising prostate-specific antigen values following surgery or radiation therapy [5]. Based on these results, it is anticipated that the 2-year MFS rate in the placebo/GM-CSF arm will be approximately 40%. For the DNA vaccine encoding PAP with rhGM-CSF arm, it is anticipated that the 2-year MFS rate is at least 70%, based on promising efficacy results from the previous phase I/II trial with the same DNA vaccine in high-risk stage D0 prostate cancer patients. A sample size of 56 subjects (28 on each arm) with stage D0 prostate was initially proposed for this trial, and this cohort of patients (accrued at UW and UCSF sites only) is specifically being evaluated under the original DOD sponsor. An additional cohort of 50 subjects (25 patients on each arm) with the same inclusion/exclusion criteria will be treated in identical fashion, but will undergo further evaluation as described above for the biomarker analyses. This cohort of subjects is not under the oversight of the original DOD sponsor, rather a separate corporate sponsor (MVI). Hence, the proposed total sample size for this extended trial is 106 subjects (53 per arm). There will be no interim analysis conducted after the initial cohort of 56 patients has been accrued. The sample size of the original study (56 patients) was chosen to detect the anticipated difference of 40% versus 70% with 80% power at the relatively large one-sided 10% significance level. For a phase II trial such as the proposed study, it is desirable to limit the type II error to be 10% or less. The proposed total sample size 106 of the extended trial will be adequate to warrant sufficient power (>90%) to detect the anticipated difference of 40% versus 70% in the 2-year MFS rate at the one-sided 5% significance level, assuming an attrition rate of up to 15%. After the last subject is randomized, each subject will be followed for at least 24 months for metastasis. The following table shows the attainable power levels to detect various differences in the 2-year MFS rates between the two study arms at the one-sided 5% significance level, assuming a sample size of 53 subjects per arm with an attrition rate of 15%.

Table: *Attainable power levels to detect differences in the 2-year MFS rates between study arms at the one-sided 5% significance level, assuming a sample size of 53 subjects per arm with an attrition rate of 15%*

| | 2-year MFS rate in DNA vaccine encoding PAP/rhGM-CSF arm | | | | |
|---|---|---|---|---|---|
| 2-year MFS rate in placebo/GM-CSF arm | 0.66 | 0.68 | 0.70 | 0.72 | 0.74 |

| | | | | | |
|---|---|---|---|---|---|
| 0.38 | 0.87 | 0.91 | 0.94 | 0.96 | 0.97 |
| 0.40 | 0.82 | 0.87 | 0.91 | 0.94 | 0.96 |
| 0.42 | 0.77 | 0.82 | 0.87 | 0.91 | 0.94 |
| 0.44 | 0.70 | 0.77 | 0.82 | 0.87 | 0.91 |

Hence, if the true 2-year MFS rate in the Placebo/GM-CSF arm is 40% or less and the true 2-year MFS rate in the DNA vaccine encoding PAP/rhGM-CSF arm is greater than 70%, then the proposed sample size of 53 subjects per arm will provide at least 91% power to detect the difference in 2-year MFS rates between arms at the one-sided 5% significance level. Analogously, a difference of 40% versus 72% will be detected with 94% power while a difference of 40% versus 66% will provide 82% power.

**Study Endpoints:**

*Primary endpoint*: Metastasis-free survival (MFS)

*Secondary endpoints*:
1) Median PSA doubling time in patients with clinical stage D0 prostate cancer, in each study arm, prior to and after treatment. Pre-randomization serum PSA doubling time (PSA DT) is calculated from all available serum PSA values provided by the same clinical laboratory using the same assay three to six months prior to randomization. This value will be obtained using the Memorial Sloan-Kettering Cancer Center nomogram (http://www.mskcc.org/mskcc/html/10088.cfm). Post-randomization serum PSA doubling time will be calculated from day 1 to the 24 month follow up (end of study), and from month 3 to month 9 in the same fashion using:

(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 ($PSADT_{3-9}$).

A two-fold increase or higher in the post-treatment ($PSADT_{0-24}$, $PSADT_{3-9}$) versus pre-treatment PSA doubling time and with a value greater than 15 months, will be considered a PSA doubling time "response" for an individual patient.

2) Safety and tolerability of the PAP-encoding DNA vaccine with GM-CSF as an adjuvant in patients with clinical stage D0 prostate cancer.

3) Median progression-free survival, and PSA progression-free survival, in patients with clinical stage D0 prostate cancer, in each study arm. Progression is defined as the appearance of metastases by radiographic imaging using 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), and median progression will be calculated from the date of randomization to the first evidence of metastatic disease. Progression will also be defined as the off-study date for those patients coming off-study prior to the 24-month time point to pursue other treatments, without evidence of metastasis. PSA progression is defined as a PSA value that is at least twice as large as its baseline (day 1) counterpart. PSA progression must be confirmed with a repeat PSA blood test at least two weeks later. The time to PSA progression will be defined as the interval between day 1 and the first time when a PSA value at least twice as large as the baseline value, also confirmed in the subsequent repeat PSA test, has been observed. All post-treatment PSA values have to been obtained from the same laboratory using the same assay. PSA progression-free survival times of subjects not experiencing PSA progression by the end of the follow-up period (24 months after the last patient has been randomized) will be censored. If lost-to-follow-up, the PSA progression-free survival time will be censored at the last available serum PSA measurement date. Similarly, if a patient experiences disease progression due to the appearance of metastases, PSA progression-free survival time will be censored at the date of the last available serum PSA measurement at the off-study time-point.

*Laboratory endpoints*:
An immune response will be defined as a PAP-specific T-cell frequency (by ELISPOT) or proliferation precursor frequency (by T-cell proliferation studies) at the completion of treatment that is at least 3-fold higher than the baseline T-cell frequency, and statistically different from media-only controls. An IgG immune response will be defined as a titratable PAP-specific IgG antibody response by ELISA, with titer of at least 1:100, detectable after immunization that was not detectable pre-immunization.

*Biomarker endpoints*:
1) Baseline immune responses specific for PAP (by antigen-specific T cell proliferation, cytokine release or regulatory response by tvDTH) will be evaluated in all subjects, blinded to treatment arm.
2) Sera obtained at baseline and at 6 months will be used to identify whether antibody responses are elicited to other prostate-associated antigens.
3) Quantitative Total Bone Imaging (QTBI) will be evaluated in a blinded fashion at baseline, 3 months and 6 months. QTBI parameters will include: Standardized Uptake Values (SUV): SUVmax, SUVtotal, SUVmean, SUVpeak and volume. These image data will be used:

1. 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)
2. To determine whether NaF18F- PET/CT reveals different patterns of disease progression with respect to treatment arm.
3. 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 ($PSADT_{0-24}$, $PSADT_{3-9}$).

**Statistical Analysis Plan**

Analysis populations:

*Intent-to-Treat (ITT) Population*: The primary population for the efficacy analysis will be the ITT population, defined as all patients who have been randomized and have received at least one dose of pTVG-HP/rhGM-CSF or rhGM-CSF treatment.

*Evaluable Efficacy Population*: The secondary population for the efficacy analysis will be the Evaluable Efficacy Population. The Evaluable Efficacy Population consists of all patients who have been treated according to protocol, i.e., all inclusion/exclusion criteria are satisfied, adequate compliance with treatment (having received all pTVG-HP/rhGM-CSF or rhGM-CSF treatment until a study endpoint was reached), and have completed all radiographic assessment (until a study endpoint was reached).

*Safety Population*: The Safety Population for assessment of safety consists of all patients who received at least one dose of pTVG-HP/rhGM-CSF or rhGM-CSF treatment.

Primary endpoint:
The 2-year MFS rates, as defined by RECIST 1.1 criteria, will be summarized in tabular format, stratified by study arm. The stratified Mantel-Haenszel test will be used to compare the 2-year MFS rates between arms. The stratification factors will include: Study cohort (initial vs. biomarker cohort), Gleason score ($\leq 7$ or $> 7$), PSA doubling time ($< 3$ months, 3-6 months, or 6-12 months), and baseline PSA at the time of screening ($\leq$ 10 ng/mL or $>$ 10 ng/mL). The survival function for metastasis-free survival will be estimated using the Kaplan-Meier method, and the comparison between the two arms will be made using a stratified logrank test at the one-sided 5% significance level. As available, meaningful quantiles of the metastasis-free survival time obtained based on Kaplan-Meier curves will be reported in each study arm. As an exploratory analysis of MFS, both univariate and multivariate Cox proportional hazards models will be used where study cohort (initial vs. biomarker cohort), Gleason score, baseline PSA doubling time and baseline PSA value will be included as covariates [62].

Secondary endpoints:
1) PSA DT response rates (based on both ($PSADT_{0\text{-}24}$ and $PSADT_{3\text{-}9}$) will be obtained for each study arm and will be summarized by standard descriptive statistics: means, standard deviations, medians and ranges. Fold changes in PSA DT from baseline to post-treatment (both ($PSADT_{0\text{-}14}$ and $PSADT_{3\text{-}9}$) will be calculated and the corresponding standard errors will be obtained by fitting a linear regression model (with log(PSA). PSA doubling times in the two study arms will be compared using a weighted (by the inverse of the standard errors) Wilcoxon Rank Sum test and a 95% confidence interval for the difference will be constructed. A logistic regression model will be used to assess the treatment effect on PSA doubling time response rates, while adjusted for key prognostic factors that include Gleason score, baseline PSA doubling time and baseline PSA.

2) Adverse events and toxicities observed will be categorized according to the most recent version of the NCI Common Terminology Criteria for Adverse Events. Each toxicity/adverse event observed will be assigned an attribution: unrelated, unlikely, possible, probable, or definite. The number and severity of observed toxicities will be analyzed descriptively in tabular format. Ninety-five percent confidence intervals of toxicity rates will be constructed for each study arm. Fisher's exact test will be used to compare toxicity rates between the two study arms.

3) As available, both median PSA PFS (PPFS) and progression-free survival (PFS) in the pTVG-HP DNA vaccine/GM-CSF arm will be compared to their counterparts in the placebo/GM-CSF arm using quantile regression methods for censored data [74]. PPFS and PFS will be summarized in each arm using Kaplan-Meier curves. For both PPFS and PFS, univariate and multivariate Cox proportional hazards models stratified by the aforementioned stratifying factors will be used to compare the two trial arms. The multivariate Cox proportional hazards model will adjust for treatment group and age at randomization.

Biomarker endpoints:

An immune response by ELISPOT or antigen-specific proliferation that is at least 3-fold higher than the baseline T-cell frequency will be analyzed as binary data, and its response rate estimated with a 95% confidence interval for each trial arm. An IgG immune response will also be analyzed as a binary data, and its response rate estimated with a 95% confidence interval for each trial arm. The comparison of immune responses between the two trial arms will be conducted using a stratified Mantel-Haenszel test.

It will be evaluated whether the presence of specific immune responses present at baseline are associated with the development of Th1-biased immune responses after treatment and with 2-year metastasis-free survival. Specifically, in order to determine if baseline immune responses specific for PAP are predictive (or not) for these immunological and clinical endpoints, logistic regression analysis and Cox proportional hazard regression analysis will be conducted.

The detection of antigen spread to other prostate associated antigens, and the identification of specific antigens recognized, will be analyzed descriptively. In addition, logistic regression analysis will be conducted to evaluate whether antigen spread is associated with the generation of PAP-specific immune responses elicited as a result of treatment. The dependent variables will be the detection of IgG responses to $\geq$ 1 "off-target" antigen at 6 months (and not present pre-treatment) while treatment arm and 6-month PAP-specific immune responses (no vs. yes) will be included as covariates. The results will be reported in terms of odds ratios and the corresponding 95% confidence intervals.

Results from the QTBI analysis will be used to determine whether individuals with identifiable bone lesions exhibit early disease progression (within 1 year) by standard bone scintigraphy and/or CT scans, and whether the time to metastatic disease is different with respect to treatment group. Specifically, the association between baseline QTBI parameters and time to disease progression will be evaluated using Cox proportional hazard analysis. Analogously, logistic regression analysis will be conducted to evaluate presence of lesions detected by NaF PET/CT at baseline is associated with early disease progression (within 1 year) rate.

Furthermore, % changes in QTBI parameters from the baseline to the 3 and 6 months assessment will be calculated and summarized in terms of means, standard deviations, medians and ranges, stratified by treatment arm. A linear mixed effects model with subject specific a random effects will be used to evaluate the association between changes in QTBI parameters and changes in PSA doubling time.

Due to the exploratory nature of the biomarker evaluations proposed in this trial, no adjustments will be made for multiple comparisons. Except for the primary endpoint which will be compared

at the one-sided 5% significance level, all other comparisons will be made using a two-sided test at a significance level 0.05 without adjustment for multiplicity. Data analysis will be conducted using SAS software (SAS Institute, Carey NC), version 9.3 or greater.

## 14. 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) and the United States Army Medical Research and Materiel Command's (USAMRMC) Office of Research Protections (ORP), Human Research Protection

Office (HRPO) grant 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 Investigators 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.

## 15. Data and Safety Monitoring Plan

A. Oversight and Monitoring Plan

For this trial there will be a designated independent medical monitor. The medical monitor (Dr. Albertini, MD) is independent of the investigative team and possesses sufficient educational and professional experience to serve as the patient advocate. The medical monitor will have the authority to stop a research study in progress, remove individual volunteers from a study, and

take whatever steps are necessary to protect the safety and well-being of research volunteers until the IRB can assess the medical monitor's report. The medical monitor will review all unanticipated problems involving risk to subjects or others, serious adverse events and subject deaths and provide an unbiased written opinion regarding the relationship to study participation and the outcome of the event or problem. The monitor must also indicate whether he concurs with the details of the report provided the UWCCC Local Principal Investigator.

The UWCCC Data and Safety Monitoring Committee (DSMC) is responsible for monitoring data quality and subject safety for all UWCCC clinical studies. A summary of DSMC activities follows:

- Review of all clinical trials conducted at the UWCCC for data integrity and safety
- Review of all serious adverse events requiring expedited reporting as defined in the protocol
- Review of reports generated by the UWCCC data quality control review process
- Submit recommendations for corrective action to the Clinical Research Committee (CRC)
- Notify the Study Chair of the DSMC recommendation to the CRC
- The committee ensures that notification is provided to external sites participating in multiple-institutional clinical trials coordinated by the UWCCC of serious adverse events requiring expedited reporting.

The following information describes the data and safety monitoring plan specific to the UWCCC clinical trial site:

**Phase II Trials**

For patients enrolled at the UWCCC, data related to these trials are discussed at regularly scheduled Disease Oriented Working Group meetings where the result of each subject's treatment is discussed and the discussion is documented in the minutes. The discussion will include the number of subjects, significant toxicities as described in the protocol, dose adjustments, and responses observed. Twice yearly summaries will be submitted to the Data and Safety Monitoring Committee for review.

B. Review and Oversight Requirements

Adverse events requiring expedited reporting (as described in the protocol) that occur at a participating institution will be reported by phone within 24 hours to the UWCCC GU Oncology Office (phone: 608-263-7107; fax: 608-265-5146, email: uwcccgu@medicine.wisc.edu). The UWCC GU Oncology Office will be responsible for reporting requirements listed below in sections B.1 and B.2

B.1. Serious Adverse Event – Reported Within 24 Hours :

Serious Adverse Events requiring expedited reporting within 24 hours (as described in the protocol) will also be reported to the Data and Safety Monitoring Committee (DSMC) via saenotify@uwcarbone.wisc.edu and to the Principal Investigator and Medical Monitor within one business day. Confirmation that all appropriate parties were notified will be done at this time. Hardcopies or electronic versions of NCI ADEERS form(#3500) and/or any other documentation available at that time will also be reviewed by the Committee Chair who will determine if immediate action is required. Within ten working days all subsequent SAE

documentation that is available will be submitted with a completed UWCCC SAE Routing form to the designated parties on the form and Committee Chair who will determine if further action is required. All information will be tracked in the UWCCC Oncore database.

If the SAE occurs on a multiple-institutional clinical trial coordinated by the UWCCC, the Outreach Coordinator will ensure that all participating sites are notified of the event and resulting action within one working day of the determination.

In addition, it will be the responsibility for UWCCC GU staff to promptly report all unanticipated problems involving risk to subjects or others, serious adverse events related to participation in the study, and subject deaths related to participation in this study that occur at any of the participating sites to the USAMRMC's Office of research Protections, Human Research Protection Office. This reporting requirement and other reporting requirements for UWCCC are detailed in Section 18: Protocol Addendum Specific for UWCCC Site for USAMRMC Requirements.

NOTE: The reporting requirements to the USAMRMC pertain only to the 56 patients initially accrued at UWCCC and UCSF for whom the USAMRMC/DOD serves as sponsor.

### B.2. Serious Adverse Event – Reported within 10 Days

Serious Adverse Events requiring expedited reports in writing within 10 working days (as described in the protocol) will be sent to the UWCCC DSMC Coordinator via datasafetymonitoring@uwcarbone.wisc.edu. Hardcopies or electronic versions of NCI ADEERS form (#3500) or other required forms will be submitted along with a copy of the SAE Routing Form. The Committee Chair will review these forms and determine if further action is required. This information will be tracked in the UWCCC ONCORE database.

If the SAE occurs on a multiple-institutional clinical trial coordinated by the UWCCC, the Outreach Coordinator will ensure that all participating sites are notified of the event and resulting action within one working day of the determination.

### B.3. Study Progress Review

Cumulative reports of serious adverse events requiring expedited reporting and any new serious adverse event requiring expedited reporting are also reviewed at the DSMC committee's meetings at 6-month intervals.

An overall assessment of accrual, toxicities as described in the protocol, and responses will enable the committee members to assess whether significant benefits or risks are occurring that would warrant study closure. This information is provided by Disease Group meeting minutes, internal audit and/or response review reports. The committee may request external DSMB reports or further information from the Disease Groups, or Study Chair.

The Data and Safety Monitoring Committee recommendations for modifications to the trial are forwarded to the Clinical Research Committee (CRC), composed of Cancer Center senior leaders that oversee all aspects of clinical research conducted at the UWCCC and makes final decisions on all issues related to clinical trials. The Study Chair is notified of this recommendation in order that he/she may alert all investigators, at the UWCCC and at external sites involved in the

trial, about the potential action. At this time the Study Chair may submit to the CRC additional information that could affect the Committee's decision. The CRC will notify the Study Chair if they concur with the Data and Safety Monitoring Committee recommendations, including suspension or closure. The Study Chair will notify all investigators involved with the study at UWCCC and external sites, the IRB, the sponsor and the funding agency and provide written documentation of these notifications to the CRC.

C. Expedited Reporting of Adverse Events

Adverse events requiring expedited reporting that occur at a participating institution will be reported by phone within 24 hours to the UWCCC GU Oncology Office (phone: 608-263-7107; fax: 608-265-5146, email: uwcccgu@medicine.wisc.edu). The UWCC GU Oncology Office will be responsible for reporting requirements listed below:

Depending on the nature, severity, and attribution of the serious adverse event an ADR report will be phoned in, submitted in writing, or both according to Table D-2 below. Expedited Serious Adverse Events must also be reported to the UWCCC Data and Safety Monitoring Committee Chair within one working day of the event. All expedited serious adverse events must also be reported to the UW IRB, and any sponsor/funding agency not already included in the list.

**E-mail initial report to:**

- Study principal investigator at dm3@medicine.wisc.edu
- Medical Monitor at uwcccgu@medicine.wisc.edu.
- GU coordinator at uwcccgu@medicine.wisc.edu (who will notify UWCCC Data and Safety Monitoring Committee Coordinator, the UW Institutional Biosafety Officer (via First Report form (http://www2.fpm.wisc.edu/biosafety/emergency_prep.htm), and the USAMRMC ORP HRPO at hsrrb@det.amedd.army.mil for the initial 56 patients) within one working day of the event

**Written reports to:**

- Study principal investigator – FAX 608-265-5146 or deliver to K4/623
- UWCC Data and Safety Monitoring Committee Coordinator – FAX 608-265-5676 or deliver to K4/6 Mailcode 6160
- UW IRB – Copy of final written report to UW Institutional Biosafety Office
- GU coordinator will submit written report to U.S. Army Medical Research and Materiel Command, ATTN: MCMR-ZB-PH, 504 Scott Street, Fort Detrick, Maryland 21702-5012 – NOTE: This pertains only to the initial 56 patients not treated on the biomarker cohort.
- FDA and NIH OBA

**Expedited Reporting Table**

Sites should follow this table for reporting adverse events to the University of Wisconsin. The University of Wisconsin will then report the event to the FDA.

Expedited Reporting Table D-2

**FDA REPORTING REQUIREMENTS FOR SERIOUS ADVERSE EVENTS (21 CFR Part 312)**

**NOTE:** Investigators **MUST** immediately report to the study PI **ANY** Serious Adverse Events, whether or not they are considered related to the investigational agent(s)/intervention (21 CFR 312.64)

An adverse event is considered serious if it results in **ANY** of the following outcomes:

1) Death
2) A life-threatening adverse event
3) An adverse event that results in inpatient hospitalization or prolongation of existing hospitalization for ≥ 24 hours
4) A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
5) A congenital anomaly/birth defect.
6) Important Medical Events (IME) that may not result in death, be life threatening, or require hospitalization may be considered serious when, based upon medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition. (FDA, 21 CFR 312.32; ICH E2A and ICH E6).

<table>
<tr><th>Hospitalization</th><th>Grade 1 Timeframes</th><th>Grade 2 Timeframes</th><th>Grade 3 Timeframes</th><th>Grade 4 & 5 Timeframes</th></tr>
<tr><td>Resulting in Hospitalization ≥ 24 hrs</td><td colspan="3">10 Calendar Days</td><td rowspan="2">24-Hour 5 Calendar Days</td></tr>
<tr><td>Not resulting in Hospitalization ≥ 24 hrs</td><td colspan="2">Not required</td><td>10 Calendar Days</td></tr>
</table>

[1]Serious adverse events that occur more than 30 days after the last administration of investigational agent/intervention and have an attribution of possible, probable, or definite require reporting as follows:

**Expedited 24-hour notification followed by complete report within 5 calendar days for:**

- All Grade 4, and Grade 5 AEs

**Expedited 10 calendar day reports for:**

- Grade 2 adverse events resulting in hospitalization or prolongation of hospitalization
- Grade 3 adverse events

**NOTE: GRADE 2 AUTOIMMUNE REACTIONS WILL BE TREATED AS GRADE 3 EVENTS FOR REPORTING PURPOSES.**

**For Hospitalization Only** – Any medical event equivalent to the CTC Grade 3,4,5 which precipitated hospitalization (or prolongation of existing hospitalization) must be reported regardless of requirements for phase of study, expected or unexpected and attribution.

Expedited reporting may not be appropriate for specific expected adverse events for certain later phase 2 and phase 3 protocols. In those situations the adverse events that will not have expedited reporting must be specified in the text of the approved protocol. An expected Grade 3 event that is using the generic reporting criteria, for instance. In a trial of investigational agents where

grade 3 diarrhea requiring hospitalization is expected, only diarrhea requiring ICU care (Grade 4) might be designated for expedited reporting.

## 16. Potential Risks and Benefits, and Procedures to Minimize Risk

### A. Potential Risks

#### A.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 monitored for 60 minutes following each treatment with evaluation of vital signs and examination of the skin site of treatment. Subjects will also be asked to keep a record of unusual site or other reactions for two days after immunization.

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 a previous phase I clinical trial with this DNA vaccine. However, 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.

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 [75]. 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.

A.2. From intradermal treatment with rhGM-CSF

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 [54]. 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 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 immunization or GM-CSF treatment.

A.3. 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

A.4. From leukapheresis

Adverse events from leukapheresis are uncommon. Bruising at the site of venipuncture for venous access is possible, as is a rare risk of infection related to this venipuncture. Anticoagulants (ACD) are used during cell collection, but these have not been associated with subsequent bleeding problems. There is a rare risk for citrate toxicity as a result of the citrate anticoagulation used during cell collection. This can cause hypocalcaemia and muscle spasms. There is no associated increased risk of infection from removing white blood cells, however a mild anemia can result for 1-2 days, and for this reason a hematocrit must be measured within 1 week of each leukapheresis; an hematocrit < 30% and/or platelet count <50,000 will be used as a contraindication for leukapheresis.

A.5. From 18F-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.

B. Potential Benefits

No benefits are guaranteed. It is hoped that individual patients treated with the DNA vaccine will derive a clinical response indicated by an increase in serum PSA doubling time and a prolonged time to PSA recurrence and the development of metastatic disease. Moreover, GM-CSF alone has been investigated as a treatment for prostate cancer. Consequently, it is possible that patients treated with GM-CSF alone will also derive clinical benefit from treatment with GM-CSF. 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. Thus, it is hoped that future patients will benefit from research participation in the current study.

## 17. Study Data Management and Procedural Issues

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

A.1. For Subjects enrolled at UWCCC

Potentially eligible subjects at the UWCCC site 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 (Jane Straus RN, Dottie Horvath RN, Mulusew Yayehyirad RN, or Mary Jane Staab RN) 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.

Assignment to treatment arm will be determined by the UW Department of Biostatistics, as determined by the protocol randomization and stratification criteria described above in Section 13. The UW Department of Biostatistics will work with the investigational pharamacies at each site to provide a code for the study arm assignment. In this fashion, pharmacies will not be blinded to arm assignment and treatment preparation, but investigators and research staff will remain blinded to treatment assignment.

A.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 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 at each institution 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 17.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 also be recorded in the clinical trials databases. All primary source documentation will be maintained in individual laboratory research charts for each subject. All laboratory analysis conducted at the UWCCC will be maintained in research charts in the laboratory of Dr. McNeel, and stored indefinitely.

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 Drs. McNeel, Liu, Cetnar, Bruce and Wilding, three GU malignancy research nurses, two 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, Dr.Jens Eickhoff, with the University of Wisconsin Department of Biostatistics.

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 the Study PI. 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 central immunological evaluation of this trial (blood specimens) will be cryopreserved and delivered in batch to the laboratory of Dr. McNeel. Other immunological evaluation will be performed at individual trial sites. 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. This research database will only be available to the study investigators, not other laboratory personnel, to maintain confidentiality. Subject sera will be maintained indefinitely in the laboratory of the study principal investigator, stored in aliquots at –20°C to –80°C. Peripheral blood mononuclear cells will be stored indefinitely in the laboratory of the study principle investigator, stored in aliquots in liquid nitrogen.

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 and USAMRMC ORP HRPO 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. Glenn Liu, and from the overall Protocol PI, 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 UWCCC study PI. Deviations that could potentially affect subject safety, such as missing safety labs, will be documented, repeated as soon as possible, and reviewed at the weekly UWCCC 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.

## 18. Protocol Addendum Specific for UWCCC (This Section Does Not Apply to Other Participating Sites)

A. UWCCC Reporting Requirements

The following are reporting requirements and responsibilities of the Principal Investigator to the United States Army Medical Research and Materiel Command's (USAMRMC) Office of Research Protections (ORP), Human Research Protection Office (HRPO), and pertain only to the initial 56 patients treated on this protocol for whom the USAMRMC/DOD serves as sponsor and for whom the USAMRM ORP HRPO has oversight.

(1) The protocol will be conducted in accordance with the protocol submitted to and approved by the USAMRMC ORP HRPO and will not be initiated until written notification of approval of the research project is issued by the USAMRMC ORP HRPO.

(2) Accurate and complete study records will be maintained and made available to representatives of the U.S. Army Medical Research and Materiel Command as a part of their responsibility to protect human subjects in research. Research records will be stored in a confidential manner so as to protect the confidentiality of subject information.

(3) All unanticipated problems involving risk to subjects or others, serious adverse events related to participation in the study and subject deaths related to participation in the study should be promptly reported by phone (301-619-2165), by email (hsrrb@det.amedd.army.mil), or by facsimile (301-619-7803) to the USAMRMC ORP HRPO. A complete written report will follow the initial notification. In addition to the methods above, the complete report will be sent to the U.S. Army Medical Research and Materiel Command, ATTN: MCMR-ZB-PH, 504 Scott Street, Fort Detrick, Maryland 21702-5012.

(4) Any deviation to the protocol that may have an effect on the safety or rights of the subject or the integrity of the study must be reported to the USAMRMC ORP HRPO as soon as the deviation is identified.

(5) Modifications to the research protocol must be submitted to the USAMRMC ORP HRPO for review and approval after review and approval by the local IRB, but before implementation.

(6) A copy of the approved annual continuing review report and the local IRB approval notification must be submitted to the USAMRMC ORP HRPO as soon as these documents become available. A copy of the approved final study report and local IRB approval notification must be submitted to the USAMRMC ORP HRPO as soon as these documents become available.

(7) The knowledge of any pending compliance inspection/visit by the OHRP or other government agency concerning clinical investigation or research, the issuance of Inspection Reports, warning letters or actions taken by any Regulatory Agencies including legal or medical actions and any instances of serious or continuing noncompliance with the regulations or requirements will be reported immediately to USAMRMC ORP HRPO.

B. Roles and Responsibilities of Study Personnel at UWCCC

Study Principal Investigator:

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 several other clinical trials, and has had formalized training in the ethics and conduct of clinical trials and human subjects protection. He will be overall responsible for the conduct of the trial and its analysis, but will not participate in patient accrual, treatment of patients on study, or interpretation of clinical data during the time that patients are on study. Dr. McNeel's involvement will center on the scientific aspects of the project, including overall trial design and analysis of samples from the trial. Dr. McNeel will participate as author on any manuscripts derived from this research.

UWCCC Local Principal Investigator:

Dr. Glenn Liu MD, Associate Professor of Medicine, is a genitourinary medical oncologist with a primary interest in genitourinary cancer clinical trials research. He will be the UWCCC local principal investigator for this study. As UWCCC local 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.

Medical Monitor:

Dr. Mark Albertini MD, Associate Professor of Medicine, is a medical oncologist with a clinical expertise in melanoma, and a laboratory expertise in tumor immunology. He has previous experience in the design and conduct of a DNA vaccine trial in melanoma, was the medical monitor for the phase I study using the current DNA vaccine, and thus has the experience necessary to anticipate and treat any unexpected adverse events. In this capacity, while he will not be directly associated with this protocol, he will be able to provide appropriate medical care to research subjects for conditions that might arise during the conduct of the study. Furthermore, he has no financial or research conflicts of interest in the outcome of the study, and hence can serve in an unbiased capacity.

Co-Investigators:

Dr. Joshua Lang MD, Assistant Professor of Medicine, is a genitourinary medical oncologist with a primary interest in phase I and genitourinary cancer clinical trials research. He will be a clinical co-investigator for this study.

Dr. Robert Jeraj PhD, Associate Professor Medical Physics, Radiology, Human Oncology, and Biomedical Engineering, serves as director of the UWCCC Translational Imaging Research Program and co-director of the UWCCC Image Analysis Core (IMAC) laboratory. He will be responsible for analysis and interpretation of the Quantitative Total Bone Imaging (QTBI) data for patients treated in the biomarker cohort.

Biostatistician:

Dr. Jens Eickhoff, PhD is a biostatistician with a primary interest in cancer 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.

Program Manager:

Ms. Mary Jane Staab, RN, has worked with the GU DOWG for over ten years. She will be responsible for directly overseeing the efforts of the research nurses, for managing budgets, and for communication with local and national regulatory agencies.

Laboratory Personnel:

Laura Johnson, PhD has worked with Dr. McNeel since 2002 and has experience with all of the methods of immune analysis proposed. She has also undergone human subjects' protection training. She will be primarily responsible for sample processing, storage, and the immunological analysis as described above.

Study Coordinator / Research Nurses:

Dorothea Horvath, RN will be the lead study coordinator for this protocol. She will be assisted by Jane Straus, RN and Mulusew Yayehyirad, RN. Ms. Horvath and Ms. Straus have worked as research nurses with the GU DOWG for over 10 years, and Ms. Yayehyirad for over 5 years. Together, they will be responsible for recruitment and consenting of subjects, maintaining study records, overseeing the administration of vaccines, and recording and reporting adverse events.

Data Managers:

UWCCC GU data managers will be responsible for entering data into the UWCCC Oncore database, and will provide administrative support for the study nurses.

## 19. References


1. Jemal, A., Siegel, R., Ward, E., Murray, T., Xu, J. and Thun, M. J. (2007). "Cancer statistics, 2007." *CA Cancer J Clin* **57**: 43-66.
2. Oefelein, M. G., Smith, N. D., Grayhack, J. T., Schaeffer, A. J. and McVary, K. T. (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., Partin, A. W., Eisenberger, M. A., Chan, D. W., Pearson, J. D. and Walsh, P. C. (1999). "Natural history of progression after PSA elevation following radical prostatectomy." *Jama* **281**: 1591-1597.
4. Freedland, S. J., Humphreys, E. B., Mangold, L. A., Eisenberger, M., Dorey, F. J., Walsh, P. C. and Partin, A. W. (2005). "Risk of prostate cancer-specific mortality following biochemical recurrence after radical prostatectomy." *Jama* **294**: 433-439.
5. Slovin, S. F., Wilton, A. S., Heller, G. and Scher, H. I. (2005). "Time to detectable metastatic disease in patients with rising prostate-specific antigen values following surgery or radiation therapy." *Clin Cancer Res* **11**: 8669-8673.
6. Lee, A. K., Levy, L. B., Cheung, R. and Kuban, D. (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**: 456-462.
7. Freedland, S. J., Humphreys, E. B., Mangold, L. A., Eisenberger, M., Dorey, F. J., Walsh, P. C. and Partin, A. W. (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**: 1765-1771.
8. McNeel, D. G. and Malkovsky, M. (2005). "Immune-based therapies for prostate cancer." *Immunol Lett* **96**: 3-9.
9. Kipp, R. T. and McNeel, D. G. (2007). "Immunotherapy for prostate cancer - recent progress in clinical trials." *Clin Adv Hematol Oncol* **5**: 465-474, 477-479.
10. McNeel, D. G. (2007). "Prostate cancer immunotherapy." *Curr Opin Urol* **17**: 175-181.
11. McNeel, D. G. (2007). "Cellular immunotherapies for prostate cancer." *Biomed Pharmacother* **61**: 315-322.
12. Gutman, A. B. and Gutman, E. B. (1938). "An acid phosphatase in the serum of patients with metastasizing carcinoma of the prostate gland." *J. Clin. Invest.* **17**: 473-479.
13. Terracio, L., Rule, A., Salvato, J. and Douglas, W. H. (1985). "Immunofluorescent localization of an androgen-dependent isoenzyme of prostatic acid phosphatase in rat ventral prostate." *Anat Rec* **213**: 131-139.
14. Fong, L., Ruegg, C. L., Brockstedt, D., Engleman, E. G. and Laus, R. (1997). "Induction of tissue-specific autoimmune prostatitis with prostatic acid phosphatase immunization; implications for immunotherapy of prostate cancer." *J. Immunol.* **159**: 3113-3117.
15. Johnson, L. E., Frye, T. P., Arnot, A. R., Marquette, C., Couture, L. A., Gendron-Fitzpatrick, A. and McNeel, D. G. (2006). "Safety and immunological efficacy of a prostate cancer plasmid DNA vaccine encoding prostatic acid phosphatase (PAP)." *Vaccine* **24**: 293-303.
16. Johnson, L. E., Frye, T. P., Chinnasamy, N., Chinnasamy, D. and McNeel, D. G. (2007). "Plasmid DNA vaccine encoding prostatic acid phosphatase is effective in eliciting autologous antigen-specific CD8+ T cells." *Cancer Immunol Immunother* **56**: 885-895.

17. Laus, R., Yang, D. M., Ruegg, C. L., Shapero, M. H., Slagle, P. H., Small, E., Burch, P. and Valone, F. H. (2001). "Dendritic cell immunotherapy of prostate cancer: preclinical models and early clinical experience." *Canc Res Ther Control* **11**: 1-10.
18. Small, E. J., Schellhammer, P. F., Higano, C. S., Redfern, C. H., Nemunaitis, J. J., Valone, F. H., Verjee, S. S., Jones, L. A. and Hershberg, R. M. (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**: 3089-3094.
19. McNeel, D. G., Dunphy, E. J., Davies, J. G., Frye, T. P., Johnson, L. E., Staab, M. J., Horvath, D. L., Straus, J., Alberti, D., Marnocha, R., Liu, G., Eickhoff, J. C. and Wilding, G. (2009). "Safety and immunological efficacy of a DNA vaccine encoding prostatic acid phosphatase in patients with stage D0 prostate cancer." *J Clin Oncol* **27**: 4047-4054.
20. Becker, J. T., Olson, B. M., Johnson, L. E., Davies, J. G., Dunphy, E. J. and McNeel, D. G. (2010). "DNA vaccine encoding prostatic acid phosphatase (PAP) elicits long-term T-cell responses in patients with recurrent prostate cancer." *J Immunother*: (in press).
21. Griffiths, J. (1980). "Prostate-specific acid phosphatase: re-evaluation of radioimmunoassay in diagnosing prostatic disease." *Clin. Chem.* **26**: 433-436.
22. Jobsis, A. C., De Vries, G. P., Anholt, R. R. and Sanders, G. T. (1978). "Demonstration of the prostatic origin of metastases: an immunohistochemical method for formalin-fixed embedded tissue." *Cancer* **41**: 1788-1793.
23. McNeel, D. G., Nguyen, L. D., Storer, B. E., Vessella, R., Lange, P. H. and Disis, M. L. (2000). "Antibody immunity to prostate cancer-associated antigens can be detected in the serum of patients with prostate cancer." *J. Urol.* **164**: 1825-1829.
24. McNeel, D. G., Nguyen, L. D., Ellis, W. J., Higano, C. S., Lange, P. H. and Disis, M. L. (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.
25. Peshwa, M. V., Shi, J. D., Ruegg, C., Laus, R. and van Schooten, W. C. (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.
26. Burch, P. A., Breen, J. K., Buckner, J. C., Gastineau, D. A., Kaur, J. A., Laus, R. L., Padley, D. J., Peshwa, M. V., Pitot, H. C., Richardson, R. L., Smits, B. J., Sopapan, P., Strang, G., Valone, F. H. and Vuk-Pavlovic, S. (2000). "Priming tissue-specific cellular immunity in a phase I trial of autologous dendritic cells for prostate cancer." *Clin. Cancer Res.* **6**: 2175-2182.
27. Small, E. J., Fratesi, P., Reese, D. M., Strang, G., Laus, R., Peshwa, M. V. and Valone, F. H. (2000). "Immunotherapy of hormone-refractory prostate cancer with antigen-loaded dendritic cells." *J. Clin. Oncol.* **18**: 3894-3903.
28. Fong, L., Brockstedt, D., Benike, C., Wu, L. and Engleman, E. G. (2001). "Dendritic cells injected via different routes induce immunity in cancer patients." *J Immunol* **166**: 4254-4259.
29. Burch, P. A., Croghan, G. A., Gastineau, D. A., Jones, L. A., Kaur, J. S., Kylstra, J. W., Richardson, R. L., Valone, F. H. and Vuk-Pavlovic, S. (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.

30. Iwasaki, A., Torres, C. A., Ohashi, P. S., Robinson, H. L. and Barber, B. H. (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., Billetta, R., Gaietta, G., Bonnin, D. B., Baird, S. M. and Albani, S. (2000). "Cell-mediated DNA transport between distant inflammatory sites following intradermal DNA immunization in the presence of adjuvant." *J Immunol* **164**: 1340-1345.
32. Corr, M., von Damm, A., Lee, D. J. and Tighe, H. (1999). "In vivo priming by DNA injection occurs predominantly by antigen transfer." *J Immunol* **163**: 4721-4727.
33. Irvine, K. R. and Restifo, N. P. (1995). "The next wave of recombinant and synthetic anticancer vaccines." *Seminars in Canc Biol* **6**: 337-347.
34. Raz, E., Tighe, H., Sato, Y., Corr, M., Dudler, J. A., Roman, M., Swain, S. L., Spiegelberg, H. L. and Carson, D. A. (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., Webster, R. G. and Woodland, D. L. (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., Sherritt, M. A., Medveczky, J., Elliott, S. L., Moss, D. J., Fernando, G. J., Brown, L. E. and Suhrbier, A. (1998). "Delivery of multiple CD8 cytotoxic T cell epitopes by DNA vaccination." *J. Immunol.* **160**: 1717-1723.
37. Cho, H. J., Takabayashi, K., Cheng, P. M., Nguyen, M. D., Corr, M., Tuck, S. and Raz, E. (2000). "Immunostimulatory DNA-based vaccines induce cytotoxic lymphocyte activity by a T-helper cell-independent mechanism." *Nat Biotechnol* **18**: 509-514.
38. Corr, M., Lee, D. J., Carson, D. A. and Tighe, H. (1996). "Gene vaccination with naked plasmid DNA: mechanism of CTL priming." *J Exp Med* **184**: 1555-1560.
39. Svanholm, C., Bandholtz, L., Lobell, A. and Wigzell, H. (1999). "Enhancement of antibody responses by DNA immunization using expression vectors mediating efficient antigen secretion." *J Immunol Methods* **228**: 121-130.
40. Rahman, F., Dahmen, A., Herzog-Hauff, S., Bocher, W. O., Galle, P. R. and Lohr, H. F. (2000). "Cellular and humoral immune responses induced by intradermal or intramuscular vaccination with the major hepatitis B surface antigen." *Hepatology* **31**: 521-527.
41. Lee, A. H., Suh, Y. S., Sung, J. H., Yang, S. H. and Sung, Y. C. (1997). "Comparison of various expression plasmids for the induction of immune response by DNA immunization." *Mol Cells* **7**: 495-501.
42. Hartmann, G., Weeratna, R. D., Ballas, Z. K., Payette, P., Blackwell, S., Suparto, I., Rasmussen, W. L., Waldschmidt, M., Sajuthi, D., Purcell, R. H., Davis, H. L. and Krieg, A. M. (2000). "Delineation of a CpG phosphorothioate oligodeoxynucleotide for activating primate immune responses in vitro and in vivo." *J Immunol* **164**: 1617-1624.
43. Disis, M. L., Bernhard, H., Shiota, F. M., Hand, S. L., Gralow, J. R., Huseby, E. S., Gillis, S. and Cheever, M. A. (1996). "Granulocyte-macrophage colony-stimulating factor: an effective adjuvant for protein and peptide-based vaccines." *Blood* **88**: 202-210.
44. Bernhard, H., Disis, M. L., Heimfeld, S., Hand, S., Gralow, J. R. and Cheever, M. A. (1995). "Generation of immunostimulatory dendritic cells from human CD34+ hematopoietic progenitor cells of the bone marrow and peripheral blood." *Cancer Res.* **55**: 1099-1104.

45. Caux, C., Dezutter-Dambuyant, C., Schmitt, D. and Banchereau, J. (1992). "GM-CSF and TNF-alpha cooperate in the generation of dendritic Langerhans cells." *Nature* **360**: 258-261.
46. Mellman, I., Turley, S. J. and Steinman, R. M. (1998). "Antigen processing for amateurs and professionals." *Trends Cell Biol* **8**: 231-237.
47. Hu, H. M., Winter, H., Urba, W. J. and Fox, B. A. (2000). "Divergent roles for CD4(+) T cells in the priming and effector/memory phases of adoptive immunotherapy." *J Immunol* **165**: 4246-4253.
48. Tarr, P. E., Lin, R., Mueller, E. A., Kovarik, J. M., Guillaume, M. and Jones, T. C. (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**: 1199-1204.
49. Dranoff, G., Jaffee, E., Lazenby, A., Golumbek, P., Levitsky, H., Brose, K., Jackson, V., Hamada, H., Pardoll, D. and Mulligan, R. C. (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.
50. Simons, J. W., Mikhak, B., Chang, J. F., DeMarzo, A. M., Carducci, M. A., Lim, M., Weber, C. E., Baccala, A. A., Goemann, M. A., Clift, S. M., Ando, D. G., Levitsky, H. I., Cohen, L. K., Sanda, M. G., Mulligan, R. C., Partin, A. W., Carter, H. B., Piantadosi, S., Marshall, F. F. and Nelson, W. G. (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.
51. Jaffee, E. M., Abrams, R., Cameron, J., Donehower, R., Duerr, M., Gossett, J., Greten, T. F., Grochow, L., Hruban, R., Kern, S., Lillemoe, K. D., O'Reilly, S., Pardoll, D., Pitt, H. A., Sauter, P., Weber, C. and Yeo, C. (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**: 1951-1971.
52. Kanellos, T. S., Sylvester, I. D., Butler, V. L., Ambali, A. G., Partidos, C. D., Hamblin, A. S. and Russell, P. H. (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**: 507-510.
53. Disis, M. L., Shiota, F. M., McNeel, D. G. and Knutson, K. L. (2003). "Soluble cytokines can act as effective adjuvants in plasmid DNA vaccines targeting self tumor antigens." *Immunobiology* **207**: 179-186.
54. McNeel, D. G., Schiffman, K. and Disis, M. L. (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.
55. Rini, B. I., Weinberg, V., Bok, R. and Small, E. J. (2003). "Prostate-specific antigen kinetics as a measure of the biologic effect of granulocyte-macrophage colony-stimulating factor in patients with serologic progression of prostate cancer." *J Clin Oncol* **21**: 99-105.
56. Dreicer, R., See, W. A. and Klein, E. A. (2001). "Phase II trial of GM-CSF in advanced prostate cancer." *Invest New Drugs* **19**: 261-265.

57. Small, E. J., Reese, D. M., Um, B., Whisenant, S., Dixon, S. C. and Figg, W. D. (1999). "Therapy of advanced prostate cancer with granulocyte macrophage colony-stimulating factor." *Clin Cancer Res* **5**: 1738-1744.
58. Rini, B. I., Fong, L., Weinberg, V., Kavanaugh, B. and Small, E. J. (2006). "Clinical and immunological characteristics of patients with serologic progression of prostate cancer achieving long-term disease control with granulocyte-macrophage colony-stimulating factor." *J Urol* **175**: 2087-2091.
59. Morisson, C., Jeraj, R. and Liu, G. (2013). "Imaging of castration-resistant prostrate cancer: development of imaging response biomarkers." *Curr Opin Urol* **23**: 230-236.
60. Zlotocha, S., Staab, M. J., Horvath, D., Straus, J., Dobratz, J., Oliver, K., Wasielewski, S., Alberti, D., Liu, G., Wilding, G., Eickhoff, J. and McNeel, D. G. (2005). "A phase I study of a DNA vaccine targeting prostatic acid phosphatase in patients with stage D0 prostate cancer." *Clin Genitourin Cancer* **4**: 215-218.
61. Disis, M. L., Grabstein, K. H., Sleath, P. R. and Cheever, M. A. (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.
62. Devereux, S., Bull, H. A., Campos-Costa, D., Saib, R. and Linch, D. C. (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**: 323-330.
63. VanBuskirk, A. M., Burlingham, W. J., Jankowska-Gan, E., Chin, T., Kusaka, S., Geissler, F., Pelletier, R. P. and Orosz, C. G. (2000). "Human allograft acceptance is associated with immune regulation." *J Clin Invest* **106**: 145-155.
64. Derks, R. A., Jankowska-Gan, E., Xu, Q. and Burlingham, W. J. (2007). "Dendritic cell type determines the mechanism of bystander suppression by adaptive T regulatory cells specific for the minor antigen HA-1." *J Immunol* **179**: 3443-3451.
65. Smith, H. A., Maricque, B. B., Eberhardt, J., Petersen, B., Gulley, J. L., Schlom, J. and McNeel, D. G. (2011). "IgG responses to tissue-associated antigens as biomarkers of immunological treatment efficacy." *J Biomed Biotech* **2011:454861**.
66. Fong, L., Kwek, S. S., O'Brien, S., Kavanagh, B., McNeel, D. G., Weinberg, V., Lin, A. M., Rosenberg, J., Ryan, C. J., Rini, B. I. and Small, E. J. (2009). "Potentiating endogenous antitumor immunity to prostate cancer through combination immunotherapy with CTLA4 blockade and GM-CSF." *Cancer Res* **69**: 609-615.
67. Zabransky, D. J., Smith, H. A., Thoburn, C. J., Zahurak, M., Keizman, D., Carducci, M., Eisenberger, M. A., McNeel, D. G., Drake, C. G. and Antonarakis, E. S. (2011). "Lenalidomide modulates IL-8 and anti-prostate antibody levels in men with biochemically recurrent prostate cancer." *Prostate*: (in press).
68. Dubovsky, J. A., Albertini, M. R. and McNeel, D. G. (2007). "MAD-CT-2 identified as a novel melanoma cancer-testis antigen using phage immunoblot analysis." *J Immunother* **30**: 675-683.
69. Hoeppner, L. H., Dubovsky, J. A., Dunphy, E. J. and McNeel, D. G. (2006). "Humoral immune responses to testis antigens in sera from patients with prostate cancer." *Cancer Immun* **6**: 1-7.
70. Dunphy, E. J., Eickhoff, J. C., Muller, C. H., Berger, R. E. and McNeel, D. G. (2004). "Identification of antigen-specific IgG in sera from patients with chronic prostatitis." *J. Clin. Immunol.* **24**: 492-501.

71. Dunphy, E. J. and McNeel, D. G. (2005). "Antigen-specific IgG elicited in subjects with prostate cancer treated with flt3 ligand." *J Immunother* **28**: 268-275.
72. Mooney, C. J., Dunphy, E. J., Stone, B. and McNeel, D. G. (2006). "Identification of autoantibodies elicited in a patient with prostate cancer presenting as dermatomyositis." *Int J Urol* **13**: 211-217.
73. Morse, M. D. and McNeel, D. G. (2010). "Prostate Cancer Patients Treated with Androgen Deprivation Therapy Develop Persistent Changes in Adaptive Immune Responses." *Hum Immunol* **71**: 496-504.
74. Koenker, R. (2008). "Censored quantile regression redux." *J. of Stat. Software* **27**: 1-25.
75. Martin, T., Parker, S. E., Hedstrom, R., Le, T., Hoffman, S. L., Norman, J., Hobart, P. and Lew, D. (1999). "Plasmid DNA malaria vaccine: the potential for genomic integration after intramuscular injection." *Hum Gene Ther* **10**: 759-768.

# APPENDIX A

## NCI Common Terminology Criteria – Version 4

http://ctep.cancer.gov/protocoldevelopment/electronic_applications/docs/ctcaev4.pdf

## APPENDIX B

## Blood Draws for Research

| | Screen[1] | Month 3 | Month 6 | Month 9 | Month 12 | End of Study |
|---|---|---|---|---|---|---|
| **Sera for Antibody** | 20mL | 10mL | 10mL | 10mL | 10mL | 10mL |
| **Blood for T-cell response** | *Leukapheresis* Or [2]200mL | 100mL | *Leukapheresis* Or [2]200mL | 100mL | 100mL | 100mL |
| **Approximate Total** | 20mL *(+Leuk.)* Or 220mL *(-Leuk.)* | 110mL | 10mL *(+Leuk.)* Or 210mL *(-Leuk.)* | 110mL | 110mL | 110mL |

[1]Blood and leukapheresis samples at screening must be collected before tetanus immunization.
[2]200mL peripheral blood draw is an alternative if leukapheresis is not feasible.

*Volumes in the table are approximate and are to be used for determining how many and which tubes are to be drawn per the protocol. Blood sera for antibody testing is to be collected in red top tubes and blood for T-Cell response is to be collected in green top heparinized tubes (or via leukapheresis at Screening and Month 6).*