9/3/15 version

Pilot Trial of Sipuleucel-T, with or without pTVG-HP DNA Booster Vaccine, in Patients with Castrate-Resistant, Metastatic Prostate Cancer

CO11816

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

STUDY SITE INFORMATION

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

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

Local Principal Investigator: Glenn Liu, M.D.
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

Other Investigators:
Christos Kyriakopoulos M.D. – Clinical Investigator
Joshua Lang, M.D. M.S. – Clinical Investigator

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 C. Eickhoff, Ph.D.
UWCCC
600 Highland Ave. K4/438 CSC
Madison, WI 53792
Tel: (608) 265-6380
eickhoff@biostat.wisc.edu

Start Date: February 11, 2013

Projected End of Accrual Date: December 31, 2015

Projected End of Study Date: December 31, 2016

TABLE OF CONTENTS

SYNOPSIS:

Primary Objectives:

1. To evaluate whether immunization with a DNA vaccine encoding prostatic acid phosphatase (PAP, pTVG-HP) can augment PAP-specific effector and memory T cells following treatment with sipuleucel-T.

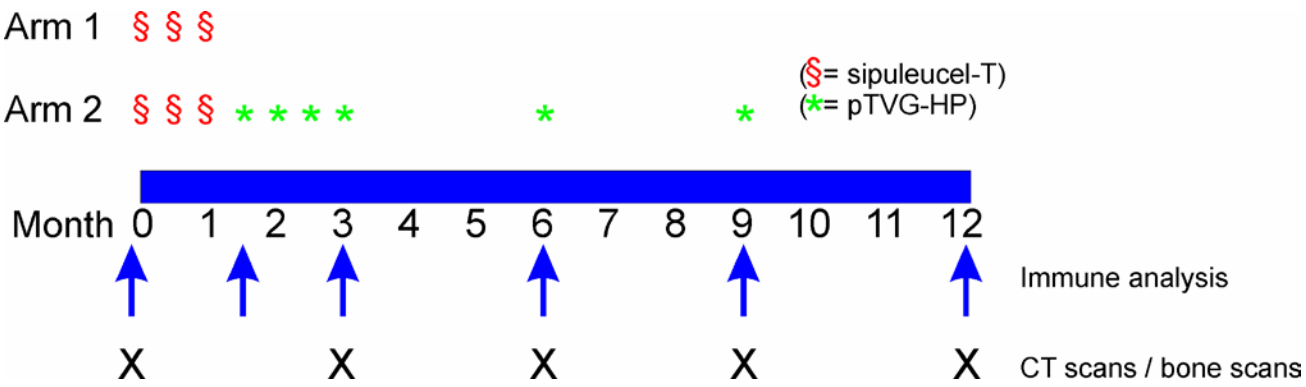
Secondary Objectives:

1. To determine 12-month radiographic progression-free survival
2. To determine median time-to-radiographic progression as a function of baseline time point (pretreatment or 3-months post treatment)
3. To evaluate effects of treatment on changes in serum PSA level and changes in PSA doubling time

Laboratory and Exploratory Objectives:

1. To determine overall survival
2. To evaluate effects of treatment on number of circulating tumor cells as determined by PSA ELISPOT
3. To evaluate PAP-specific antibody and T-cell immune responses following treatment with sipuleucel-T and DNA vaccine
4. To determine whether PAP-specific immune response is associated with prolonged (1 year) radiographic progression-free survival
5. To determine whether baseline immune responses (Th1 type or regulatory type) predict for immune responses elicited/augmented following treatment with sipuleucel-T +/- DNA vaccine
6. To determine whether treatment elicits immunologic antigen spread to other prostate associated antigens

Study Scheme:



Study Calendar:

Vaccine / Treatment Visit:	Pre-Screen (within 4 weeks of Screen)	Screen/Randomization (within 2 weeks of day 1)	Day 1 – sipuleucel-T collection #1	Day 4 – sipuleucel-T infusion #1	Day 15 – sipuleucel-T collection #2	Day 18 – sipuleucel-T infusion #2	Day 29 – sipuleucel-T collection #3	Day 32 – sipuleucel-T infusion #3	Day 43 – sipuleucel-T follow-up / DNA imm #1[a]	Day 57 – DNA imm #2[a]	Day 71 – DNA imm #3[a]	3-Month Followup (Day 85) – DNA imm #4[a]	6-Month Follow up (Week 24) – DNA imm #5[a]	9-Month Follow-up (Week 36) – DNA imm #6[a]	12-Month (Week 48) Follow-up and/or End of study visit
History & Physical Exam															
History	X														
Consent	X														
Physical Exam, ECOG PS		X			X		X		X			X	X	X	X
Vital signs		X		X	X	X	X	X							
Toxicity assessment [b]		X			X		X		X			X	X	X	X
Injection site inspection									X	X	X	X	X	X	X
Lab Tests															
CBC with differential and platelets [c]		X			X		X		X			X	X	X	X
Creatinine, alk phos, SGOT, total bilirubin, amylase, LDH		X							X			X	X	X	X
Serology for HIV Ab/Ag combo, Hepatitis B Surface Ag, Hepatitis C AB		X													
Serum testosterone, ALT		X													
PSA[d] and PAP		X							X			X	X	X	X
Procedures															
CT abdomen/pelvis [e]	X											X[f]	X	X	X[g]
Bone scan [e]	X											X[f]	X	X	X[g]
Leukapheresis		X[h]	X		X		X						X[h,i]		
Sipuleucel-T infusion				X		X		X							
Tetanus immunization		X													
DNA immunization –ARM 2 PATIENTS ONLY [a]									X	X	X	X	X	X	
Blood for CTC analysis		X							X			X	X	X	X
Blood for immune studies [j]		X							X			X	X	X	X[k]

Note: Grey regions indicate those tests or procedures or visits only applicable to subjects treated on Arm 2. Acceptable delays in study treatments or procedures may result in corresponding delays of subsequent visits. Annual long term follow up will occur for up to five years. See Section 9 for more details.

[a] ARM 2 Only: Biweekly DNA immunization (#1-3) can be +/- 3 days; quarterly DNA immunization (#4-6) can be +/- 7 days from expected date determined from prior visit.

[b] Study coordinator or research nurse review of systems, including ECOG PS

[c] CBC with differential and platelets to be performed within 72 hours prior to leukapheresis (for Sipuleucel-T treatment) unless an event occurs that would require it be drawn sooner for safety considerations, or if required sooner per institutional requirements or MD discretion. A CBC result within 7 days prior to leukapheresis for research is ideal.

[d] PSA measurements must be performed at the 3-month intervals defined, but can be more frequent if clinically indicated.

[e] Radiographic studies (CT scans and bone scans) can be performed +/- 7 days of the study visit

[f] Staging studies (CT and bone scan): scan progression between Screening and Month 3 will not be used to define radiographic progression for the purposes of this study and will not necessitate subjects to come off study. Patients should come off study at this time point only due to symptomatic progression requiring other treatment for prostate cancer.

[g] 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 12 weeks

[h] Leukapheresis collected (+/- 7 days) for research, not preparation of sipuleucel-T product

[i] If leukapheresis is not able to be obtained at Month 6 visit, then up to 150 ml may be obtained via peripheral blood draw as an alternative.

[j] Blood for immune studies is to be collected per calendar (+/-7 days). This should still be collected for subjects that come off study early, however the quarterly interval time points can correspond to the patient's standard of care visits.

[k] Blood for immune studies is not necessarily collected at "End of Study" visit; it's collected at quarterly intervals for up to one year.

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, over 30,000 U.S. men are estimated to have died as a result of prostate cancer in 2010 [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]. Prostate cancer, once it becomes metastatic, is not curable and is generally initially treated with androgen deprivation, and androgen deprivation remains the cornerstone on which other therapies are added [3]. Unfortunately, within typically 2-3 years the disease becomes refractory to androgen deprivation, and castrate-resistant prostate cancer is the lethal phenotype of the disease. Within the last several years, several therapies have been approved by FDA based on their ability to prolong overall survival in this population of patients. Specifically, docetaxel was approved in 2004 after two large randomized trials showed a 2-3 month median improvement in overall survival compared with mitoxantrone [4, 5]. Cabazitaxel was approved in 2010 for patients with disease refractory to docetaxel after an international trial demonstrated a similar 2-3 month median improvement in overall survival compared with mitoxantrone [6]. Also in 2010, sipuleucel-T (Provenge®, Dendreon Corporation) was approved for patients with minimally symptomatic castrate-resistant, metastatic prostate cancer, based on the results of a prospectively randomized, blinded, phase III placebo-controlled clinical trial, and supporting data from previous phase III clinical trials, demonstrating a median 4-month improvement in overall survival [7]. Finally, in 2011, abiraterone, an oral androgen synthesis inhibitor, was similarly approved in the setting of docetaxel-refractory, castrate-resistant metastatic prostate cancer following a prospectively randomized, blinded, placebo-controlled trial demonstrating nearly a 4-month median improvement in overall survival [8]. These recent advances have clearly improved the situation for patients with advanced prostate cancer; however have presented new challenges in terms of the optimal sequence and approach to the management of castrate-resistant disease.

Despite the impact of chemotherapies for advanced prostate cancer, many patients and treating physicians believe that the small overall survival benefit provided by chemotherapy may not justify its use in all patients, in part due to potential side effects [9]. Vaccine-based strategies, also known as active immunotherapies, are particularly appealing as potentially safer treatments, and the trials that led to the approval of sipuleucel-T showed markedly fewer adverse events than are typically seen with chemotherapy agents [7]. Many vaccines for prostate cancer are in clinical development, all of which have demonstrated similar safety profiles, and some of which have demonstrated anti-tumor activity, as we have recently reviewed [10-13]. Consequently, evaluating active immunotherapies in combination with other immunotherapy or traditional agents, or earlier in the course of disease, are rational directions to further improve on these therapies to attempt to further improve on the survival benefit observed [14].

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 [15]. 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 [16]. 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 [17-20]. PAP is the target antigen of the autologous antigen-presenting cell sipuleucel-T vaccine, in which autologous peripheral blood mononuclear cells are loaded *ex vivo* with a PAP-GM-CSF fusion protein. A separate phase I clinical trial evaluated dendritic cells loaded with a murine homologue of PAP, and demonstrated immunogenicity of this approach [21]. In rodent studies, we have similarly demonstrated that PAP can be immunologically targeted using genetic vaccines, and a plasmid DNA vaccine in particular [18, 19]. We have previously reported the results of a phase I/II trial conducted in patients with early, PSA-recurrent (clinical stage D0) prostate cancer using this same DNA vaccine (pTVG-HP). No significant adverse events were observed in 22 subjects treated over a 12-week period of time. Moreover, several patients developed evidence of PAP-specific CD4+ and CD8+ T-cells, and several patients experienced a prolongation in the PSA doubling time, demonstrating immunological efficacy and suggesting a possible anti-tumor effect [22]. The presence of long-term IFNγ-secreting immune responses to PAP, detectable at multiple times months after immunization, were associated with increases in PSA doubling time, suggesting this might serve as a rational biomarker for efficacy [23]. Moreover, it was found that immune responses could be augmented months later with repeated immunizations, suggesting that DNA vaccines might provide a simple means of boosting immune responses from other priming immunizations [23]. These findings have justified further evaluation of this vaccine in a randomized phase II clinical trial, a study currently underway, and also specifically suggest that the pTVG-HP might be used in combination with sipuleucel-T to augment PAP-specific T-cell responses primed by that immunization strategy, as both vaccine approaches target the same antigen. This serves as the primary rationale for the current trial.

2. Background and Rationale

A. PAP is a tumor antigen in prostate cancer and PAP-specific CD8+ CTL can lyse prostate cancer cells

PAP was first identified in 1938 and was initially used as a serum marker for the detection of prostate cancer [15, 24]. 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 [25]. 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* [26, 27]. 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 [28]. Moreover, we have previously demonstrated that CD8+ T cells specific for PAP, with cytolytic activity for prostate cancer cells, can exist in patients with prostate cancer, and can be augmented with vaccination [23, 28].

B. Sipuleucel-T: The first anti-tumor vaccine FDA approved for the treatment of cancer

We have previously reviewed the preclinical and early clinical development of sipuleucel-T elsewhere [14]. Sipuleucel-T has been evaluated in three Phase III clinical trials in patients with metastatic, castrate-resistant prostate cancer. In the first phase III trial (D9901), Small and colleagues enrolled 127 men with asymptomatic disease, randomized 2:1 to receive three biweekly infusions of sipuleucel-T versus placebo. Patients on the sipuleucel-T arm received as many autologous cells, cultured *in vitro* with PA2024 (PAP-GM-CSF fusion protein), as could be prepared from a standard leukapheresis product (with a median of $3.65x10^9$ nucleated cells per infusion [29]). The patients receiving placebo underwent a leukapheresis, and cells were re-infused without being pulsed with PA2024. In this study, the primary endpoint was TTP (either radiographic or development of pain). In this study, treatment with sipuleucel-T was found to be well tolerated. The most common side effects associated with sipuleucel-T infusion were Grade 1 or 2 rigors and pyrexia (54.9% and 37.8% of patients, respectively), with a lower frequency of patients having Grade 1 or 2 tremors (26.8%) [29]. T-cell proliferative responses were detected to the PA2024 fusion protein [29]. However, while serum PSA decreases of greater than 25% were detected in 6.8% of patients receiving sipuleucel-T (with no decreases in the control patients), the primary endpoint of TTP did not reach statistical significance: patients receiving sipuleucel-T had a median TTP of 11.7 weeks (95% confidence interval (CI): 9.1-16.6) compared with 10.0 weeks for patients receiving placebo (95% CI: 8.7-13.1) [29].

While the D9901 trial was ongoing, a second companion phase III trial was also underway, accruing patients with asymptomatic metastatic, castrate-resistant disease (D9902A). This trial was being conducted as a confirmatory study with the same population, study design, and randomization scheme. While this trial was still accruing patients, the results from the D9901 trial showing a lack of difference in TTP were unblinded, and due to lack of significance in the primary endpoint of TTP, the D9902A trial was abandoned.

A subset analysis of D9901 found that patients with a Gleason score ≤ 7 treated with sipuleucel-T had a significant increase in TTP compared with those receiving placebo. This led to another Phase III trial (D9902B), which was designed similarly to the D9901 trial with 127 patients and a primary endpoint of TTP, but with the plan to only accrue asymptomatic metastatic, castrate-resistant patients with a Gleason score of 7 or less. However, as this trial was beginning to accrue, a later analysis of the D9901 trial revealed a significant difference in overall survival, with patients receiving sipuleucel-T having a three-year overall survival of 25.9 months, compared with 21.4 months of patients receiving placebo (hazard ratio (HR) 1.43, p=0.01) [30]. Additionally, as described above, it was observed that nearly half of progression events occurred within the first 12 weeks of the study, possibly before an optimal immunologic effect could have occurred, and therefore possibly accounting for a lack of difference in TTP [29, 30]. Furthermore, an integrated analysis of the D9901 and D9902A trials suggested that patients receiving sipuleucel-T had a median survival of 23.2 months (95% CI: 19.0-31.0) versus 18.9 months (95% CI: 13.5-25.3) in the placebo control group (HR: 1.5, p=0.011) [30]. Due to these findings, the D9902B protocol was amended, changing the primary endpoint from TTP to overall survival, and increasing the planned patient enrollment from 127 to 512 to sufficiently power this study to detect a difference in overall survival. This trial (IMPaCT, Immunotherapy for Prostate Adenocarcinoma Treatment) confirmed that treatment with sipuleucel-T provided a significant increase in overall survival: patients receiving an infusion of PA2024-pulsed cells had a median survival of 25.8 months compared to 21.7 months in the placebo group, consistent with the previous trials [7]. This survival difference reflected a significant difference in the risk of death

in patients treated with sipuleucel-T compared to the placebo group (hazard ratio (HR), 0.78. 95% CI: 0.61-0.96, p=0.03) [7]. This survival benefit was also present regardless of prior or subsequent treatment with docetaxel and prednisone. Similar to previous trials, no difference in time to progression was observed (14.6 weeks in the sipuleucel-T group versus 14.4 weeks in the placebo group, p=0.63), contradicting trends observed in the earlier phase studies [7]. With the significant survival benefit detected in the IMPACT phase III trial, FDA approved sipuleucel-T in April 2010 for the treatment of asymptomatic or minimally symptomatic metastatic, castrate-resistant prostate cancer.

C. 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 [31-33], antigen presentation occurs through naturally processed epitopes, and does not require autologous cell processing. 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 [19, 34]. 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 [35] and CD8+ T-cells specific for the targeted antigen [31, 36-39]. In animal models, the use of an intradermal route of vaccine administration, in particular, tends to promote this Th1/CTL-biased immune response [35, 40, 41]. It should also be acknowledged that the clinical efficacy of a DNA vaccine encoding tyrosinase for the treatment of canine melanoma, delivered with GM-CSF as a vaccine adjuvant, was approved by the USDA in early 2010 based on the results of clinical studies demonstrating an improved survival of companion dogs with oral melanoma. In fact, this represents the first vaccine approved in the U.S. for the treatment of existing cancer. Thus, this approach bears further investigation in human clinical trials.

D. DNA vaccine encoding PAP (pTVG-HP) can elicit antigen-specific CD4+ and CD8+ T cells in patients with prostate cancer

We have previously reported that DNA vaccines encoding either the human or rat homologue of PAP can elicit PAP-specific CD4+ and CD8+ T cells in rats, suggesting a feasible means of eliciting PAP-specific anti-tumor immune responses in patients [18, 19]. We have subsequently reported the results from a phase I/II clinical trial in which subjects with non-castrate, non-metastatic prostate cancer were immunized six times at two-week intervals with this same DNA vaccine. The primary objectives of that trial were to evaluate the safety and immunological efficacy of the pTVG-HP DNA vaccine in patients with clinical stage D0 prostate cancer [42]. 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, similar to the approach used in the canine melanoma vaccine trials, 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 determined to not be treatment-related. No other events > grade 2 were observed in any of the 22 subjects treated. Common events observed were 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 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 previously reported, 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 [22]. 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 [22]. 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; the presence of this persistent immunity was associated with favorable changes in PSA doubling time [23]. In addition, one patient went on to receive booster immunizations with 100 µg pTVG-HP at monthly intervals. As shown in Figure 1, booster immunizations were able to augment PAP-specific CD4+ and CD8+ proliferative T cells, as well as PAP epitope-specific CD8+ T cells (Figure 2) 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.

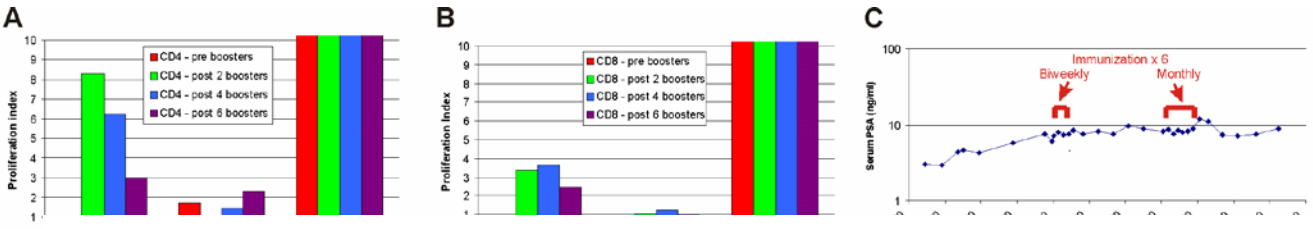


Figure 1: PAP-specific cellular immune responses can be boosted months after initial immunization**.** Peripheral blood mononuclear cells were obtained from a patient 18 months after the initial immunization series (pre boosters), and after 2, 4, or 6 monthly booster immunizations with 100 µg pTVG-HP. 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.

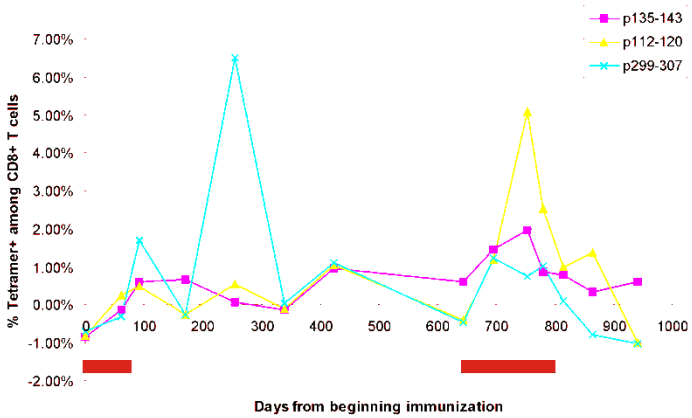


Figure 2: PAP-derived epitope-specific cellular immune responses can be detected long-term after initial immunization**.** Peripheral blood mononuclear cells were obtained from an HLA-A2-expressing patient at multiple intervals following an initial 6 biweekly immunization series and 6 monthly booster 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.).

Given the ability of a DNA vaccine to elicit PAP-specific T cells in some patients with prostate cancer, the observation that immune responses elicited tended to be after multiple immunizations, and the further observation that in at least one individual these immune responses could be boosted with subsequent reimmunization [23], a second pilot trial was initiated evaluating different schedules of immunization in patients with castrate-resistant, non-radiographically metastatic prostate cancer. This trial was designed to answer the question of whether six immunizations were insufficient in some individuals to develop an immune response, and whether ongoing repetitive immunization might be necessary. While this trial is still underway and results are preliminary, to date we have identified that many patients developed PAP-specific immune responses after as few as 3-6 immunizations (Figure 3, subject #2), and some individuals (Figure 3, subject #1), no immune responses were detectable after even 24 biweekly immunizations. These findings suggest that more potent immunization strategies might be necessary to elicit PAP-specific immunity in some individuals that could be further boosted with a DNA vaccine, and hence a rationale for the current trial. In addition, the findings from both of the trials above have provided the rationale for a randomized phase II clinical trial evaluating this same DNA vaccine to determine whether vaccination prolongs time to disease progression – this trial is currently underway.

E. Summary

The current protocol will examine the use of a plasmid DNA vaccine encoding PAP to induce and/or augment T-cell immune responses to PAP in patients with castrate-resistant, metastatic prostate cancer following treatment with sipuleucel-T. Based on published results from trials conducted with sipuleucel-T, PA2024 and PAP antibody and proliferative T cell responses have been observed, suggesting a possible CD4+ T-cell-biased immune response. In 25 individuals who have received multiple immunizations with the pTVG-HP DNA vaccine, no PAP-specific antibody responses have been observed, and multiple individuals have had detectable Th1-biased PAP-specific CD4+ and CD8+ responses. These results suggest that different types of immune responses to the same antigen might be elicited depending on the type of immunization, and hence a combination able to elicit a broad immune response might have greater anti-tumor effect. Given that a Th1/CD8+/CTL response would be predicted to be the most effective immune response elicited capable of lysing PAP-expressing prostate cancer cells, the identification of these specific subsets will be the primary endpoint of this pilot trial. The trial will be conducted in patients with minimally symptomatic, castrate-resistant, metastatic prostate cancer, as this is the approved indication for treatment with sipuleucel-T. In addition, given previous observations from clinical trials using sipuleucel-T, and multiple other anti-tumor vaccine trials, suggesting that clinical effects (unlike with standard cytotoxic therapies) might occur months after treatment

concurrent with the generation of immune responses, exploratory clinical endpoints will be to determine whether treatment affects progression-free survival using a delayed (12-week) time point as the baseline for subsequent radiographic comparisons. Additional clinical endpoints will be to examine the effects of treatment on circulating tumor cell number, PSA kinetics, and whether favorable delays in disease progression are associated with the detection of persistent immune responses to PAP or other "off-target" prostate cancer antigens after treatment, or negatively associated with unfavorable tolerant-type immune responses existing pre-treatment. If successful, this trial could provide rational clinical endpoints and immune biomarkers that could be used in the design of future studies using sipuleucel-T in combination with other agents, and/or a larger, confirmatory study in combination with booster DNA vaccines.

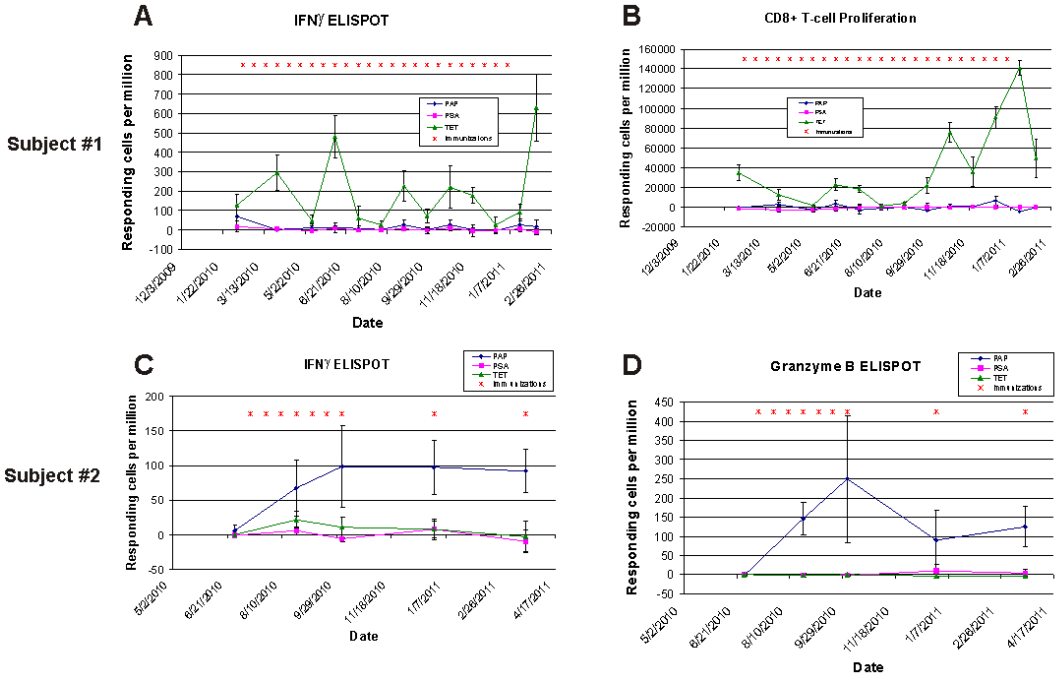


Figure 3: Patients with the same stage of prostate cancer respond differently to treatment with a DNA vaccine. Patients with castrate-resistant, non-metastatic prostate cancer were immunized at two-week intervals with a DNA vaccine encoding PAP 24 times (subject 1, panels A and B) or 6 times, followed by immunization at 3-month intervals (subject 2, panels C and D), as indicated by the red asterisks. Immune response was assessed using freshly obtained PBMC, without *in vitro* stimulation by T-cell proliferation using PKH26 dye dilution, using as stimulator antigens PAP (blue), PSA (pink, negative control), and tetanus toxoid (green), and assessed by flow cytometry after 7 days in antigen-specific culture (panel B). Precursor frequency of antigen-specific CD8+ T cells was estimated using ModFit software. In addition, immune response was assessed by antigen-specific IFNγ (panel A and C) or granzyme B (panel D) release by ELISPOT after 48- or 24-hour antigen-specific culture without *in vitro* stimulation.

3. Objectives

A. Primary Objectives

1. To evaluate whether immunization with a DNA vaccine encoding prostatic acid phosphatase (PAP, pTVG-HP) can augment PAP-specific effector and memory T cells following treatment with sipuleucel-T.

B. Secondary Objectives:

1. To determine 12-month radiographic progression-free survival

2. To determine median time-to-radiographic progression as a function of baseline time point (pretreatment or 3-months post treatment)

3. To evaluate effects of treatment on changes in serum PSA level and changes in PSA doubling time

C. Laboratory and Exploratory Objectives:

1. To determine overall survival

2. To evaluate effects of treatment on number of circulating tumor cells as determined by PSA ELISPOT

3. To evaluate PAP-specific antibody and T-cell immune responses following treatment with sipuleucel-T and DNA vaccine

4. To determine whether PAP-specific immune response is associated with prolonged (1 year) radiographic progression-free survival

5. To determine whether baseline immune responses (Th1 type or regulatory type) predict for immune responses elicited/augmented following treatment with sipuleucel-T +/- DNA vaccine

6. To determine whether treatment elicits immunologic antigen spread to other prostate associated antigens

4. Product Information

A. Sipuleucel-T

PA2024

PA2024 is a recombinant fusion protein consisting of human PAP and GM-CSF. GM-CSF acts as a growth factor and differentiation factor for antigen-presenting cells, including dendritic cells; the purpose of the fusion protein is to direct the PAP antigen to antigen-presenting cells *in vitro*, and promote antigen uptake and processing of the PAP antigen.

Preparation of sipuleucel-T

Patients will undergo a standard 1.5-2.0 blood volume leukapheresis to harvest peripheral blood mononuclear cells. This procedure will be performed either at the UWHC Infusion Center or a contract provider authorized by Dendreon Corporation. Immediately after collection, the product

will be transported by air carrier to a Dendreon-approved national cell processing facility. There, the cell product will be enriched for antigen-presenting cells by proprietary buoyant density centrifugation techniques, and cultured *in vitro* in the presence of PA2024 for 36-44 hours. After the culture period, the cells will be washed and resuspended in Lactated Ringer's Injection, USP. The sipuleucel-T cell product will then be shipped back to the UWHC for infusion at the UWHC Infusion Center. During shipment, sterility testing and cell count data are gathered for final product release for re-infusion. These procedures will all be performed as part of the standard-of-care for the FDA-approved sipuleucel-T product and re-infusion.

B. Plasmid DNA vaccine

pTVG-HP (pTVG4 vector containing cDNA for human PAP)

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

GM-CSF

GM-CSF (Leukine®, Sargramostim), will be obtained from commercial vendor (Genzyme, Cambridge, MA) and used as a vaccine adjuvant and will be provided without 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, 44]. The use of GM-CSF is associated with little toxicity [45-47]. 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 [48]. In previous clinical trials with this DNA vaccine and GM-CSF, transient dyspnea and chest pain have been observed, occurring within 45 minutes of treatment and resolving within 10 minutes without treatment; these reactions have been attributed to the GM-CSF. One allergic reaction with tongue swelling and lip edema has been observed in one patient following 11 immunizations, resolving following treatment with antihistamines, and without sequelae. 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 [47].

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

5. Patient Selection

A. Inclusion Criteria

1. Age ≥ 18 years.
2. Histologically confirmed diagnosis of prostate cancer (adenocarcinoma of the prostate)
3. Metastatic disease as evidenced by the presence of soft tissue and/or bone metastases on imaging studies (CT of abdomen/pelvis, bone scintigraphy)
4. Castrate-resistant disease, defined as follows:
    a. All patients must have received standard of care androgen deprivation treatment before trial entry (surgical castration versus GnRH analogue or antagonist treatment), and subjects receiving GnRH analogue or antagonist must continue this treatment throughout the time on this study.
    b. Patients may have been treated previously with a nonsteroidal antiandrogen, with evidence of disease progression (defined below) subsequently. Subjects must be off use of anti-androgen for at least 4 weeks (for flutamide) or 6 weeks (for bicalutamide or nilutamide) prior to registration.

        Subjects who demonstrate an anti-androgen withdrawal response, defined as a ≥ 25% decline in PSA within 4-6 week of stopping a nonsteroidal antiandrogen are not eligible until the PSA rises above the nadir observed after antiandrogen withdrawal.
    c. Castration levels of testosterone (< 50 ng/dL) within 2 weeks of registration
5. Progressive disease while receiving androgen deprivation therapy defined by any one of the following as per the Prostate Cancer Clinical Trials Working Group 2 (PCWG2) bone scan criteria [49] or RECIST 1.1 during or after completing last therapy:
    a. PSA: At least two consecutive rises in serum PSA, obtained at a minimum of 1-week intervals, and each value ≥ 2.0 ng/mL.
    b. Measurable disease: ≥ 50% increase in the sum of the cross products of all measurable lesions or the development of new measurable lesions. The short axis of a target lymph node must be at least 15mm by spiral CT to be considered a target lesion.
    c. Non-measurable (bone) disease: The appearance of two or more new areas of uptake on bone scan consistent with metastatic disease compared to previous imaging during castration therapy. The increased uptake of pre-existing lesions on bone scan will not be taken to constitute progression, and ambiguous results must be confirmed by other imaging modalities (e.g. X-ray, CT or MRI).

6. Must have ≥ 3 serum PSA values obtained over at least a 12 week period of time prior to registration, including the day of screening, to calculate a PSA doubling time (http://www.mskcc.org/applications/nomograms/prostate/PsaDoublingTime.aspx). Note: PSA's are not required to be obtained at the same laboratory. Use all PSA values that have been done in last 6 months to calculate PSA doubling time.
7. Life expectancy of at least 6 months
8. Patients must have an ECOG performance status of 0, 1, or 2.
9. Adequate hematologic, renal and liver function as evidenced by the following within 14 days of first peripheral blood collection for Sipuleucel-T.

WBC	≥ 2000 / mm^3
ANC	≥ 1000 / mm^3
HgB	≥ 9.0 gm/dL
Platelets	≥ 100,000 / mm^3
Creatinine	≤ 2.0 mg/dL
AST, ALT	≤ 2.5 x institutional upper limit of normal

10. Negative serology tests for HIV Ab/Ag Combo and for active Hepatitis B and Hepatitis C, within 14 days of first peripheral blood collection for Sipuleucel-T.
11. Patients must be at least 4 weeks from any prior treatments and have recovered (to < Grade 2) from acute toxicity attributed to this prior treatment
12. 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

B. Exclusion Criteria

1. Small cell or other variant prostate cancer histology
2. Patients may not be receiving other investigational agents or be receiving concurrent anticancer therapy other than androgen deprivation
3. Symptomatic metastatic disease, as defined by the need for opioid analgesics for the treatment of pain attributed to a prostate cancer metastatic lesion; patients receiving opioids must receive approval from the PI for eligibility
4. Patients may not have been treated with prior sipuleucel-T
5. Treatment with any of the following medications within 28 days of registration, or while on study, is prohibited:
   - Systemic corticosteroids (at doses over the equivalent of 1 mg prednisone daily); inhaled, intranasal or topical corticosteroids are acceptable
   - PC-SPES
   - Saw Palmetto
   - Megestrol
   - Ketoconazole
   - 5-α-reductase inhibitors – patients already taking 5-α-reductase inhibitors prior to 28 days prior to registration may stay on these agents throughout the course of therapy, but these should not be started while patients are on study
   - Diethyl stilbesterol
   - Abiraterone
   - Any other hormonal agent or supplement being used with the intent of cancer treatment

6. External beam radiation therapy within 4 weeks of registration is prohibited, or anticipated need for radiation therapy (e.g. imminent pathological fracture or spinal cord compression) within 3 months of registration.
7. Major surgery within 4 weeks of registration is prohibited
8. Prior cytotoxic chemotherapy (e.g. docetaxel, mitoxantrone, cabazitaxel) within 6 months of registration is prohibited
9. Patients with a history of life-threatening autoimmune disease
10. Patients who have undergone splenectomy
11. Patients must not have other active malignancies other than non-melanoma skin cancers or carcinoma in situ of the bladder. Subjects with a history of other cancers who have been adequately treated and have been recurrence-free for $\geq$ 3 years are eligible.
12. Patients with known brain metastases
13. Any antibiotic therapy or evidence of infection within 1 week of registration
14. Any other medical intervention or condition, which, in the opinion of the PI could compromise patient safety or adherence with the study requirements
15. Patients cannot have concurrent enrollment on other phase I, II, or III investigational treatment studies.

6. Experimental Design

This will be a 1:1 randomized, open-label, single institution pilot trial designed to evaluate the immunological and clinical effect of booster immunizations with a DNA vaccine encoding PAP with rhGM-CSF adjuvant following treatment with sipuleucel-T. Study arms will be defined as follows:

Arm 1: sipuleucel-T – administered intravenously (i.v.) per standard of care biweekly, weeks 0, 2, and 4

Arm 2: sipuleucel-T – administered intravenously (i.v.) per standard of care biweekly, weeks 0, 2, and 4
pTVG-HP (100 µg) with rhGM-CSF (208 µg) administered intradermally (i.d.) biweekly 4 times, then every 3 months to complete a 1-year treatment period (i.e. weeks 6 (day 43), 8 (day 57), 10 (day 71), 12 (month 3), then months 6 and 9)

A. Study Arm Assignment and Toxicity Assessment

Patients with asymptomatic-to-minimally-symptomatic, castrate-resistant, metastatic prostate cancer, with evidence of progressive disease, and a calculable PSA doubling time with at least 3 serum PSA values collected over a minimum of 3 months, will be invited to participate. These are subjects who would be eligible to receive sipuleucel-T as routine standard of clinical care. Subjects will be randomly assigned to a treatment group, stratified by pre-treatment estimated survival (< 18 months, $\geq$ 18 months), using the Halabi nomogram [50], and using laboratory/clinical data collected at screening for this determination. This stratification was chosen given the identification in a previous clinical trial that patients with more indolent disease characteristics appeared to have a greater benefit from vaccine treatment in terms of overall

survival, and the specific use of an 18-month cutoff is currently being used in prospective evaluation of other vaccine trials [51]. Given the absence of significant adverse events observed in previous clinical trials with either sipuleucel-T or pTVG-HP, 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.

Evidence that the toxicity rate is excessive will be considered sufficient if the lower limits of the 90% one-sided confidence intervals for the estimates of the true toxicity rates exceed the toxicity limits defined above (i.e., 20% for Grade 3 events and 10% for Grade 4 events), after the first 5 patients have been accrued. Operationally, this will occur if any of the following events occurs within a study arm: Grade ≥ 3 events (or Grade >1 autoimmune events) are present in at least 3 out of 5, 4 out of 9 (or less), 5 out of 13 (or less), or 6 out of 15 (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 2 out of 5, 3 out of 11 (or less), or 4 out of 15 (or less) of the subjects accrued (within a study arm).

If one of these thresholds is reached, accrual will be temporarily suspended and the study will be reviewed by the UW DSMC (data and safety monitoring committee) to consider safety and dose modifications with respect to treatment 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 radiographic disease progression, with progression being defined only AFTER the first 3-month staging evaluation, 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. The 6 month visit, in particular, is important to collect for the purpose of endpoint analysis. Subjects who have not come off study earlier for these reasons will come off study at the end of the one-year treatment period.

The primary endpoint of the trial will be an immunological endpoint – the detection of augmented numbers of PAP-specific effector (IFNγ-secreting and/or granzyme B-secreting) and memory (proliferating) T-cell immune response after immunization, using blood obtained at 6 months for the primary analysis compared with pre-treatment. Immune response will be determined as a static time point at 6 months, and "durable" immune responses will also be determined by evaluation at 3, 9, and 12 months after study entry.

All subjects will undergo radiographic imaging (CT of abdomen and pelvis and bone scan) prior to treatment, and at 3-month intervals (or as clinically indicated). Radiographic disease progression will be defined using both RECIST 1.1 and PCWG2 published criteria [49], as detailed in Section 7. Based on the prior trials leading to the approval of sipuleucel-T, and because immune therapies are expected to require up to several months before radiographic responses or stabilization might be observed, the radiographic studies obtained at 3 months will not be used to define progression, rather will be used as the baseline for future comparison. That is, while baseline studies will be obtained prior to study registration, and radiographic progression may be expected in 50% of patients at 3 months, this will not be used to define progression necessitating patients come off study, rather disease progression occurring after this initial 3-month period. Time to progression will be determined from registration for all subjects. 1-year progression-free survival will be calculated for each treatment arm, as well as the median progression-free survival.

PSA doubling times will be calculated from all available values obtained within 3-6 months from the same clinical laboratory (when feasible) prior to study enrollment, up to and including the baseline value, and used to determine a pre-treatment PSA doubling time. Similarly, a post-treatment doubling time will be calculated from all PSA values obtained beginning with the PSA value at day 1 and continuing until the end-of study (or 1-year) value.

7. Measurement of Effect

A. Malignant Disease Evaluation

To assess objective response, it is necessary to estimate the overall tumor burden at baseline (for purposes of comparison, "baseline" imaging will be that obtained at 3-month time point) to which subsequent measurements will be compared. Measurable disease is defined by the presence of at least one measurable lesion.

All measurements should be recorded in metric notation by use of a ruler or calipers. The same method of assessment and the same technique should be used to characterize each identified lesion at baseline and during follow-up. All pre-treatment evaluations should be performed as closely as possible to the beginning of treatment and not more than four weeks before registration.

The term evaluable in reference to measurability will not be used because it does not provide additional meaning or accuracy.

At baseline, tumor lesions will be characterized as either measurable or non-measurable.

NOTE: While progression will be documented from pre-treatment staging studies in all individuals, the intent of these measurements in this trial will be to determine whether "baseline" evaluation for immune-based treatments is preferable at a later time point after the therapy has been given the opportunity to exert an anti-tumor effect. Hence, the "baseline" evaluation for determining progression will be the staging studies performed at day 85 (3 months). Objective progression at Month 3 from the pre-treatment scans will not be used to alter treatment unless the

patient has symptomatic disease requiring other therapy and thereby needs to discontinue treatment.

1. Measurable

   Lesions that can be accurately measured in at least one dimension (longest diameter to be recorded) as ≥ 20 mm (2.0 cm) with conventional techniques or as > 10 mm (1.0 cm) with **spiral** CT scan. For a lymph node to be considered pathologically enlarged and measurable, it must be ≥ 15mm in short axis when assessed by spiral CT.

   If the measurable disease is restricted to a solitary lesion, its neoplastic nature should be confirmed by cytology/histology.

   Tumor lesions that are situated in a previously irradiated area are not considered measurable.

2. Non-Measurable

   All other lesions, including small lesions [longest diameter < 20 mm (2.0 cm) with conventional techniques or < 10 mm (1.0 cm) with **spiral** CT scan], lymph nodes < 15mm in short axis, and truly non-measurable lesions.

   Lesions considered to be truly non-measurable include the following: bone lesions, leptomeningeal disease, ascites, pleural/pericardial effusion, lymphangitis cutis/pulmonis, abdominal masses that are not confirmed and followed by imaging techniques, and cystic lesions.

B. Definitions of Response

1. Target Lesions

   All measurable lesions, up to a maximum of two lesions per organ and five lesions in total, representative of all involved organs. Target lesions should be selected on the basis of their size (those with the largest diameters) and their suitability for accurate repeated measurements.

   The sum of the diameters (long axis for non-nodal, short axis for nodal) of all target lesions will be calculated at baseline and reported as the baseline sum diameter. The sum diameter will be used to characterize the objective tumor response.

   a. Complete Response (CR)

   The disappearance of all target lesions. To be assigned a status of complete response, changes in tumor measurements must be confirmed by repeat assessments performed **no less than four weeks** after the criteria for response are first met. PSA must also be ≤ 0.2 ng/mL. All disease must be assessed using the same technique as at baseline. Lymph nodes that shrink to less than 1.0 cm are considered normal.

   b. Partial Response (PR)

   At least a 30% decrease in the sum of the diameters of target lesions (long axis for non-nodal lesions, short axis for nodal lesions), taking as reference the baseline sum

diameter. To be assigned a status of partial response, changes in tumor measurements must be confirmed by repeat assessments performed **no less than four weeks** after the criteria for response are first met. There must be no new lesions. All target measurable lesions must be assessed using the same techniques as at baseline.

c. Progressive Disease (PD)

At least a 20% increase in the sum of the diameters of target lesions (long axis for non-nodal lesions, short axis for nodal lesions), and a 0.5 cm absolute minimum increase, taking as reference the smallest sum diameter recorded since the Month 3 baseline measurements, or the appearance of one or more new lesion(s).

d. Stable Disease (SD)

Change in tumor size is less than that required to identify a partial response or progressive disease. To be assigned a status of stable disease, measurements must have met the stable disease criteria at least once after study entry at a minimum interval of 12 weeks.

2. Nontarget Lesions

All other lesions or sites of disease that do not meet the criteria for target lesions, including those on bone scintigraphy. Measurements of these lesions are not required, but the presence or absence of each should be noted throughout the study.

a. Complete Response (CR)

The disappearance of all nontarget lesions and undetectable PSA tumor marker levels. To be assigned a status of complete response, changes in tumor measurements must be confirmed by repeat assessments performed **no less than four weeks** after the criteria for response are first met.

b. Incomplete Response/Stable Disease (SD)

The persistence of one or more nontarget lesion(s) and/or the persistence of detectable serum PSA tumor marker levels. To be assigned a status of stable disease, measurements must have met the stable disease criteria at least once after study entry at a minimum interval of 12 weeks.

c. Progressive Disease (PD)

The appearance of one or more new lesion(s) and/or unequivocal progression of existing nontarget lesions.

NOTE: For lesions only detectable by bone scan, the appearance of $\geq$ 2 new lesions, with symptoms, will constitute disease progression. Without symptoms, and if no other evidence of disease progression (no progressive disease by PSA or measurable disease criteria), progression must be documented with repeat bone scintigraphy at least 6 weeks later demonstrating $\geq$ 2 new lesions. This is to eliminate the possibility of flair responses seen on bone scans.

3. Symptomatic Deterioration

Patients with a global deterioration of health status requiring discontinuation of treatment without objective evidence of disease progression at that time should be classified as having symptomatic deterioration.

4. PSA Progression

While PSA values will be collected, and PSA kinetics (PSA responses and maximal changes in PSA by waterfall plots) monitored as secondary endpoints, PSA rise will not be used to determine objective disease progression. Similar to the radiographic studies above, PSA progression or response will be defined using both pre-treatment (study entry) and 3-month values as baseline values.

a. **PSA Complete Response:** Decrease in PSA to <0.2 ng/mL and confirmed with PSA measurement a minimum of four weeks later (confirmed PSA CR). There must be no evidence of radiographic progression.

b. **PSA Partial Response**: Greater than or equal to 50% reduction in baseline PSA. There must be no evidence of radiographic progression.

c. **Best Response**: This is calculated from the sequence of objective statuses. The date of response will be defined as the first date at which PSA declined from baseline or normalized.

i. **Confirmed PSA PR**: Two or more objective statuses of PSA PR or better a minimum of four weeks apart documented before progression. Best response for objective disease must be stable/no response or better.

ii. **Unconfirmed PSA PR**: One objective status of PSA PR documented before progression, but not qualifying for confirmed PSA PR. Best response for objective disease must be Stable/no response or better.

iii. **No PSA Response**: Objective PSA status does not qualify as a PSA PR or unconfirmed PSA PR.

iv. **Inadequate Assessment, response unknown**: When best response for objective disease is inadequate or unknown or when PSA has been inadequately assessed, then PSA response will be coded likewise.

d. **PSA Progression**: Although not considered progression per the primary objective of this study, time to PSA progression will be used for secondary analyses. PSA progression will be defined as a **50%** increase in PSA over the nadir PSA, and $\geq$ 2 ng/mL above the nadir, confirmed by a second value 3 or more weeks later (i.e. confirmed rising trend). If no on-study reduction has occurred, nadir would be the baseline value (pre-treatment or month 3 time point for analyses).

C. Evaluation of Patient's Best Overall Response

The best overall response is the best response recorded from baseline until disease progression/recurrence, taking as reference for progressive disease the smallest measurements recorded after baseline. The table below provides overall responses for all possible combinations of tumor responses in target and nontarget lesions, with or without new lesions.

To be assigned a status of complete or partial response, changes in tumor measurements must be confirmed by repeat assessments performed **no less than four weeks** after the criteria for response are first met.

To be assigned a status of stable disease, measurements must have met the stable disease criteria at least once after study entry at a minimum interval of 12 weeks.

Overall Response for all Possible Combinations of Tumor Response

Target Lesions	**Nontarget Lesions**	**New Lesions**	**Overall Response**
CR	CR	No	CR
CR	Incomplete response/SD	No	PR
PR	Non-PD	No	PR
SD	Non-PD	No	SD
PD	Any	Yes or No	PD
Any	PD	Yes or No	PD
Any	Any	Yes	PD

CR = complete response; PR = partial response; SD = stable disease; PD = progressive disease

1. First Documentation of Response
   The time between initiation of therapy and first documentation of PR or CR.

2. Confirmation of Response
   To be assigned a status of complete or partial response, changes in tumor measurements must be confirmed by repeat assessments performed **no less than four weeks** after the criteria for response are first met.

3. Duration of Response
   Duration of overall response – the period measured from the time that measurement criteria are met for complete or partial response (whichever status is recorded first) until the first date that recurrent or progressive disease is objectively documented, taking as reference the smallest measurements recorded since treatment started.

   a. Duration of Overall Complete Response
   The period measured from the time measurement criteria are met for complete response until the first date that recurrent disease is objectively documented.

   b. Duration of Stable Disease
   A measurement from baseline (3 month) until the criteria for disease progression is met, taking as reference the smallest measurements recorded since baseline. To be

assigned a status of stable disease, measurements must have met the stable disease criteria at least once after study entry at a minimum interval of 12 weeks.

4. Survival

   Overall survival will be defined as the time interval from randomization to death from any cause or to the last follow-up in censored patients.

5. Time to Objective Disease Progression

   Time to progression will be defined as a function of baseline time points in two ways:

   (1) *Time to progression from randomization*: Time elapsed from randomization until disease progression or death. If a patient doesn't experience a disease progression or death event before the end of the follow-up period, then the observation for this patient will be censored.

   (2) *Time to progression from 3 months post-randomization*: Time elapsed from the 3 months disease progression assessment until disease progression or death. If a patient doesn't experience a disease progression or death event before the end of the follow-up period, then the observation for this patient will be censored.

The intent of defining time to progression as a function of baseline time points (date of randomization and 3-month disease assessment) is to determine if immunization prolongs the time to progression using a delayed baseline evaluation after the treatment has been given time to elicit an anti-tumor response.

6. Methods of Measurement

   Imaging based evaluation is preferred to evaluation by clinical examination. The same imaging modality must be used throughout the study to measure disease.

   a. CT and MRI

   CT and magnetic resonance imaging (MRI) are the best currently available and most reproducible methods for measuring target lesions. Conventional CT and MRI should be performed with contiguous cuts of 10 mm or less in slice thickness. Spiral CT should be performed by use of a 5 mm contiguous reconstruction algorithm. This specification applies to tumors of the chest, abdomen, and pelvis, while head and neck tumors and those of the extremities require specific procedures. Ultrasound should not be used for measurement methods.

   b. Tumor Markers

   The PSA tumor marker alone will not be used to assess response. PSA values will be collected for separate reporting of PSA kinetics, as described above. In addition, PSA must decline to < 0.2 ng/mL for a patient to be considered in complete clinical response when all tumor lesions have disappeared.

c. Clinical Examination

Clinically detected lesions will only be considered measurable when they are superficial (e.g., skin nodules and palpable lymph nodes). For skin lesions, documentation by color photography, including a ruler to estimate size of the lesion, is recommended. Photographs should be retained at the institution.

8. 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) with an attribution of at least possibly related to the study or treatment procedures, and occurring between the pre-study visit and within one month of the final 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 Grade 2 autoimmune toxicity) that is believed at least possibly due to treatment, 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 initial 12 weeks of treatment, 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 3-month 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 study and local PIs. The following dose schedule could 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.

9. Plan of Treatment

The following section describes the schedule for prescreening, sipuleucel-T collections and infusions, vaccine inoculations, and clinical and laboratory evaluations.

When a chemistry panel is indicated the following tests are performed: creatinine, SGOT, total bilirubin, alkaline phosphatase, amylase, and LDH. Whenever a CBC is indicated, this shall include differential and platelet count.

Sipuleucel-T infusions should be 14 days apart, but may be up to 21 days apart to accommodate scheduling difficulties. The study calendar will then be delayed accordingly by the same amount of time. For a delay greater than a week, the nature of this delay will be discussed with the medical monitor or PI to determine the appropriate course of action. For subjects on Arm 2, biweekly DNA vaccination (Immunization #1-3) can be +/- 3 days and quarterly vaccinations (Immunizations #4-6) can be +/- 7 days from the expected date (determined from prior visit).

All subjects should have blood drawn for immune monitoring per study calendar (+/- 7 days) unless they come off study early. Subjects that come off study early should still have all of the immune draws done, but the blood draws can correspond with standard of care visits (i.e. once off study, they don't need to come to clinic just for the immune draw). This plan is summarized in the Study Schema (pg. 4), Study Calendar (pg. 5), and Blood Draws for Research table (Appendix B)

A. Prescreen (performed within 4 weeks of Day 1)

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

B. Screening Evaluation (performed within 2 weeks of 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 differential and platelets), chemistry panel, serum prostate specific antigen (PSA), serum PAP, serum testosterone, serology tests for HIV1, HIV2, Hepatitis B, and Hepatitis C. Tests ordered are the following: serology for HIV Ab/Ag Combo, Hepatitis B Surface Ag, and Hepatitis C AB
3. Determination of predicted survival (< 18 months, ≥ 18 months) for stratification purposes using laboratory values and clinical data (ECOG performance score, presence or absence of visceral metastases, Gleason score, screening PSA, screening LDH, screening alkaline phosphatase, and screening hemoglobin) and the Halabi nomogram [50].
4. Randomization – treatment arm assignment
5. Baseline research leukapheresis (50-100 mL approximate total volume, for immunological monitoring, not for preparation of sipuleucel-T) – to take place 2 weeks (+/- 7 days) prior to day 1.
6. Tetanus immunization (to take place after baseline leukapheresis and prior to day 1)

7. 20 mL peripheral blood (red-top tubes) for sera to evaluate baseline antibody responses, and 20 mL peripheral blood (green-top tubes) for CTC baseline evaluation

C. Peripheral Blood Collection for Sipuleucel-T – Day 1

1. Standard 1.5-2.0 liter peripheral blood cell leukapheresis – conducted at Dendreon-authorized center for sipuleucel-T collection.

D. Sipuleucel-T Infusion #1 – Day 4

1. Reinfusion of sipuleucel-T product – conducted at UWHC – standard-of-care protocol
2. Monitor patient for 30 minutes after completing infusion. Monitoring should include vital signs (including temperature, heart rate and blood pressure) and brief symptoms assessment by nursing staff prior to infusion and 30 minutes after infusion complete.

E. Peripheral Blood Collection for Sipuleucel-T – Day 15

1. Physical examination, symptoms assessment, and ECOG performance score (these can occur up to 72 hours prior to leukapheresis)
2. Blood draw for CBC with differential and platelets (can occur up to 72 hours prior to leukapheresis)
3. Standard 1.5-2.0 peripheral blood cell leukapheresis – conducted at Dendreon-authorized center for sipuleucel-T collection.

F. Sipuleucel-T Infusion #2 – Day 18

1. Reinfusion of sipuleucel-T product – conducted at UWHC – standard-of-care protocol
2. Monitor patient for 30 minutes after completing infusion. Monitoring should include vital signs (including temperature, heart rate and blood pressure) and brief symptoms assessment by nursing staff prior to infusion and 30 minutes after infusion complete.

G. Peripheral Blood Collection for Sipuleucel-T – Day 29

1. Physical examination, symptoms assessment, and ECOG performance score (can occur up to 72 hours prior to leukapheresis)
2. Blood draw for CBC with differential and platelets (can occur up to 72 hours prior to leukapheresis)
3. Standard 1.5-2.0 peripheral blood cell leukapheresis – conducted at Dendreon-authorized center for sipuleucel-T collection.

H. Sipuleucel-T Infusion #3 – Day 32

1. Reinfusion of sipuleucel-T product – conducted at UWHC – standard-of-care protocol
2. Monitor patient for 30 minutes after completing infusion. Monitoring should include vital signs (including temperature, heart rate and blood pressure) and brief symptoms assessment by nursing staff prior to infusion and 30 minutes after infusion complete.

I. Sipuleucel-T Follow-Up Visit Day 43 (+/- 3 days)

1. Physical examination, symptoms assessment, and ECOG performance score
2. Blood draw for CBC with differential and platelets, chemistry panel, PSA and 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 and CTC analysis

ARM 2 SUBJECTS ONLY: DNA Immunization #1:

4. Subjects will receive pTVG-HP DNA immunizations intradermally on the deltoid area of the lateral arm (preferentially left arm) with 0.25 mL administered at each of two adjacent sites.
5. Following vaccinations, the subjects will be observed for 60 minutes for unanticipated adverse events and injection site inspection.

J. ARM 2 SUBJECTS ONLY: DNA Immunization #2 – Day 57 (+/- 3 days)

1. Immunization/GM-CSF and post-treatment monitoring as described above in section 9.I.4-5

K. ARM 2 SUBJECTS ONLY: DNA Immunization #3 – Day 71 (+/- 3 days)

1. Immunization/GM-CSF and post-treatment monitoring as described above in section 9.I.4-5

L. 3-Month Follow-up – Day 85 (+/- 7 days)

1. Physical examination, symptoms assessment, and ECOG performance score.
2. Blood draw for CBC with differential and platelets, chemistry panel, serum PSA and 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 and CTC analysis
4. CT scan of abdomen and pelvis, bone scintigraphy (can be performed +/- 7 days of this visit)

ARM 2 SUBJECTS ONLY: DNA Immunization #4:

5. Immunization/GM-CSF and post-treatment monitoring as described above in section 9.I.4-5

M. 6-month Follow-up – Week 24 (+/- 7 days)

1. Physical examination, symptoms assessment, and ECOG performance score
2. Blood draw for CBC with differential and platelets, chemistry panel, serum PSA and PAP (can occur up to 7 days prior to leukapheresis)
3. CT scan of abdomen/pelvis and bone scintigraphy (can be performed +/- 7 days of this visit)
4. 10 ml peripheral blood (red-top tubes) for sera to evaluate antibody responses, and 20 ml peripheral blood (green-top heparinized tubes) for CTC analysis
5. Leukapheresis (~1.0 blood volume, 100 mL collection) for T-cell response evaluation (can be performed +/- 7 days of this study visit). If leukapheresis is not able to be obtained, then up to 150 ml of peripheral blood (green-top heparinized tubes) may be obtained via peripheral blood draw for the T-cell response evaluation.

ARM 2 SUBJECTS ONLY: DNA Immunization #5

6. Immunization/GM-CSF and post-treatment monitoring as described above in section 9.I.4-5

N. 9-Month Follow-up – Week 36 (+/- 7 days)

1. Physical examination, symptoms assessment, and ECOG performance score.
2. Blood draw for CBC with differential and platelets, chemistry panel, serum PSA and 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 and CTC analysis
4. CT scan of abdomen and pelvis, bone scintigraphy (can be performed +/- 7 days of this visit)

ARM 2 SUBJECTS ONLY: DNA Immunization #6

5. Immunization/GM-CSF and post-treatment monitoring as described above in section 9.I.4-5

O. OFF STUDY VISIT AND/OR 12-Month Follow-up – (week 48 +/- 7 days)

1. Physical examination, symptoms assessment, and ECOG performance score.
2. Blood draw for CBC with differential and platelets, chemistry panel, serum PSA and 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 and CTC analysis.
4. CT scan of abdomen and pelvis, bone scintigraphy (can be performed +/- 7 days of this visit, and does not need to be repeated for off-study visit if progression of disease already confirmed within 1 month)

P. Long-term Follow-Up

1. Subjects will be contacted by telephone (if not already being seen in clinic) annually (from registration) for up to 5 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

10. Response Monitoring

A. Immunological Monitoring

Blood will be collected by either peripheral blood draw (up to 150 mL) or leukapheresis (50-100 mL) pre-immunization, after the initial three sipuleucel-T treatments, and at quarterly intervals up to one year, for immunological monitoring. From the heparinized blood, peripheral blood mononuclear cells (PBMC) will be prepared by density centrifugation over Ficoll-Paque using standard techniques. PBMC will be used directly for analysis, and residual material 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. IFNγ and granzyme B ELISPOT analysis, PAP-specific T-cell proliferation, flow cytometric assays of antigen-specific cytokine secretion, and ELISA tests for antigen-specific antibodies, will be the primary methods of analysis. The primary antigens tested will be PAP (experimental), PSA (negative control), and tetanus toxoid (positive control). The primary immune analysis will be conducted at the 6-month time point, and compared with the pre-treatment time point, and for patients to be evaluable for immune response (primary endpoint), blood (PBMC and serum) from this time point must be available for analysis. However, immune monitoring will be conducted at the other time points indicated in secondary analyses to evaluate kinetic measures of immunity, and evaluate whether durable immune responses of particular phenotypes are elicited and/or maintained. Assays may be conducted at the time of sample collection (fresh) and/or batched and performed at one time from multiple cryopreserved samples collected at different time points. Other methods of effector and regulatory T-cell response to PAP and other human tissue antigens may be used. Some assays may assess immune responses to the PA2024 (PAP-GM-CSF) fusion antigen or to GM-CSF.

A.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 [23]. IFNγ and granzyme B will be preferred analytes evaluated, as these are specifically associated with inflammatory/tissue-destructive (Th1-type, cytolytic) immune responses (see Figure 3). Specifically, cryopreserved PBMC from subjects at the various time points will be thawed, rested, 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.

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

A.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 PKH26 dye dilution assay (see Figure 3). Specifically, PBMC will be labeled *in vitro* with PKH26 dye (Sigma, St. Louis, MO) according to manufacturer's recommendation. 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 a pool of peptide spanning the amino acid sequence of PAP, 2 µg/ml PAP protein (Research Diagnostics Inc., Flanders, NJ), 2 µg/ml PSA (Research Diagnostics Inc.), 250 ng/ml tetanus toxoid, and 2.5 µg/ml phytohemaglutinin (PHA). After 6-7 days of culture at 37°C/5% CO_2, cell surface markers will be stained to characterize the T-cell phenotype and memory phenotype of proliferating cells (CD45RO, CCR7). Flow

cytometery will be used to identify and enumerate CD3+CD4+ and CD3+CD8+ T cells co-staining for PKH26. The frequency of antigen-specific CD3+CD4+ and CD3+CD8+ T cells will be determined by determining the precursor frequency of PKH26+ events among CD4+ or CD8+ events (estimated from the number of cells and number of cell divisions by dye dilution using ModFit software, Verity Software House, Topsham, ME), and subtracting the mean precursor frequency of proliferating cells under media-only conditions.
REPORTING AND RESPONSE DEFINITION: Data will be reported as a mean and standard deviation of antigen-specific (PAP-specific) proliferative precursors per 10^6 PBMC using triplicate assessments for each antigen-stimulation condition. Comparison of experimental wells with control, no antigen, wells will be performed using a two-tailed Student's t test, with $p < 0.05$ defined as a significant antigen-specific proliferative T-cell response. A significant antigen-specific response resulting from immunization will then be defined as a PAP-specific response detectable at the 6-month post-treatment time point (or other post-treatment time point evaluated) that is significantly higher than to media only (as above), at least 3-fold higher than the mean baseline value, and with a frequency > 100 per 10^6 CD4+ or CD8+ T cells.

A.3. 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 [26]. 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^oC. 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 [26].
REPORTING AND RESPONSE DEFINITION: These are not strictly quantitative assays. IgG response will be reported graphically demonstrating sera dilution curves, and by titer – defined as the highest sera dilution at which IgG responses are detectable above the mean + 3 standard deviations of the negative control. A positive IgG response resulting from immunization will be defined as an antigen-specific (anti-PAP) IgG titer at least 4-fold higher than the baseline titer detectable at the 6-month post-treatment time point (or other post-treatment time point evaluated).

A.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 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 [52]. 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 [53]. 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.

A.5. Assessment of antigen-spread to other prostate-associated antigens

High-throughput immunoblot (HTI): A secondary 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. Exploratory studies will 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 [54-56]. IgG specific for 126 antigens, including 29 cancer-testis antigens [57, 58] and 97 prostate antigens frequently immunologically recognized [59-62] will be identified by screening a high-density phage array expressing these individual antigens, as we have previously reported [54, 56, 57]. 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 if 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. 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.

B. Circulating Tumor Cell (CTC) Enumeration

CTC will be enumerated at the same time points as for immune evaluation (pre-treatment, after 6 weeks, and at quarterly intervals for up to one year) using an ELISPOT methodology as described above, however using capture and detection antibodies specific for PSA [63]. This methodology has been used to count PSA-secreting cells directly from peripheral blood with a frequency < 1 per million PBMC. Given the rarity of these cells, they will not be normally distributed among individual wells of an ELISPOT plate. Hence, PBMC will be plated at

200,000 cells/well of a 96-well plate, and all spots will be counted (i.e. screening for events among ~20 x 10^6 total PBMC). Multiple replicates to determine standard deviation are not feasible. Other methods of CTC enumeration, such as by flow cytometry, may also be used.

C. Serum PSA Doubling Time Response

See Section 12 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 for the three-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 serum PSA doubling time will be calculated using the same algorithm and using all serum PSA values available during the time patients are on study beginning with the PSA value obtained at day 1.
REPORTING AND RESPONSE DEFINITION: PSA doubling times for the pre-treatment and post-treatment periods will be defined as above. An increase in the PSA doubling time to at least double the baseline value will be defined as a PSA doubling time "response" [64].

11. Reporting Adverse Events

Please see section 14 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 adverse events will be recorded from time of informed consent through 30 days after the final dose of study drug, as well as any time after these 30 days that the event is believed to be at least possibly related to study treatment. Serious adverse events are any events occurring that result in any of the following outcome:

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 14.C.

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.

12. Statistical Considerations

A. Overview

This a pilot trial to evaluate the immunological and clinical effect of booster immunizations with a DNA vaccine encoding PAP with rhGM-CSF adjuvant following treatment with sipuleucel-T. The rationale for conducting this trial is summarized in Section 2 (E).

B. Objectives

The primary objective of this study is to evaluate whether immunization with a DNA vaccine encoding prostatic acid phosphatase (PAP, pTVG-HP) can augment PAP-specific effector and memory T cells following treatment with sipuleucel-T.

The secondary objectives are:

1. To determine 12-month progression-free survival
2. To determine median time-to-progression as a function of baseline time point (pretreatment or 3-months post treatment)
3. To evaluate effects of treatment on changes in serum PSA level and changes in PSA doubling time

The laboratory and clinical exploratory objectives are:

1. To determine overall survival

2. To evaluate effects of treatment on number of circulating tumor cells as determined by PSA ELISPOT
3. To evaluate PAP-specific antibody and T-cell immune responses following treatment with sipuleucel-T and DNA vaccine
4. To determine whether PAP-specific immune response is associated with prolonged (1 year) progression-free survival
5. To determine whether baseline immune responses (Th1 type or regulatory type) predict for immune responses elicited/augmented following treatment with sipuleucel-T +/- DNA vaccine
6. To determine whether treatment elicits immunologic antigen spread to other prostate associated antigens

C. Definitions/Endpoints

C.1. Primary Immunological Endpoint: Immune Response

An immune response will be defined based on the detection of augmented numbers of PAP-specific effector (IFNγ-secreting and/or granzyme B-secreting) and/or memory (proliferating) T-cell immune response after immunization, using blood obtained at 6 months for the primary analysis compared with pre-treatment. Specifically, a positive antigen-specific response resulting from immunization will be defined as a PAP-specific response by two or more of these three tests detectable at the 6-month post-treatment time point that is significantly ($p<0.05$, two-sided using a two-sample t-test) higher than to media only, at least 3-fold higher than the mean baseline value, and with a frequency > 10 per 10^6 PBMC, based on 8-well replicate assays (for ELISPOT-based assays) or triplicate assays (for proliferation analysis). Otherwise, a patient will be declared as an immune non-responder.

Immune response will be determined as a static time point at 6 months, and "durable" immune responses will also be determined by the presence of a PAP-specific immune response by the criteria above detectable at 3, 9, or 12 months after study entry.

C.2. Secondary Clinical and Immunological Endpoints

C.2.1. Time to Progression

Time to progression will be defined as a function of baseline time points in two ways:

(1) *Time to progression from randomization*: Time elapsed from randomization until disease progression or death. If a patient doesn't experience a disease progression or death event before the end of the follow-up period, then the observation for this patient will be censored.

(2) *Time to progression from 3 months post-randomization*: Time elapsed from the 3 months disease progression assessment until disease progression or death. If a patient doesn't experience a disease progression or death event before the end of the follow-up period, then the observation for this patient will be censored.

The rationale for defining time to progression as a function of baseline time points (date of randomization and 3-month disease assessment) is described in Section 6 (B).

C.2.2. Twelve-month Progression Free Survival Rate

The 12-month progression free survival rate will be defined as the proportion of patients progression free at the 12 month (post-randomization) disease assessment time point.

C.2.3. Overall Survival

Overall survival will be defined as the time interval from randomization to death from any cause or to the last follow-up in censored patients.

C.2.4. PSA Response

PSA complete response will be defined a decrease in PSA to <0.2 ng/mL. The decrease must be confirmed with PSA measurement a minimum of four weeks later (confirmed PSA CR). PSA partial response will be defined as reduction of at least 50% in baseline PSA.

C.2.5. PSA Doubling Time

PSA doubling time (PSADT) will be defined as the time it takes for the PSA value to double. PSADT will be calculated for each patient pre-treatment and post-treatment. The pre-treatment PSADT will be calculated from all available values obtained within 3-6 months prior to study enrollment, up to and including the baseline value. The post-treatment PSADT will be calculated from all PSA values obtained beginning with the PSA value at day 1 and continuing until the end-of study value. The pre-treatment and post-treatment PSADT will be calculated for each patient as the log 2 divided by the slope of the log PSA line, based on the linear regression model of log (PSA value) on time.

C.2.6. Evaluable for Immune Response

All patients who complete sipuleucel-T treatment at weeks 0, 2, and 4, and who have an immune response assessment at month 6 will be evaluable for immune response.

C.2.7. Analysis Populations

The ITT population consists of all patients who are randomized. The per-protocol population is defined as all patients who have been treated according to protocol, i.e., all inclusion/exclusion criteria are satisfied, and adequate treatment compliance of sipuleucel-T treatment and vaccine (pTVG-HP) is achieved.

D. Study Design

The study is designed as a randomized, two-arm, open-label, single institution pilot trial. Study arms will be defined as follows:

Arm 1: sipuleucel-T – administered intravenously (i.v.) per standard of care biweekly, weeks 0, 2, and 4.

Arm 2: sipuleucel-T – administered intravenously (i.v.) per standard of care biweekly, weeks 0, 2, and 4
pTVG-HP (100 μg) with rhGM-CSF (208 μg) administered intradermally (i.d.) biweekly 4 times, then every 3 months to complete a 1-year treatment period (i.e. weeks 6, 8, 10, 12, then months 6, 9)

Eligible subjects will be randomly assigned in 1:1 fashion to either Arm 1 or Arm 2.

E. Randomization and Stratification

Eligible patients will be randomized to the two study arms with an allocation ratio of 1:1. The randomization will be based on permutated blocks of size 2-4, stratified by estimated survival ($<$ 18 months, $\geq$ 18 months) using the Halabi nomogram [50].

F. Sample Size Estimation and Accrual

The endpoint of this pilot trial will be an immunological endpoint, i.e., the immune response rate at 6 months. Based on published results from a randomized trial using sipuleucel-T only in this population, in which proliferative T-cell responses specific for PAP were detected in 15 of 55 (27.3%) patients after immunization [7], and results from a phase I trial with this DNA vaccine only in which PAP-specific cytolytic T-cell responses were amplified after immunization in 7 out 12 (58%) evaluable patients [23], it is anticipated that the immune response rate in the sipuleucel-T alone arm (Arm 1) will be 25% and that the addition of the pTVG-HP vaccine (Arm 2) will boost the response rate to at least 75%. An increase of 50% in the immune response rate will be considered as a clinically important difference. The following table shows the required sample sizes (per arm) to detect various differences in the immune response rates between the two arms with 80% power at the one-sided 5% significance level. These calculations are based on the stratified Mantel-Haenszel test with exact p-values.

Table 12.1 *Required sample sizes (per arm) to detect various differences in the immune response rates between the two arms with 80% power at the two-sided 5% significance level*

	Immune Response Rate: Arm 2 (sipuleucel-T + pTVG-HP vaccine arm)			
Immune Response Rate Arm 1 (sipuleucel-T alone arm)	70%	75%	80%	85%
15%	12	10	9	9
20%	15	12	10	9
25%	18	14	12	10
30%	23	18	15	12

A sample size of 14 patients per arm is proposed for this trial. This sample size is sufficient to detect an anticipated difference in the immune response rate of at least 50% (i.e., ≥75% in Arm 2 vs. 25% in Arm 1) with adequate power (≥80%) at the one-sided 5% significance level. The following table shows the attainable power levels to detect various differences in the immune response rates between arms at the one-sided 5% significance level, assuming a sample size of 28 evaluable (for immune response) patients.

Table 12.2: *Attainable power levels to detect various differences in the immune response rates between the two arms at the one-sided 5% significance level, assuming a sample size of 14 evaluable patients per arm*

	Immune Response Rate: Arm 2 (sipuleucel-T + pTVG-HP vaccine arm)			
Immune Response Rate Arm 1 (sipuleucel-T alone arm)	70%	75%	80%	85%
15%	85%	91%	96%	98%
20%	76%	85%	91%	96%
25%	65%	80%	84%	91%
30%	53%	65%	76%	85%

A difference in the immune response rate of 55% between the two arms will be detected with at least 84% power. Analogously, a difference in the efficacy response rate of only 40% between the two arms will be detected with 53% power at the one-sided 5% significance level. In order to account for unevaluable patients, 30 eligible patients (15 per arm) will be accrued and randomized. Based on past experience with this patient population treated at our institution, we anticipate that the proposed total number of 30 patients will be accrued within 18 months. Each patient will be followed for at least 12 months.

G. Statistical Analysis Plan

G.1. General

This section outlines the statistical analysis strategy and procedures for the study. If after the study has begun changes are made to primary and/or key secondary objectives, or the statistical methods related to those objectives, then the protocol will be amended.

Summary tabulations will be presented that will display the number of observations, mean, standard deviation, median, range, minimum, and maximum for continuous variables and the number and percent per category for ordinal and categorical data. All endpoints of this study will be presented graphically where possible using boxplots and histograms for continuous variables and (stacked) bar charts for categorical variables. Data analysis will be performed using SAS® (SAS Institute Inc., Cary, North Carolina) version 9.2 or greater.

There are many comparisons made between the two trial arms for multiple measurements, both clinical and laboratory. However, since this a pilot study, no adjustment will be made for

multiple comparisons. Except for the primary immunological endpoint, where the comparison between arms will be conducted at the one-sided 0.05 significance level, all other comparisons will be made using a two-sided test at a significance level of 0.05 without adjustment for multiplicity.

G.2. Baseline Comparability

All measurements (variables) collected at baseline will be summarized and compared between study arms. These include demographic variables, lab parameters (CBC and platelets, creatinine, alk phos, SGOT, total bilirubin, amylase, LDH, serum testosterone and PSA and PAP) and medical history information. Comparisons between study arms of baseline variables on a continuous scale will be performed using a two-sample t-test and/or nonparametric Wilcoxon Rank Sum test. Comparisons between study arms of baseline variables on a categorical scale will be performed using Fisher's exact test.

G.3. Analysis of the Primary Immunological Endpoint: Immune Response Rate

Immune responses, obtained at 6 months, will be summarized in tabular format. Ninety-five percent confidence intervals for the proportions of patients with an immune response will be constructed for each study arm. The comparison of immune response rates between study arms will be performed using the Cochran-Mantel-Haenszel test**,** stratified by estimated survival (< 18 months, ≥ 18 months) using the Halabi nomogram [50]. Due to the small sample size, exact p-values will be computed using Zelen's exact test for equal odds ratios for stratified 2 X 2 tables.

As a secondary analysis, "durable" immune responses will be determined by the presence of immune responses at one or more of the 3, 9, and 12 month time points evaluated after randomization. Immune responses determined at the 3, 9 and 12 month time points will be summarized in tabular format and compared between study arms using the Cochran-Mantel-Haenszel test**,** stratified by estimated survival (< 18 months, ≥ 18 months) using the Halabi nomogram [50]. Furthermore, a generalized linear mixed effects model for binomial data with a logit link function will be employed to evaluate the longitudinal effects of immune responses measured at the 3-month intervals.

G.4. Analysis of Secondary and Exploratory Clinical and Immunological Endpoints

G.4.1. Analysis of 12-month Progression-Free Survival Rate

The 12-month progression-free survival rate will be estimated for each arm using the Kaplan-Meier method. The 12-month progression-free survival rate, along with the corresponding two-sided 95% confidence intervals, will be reported for each arm. In order to evaluate the 12-month progression-free rate as a function of baseline time point (pretreatment or 3-months post treatment, the analysis will be conducted using two different baseline values: (1) Date of randomization, and (2) 3-months disease assessment.

G.4.2. Analysis of Progression-Free Survival

Progression-free survival will be estimated for each arm using the Kaplan-Meier method. The median progression-free survival, along with the corresponding two-sided 95% confidence intervals, will be reported for each arm. The comparison of progression-free survival between study arms will be performed using the stratified (by estimated survival at baseline, < 18 months vs. ≥ 18 months) log-rank test. In order to evaluate progression-free rate as a function of baseline time point (pretreatment or 3-months post treatment, the analysis will be conducted using two different baseline values: (1) Date of randomization, and (2) 3-months disease assessment.

G.4.3. Analysis of Overall Survival

Overall survival will be estimated for each arm using the Kaplan-Meier method. The median overall survival, along with the corresponding two-sided 95% confidence intervals, will be reported for each arm. The comparison of overall survival between study arms will be performed using the stratified log-rank test.

G.4.4. Analysis of Changes in PSA Values, PSA Kinetics and PSA Doubling Time

PSA values will be summarized using standard descriptive statistics for each defined assessment time point (baseline, 3, 6, 9, and 12 months) in terms of number of observations, means, standard deviations, medians and ranges, stratified by study arm. Percentage changes from baseline to the 3, 6, 9, and 12 months assessment will be calculated. Changes within each arm will be evaluated using a paired t-test or nonparametric Wilcoxon Signed Rank test, if the percentage changes are not normally distributed. Analysis of variance, which will include study arm and baseline predicted survival (< 18 months vs. ≥ 18 months) will be used to compare percentage changes in PSA values between study arms. If the percentage changes are not normally distributed and if a normality-improving data transformation is unavailable, then the stratified, nonparametric Wilcoxon rank-sum tests will replace the planned ANOVA. Changes in PSA levels will be presented graphically using waterfall plots.

PSA response rates (CR / PR) will be summarized in tabular format and compared between study arms using the Cochran-Mantel-Haenszel test**,** stratified by baseline estimated survival (< 18 months vs. ≥ 18 months).

PSA doubling time for each patient is estimated as: PSADT = log(2)/b, where b is the OLS estimator of slope parameter of the linear regression model $\log(PSA) = \alpha + \beta * month + \varepsilon$. This method may result in negative PSA doubling time estimates. For analysis purposes, negative PSA doubling time estimates will be replaced by fixed positive PSA doubling time values (e.g., by the largest observed positive PSA doubling time value). Serum PSA doubling time will be summarized for each study arm by standard descriptive statistics in terms of means, standard deviations, medians and ranges. Percentage changes between pre-treatment and post-treatment PSA doubling times will be computed. Changes between pre- and post-treatment serum PSA doubling time within each arm will be assessed using a paired t-test and/or non-parametric Wilcoxon Signed Rank test. A two-sample t-test or non-parametric Wilcoxon Rank Sum test will be used to compare the changes in PSA doubling time from the pre- to the post-treatment assessments between study arms.

G.5. Correlative Analyses

G.5.1. Analysis for evaluating effects of treatment on the number of circulating tumor cells as determined by PSA ELISPOT

The number of circulating tumor cells per million PBMC will be summarized in terms of medians and ranges, stratified by study arm and assessment time point. The nonparametric Wilcoxon Signed Rank test will be used to evaluate the effects of treatment on the number of circulating tumor cells within each study arm. The comparison of changes in the number of circulating tumor cells between study arms will be performed using the nonparametric, stratified, Wilcoxon Rank Sum test. Results will be presented graphically over time, and trends (increase, decrease, stable) will be assessed over time.

G.5.2. Analysis for evaluating PAP-specific antibody and T-cell immune responses following treatment with sipuleucel-T and DNA vaccine

PAP-specific antibody and T-cell immune responses, evaluated by multiple different measures described in Section 10.A, following treatment with sipuleucel-T and DNA vaccine will be summarized in tabular format.

G.5.3. Analysis for evaluating whether PAP-specific immune response is associated with prolonged (1 year) progression-free survival

Logistic regression analysis will be conducted to evaluate whether PAP-specific immune response is associated with prolonged (1-year) progression-free survival. The dependent variables will be 12-month progression free survival while treatment arm and 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.

G.5.4. Analysis for evaluating whether baseline immune responses (Th1 type or regulatory type) predict for immune responses elicited/augmented following treatment with sipuleucel-T +/- DNA vaccine

Logistic regression analysis will be conducted to evaluate whether baseline immune responses (Th1-type or regulatory type) predict for immune responses elicited/augmented following treatment with sipuleucel-T +/- DNA vaccine. 6-month and durable immune response at 3,6 or 12 months will be included as dependent variables in this analysis while treatment arm and baseline PAP-specific immune responses (no vs. yes) will be included as covariates. Odds ratios and the corresponding 95% confidence intervals will be reported.

G.5.5. Analysis for evaluating whether treatment elicits immunologic antigen spread to other prostate associated antigens

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.

13. 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 the research laboratory of the PI for immune analysis.

B. 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 16 below.

C. Consent

The local Principal Investigator, co-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.

14. Data and Safety Monitoring Plan

A. Oversight and Monitoring Plan

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 CRC

- Notify the Local Principal Investigator of the DSMC's recommendation to the CRC
- Work in conjunction with the Health Sciences IRB in the review of protocol deviations, violations and unanticipated problems reported by the UWCCC DOWGs.
- The committee ensures that notification is provided to all external sites participating in multiple-institutional clinical trials coordinated by the UWCCC of serious adverse events requiring expedited reporting.

Phase II Trials

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 for each treatment arm/dose level, the number of subjects, significant toxicities as described in the protocol, dose adjustments, and responses observed. Protocol Summary Reports are required for submission to the Data and Safety Monitoring Committee for review.

B. Review and Oversight Requirements

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

Serious Adverse Events requiring reporting within 24 hours (as described in Table D-2) must also be reported to the Data and Safety Monitoring Committee (DSMC) Chair via an email to saenotify@uwcarbone.wisc.edu within one business day. A 24 hr. initial "SAE Details" Report, generated in the UWCCC database, must be attached to the email along with any pertinent information available at the time of initial reporting. The DSMC Chair will review the information and determine if immediate action is required. Within 10 working days, all subsequent SAE documentation must be submitted electronically along with a 24 hour follow-up "SAE Details" Report and a completed UWCCC SAE Routing Form to saenotify@uwcarbone.wisc.edu. All information is entered and tracked in the UWCCC 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.

See Section D for detailed instructions on SAE reporting.

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

Serious Adverse Events requiring reporting within 10 working days (as described Table D-2l) will also be sent to the UWCCC DSMC Chair via email to saenotify@uwcarbone.wisc.edu. A 10 day "SAE Details" report, generated in the UWCCC database must be attached to the email along with pertinent information regarding the SAE and the UWCCC SAE Routing Form. The Committee Chair will review the information and determine if further action is required. This information is entered and tracked in the UWCCC 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.

See Section D for detailed instructions on SAE reporting.

C. Study Progress Review

Protocol Summary Reports (PSR) are required to be submitted to the DSMC commensurate with the Phase of the study. The PSR provides a cumulative report of serious adverse events, as well as any protocol violations, deviations or unanticipated problems, toxicities and responses that have occurred on the protocol in the timeframe specified. PSRs are reviewed at each DSMC meeting.

Protocol Summary Reports enable DSMC committee members to assess whether significant benefits or risks are occurring that would warrant study closure. This information is also provided by Disease Oriented Working Group meeting minutes, internal audit and/or response review reports. In addition, the DSMC requires the DOWG or Local Principal Investigator to submit external DSMB reports or any other significant study-related information.

In the event that there is significant risk warranting study suspension or closure, the DSMC will notify the Local PI of the DSMC findings. The DSMC ensures that the Local PI reports any temporary or permanent suspension of a clinical trial to the sponsor (e.g., NCI Program Director, Industry Supporter(s), Cooperative Group Study Chair, etc.) and other appropriate agencies.

D. Expedited Reporting of Adverse Events

Depending on the nature, severity, and attribution of the serious adverse event an SAE report will be phoned in, submitted in writing, or both according to Table D-2 below. All serious adverse events must also be reported to the UWCCC Data and Safety Monitoring Committee Chair. All serious adverse events must also be reported to the UW IRB (if applicable), and any sponsor/funding agency not already included in the list.

Determine the reporting time line for the SAE in question by using the following table D-2.

D.1. SAE Requiring 24 Hour Reporting Occurs at UWCCC:

a. To the FDA and NIH OBA*:*

Report the SAE to the FDA using the FDA Med Watch form available online. Print the completed Med Watch form and fax it to FDA at (800) 332-0178.

Report the SAE to the National Institute of Health Office of Biotechnology Activities using their reporting form available online. Completed reports may be sent either e-mail or facsimile to the addresses listed on the form.

b. To the Industry Supporter:

All SAEs that occur from the signing of the study specific consent through the duration of the post-therapy adverse event collection period must be reported to Dendreon within 24 hours of being made aware of the SAE. Notification can be

made via phone or telefacsimile using an SAE Report Form to be provided by Dendreon.

Dendreon Corporation
Attn: Safety Manager
Facsimile: (206) 829-1647
Phone: (206) 219-7189
After Hours: (206) 274-6774

Significant new information regarding an ongoing SAE and the resolution must be sent to Dendreon within 3 business days of awareness of the new information to Dendreon on the SAE Report Form.

c. To the IRB:

Consult the UW-IRB website for reporting guidelines.

d. To the UWCCC:

Reference the **SAE SOP** (Standard Operating Procedure) and the **SAE Reporting Workflow for DOWGs** on the UWCCC website (http://www.uwccc.wisc.edu) for specific instructions on how and what to report to the UWCCC for 24-hour initial and follow-up reports. **A follow-up report is required to be submitted within 10 days of the initial 24-hour report.**

For this protocol, the following entities are required to be notified:
1. saenotify@uwcarbone.wisc.edu
2. Any other appropriate parties listed on the SAE Routing Form (for follow-up reports only)
3. UW Institutional Biosafety Officer (via First Report form (http://www2.fpm.wisc.edu/biosafety/emergency_prep.htm) within one working day of the event

D.2. <u>SAE Requiring 10 Day Reporting Occurs at UWCCC:</u>

a. To the FDA and NIH OBA

Report the SAE to the FDA using the FDA Med Watch form available online. Print the completed Med Watch form and fax it to FDA at (800) 332-0178.

Report the SAE to the National Institute of Health Office of Biotechnology Activities using their reporting form available online. Completed reports may be sent either e-mail or facsimile to the addresses listed on the form.

b. To the Industry Supporter:
All SAEs that occur from the signing of the study specific consent through the duration of the post-therapy adverse event collection period must be reported to

Dendreon within 24 hours of being made aware of the SAE. Notification can be made via phone or telefacsimile using an SAE Report Form to be provided by Dendreon.

Dendreon Corporation
Attn: Safety Manager
Facsimile: (206) 829-1647
Phone: (206) 219-7189
After Hours: (206) 274-6774

Significant new information regarding an ongoing SAE and the resolution must be sent to Dendreon within 3 business days of awareness of the new information to Dendreon on the SAE Report Form.

c. To the IRB:

Consult the UW-IRB website for reporting guidelines.

d. To the UWCCC:

Reference the **SAE SOP** and the **SAE Reporting Workflow for DOWGs** on the UWCCC website (http://www.uwccc.wisc.edu) for specific instructions on how and what to report to the UWCCC for 10-day reports.

For this protocol, the following entities are required to be notified:
1. saenotify@uwcarbone.wisc.edu
2. Any appropriate parties listed on SAE Routing Form
3. UW Institutional Biosafety Officer (via First Report form (http://www2.fpm.wisc.edu/biosafety/emergency_prep.htm) within one working day of the event

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).

Hospitalization	Grade 1 Timeframes	Grade 2 Timeframes	Grade 3 Timeframes	Grade 4 & 5 Timeframes
Resulting in Hospitalization ≥ 24 hrs	10 Calendar Days			24-Hour 5 Calendar Days
Not resulting in Hospitalization ≥ 24 hrs	Not required		10 Calendar Days	

[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: FOR THIS STUDY, GRADE 2 AUTOIMMUNE REACTIONS WILL BE TREATED AS GRADE 3 EVENTS FOR REPORTING PURPOSES.

15. 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 no autoimmune treatment-limiting adverse events have been observed to date, it is conceivable that by using a prime-boost strategy with different immunization approaches targeting the same PAP antigen could lead to more autoimmune events than either alone. For this reason, these blood tests will be continued to be monitored in this trial.

While there has been a suggestion that the plasmid DNA could insert into the host chromosomal DNA, this has not been documented in any other study, and several laboratory investigations have suggested the possibility of this occurring is less than the spontaneous mutation rate, and therefore not a real risk [65]. 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. <u>From intradermal treatment with rhGM-CSF</u>

GM-CSF, administered intradermally with the vaccine, is being used as a vaccine adjuvant, and has demonstrated safety from multiple other vaccine trials in humans. Common side effects include erythema and induration at the site of immunization (lasting for several days), and a transient decrease in peripheral white blood cell counts (lasting for several hours), mild flu-like symptoms (lasting for several hours). It has also been observed and reported that T-cell and antibody responses can occur to GM-CSF, but without any known clinical sequelae [47]. Uncommon and rare side effects, which have been less frequently observed with daily

administration of higher doses of GM-CSF, include: vomiting; diarrhea; fatigue; weakness; headache; decreased appetite; feeling of faintness; facial flushing; pain in the bones, muscles, chest, abdomen, or joints; blood clots or unusual bleeding symptoms; rapid or irregular heartbeat or other heart problems; kidney and liver dysfunction; fluid accumulation or worsening of pre-existing fluid accumulation in arms and legs, in the lungs, and around the heart that may result in breathing problems and heart failure; allergic reactions; sloughing of skin; liver enlargement; Guillain-Barré syndrome; hypotension; loss of consciousness; dyspnea. In a previous phase I trial with this DNA vaccine and GM-CSF, two patients experienced chest and back pain, attributed to the GM-CSF, which lasted less than 10 minutes and occurred within one hour of receiving GM-CSF. For this reason, all subjects will be monitored for one hour after receiving each DNA immunization treatment.

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 tetanus immunization

A tetanus booster vaccine will be delivered prior to receiving sipuleucel-T as an immunological positive control for the laboratory analysis. While this is a standard, well tolerated immunization, rare potential side effects of this vaccine could include: difficulty in breathing or swallowing; hives; swelling of the eyes, face or inside of the nose; confusion; convulsions; headaches; sleepiness; lymphadenopathy; and vomiting. Other mild adverse effects not requiring medical attention include: chills; fever; mild irritability or tiredness; skin rash; or pain, tenderness, redness, itching or swelling at the site of injection.

A.6. From sipuleucel-T

Sipuleucel-T has been given to over 1000 patients with castrate-resistant metastatic prostate cancer. In the phase III IMPACT trial that led to its approval, the primary adverse events experienced by patients receiving sipuleucel-T more commonly than placebo included fever, chills, nausea, emesis, headache, dizziness, pain, myalgias, hypertension, sweating, and influenza-like symptoms, all typically of limited (1-2 day) duration. Given that sipuleucel-T will

be given in the same population of subjects, and prior to the other experimental treatment, we do not expect other unusual adverse events from this treatment.

B. Potential Benefits

No benefits are guaranteed. It is hoped that individual patients treated with the DNA vaccine following sipuleucel-T will derive a clinical response as suggested by a delay in time to disease progression. In addition, sipuleucel-T is the standard of care for patient with this stage of disease, based on a demonstrated improvement in overall survival. Hence it is hoped that further booster immunization will provide additional benefit that could be demonstrated by an improvement in time to disease progression. 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.

16. Study Data Management and Procedural Issues

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

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 based on the randomization list which will be generated by the study biostatistician.

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.

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

Long-term follow-up form

All clinical safety and clinical response data will be collected by the study coordinators on electronic case report forms as noted in section 16.B.

Research data evaluating immune responses will 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 a minimum of 7 years.

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

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

C. Description of Procedures to Maintain Confidentiality of Research Specimens

All specimens obtained for the immunological evaluation of this trial (blood specimens) will be delivered to the laboratory of Dr. McNeel. Specimens received by Dr. McNeel's lab will only be handled by laboratory personnel who have undergone HIPAA training and annual UWHC-mandated blood-borne pathogen safety training. Receipt of specimens will be entered into a database that will provide a unique code for each specimen. All stored specimens (sera,

peripheral blood mononuclear cells) will be labeled with this unique code and the date of preparation. Thus, all patient identifiers will be removed from the final stored samples, and any data generated will contain only the unique code as identifier. A database will be maintained to link individual coded specimens with an individual subject (name, hospital medical record number, date/time point) in order to be able to compare information from samples obtained at different time points from a specific individual, and ultimately for transfer of research data to the clinical trials database. 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 principal 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.

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

E. Withdrawal from Study Protocol

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

F. Roles and Responsibilities of Study Personnel at UWCCC

Study Principal Investigator:

Dr. Douglas McNeel MD PhD, Associate 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. 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.

17. References:

1. Jemal A., Siegel R., Xu J., and Ward E. (2010) "Cancer statistics, 2010." *CA Cancer J Clin* **60**:277-300.
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. Crawford E. D., Eisenberger M. A., McLeod D. G., Spaulding J. T., Benson R., Dorr F. A., Blumenstein B. A., Davis M. A., and Goodman P. J. (1989) "A controlled trial of leuprolide with and without flutamide in prostatic carcinoma." *N Engl J Med* **321**:419-424.
4. Tannock I. F., de Wit R., Berry W. R., Horti J., Pluzanska A., Chi K. N., Oudard S., Theodore C., James N. D., Turesson I., Rosenthal M. A., and Eisenberger M. A. (2004) "Docetaxel plus prednisone or mitoxantrone plus prednisone for advanced prostate cancer." *N Engl J Med* **351**:1502-1512.
5. Petrylak D. P., Tangen C., Hussain M., Lara P., and al. e. (2004) "SWOG 99-16: Randomized phase III trial of docetaxel (D)/estramustine (E) versus mitoxantrone (M)/prednisone (p) in men with androgen-independent prostate cancer (AIPCA) (abstract #3)." *Proc Am Soc Clin Oncol* **22**
6. de Bono J. S., Oudard S., Ozguroglu M., Hansen S., Machiels J. P., Kocak I., Gravis G., Bodrogi I., Mackenzie M. J., Shen L., Roessner M., Gupta S., and Sartor A. O. (2010) "Prednisone plus cabazitaxel or mitoxantrone for metastatic castration-resistant prostate cancer progressing after docetaxel treatment: a randomised open-label trial." *Lancet* **376**:1147-1154.
7. Kantoff P. W., Higano C. S., Shore N. D., Berger E. R., Small E. J., Penson D. F., Redfern C. H., Ferrari A. C., Dreicer R., Sims R. B., Xu Y., Frohlich M. W., and Schellhammer P. F. (2010) "Sipuleucel-T immunotherapy for castration-resistant prostate cancer." *N Engl J Med* **363**:411-422.
8. de Bono J. S., Logothetis C. J., Molina A., Fizazi K., North S., Chu L., Chi K. N., Jones R. J., Goodman O. B., Jr., Saad F., Staffurth J. N., Mainwaring P., Harland S., Flaig T. W., Hutson T. E., Cheng T., Patterson H., Hainsworth J. D., Ryan C. J., Sternberg C. N., Ellard S. L., Flechon A., Saleh M., Scholz M., Efstathiou E., Zivi A., Bianchini D., Loriot Y., Chieffo N., Kheoh T., Haqq C. M., and Scher H. I. (2011) "Abiraterone and increased survival in metastatic prostate cancer." *N Engl J Med* **364**:1995-2005.
9. Kirk T. N. and Moyad M. A. (2006) "National survey of advanced prostate cancer patients reveals disparity between perceptions and reality of treatment." *Proc. Amer. Soc. Clin. Oncol.* **24**:490s, abstract #8590.
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. Ward J. E. and McNeel D. G. (2007) "GVAX: an allogeneic, whole-cell, GM-CSF-secreting cellular immunotherapy for the treatment of prostate cancer." *Expert Opin Biol Ther* **7**:1893-1902.
13. Alam S. and McNeel D. G. (2010) "DNA vaccines for the treatment of prostate cancer." *Expert Rev Vaccines* **9**:731-745.
14. Olson B. M. and McNeel D. G. (2011) "Sipuleucel-T: immunotherapy for advanced prostate cancer." *Open Acc. J. Urol.* **3**:49-60.

15. 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.
16. 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.
17. 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.
18. 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.
19. 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.
20. 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.
21. Fong L., Brockstedt D., Benike C., Breen J. K., Strang G., Ruegg C. L., and Engleman E. G. (2001) "Dendritic Cell-Based Xenoantigen Vaccination for Prostate Cancer Immunotherapy." *J Immunol* **167**:7150-7156.
22. 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.
23. 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* **33**:639-647.
24. Griffiths J. (1980) "Prostate-specific acid phosphatase: re-evaluation of radioimmunoassay in diagnosing prostatic disease." *Clin. Chem.* **26**:433-436.
25. 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.
26. 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.
27. 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.
28. Olson B. M., Frye T. P., Johnson L. E., Fong L., Knutson K. L., Disis M. L., and McNeel D. G. (2010) "HLA-A2-restricted T-cell epitopes specific for prostatic acid phosphatase." *Cancer Immunol Immunother* **59**:943-953.
29. 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.
30. Higano C. S., Schellhammer P. F., Small E. J., Burch P. A., Nemunaitis J., Yuh L., Provost N., and Frohlich M. W. (2009) "Integrated data from 2 randomized, double-

blind, placebo-controlled, phase 3 trials of active cellular immunotherapy with sipuleucel-T in advanced prostate cancer." *Cancer* **115**:3670-3679.

31. 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.
32. 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.
33. 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.
34. Irvine K. R. and Restifo N. P. (1995) "The next wave of recombinant and synthetic anticancer vaccines." *Seminars in Canc Biol* **6**:337-347.
35. 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.
36. 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.
37. 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.
38. 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.
39. 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.
40. 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.
41. 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.
42. 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.
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. 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.
45. 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.

46. 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.
47. 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.
48. 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.
49. Scher H. I., Halabi S., Tannock I., Morris M., Sternberg C. N., Carducci M. A., Eisenberger M. A., Higano C., Bubley G. J., Dreicer R., Petrylak D., Kantoff P., Basch E., Kelly W. K., Figg W. D., Small E. J., Beer T. M., Wilding G., Martin A., and Hussain M. (2008) "Design and end points of clinical trials for patients with progressive prostate cancer and castrate levels of testosterone: recommendations of the Prostate Cancer Clinical Trials Working Group." *J Clin Oncol* **26**:1148-1159.
50. Halabi S., Small E. J., Kantoff P. W., Kattan M. W., Kaplan E. B., Dawson N. A., Levine E. G., Blumenstein B. A., and Vogelzang N. J. (2003) "Prognostic model for predicting survival in men with hormone-refractory metastatic prostate cancer." *J Clin Oncol* **21**:1232-1237.
51. Gulley J. L., Arlen P. M., Madan R. A., Tsang K. Y., Pazdur M. P., Skarupa L., Jones J. L., Poole D. J., Higgins J. P., Hodge J. W., Cereda V., Vergati M., Steinberg S. M., Halabi S., Jones E., Chen C., Parnes H., Wright J. J., Dahut W. L., and Schlom J. (2010) "Immunologic and prognostic factors associated with overall survival employing a poxviral-based PSA vaccine in metastatic castrate-resistant prostate cancer." *Cancer Immunol Immunother* **59**:663-674.
52. 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.
53. 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.
54. 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**
55. 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.
56. 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).

57. 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.
58. 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.
59. 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.
60. 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.
61. 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.
62. 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.
63. Alix-Panabieres C., Rebillard X., Brouillet J. P., Barbotte E., Iborra F., Segui B., Maudelonde T., Jolivet-Reynaud C., and Vendrell J. P. (2005) "Detection of circulating prostate-specific antigen-secreting cells in prostate cancer patients." *Clin Chem* **51**:1538-1541.
64. 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.
65. 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	Day 43	Month 3	Month 6	Month 9	Month 12
Research Leukapheresis **OR** **Alternative*** **(150mL – peripheral blood)**	50-100mL N/a			50-100mL -----or---- 150mL*		
Sera for Antibody	20mL	10mL	10mL	10mL	10mL	10mL
Blood for CTC and/or T-cell response	20 mL	100mL	100mL	20mL	100mL	100mL
Approximate Total	90-190mL	110mL	110mL	80-180mL	110mL	110mL

***** Peripheral blood draw may be done as an alternative to leukapheresis only at Month 6. Patients should not go on study if leukapheresis cannot be done at screening.

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 CTC analysis/T-Cell response is to be collected in green top heparinized tubes.