Vaccine Therapy and Pembrolizumab in Treating Patients with Hormone-Resistant, Metastatic Prostate Cancer
NCT02499835

PI: Glenn Liu, MD
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Pilot trial of pTVG-HP DNA Vaccine and Pembrolizumab in Patients with Castration-Resistant, Metastatic Prostate Cancer

UW15014

INVESTIGATIONAL AGENT:

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

STUDY SPONSOR / PROTOCOL PRINCIPAL INVESTIGATOR:
Douglas G. McNeel, MD PhD

STUDY SUPPORTER:
Madison Vaccines, Inc., 505 S. Rosa Road, Madison, WI
Prostate Cancer Foundation (MSN 175454)

STUDY SITE INFORMATION

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

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
Hamid Emamekhoo, M.D. – Clinical Investigator
Robert Jeraj, Ph.D. – Physicist, molecular imaging
Scott Perlman, M.D. – Nuclear Medicine
Humberto, Rosas M.D. – Interventional Radiology

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

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Projected End of Study Date: September 1, 2019

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SYNOPSIS:

Primary Objectives:

1. To evaluate the safety of pembrolizumab in combination with pTVG-HP in patients with castration-resistant, metastatic prostate cancer
2. To determine the 6-month progression-free survival and median time to radiographic progression in patients with castration-resistant metastatic prostate cancer treated with pembrolizumab in combination with pTVG-HP
3. To evaluate the anti-tumor response rates (objective response rate and PSA response rate, using PCWG2 criteria) in patients with castration-resistant metastatic prostate cancer treated with pembrolizumab in combination with pTVG-HP

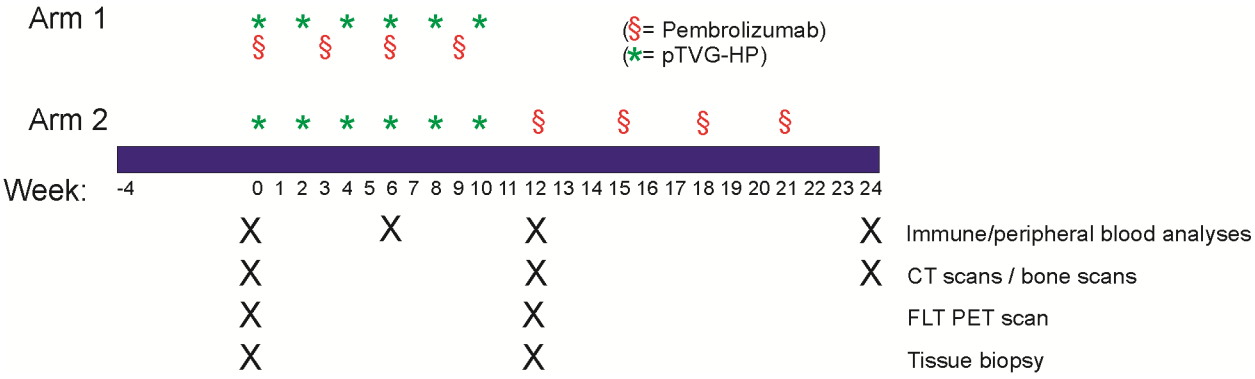
Secondary Objectives:

1. To determine whether either treatment sequence, or PAP-specific immune response, is associated with prolonged (6-month) radiographic progression-free survival
2. To evaluate effects of schedule (concurrent versus delayed administration of pembrolizumab) on the magnitude of PAP-specific T-cell responses, PD-1 expression on circulating T cells, and PD-L1 expression on circulating epithelial cells (CEC) and on tumor biopsies
3. To determine the median time to radiographic progression using concurrent administration schedules

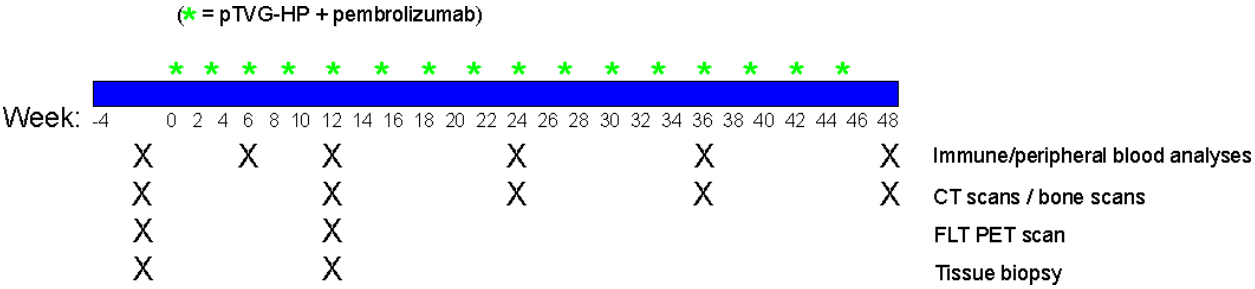
Exploratory Objectives:

1. To evaluate effects of treatment on the number of circulating tumor cells
2. To evaluate PAP-specific antibody responses following treatment with pembrolizumab and pTVG-HP DNA vaccine
3. To determine whether treatment with either sequence elicits immunologic antigen spread to other prostate associated antigens
4. To determine whether pre-existing or vaccine-induced PD-L1 expression on CEC or tumor biopsies is predictive of objective clinical response
5. To determine whether treatment elicits expression of other regulatory molecules on tumor-specific T cells (e.g. TIM3, BTLA, and LAG3) or tumor cells (e.g. HVEM, phosphatidyl serine, PD-L2)
6. To determine whether PD-1-regulated antigen-specific T-cells identified by *trans vivo* DTH testing can identify patients who develop objective clinical responses with PD-1 blockade therapy in combination with pTVG-HP
7. To determine whether changes in lymph nodes and soft tissue tumor lesions are observed by FLT PET/CT after treatment with vaccine with or without pembrolizumab
8. To determine if PD-1 inhibitor therapy in combination with pTVG-HP will change number and activity (SUV) in osteoblastic metastases as measured by NaF PET/CT.

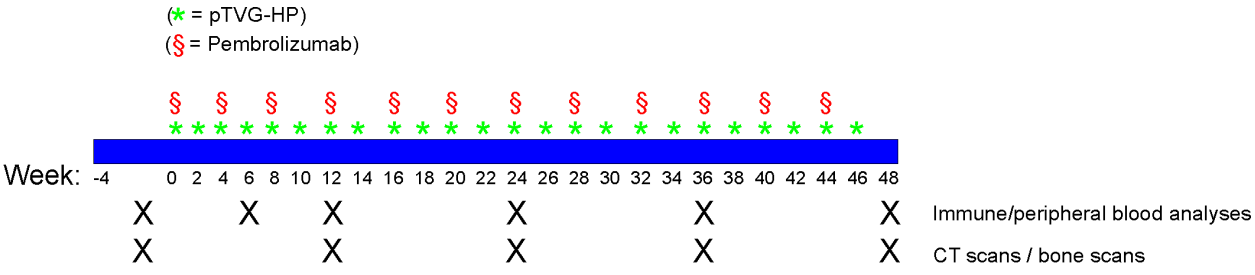
Study Scheme:



Extended treatment Arm 3:



Extended treatment Arm 4:



Plan of Treatment: ARM 1 (Dark grey indicates those days and procedures specific to Arm 1)

Vaccine / Treatment Visit:	SCREENING																
	Within 6 weeks of day 1	Within 4 weeks of day 1	Day 1	Day 15 (+/- 3 days)	Day 22 (7 +/- 3 days after day 15)	Day 29 (14 +/3 days after day 15)	Day 43 (14 +/- 3 days after day 29)	Day 57 (14 +/- 3 days after day 43)	Day 64 (7 +/- 3 days after day 57)	Day 71 (14 +/- 3 days after day 57)	Day 85 (14 +/- 3 days after day 71) – 3 month	Da 106 (21 +/- 3 days after day 85)	Da 127 (21 +/- 3 days after day 106)	Day 148 (21 +/- 3 days after day 127)	Day 169 (21 +/- 3 days after day 148) – 6 m	9-month F/U (84 +/- 10 days after day 169	OF F STUDY 12-month F/U – (84 +/- 10 days after 9moth
History and Physical Exam																	
History	X																
Consent	X																
Physical Exam, ECOG PS		X	X		X		X		X		X	X	X	X	X	X	X
Tox assessment[a]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Lab Tests																	
CBC	X		X		X		X		X		X[g]	X	X	X	X	X	X
Chemistry panel[f]	X		X		X		X		X		X[g]	X	X	X	X	X	X
PT/INR		X[k]									X[k]						
Serum testosterone	X																
PSA and PAP	X		X		X		X		X		X[g]	X	X	X	X	X	X
Procedures																	
CT chest/ abdomen/pelvis	X										X[gj]				X[hj]	X[hj]	X[ij]
Bone scan[l]	X										X[g]				X[h]	X[h]	X[i]
Leukapheresis		X[b]													X[bh]		
FLT PET scan[m]		X[c]									X[g]						
Tissue biopsy[m]		X[d]									X[g]						
Tetanus immunization		X[e]															
Blood for immune analyses		X					X				X				X	X	X
Blood for CTC analysis		X					X				X				X	X	X
Treatments																	
DNA immunization			X	X		X	X	X		X							
Pembrolizumab infusion			X		X		X		X								

[a] Study coordinator or research nurse review of systems
[b] For patients unable to undergo leukapheresis, a 200-mL blood draw (green top tubes) may be performed instead
[c] FLT PET scan – within 4 weeks (28 days) of day 1 – to provide baseline study for comparison prior to treatment
[d] Biopsy within 2 weeks prior to day 1
[e] Tetanus immunization to take place any time after leukapheresis and prior to day 1
[f] Where chemistry labs indicated, the following serum tests are to be performed: Chem 7 (sodium, potassium, bicarbonate, BUN, creatinine, glucose), ALT, AST, bilirubin, alkaline phosphatase, amylase, TSH (thyroid stimulating hormone), and LDH
[g] Procedures can be +/- 7 days of this visit, scans to be done prior to biopsy (and study treatment for Arm 2)
[h] Can be +/- 7 days of this visit
[i] Can be +/- 7 days of this visit, and not required if this is off-study visit and previous scans already confirmed disease progression
[j] CT of chest at this time point only as clinically indicated
[k] PT/INR only required for those subjects who will be undergoing a biopsy. May be performed up to 30 days prior to biopsy, required same day if subject is on coumadin
[l] If NaF PET/CT is available as standard of care procedure, this may be substituted for bone scan (bone scintigraphy), however all pre-treatment and post-treatment tests must use the same modality (NaF PET/CT or bone scintigraphy)
[m] See section 9 for whether or not biopsy and FLT PET is required

Plan of Treatment: ARM 2

Vaccine / Treatment Visit:	SCREENING														
	Within 6 weeks of day 1	Within 4 weeks of day 1	Day 1	Day 15 (+/- 3 days)	Day 29 (14 +/3 days after day 15)	Day 43 (14 +/- 3 days after day 29)	Day 57 (14 +/- 3 days after day 43)	Day 71 (14 +/- 3 days after day 57)	Day 85 (14 +/- 3 days after day 71) – 3 month	Da 106 (21 +/- 3 days after day 85)	Da 127 (21 +/- 3 days after day 106)	Day 148 (21 +/- 3 days after day 127)	Day 169 (21 +/- 3 days after day 148) – 6 m	9-month F/U (84 +/- 10 days after day 169	OF F STUDY 12-month F/U – (84 +/- 10 days after 9moth F/U)
History and Physical Exam															
History	X														
Consent	X														
Physical Exam, ECOG PS		X	X			X			X	X	X	X	X	X	X
Tox assessment[a]		X	X	X	X	X	X	X	X	X	X	X	X	X	X
Lab Tests															
CBC	X		X			X			X[g]	X	X	X	X	X	X
Chemistry panel[f]	X		X			X			X[g]	X	X	X	X	X	X
PT/INR		X[k]							X[k]						
Serum testosterone	X														
PSA and PAP	X		X			X			X[g]	X	X	X	X	X	X
Procedures															
CT chest/ abdomen/pelvis	X								X[gj]				X[hj]	X[hj]	X[ij]
Bone scan[l]	X								X[g]				X[h]	X[h]	X[i]
Leukapheresis		X[b]											X[bh]		
FLT PET scan[m]		X[c]							X[g]						
Tissue biopsy[m]		X[d]							X[g]						
Tetanus immunization		X[e]													
Blood for immune analyses		X				X			X[g]				X	X	X
Blood for CTC analysis		X				X			X[g]				X	X	X
Treatments															
DNA immunization			X	X	X	X	X	X							
Pembrolizumab infusion									X	X	X	X			

[a] Study coordinator or research nurse review of systems
[b] For patients unable to undergo leukapheresis, a 200-mL blood draw (green top tubes) may be performed instead
[c] FLT PET scan – within 4 weeks (28 days) of day 1 – to provide baseline study for comparison prior to treatment
[d] Biopsy within 2 weeks prior to day 1
[e] Tetanus immunization to take place any time after leukapheresis and prior to day 1
[f] Where chemistry labs indicated, the following serum tests are to be performed: Chem 7 (sodium, potassium, bicarbonate, BUN, creatinine, glucose), ALT, AST, bilirubin, alkaline phosphatase, amylase, TSH (thyroid stimulating hormone), and LDH
[g] Procedures can be +/- 7 days of this visit; scans to be done prior to biopsy (and study treatment for Arm 2)
[h] Can be +/- 7 days of this visit
[i] Can be +/- 7 days of this visit, and not required if this is off-study visit and previous scans already confirmed disease progression
[j] CT of chest at this time point only as clinically indicated
[k] PT/INR only required for those subjects who will be undergoing a biopsy. May be performed up to 30 days prior to biopsy, required same day if subject is on coumadin[l] If NaF PET/CT is available as standard of care procedure, this may be substituted for bone scan (bone scintigraphy), however all pre-treatment and post-treatment tests must use the same modality (NaF PET/CT or bone scintigraphy)
[m] See section 9 for whether or not biopsy and FLT PET is required.

Plan of Treatment: ARM 3 – EXTENDED TREATMENT COHORT

Vaccine / Treatment Visit:	SCREENING: Within 6 wks of day 1	SCREENING: Within 4 wks of day 1	Day 1	Week 3 (21 +/- 3 days from Day 1)	Week 6 (21 +/- 3 days from week 3)	Week 9 (21 +/- 3 days from week 6)	Week 12 (21 +/- 3 days from week 9)	Week 15 (21 +/- 3 days from week 12)	Week 18 (21 +/- 3 days from week 15)	Week 21 (21 +/- 3 days from week 18)	Week 24 (21 +/- 3 days from week 21)	Week 27 (21 +/- 3 days from week 24)	Week 30 (21 +/- 3 days from week 27)	Week 33 (21 +/- 3 days from week 30)	Week 36 (21 +/- 3 days from week 33)	Week 39 (21 +/- 3 days from week 36)	Week 42 (21 +/- 3 days from week 39)	Week 45 (21 +/- 3 days from week 42)	END OF STUDY Week 48 (21 +/- 3 days after week 45)	28-Day Follow Up (28 +/- 7 days after off-treatment visit)
History and Physical Exam																				
History	X																			
Consent	X																			
Physical Exam, ECOG PS		X	X		X		X		X		X		X		X		X		X	X
Tox assessment[a]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Lab Tests																				
CBC	X		X	X[i]	X[i]	X[i]	X[i]	X[i]	X[i]	X[i]	X[i]	X[i]	X[i]	X[i]	X[i]	X[i]	X[i]	X[i]	X[i]	X
Chemistry panel[f]	X		X	X[i]	X[i]	X[i]	X[i]	X[i]	X[i]	X[i]	X[i]	X[i]	X[i]	X[i]	X[i]	X[i]	X[i]	X[i]	X[i]	X
PT/INR		X[k]					X[k]													
Serum testosterone	X																			
PSA and PAP	X		X	X[i]	X[i]	X[i]	X[i]	X[i]	X[i]	X[i]	X[i]	X[i]	X[i]	X[i]	X[i]	X[i]	X[i]	X[i]	X[i]	X
Procedures																				
CT chest/ abdomen/pelvis	X						X[gj]				X[hj]				X[hj]				X[hj]	
Bone scan	X						X[g]				X[h]				X[h]				X[h]	
Leukapheresis		X[b]									X[bh]									
FLT PET scan[m]		X[c]					X[g]													
NaF PET/CT scan[m]		X			X[g]		X[g]													
Tissue biopsy[m]		X[d]					X[g]													
Tetanus immunization		X[e]																		
Blood for immune analyses		X			X		X				X				X				X	(X)[n]
Blood for CTC analysis		X			X		X				X				X				X	(X)[n]
Treatments																				
DNA immunization			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		
Pembrolizumab infusion			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		

[a] Study coordinator or research nurse review of systems
[b] For patients unable to undergo leukapheresis, a 200-mL blood draw (green top tubes) may be performed instead
[c] FLT PET scan – within 4 weeks (28 days) of day 1 – to provide baseline study for comparison prior to treatment
[d] Biopsy within 2 weeks prior to day 1
[e] Tetanus immunization to take place any time after leukapheresis and prior to day 1
[f] Where chemistry labs indicated, the following serum tests are to be performed: Chem 7 (sodium, potassium, bicarbonate, BUN, creatinine, glucose), ALT, AST, bilirubin, alkaline phosphatase, amylase, TSH (thyroid stimulating hormone), and LDH
[g] Procedures can be +/- 7 days of this visit; scans to be done prior to biopsy
[h] Can be +/- 7 days of this visit
[i] Blood draws can be up to 3 days prior to this visit
[j] CT of chest at this time point only as clinically indicated
[k] PT/INR only required for those subjects who will be undergoing a biopsy. May be performed up to 30 days prior to biopsy, required same day if subject is on coumadin
[m] See section 9 for whether or not biopsy, FLT, or NaF PET is required.
[n] Blood collected for immune analysis only if not collected within previous 60 days

Plan of Treatment: ARM 4 – EXTENDED TREATMENT COHORT

Vaccine / Treatment Visit	Screening		Day 1	Wk 2 (14 +/- 3 days from Day 1)	Wk 4 (14 +/- 3 days from Wk 2)	Wk 6 (14 +/- 3 days from Wk 4)	Wk 8 (14 +/- 3 days from Wk 6)	Wk 10 (14 +/- 3 days from Wk 8)	Wk 12 (14 +/- 3 days from Wk 10)	Wk 14 (14 +/- 3 days from Wk 12)	Wk 16 (14 +/- 3 days from Wk 14)	Wk 18 (14 +/- 3 days from Wk 16)	Wk 20 (14 +/- 3 days from Wk 18)	Wk 22 (14 +/- 3 days from Wk 20)	Wk 24 (14 +/- 3 days from Wk 22)	Wk 26 (14 +/- 3 days from Wk 24)	Wk 28 (14 +/- 3 days from Wk 26)	Wk 30 (14 +/- 3 days from Wk 28)	Wk 32 (14 +/- 3 days from Wk 30)	Wk 34 (14 +/- 3 days from Wk 32)	Wk 36 (14 +/- 3 days from Wk 34)	Wk 38 (14 +/- 3 days from Wk 36)	Wk 40 (14 +/- 3 days from Wk 38)	Wk 42 (14 +/- 3 days from Wk 40)	Wk 44 (14 +/- 3 days from Wk 42)	Wk 46 (14 +/- 3 days from Wk 44)	END OF TREATMENT Wk 48 (14 +/- 3 days from Wk 46)	28-Day Follow Up (28 +/- 7 days after off-treatment visit)
	Within 6 wks of day 1	Within 4 wks of day 1																										
History and Physical Exam																												
History	X																											
Consent	X																											
Physical Exam, ECOG PS		X	X		X		X		X		X		X		X		X		X		X		X		X		X	X
Tox assessment[a]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Lab Tests																												
CBC	X		X		X[f]		X[f]		X[f]		X[f]		X[f]		X[f]		X[f]		X[f]		X[f]		X[f]		X[f]		X[f]	X[f]
Chemistry panel[d]	X		X		X[f]		X[f]		X[f]		X[f]		X[f]		X[f]		X[f]		X[f]		X[f]		X[f]		X[f]		X[f]	X[f]
Serum testosterone	X																											
PSA and PAP	X		X		X[f]		X[f]		X[f]		X[f]		X[f]		X[f]		X[f]		X[f]		X[f]		X[f]		X[f]		X[f]	X[f]
Procedures																												
CT chest/ abdomen/pelvis	X								X[eg]						X[eg]						X[eg]						X[eg]	
Bone scan	X								X[e]						X[e]						X[e]						X[e]	
NaF PET/CT scan[i]		X				X[e]			Xe																			
Leukapheresis[b]		X[b]													X[be]													
Tetanus immunization[c]		X[c]																										
Blood for immune analyses		X				X			X						X						X						X	(X)[h]
Blood for CTC analysis		X				X			X						X						X						X	(X)[h]
Treatments																												
DNA immunization			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X		
Pembrolizumab infusion			X		X		X		X		X		X		X		X		X		X		X		X			

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[e] Can be +/- 7 days of this visit
[f] Blood draws can be up to 3 days prior to this visit
[g] CT of chest at this time point only as clinically indicated
[h] Blood collected for immune analysis only if not collected within previous 60 days
[i] There will be up to 10 subjects total between Arms 3 and 4 who will undergo NaF PET/CT scan.

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 die as a result of prostate cancer in 2014 [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 and 2013, abiraterone and enzalutamide, agents targeting androgen synthesis or signaling, were similarly approved in the setting of docetaxel-refractory, castrate-resistant metastatic prostate cancer following prospectively randomized, blinded, placebo-controlled trial demonstrating a 2-3-month median improvement in overall survival [8, 9]. 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 [10]. 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 [11, 12, 13, 14]. There has also been interest in developing antigen non-specific immune-activating therapies, such as through T-cell checkpoint blockade. Inhibitors of PD/PD-L1 or CTLA-4, for example, have demonstrated remarkable efficacy alone or in combination for metastatic melanoma [15, 16, 17], and PD/PD-L blockade inhibitors have demonstrated activity as single agents for several solid tumor types [18, 19]. Targeting PD-1, in particular, should be a universal therapy, as it targets the T-cell compartment rather than the tumor directly. However, in separate phase I trials with PD-1 blocking antibodies, there has been no objective response observed to patients (n=25) with metastatic prostate cancer [18, 19]. The difference in response to certain tumors, with higher frequencies of responses observed in patients with renal cell cancer, melanoma and non-small cell lung cancer compared to prostate cancer, for example, suggest that differences are likely due to

differences in the T-cells of responding and non-responding patients. In addition, early phase clinical trials using PD-1 or PD-L1 have identified that the expression of at least one of the ligands for PD-1 (PD-L1) on the target tumor cell by biopsy is associated with clinical response to therapy [18]. This is expected, given that tissue-infiltrating T cells can induce the expression of PD-L1 via the expression of IFNγ. Notwithstanding, some tumors that are known to express PD-L1, such as prostate cancer [20], have demonstrated little response to treatment with PD-1 blockade as single-agent therapies in early phase trials [18, 19].

In preliminary studies (further described below), we have recently identified in a murine model using a vaccine targeting a tumor antigen that DNA immunization leads to the generation of CD8+ T cells with cytolytic activity, and that these cells express PD-1. Moreover, immunization leads to a compensatory response in the tumor to increase expression of PD-L1. In further studies, efforts to increase the number and avidity of CD8+ T cells with cytolytic activity by means of changes introduced into the DNA vaccine led to an unanticipated ***inferior*** anti-tumor immune response due to this PD-1/PD-L1 upregulation. Blockade of this pathway using antibodies blocking either PD-1 or PD-L1 restored the anti-tumor activity of the vaccine encoding the tumor-specific target antigen in this murine model, an effect not observed with anti-PD-1 treatment alone, as monotherapy had no demonstrable effect. Importantly, immunization with the vaccine encoding the native (unmodified) antigen in combination with an antibody blocking PD-1 similarly demonstrated an improved anti-tumor response compared to vaccination alone. As a result, evaluating active immunotherapies in combination with PD-1 checkpoint inhibitors are a rational direction to further improve on the efficacy of these therapies and may have durable, objective anti-tumor responses in patients with metastatic cancer [21].

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 [22]. 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 [23]. 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 [24, 25, 26, 27]. 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 [28]. In rodent studies, we have similarly demonstrated that PAP can be immunologically targeted using genetic vaccines, and a plasmid DNA vaccine in particular [25, 26]. 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 [29]. 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 [30]. 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 eliciting tumor-specific CD8+ T cells [31]. These

findings have justified further evaluation of this vaccine in a randomized phase II clinical trial, a study currently underway (NCT01341652). Finally, further preliminary studies have suggested that patients previously treated with pTVG-HP have circulating PAP-specific CD8+ T cells with PD-1 expression and circulating EpCam+ circulating epithelial cells (CEC) with PD-L1 expression, analogous to our findings in murine models. These finding suggest that the pTVG-HP vaccine might specifically be used to elicit CD8+ T cells specific for prostate tumors, the efficacy of which could be augmented with concurrent treatment with PD-1 blockade. Collectively, these findings form the 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 [22, 32]. 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 [33]. 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* [34, 35]. 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 [36]. 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 [30, 36].

B. PD-1 blockade

A major mechanism by which tumors can avoid immune detection is by expression of PD-L1 or PD-L2, ligands for a receptor on T cells (PD-1), activation of which can decrease T-cell function and lead to immune tolerance. There is currently great enthusiasm to specifically develop PD/PD-L blockade inhibitors given the relative paucity of adverse events observed with these agents in clinical trials, and long-term disease response observed in some instances in early phase clinical trials. Targeting PD-1, in particular, should be a universal therapy, as it targets the T-cell compartment rather than the tumor directly. However, clinical trial experience to date suggests that patients with some solid tumor types (notably renal cell cancer, melanoma and non-small cell lung cancer) experience more benefit than patients with other histologies, including prostate cancer [18, 19]. This disparity suggests that differences are due to differences in the T-cells of responding and non-responding patients. In particular, higher frequencies of tumor-infiltrating lymphocytes (TIL) are typically observed in patients with renal cell cancer and melanoma than prostate cancer [37]. In addition, early phase clinical trials using PD-1 or PD-L1 have identified that the expression of at least one of the ligands for PD-1 (PD-L1) on the target tumor cell by biopsy is associated with clinical response to therapy [18]. This is expected, given that tissue-infiltrating T cells can induce the expression of PD-L1 via the expression of IFNγ, and ligand binding of PD-1

leads to decrease in T-cell effector function. It has been demonstrated that prostate cancers can express PD-L1, and can have infiltrating PD-1-expressing T cells [20]. Taken together, these results suggest that the efficacy of anti-tumor immunotherapy could be increased for prostate cancer by combining agents able to increase the number of tumor-specific T cells, such as through vaccination, and by PD-1/PD-ligand blockade.

Given the anti-tumor responses observed in early phase clinical trials with antibodies targeting either PD-1 or PD-L1, several pharmaceutical companies have been developing related agents. At the time of this writing, only one agent has yet been approved as a therapy, pembrolizumab (Keytruda®, Merck). Specifically, pembrolizumab was approved in September 2014 for the treatment of ipilimumab-refractory advanced melanoma as a "breakthrough" therapy on the basis of an open-label, international, multicenter expansion cohort of a phase I trial of patients with advanced (metastatic) melanoma whose disease had progressed following treatment with ipilimumab [38]. In that trial, 173 patients received pembrolizumab at one of two doses (2 mg/kg or 10 mg/kg) at 3-week intervals until disease progression or intolerable toxicity. An overall response rate of 26% was observed, irrespective of dose [38]. Grade 3 fatigue was the only drug-related grade 3 or 4 adverse event reported in more than one patient. Given these findings, pembrolizumab is currently FDA approved for the treatment of patients with ipilimumab-refractory melanoma, dosed at 2 mg/kg intravenously every 3 weeks until disease progression or intolerable adverse effects. Of note, however, earlier phase clinical trials have suggested that treatment can lead to prolonged responses even after discontinuing treatment [15].

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 [39, 40, 41], 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 [26, 42]. 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 [43] and CD8+ T-cells specific for the targeted antigen [39, 44, 45, 46, 47]. In animal models, the use of an intradermal route of vaccine administration, in particular, tends to promote this Th1/CTL-biased immune response [43, 48, 49]. It should also be acknowledged that the clinical efficacy of a DNA vaccine encoding tyrosinase for the treatment of canine melanoma 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 [50, 51]. 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 [25, 26]. 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 [52]. This was a dose-escalation study, with an expanded cohort of subjects treated at the maximum tolerated dose. In the dose-escalation portion, nine subjects were treated in three dose cohorts of 100 μg, 500 μg, or 1500 μg DNA, administered intradermally every two weeks for six total immunizations. 200 μg GM-CSF was co-administered as a vaccine adjuvant with each immunization. 13 additional subjects were treated in an expanded cohort at the 1500 μg DNA dose. No serious adverse events were observed, and no significant laboratory anomalies were observed. Common events observed were grade 1/2 fevers, chills, and local site reactions lasting typically less than 24 hours. 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. 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 [29]. 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 [30].

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. This trial demonstrated that PAP-specific immune responses developed in some individuals after as few as 3-6 immunizations, and some individuals developed no detectable immune responses after even 24 biweekly immunizations [31]. Favorable changes in PSA doubling time were again observed, and tended to be greatest in patients with evidence of long-term immunity [31]. 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, a multicenter trial that is currently underway (NCT01341652).

E. DNA vaccine with PD pathway blockade elicits anti-tumor response in murine model

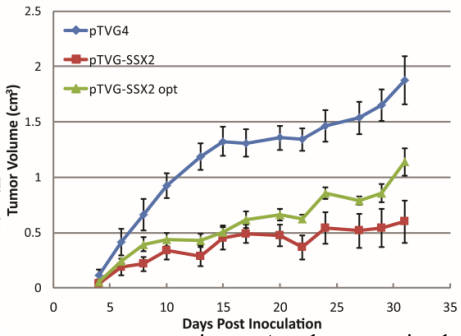
Given our findings in human trials that some patients did not develop evidence of immune response, and even those that did still showed evidence of disease progression, we have sought to evaluate methods to increase the efficacy of DNA vaccines and evaluate potential mechanisms of tumor escape. For these efforts we have focused on vaccine strategies targeting SSX2, a "neoantigen," and one for which we have identified that there are only two HLA-A2-restricted

epitopes, one dominant (p103-111) and one subdominant (p41-49), providing a convenient preclinical model [53]. Using the HHD-II strain of mice, which expresses human HLA-A2 and HLA-DR1 and is knocked out for murine MHC class I and II [54], we have demonstrated that immunization with a DNA plasmid encoding SSX2 elicits robust CD8+ T cells that can be identified by tetramer staining and IFNγ ELISPOT [36, 53, 55]. We have also generated a syngeneic sarcoma cell line in this HHD-II strain, and transfected this to express SSX2 (Sarc/SSX2). Using this system, we have explored whether the CD8+ effector T-cell response can be augmented using plasmid DNA vaccines modified to encode stronger MHC class I binding epitopes. Using peptides with point mutations demonstrated to increase HLA-A2 binding affinity, we demonstrated that direct immunization could elicit CD8+ T cells with cross-reactive recognition for the native epitope, and that these cells could lyse peptide-loaded target cells, and human prostate tumor cells expressing native SSX2 and HLA-A2 [56]. Having identified altered peptide ligands (APL) for each epitope that elicited a higher frequency of epitope-specific T cells, we next encoded these APL within the coding sequence of an SSX2 plasmid DNA vaccine. HHD-II mice, without tumors, were immunized with plasmids encoding one or two modified epitopes, modified at single amino acids. We have reported that immunization with a plasmid encoding both modified epitopes elicited a greater frequency of T cells specific for both epitopes compared with the native vaccine (pTVG-SSX2) [56]. Immunization with a DNA vaccine with point mutations ablating HLA-A2 binding elicited no peptide-specific T cells. The plasmid DNA with two HLA-A2-optimized point mutations (pTVG-SSX2^{opt}) was then evaluated for anti-tumor efficacy in studies in which Sarc/SSX2 tumors were implanted prior to immunization. As shown in Figure 1, animals receiving the native SSX2 gene by DNA immunization had a marked decrease in tumor growth rate, but complete tumor eradication was not observed. Unexpectedly, and replicated in several additional studies, tumor-bearing mice receiving the pTVG-SSX2^{opt} DNA vaccine had greater tumor growth compared with the native pTVG-SSX2 vaccine. To evaluate possible means of immune avoidance, we evaluated tumor cells for expression of PD-L1. Sarc/SSX2 tumor cells treated with IFNγ *in vitro* upregulated PD-L1 as detected by flow cytometry (not shown). Moreover, as shown in Figure 2, Sarc/SSX2 tumors from mice immunized with either pTVG-SSX2 or pTVG-SSX2^{opt}, but not vector control, upregulated PD-L1 expression, suggesting that PD-L1 expression was induced by the antigen-specific T cells. This was further demonstrated by culturing splenocytes from mice immunized with pTGV-SSX2^{opt} with tumor cells *in vitro* – PD-L1 expression was increased compared with treatment of tumor cells with splenocytes from control-immunized mice (data not shown). Next, as shown in Figure 3, antigen-specific CD8+ T cells detected by tetramer staining were highly PD-1 expressing in mice immunized with pTVG-SSX2^{opt} compared with mice immunized with the native pTVG-SSX2 vector. Finally, as shown in Figure 4, combining vaccine treatment with an antibody blocking PD-1 (clone G4, courtesy of Dr. Leiping Chen) was found to elicit a greater anti-tumor response than treatment with either vaccine or anti-PD1 alone. Similar results were demonstrated in separate studies with the native vaccine alone in combination with anti-PD-1 treatment (not shown), and tumor eradication was observed in 5/10 animals treated with either vaccine combination (not shown). *Taken together, these findings demonstrate that PD-L1 expression by tumors, induced by antigen-specific CD8+ T cells, is a mechanism of tumor immune escape, and this can be abrogated by PD-1 blockade.*

Given these findings, we next asked whether other immune regulatory markers were increased following immunization. Splenocytes from animals treated with a DNA vaccine encoding native or optimized SSX2 were collected and antigen-specific CD3+CD8+ T cells were assessed for

expression of PD-1, TIM-3, LAG3, BTLA, CD160, or CD244. As shown in Figure 5, in addition to PD-1, expression of TIM-3 and LAG3 were also increased on T cells following immunization with the modified construct. Expression of CD244 was higher on tetramer+ cells from animals immunized with the native vaccine than with the control or modified construct. These findings demonstrate that combining T-cell regulatory receptor blockade with anti-PD-1 with a T-cell activating therapy (DNA vaccine) is a rational clinical direction, and this is the primary endpoint of this trial. Moreover, our murine studies suggest that other regulatory receptors might also be affected by immunization, and hence the evaluation of this will be an exploratory endpoint of this trial.

In further preclinical studies to evaluate the possible role of PD-1 in patients who previously received a DNA vaccine encoding PAP, we used a delayed-type hypersensitivity (DTH) model (developed by UW investigator Dr. William Burlingham, PhD) to evaluate mechanisms of immune regulation, similar to studies we have previously reported [57]. While a small number, we have found in 2 of 2 patients evaluated prior to and after DNA vaccination that PD-1 blockade, like CTLA-4 blockade, could restore effector function (as measured by DTH) of PAP-specific T cells elicited with vaccination (Figure 6). We also found in a patient previously treated with pTVG-HP, who then subsequently received sipuleucel-T for metastatic disease, that PD-L1 expression could be directly detected on circulating $CD45^{-}EpCAM^{+}$ cells (Figure 7). These studies form the basis for further exploratory studies proposed, to determine whether the *trans vivo* DTH method used can identify patients with antigen-specific PD-1-mediated regulation as either a predictive biomarker or measure of response, and whether similarly the presence of PD-L1 can be detected on circulating epithelial (tumor) cells as a predictive biomarker or measure of response, similar to what has been proposed for other studies using PD-1 blocking antibodies [18].



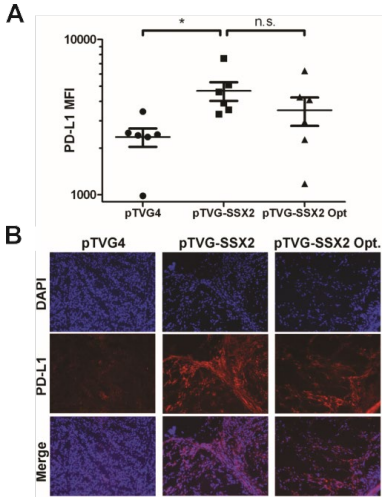


Figure 1: Modificatio
by DNA vaccine do
Groups of 6 HHD-
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(pTVG-SSX2, red),
"optimized" epitopes (pTVG-SSX
(pTVG4, blue). Tumor growth
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2+ tumor-bearing
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expression. As shown, animals immunized with either DNA vaccine encoding SSX2 (native or both modified epitopes) had increased PD-L1 expression on tumors relative to controls.

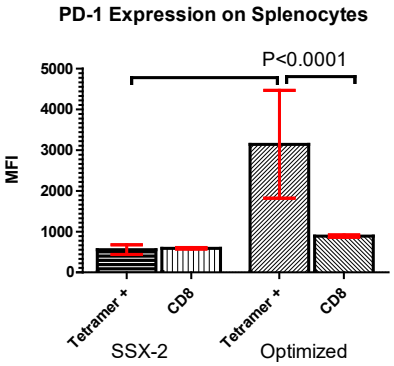

Figure 3: Immunization of mice with optimized SSX2-encoding DNA vaccine elicits higher PD-1 expression on antigen-specific CD8+ T cells. Splenocytes from animals immunized with pTVG-SSX2 (SSX-2) or pTVG-SSX2^{opt} (Optimized) were stained for p103 and p41 (HLA-A2) tetramers and PD-1. Shown is the mean fluorescence intensity (MFI) of PD-1 expression on antigen-specific CD3+CD8+tetramer+ (tetramer+) cells compared with CD3+CD8+tetramer- (CD8) popula-tions. As shown, animals immunized with the optimized vaccine had increased PD-1 expression on antigen-specific CD8+ T cells.

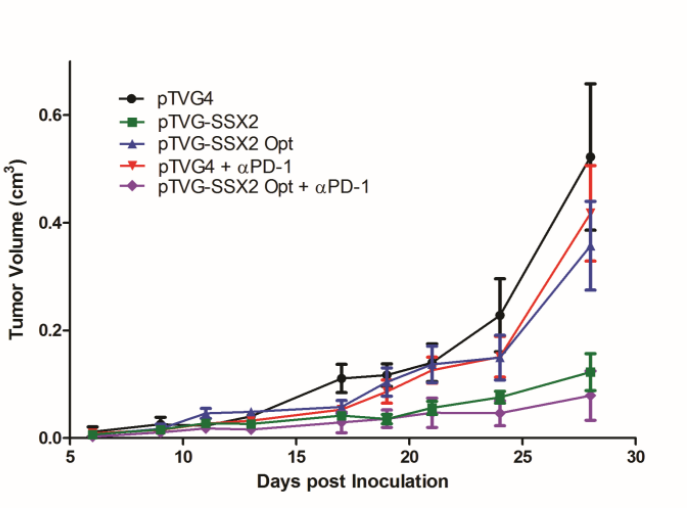

Figure 4: Immunization of mice with optimized SSX2-encoding DNA vaccine with anti-PD-1 elicits greater anti-tumor response. Groups of 4-6 HLA-A2 transgenic mice were implanted subcutaneously with 2x10^4 Sarc/SSX2 tumor cells and then immunized weekly with 100 µg plasmid DNA encoding SSX2 (pTVG-SSX2), or encoding SSX2 containing both "optimized" epitopes (pTVG-SSX2^{opt}), or vector control (pTVG4), as in Figure 4, and with or without 100 µg anti-mouse PD-1 (clone G4) administered 24 hours after each immunization. Tumor growth was followed over time. As shown, the anti-tumor efficacy of the "optimized" vaccine was improved with anti-PD1, and no anti-tumor effect was seen with anti-PD1 treatment alone.

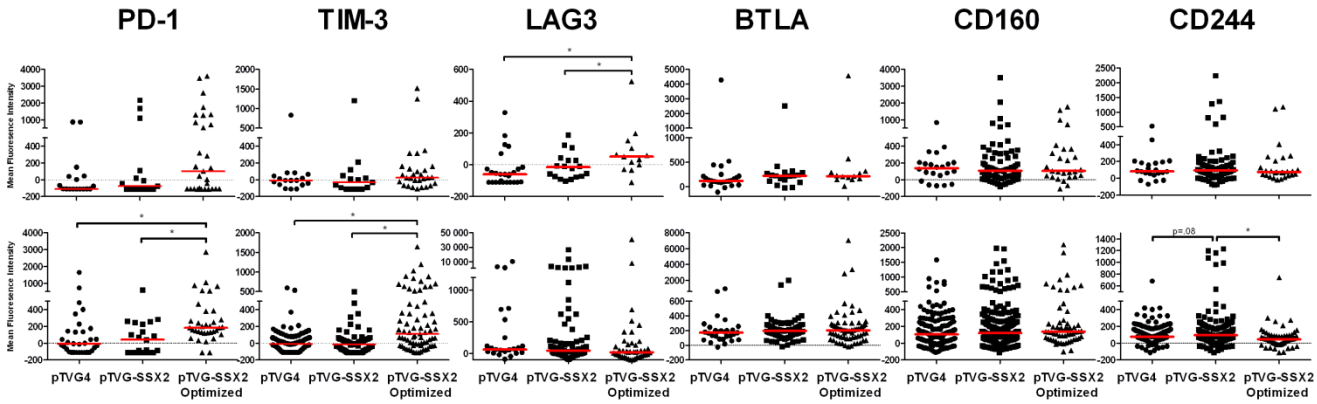

Figure 5: Immunization of mice with APL-modified SSX2-encoding DNA vaccine elicits PD-1 and TIM-3 expressing tetramer+ CD8+ T cells. Groups of 4-6 HLA-A2 transgenic mice were implanted subcutaneously with 2x10^4 Sarc/SSX2 tumor cells and then immunized weekly with 100 µg plasmid DNA encoding SSX2 (pTVG-SSX2), or encoding SSX2 containing both APL-modified epitopes (pTVG-SSX2^{opt}), or vector control (pTVG4), as in Figure 4. Splenocytes recovered from animals were stained for tetramer positive events among CD8+ T cells and costained for the markers indicated. Top panels represent p41-specific tetramer positive events, bottom panels represent p103-specific tetramer positive events. Of note, tetramer positive events are detectable in mice with Sarc/SSX2 tumors even with control immunization (pTVG4); these events not detectable in non-tumor bearing mice (not shown).

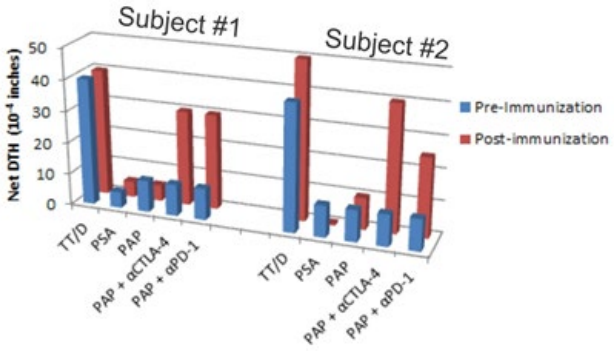

Figure 6: Patients treated with pTVG-HP develop T cell responses to PAP, the function of which is regulated by PD-1. PBMC obtained from 2 patients pre (blue) or 2 weeks post (red) a 12-week treatment with pTVG-HP were placed in the footpads of SCID mice with the 1 µg of the antigens listed (TT/D = tetanus toxoid, positive control, PSA = negative control), with or without 1 µg of anti-CTLA-4 or anti-PD1. DTH responses were read at 24 hours. As shown, both patients had responses to PAP after immunization that were "uncovered" by either CTLA-4 or PD-1 blockade.

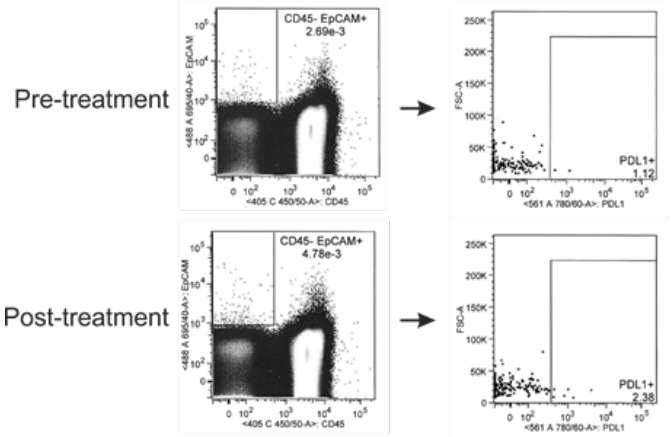

Figure 7: PD-L1 can be detected directly on circulating epithelial cells (presumed circulating tumor cells) by flow cytometry. PBMC were obtained from a patient who had previously received pTVG-HP, and then subsequently received standard sipuleucel-T for the treatment of metastatic prostate cancer. Cells were stained for CD45, EpCAM, and PD-L1. The gating shows live ($DAPI^-$) $CD45^-EpCAM^+$ cells (a population not detectable in non-cancer population – not shown)for their PD-L1 expression.

F. Summary

The current protocol will examine the use of a plasmid DNA vaccine encoding PAP to induce and/or augment therapeutic T-cells specific for the prostate tumor antigen PAP in patients with castrate-resistant, metastatic prostate cancer with concurrent or subsequent treatment with a PD pathway inhibitor, pembrolizumab. The primary endpoint, objective response, is based on observations from previous early phase clinical trials demonstrating no objective disease response in patients treated with PD-1 blockade alone, and our preliminary studies demonstrating that in a similar murine model of tumor cells that express PD-L1 that there was no evidence of tumor growth delay or eradication unless PD pathway blockade was combined with tumor antigen-specific vaccination. This trial will explore whether there is a preferred sequence of treatment, and will in secondary and exploratory endpoints evaluate whether sequential or concurrent treatment affects the character and magnitude of the immune response. Further studies will explore whether the identification of antigen-specific PD-1-regulated T-cells is predictive for response to combined treatment. An additional exploratory endpoint will be to identify whether changes in FLT PET scans, particularly in systemic lymph nodes or lymph nodes in the area of immunization, might serve as a molecular imaging tool to identify immunological activation following immunization with or without pembrolizumab. The trial will be conducted in patients with castrate-resistant, metastatic prostate cancer, as this is the lethal form of prostate cancer and one for which there is a high need for therapies with a greater safety profile and greater magnitude of benefit. Moreover, based on published studies, the median time to disease progression in this stage

of disease is on the order of 3 months, and few PSA responses have been observed following vaccination alone in published studies in this stage of disease [7, 58], simplifying the identification of potential benefit, and potential magnitude of such benefit, for planning future confirmatory clinical trials.

In May 2017 this trial will be modified to further explore the concurrent administration of these two agents. This is based on emerging data from this trial that suggests that this treatment has evidence of anti-tumor efficacy, but is limited by being only delivered over a 3-month period of time. As such, after completion of the two-arm trial with at least 12 evaluable subjects per study arm, those arms will be closed to further accrual, with an extended treatment cohort (Arm 3) that will be opened to accrual as described below.

In early 2018 this trial will be modified again to further explore a schedule of concurrent administration of the two agents, but with less frequent pembrolizumab dosing. This is based on emerging immunological biomarker data from the trial that the vaccine schedule at every-two-week intervals may be preferred, and with the goal of minimizing the frequency of adverse events related to pembrolizumab observed in Arm 3. In January 2018, 11 patients had been accrued to Arm 3 and had received at least one treatment with DNA and pembrolizumab. At that point, 2 of 11 patients treated in Arm 3 had no evidence of PSA decline from baseline, which was lower than expected from what had been observed in Arm 1 (in which 8 of 13 patients had some PSA decline from baseline, p=0.04). In addition, while not a clinical parameter, the frequency of immune response to the PAP target antigen was observed in the laboratory to be overall lower than what had been observed in either Arm 1 or Arm 2. Given these findings, and given that the primary change from Arm 1 to Arm 3 was in changing the vaccine schedule from every 2 weeks to every 3 weeks, it was decided to add an additional treatment arm that would keep the vaccine schedule at 2-week intervals, but continuing combined treatment beyond 12 weeks. The schedule for pembrolizumab was increased to every 4 weeks for patient convenience and with the goal of reducing toxicity, since adverse events > grade 2 experienced to date were all attributed to pembrolizumab. This extended treatment cohort (Arm 4) will open to accrual after completion of enrollment to Arm 3.

3. Objectives

A. Primary Objectives

1. To evaluate the safety of pembrolizumab in combination with pTVG-HP in patients with castration-resistant, metastatic prostate cancer

2. To determine the 6-month progression-free survival and median time to radiographic progression in patients with castration-resistant metastatic prostate cancer treated with pembrolizumab in combination with pTVG-HP

3. To evaluate the anti-tumor response rates (objective response rate and PSA response rate, using PCWG2 criteria) in patients with castration-resistant metastatic prostate cancer treated with pembrolizumab in combination with pTVG-HP

B. Secondary Objectives:

1. To determine whether either treatment sequence, or PAP-specific immune response, is associated with prolonged (6-month) radiographic progression-free survival

2. To evaluate effects of schedule (concurrent versus delayed administration of pembrolizumab) on the magnitude of PAP-specific T-cell responses, PD-1 expression on circulating T cells, and PD-L1 expression on circulating epithelial cells (CEC) and on tumor biopsies

3. To determine the median time to radiographic progression using concurrent administration schedules

C. Laboratory and Exploratory Objectives:

1. To evaluate effects of treatment on number of circulating tumor cells

2. To evaluate PAP-specific antibody responses following treatment with pembrolizumab and pTVG-HP DNA vaccine

3. To determine whether either treatment sequence elicits immunologic antigen spread to other prostate-associated antigens

4. To determine whether pre-existing or vaccine-induced PD-L1 expression on CEC or tumor biopsies is predictive of objective clinical response

5. To determine whether treatment elicits expression of other regulatory molecules on tumor-specific T cells (e.g. TIM3, BTLA, and LAG3) or tumor cells (e.g. HVEM, phosphatidyl serine, PD-L2)

6. To determine whether PD-1-regulated antigen-specific T cells identified by *trans vivo* DTH testing can identify patients who develop objective clinical responses with PD-1 blockade therapy in combination with pTVG-HP

7. To determine whether changes in lymph nodes and soft tissue tumor lesions are observed by FLT PET/CT after treatment with vaccine with or without pembrolizumab

8. To determine if PD-1 inhibitor therapy in combination with pTVG-HP will change number and activity (SUV) in osteoblastic metastases as measured by NaF PET/CT.

4. Product Information

A. Anti-PD-1 monoclonal antibody (Pembrolizumab, Keytruda®)

Pembrolizumab (Keytruda®, Merck) is a human programmed death receptor-1 (PD-1)-blocking antibody indicated for the treatment of patients with unresectable or metastatic melanoma and

disease progression following ipilimumab and, if BRAF V600 mutation positive, a BRAF inhibitor. While this agent is commercially available and will be purchased for use, its use is investigational under this protocol. The same dose and schedule will be used in this protocol as for the approved melanoma indication. Specifically, it will be administered at 2 mg/kg as an intravenous infusion over 30 minutes every 3 weeks (up to four maximum doses). Actual current body weight will be used to calculate the dose for each treatment. Pembrolizumab will be administered as an intravenous infusion over 30 minutes using an IV line containing a sterile, non-pyrogenic, low-protein binding 0.2 μm to 5 μm in-line or add-on filter, and according to its package insert.

NOTE: For the expansion cohort (Arm 3), this will similarly use a dose of 2 mg/kg pembrolizumab, but with 200 mg maximum dose, administered as an intravenous infusion over 30 minutes every 3 weeks for up to a maximum of 16 doses. For the second expansion cohort (Arm 4), this will similarly use a dose of 2 mg/kg pembrolizumab, but with 200 mg maximum dose, and administered as an intravenous infusion over 30 minutes every 4 weeks for up to a maximum of 12 doses.

The most common adverse reactions (reported in $\geq$ 20% of patients) included fatigue, cough, nausea, pruritis, rash, decreased appetite, constipation, arthralgia, and diarrhea. Immune-mediated adverse reactions have also been observed, including immune-mediated pneumonitis, colitis, hepatitis, hypophysitis, nephritis, hyperthyroidism and hypothyroidism. The management of suspected adverse reactions is discussed in Section 8 below.

B. Plasmid DNA vaccine

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

pTVG-HP is a plasmid DNA, produced in *E. coli,* that encodes the cDNA for human prostatic acid phosphatase (PAP). DNA from the master cell bank bacterial strain has been confirmed by standard DNA sequencing to confirm its identity. 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 are 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 lot release. The vaccine will be supplied in single-use vials containing at least 0.6 mL 0.2 mg/mL pTVG-HP in phosphate-buffered saline. Vials will be stored at <–60°C until the day of use.

GM-CSF

GM-CSF (Leukine®, Sargramostim), will be obtained from commercial vendor (such as Genzyme, Cambridge, MA, or other commercial vendor) 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 [59, 60]. The use of

GM-CSF is associated with little toxicity [61, 62, 63]. GM-CSF is a sterile, white, preservative-free, lyophilized powder supplied in 250 µg-dose vials.

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

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

Administration of rhGM-CSF may aggravate fluid retention in patients with pre-existent edema, capillary leak syndrome, or pleural or pericardial effusions. In some patients with pre-existing renal or hepatic dysfunction, elevation of the serum creatinine or bilirubin and hepatic enzymes has occurred during the administration of rhGM-CSF. Dose reduction or interruption of rhGM-CSF administration has resulted in a decrease to pretreatment values. Occasional transient and reversible supraventricular arrhythmia has been reported in uncontrolled studies, particularly in patients with a previous history of cardiac arrhythmia.

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

Because GM-CSF will only be administered at most once every two weeks on the current protocol, most of these side effects are not anticipated. In previous vaccine studies using rhGM-CSF as an adjuvant in an identical fashion, local skin reactions consisting of erythema and induration have occurred in many patients. These resolved without need for treatment over a 2-3 week period for some, and over 2-3 days for most. Transient leukopenia (lasting only a couple of hours) has been observed in some patients, and resolved without treatment and without clinical sequelae [64]. 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 [63].

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.

The vaccine will then be administered intradermally, on the lateral aspect of the arm (left arm, preferentially) in two adjacent sites, with the total syringe contents (0.25 to 0.3 mL) administered at each site.

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 (and be receiving) standard of care androgen deprivation treatment (surgical castration versus GnRH analogue or antagonist treatment); subjects receiving GnRH analogue or antagonist must continue this treatment throughout the time on this study.
    b. Patients may or may not have been treated previously with a nonsteroidal antiandrogen. For patients previously treated with an antiandrogen, they must be off use of anti-androgen for at least 4 weeks (for flutamide) or 6 weeks (for bicalutamide or nilutamide) prior to registration. Moreover, 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. Patients must have a castrate serum level of testosterone (< 50 ng/dL) within 6 weeks of day 1
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 [65] 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, with the final value $\geq$ 2.0 ng/mL.
b. Measurable disease: $\geq$ 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 (or NaF PET/CT) 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. Prior treatment with abiraterone or enzalutamide is permitted, but patients must have been off prior corticosteroid treatment for at least 28 days.
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 6 weeks of day 1:

WBC	$\geq 2000 / mm^3$
ANC	$\geq 1000 / mm^3$
HgB	$\geq$ 9.0 gm/dL
Platelets	$\geq 100{,}000 / mm^3$
Creatinine	$\leq$ 2.0 mg/dL
AST, ALT	$\leq$ 2.5 x institutional upper limit of normal

10. No known history of HIV 1 and 2, HTLV-1, or active Hepatitis B or Hepatitis C
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, unless considered chronic
12. Patients must be willing and able (in the opinion of the treating physician) to undergo two research biopsies for the investigational component of this trial. See section 6.A for more details.
13. Patients must be willing to undergo two leukapheresis procedures for the investigational component of this trial
14. Patients must be willing to undergo FLT PET/CT or NaF PET/CT scans for the investigational component of this trial and have no known allergies to FLT or NaF. See section 9 for more details.
15. For those patients who are sexually active, they must be willing to use barrier contraceptive methods during the period of treatment on this trial (and for four weeks after the last DNA immunization treatment for patients in Arm 1)
16. 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 (non-adenocarcinoma) prostate cancer histology, unless there is evidence that the tumor expresses PAP
2. Patients may not be receiving other investigational agents or be receiving concurrent anticancer therapy other than standard androgen deprivation therapy

3. Concurrent bisphosphonate therapy is not excluded, however patients should not start bisphosphonate therapy while on this study; those patients already receiving bisphosphonate therapy should continue at the same dosing and schedule as prior to study entry
4. Rapidly progressive symptomatic metastatic disease, as defined by the need for increased opioid analgesics within one month of registration for the treatment of pain attributed to a prostate cancer metastatic lesion; patients receiving opioids must receive approval from the PI for eligibility
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 5 mg prednisone daily) – not permitted within 1 month of registration; 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
    - Enzalutamide
    - Radium 223 (Xofigo®)
    - 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 (for example, but not limited to, docetaxel, mitoxantrone, cabazitaxel) within 6 months of registration is prohibited
9. Patients with a history of life-threatening autoimmune disease
10. Patients with a history of allergic reactions to GM-CSF or the tetanus vaccine
11. Patients who have undergone splenectomy
12. Patients must not have other active malignancies other than non-melanoma skin cancers or superficial bladder cancer. Subjects with a history of other cancers who have been adequately treated and have been recurrence-free for $\geq$ 3 years are eligible.
13. Patients with known brain metastases
14. Any antibiotic therapy or evidence of infection within 1 week of registration
15. Any other medical intervention or condition, which, in the opinion of the PI or treating physician, could compromise patient safety or adherence with the study requirements (including biopsies or leukapheresis procedures) over the primary 3-6 month treatment period.
16. Patients cannot have concurrent enrollment on other phase I, II, or III investigational treatment studies.

17. NOTE: There is no exclusion for prior immune-based therapy. This includes patients previously treated on Arms 1 or 2 who are otherwise eligible for treatment on Arm 3 or 4.

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 a DNA vaccine encoding PAP with rhGM-CSF adjuvant given with (concurrently or sequentially) pembrolizumab. Study arms will be defined as follows:

Arm 1: pTVG-HP (100 µg) with rhGM-CSF (208 µg) administered intradermally (i.d.) biweekly 6 times
Pembrolizumab 2 mg/kg, administered intravenously every 3 weeks 4 times, beginning on day 1 after the first pTVG-HP vaccination

Arm 2: pTVG-HP (100 µg) with rhGM-CSF (208 µg) administered intradermally (i.d.) biweekly 6 times
Pembrolizumab 2 mg/kg, administered intravenously every 3 weeks 4 times, the first dose administered two weeks after the last pTVG-HP vaccination

Extended treatment Arm 3:
pTVG-HP (100 µg) with rhGM-CSF (208 µg) administered intradermally (i.d.) every 3 weeks, for a maximum of 16 doses
Pembrolizumab 2 mg/kg, with a maximum dose of 200 mg, administered intravenously every 3 weeks, for a maximum of 16 doses, beginning on day 1 after the first pTVG-HP vaccination

Extended treatment Arm 4:
pTVG-HP (100 µg) with rhGM-CSF (208 µg) administered intradermally (i.d.) every 2 weeks, for a maximum of 24 doses
Pembrolizumab 2 mg/kg, with a maximum dose of 200 mg, administered intravenously every 4 weeks, for a maximum of 12 doses, beginning on day 1 after the first pTVG-HP vaccination

A. Study Arm Assignment and Toxicity Assessment

Patients with castrate-resistant, metastatic prostate cancer, with evidence of progressive disease by scans (but not by pain symptoms) or rising PSA, who may or may not have been previously treated with other therapies (including chemotherapy, abiraterone, or enzalutamide), will be invited to participate. Subjects will be randomly assigned to a treatment group for the first two study arms. Once at least 12 evaluable subjects have been accrued to Arms 1 and 2, and once Arm 3 is open to accrual, Arms 1 and 2 will be closed and all further accrual will be to Arm 3. Given the absence of significant adverse events observed in previous clinical trials with pTVG-HP, no adverse events > grade 2 are anticipated related to that agent. While not common, previous immune-associated toxicities have been observed with pembrolizumab, including immune-mediated pneumonitis, colitis, hepatitis, hypophysitis, nephritis, hyperthyroidism, and hypothyroidism. Hence these may

be anticipated events. In 89 patients treated with pembrolizumab at 2 mg/kg every 3 weeks in a study of patients with unresectable or metastatic melanoma, < 20% of patients experienced any adverse reaction > grade 2; the most common adverse reaction was fatigue (7% of patients). Three grade 4 laboratory abnormality events (hyperglycemia, increased ALT, and anemia) were reported. In a larger trial of 411 patients with advanced melanoma, 9% of patients discontinued treatment due to adverse reactions. However, patients in the approval trial received a median of 9 doses, more doses than will be used in the current trial. In the current trial patients will be evaluated for evidence of toxicity, and further accrual to a specific treatment arm will be discontinued if the toxicity rate is deemed to be excessive for that arm. Given the more limited exposure to this agent (4 doses total), a toxicity rate of 35% for Grade 3 events or 20% 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 from vaccination.

Evidence that the toxicity rate is excessive will be considered sufficient if, within any arm, 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., 35% for Grade 3 events and 20% 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 are present in at least 4 out the first of 5, 5 out of the first 7 (or less), 6 out of the first 10 (or less), or 7 out of the first 12 (or less) or 8 out the first 14 (or less) or 9 out of the first 16 (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 3 out of the first 5, 4 out of 9 (or less), 5 out of the first 13 (or less) or 6 out of 16 (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 for safety, dose modification and safety modification, and the study will be reviewed for safety by the UW DSMC (data and safety monitoring committee) 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 (CT and/or bone scintigraphy) 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 continuing treatment is not in the best interest of the patient and/or that other standard therapies for prostate cancer are warranted due to symptomatic progression. Patients should be discouraged from discontinuing protocol treatment for PSA rise only. NaF and FLT PET/CT scans will be investigational and will not be used for making individual subject treatment decisions with respect to progression necessitating continuing or discontinuing protocol treatment. However, findings on NaF or FLT PET/CT that might pose medical risk to individual subjects will be communicated to the treating physician such that dedicated clinical imaging/intervention could be pursued, if necessary. Such findings on NaF or FLT PET/CT, by themselves, will not be used to define disease progression necessarily requiring patients to come off trial. Patients who, in the discretion of the treating physician, appear to be benefiting (e.g. declining serum PSA and/or

improvement in symptoms despite new radiographic lesions), may continue in protocol treatment/observation after discussion with the PI and study sponsor. 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. Patients who undergo an initial research biopsy and/or NaF/FLT PET scan should also undergo the subsequent scans, as long as they are willing, even if they are off study prior to Month 3. In particular, evaluations performed at 6-month time point should be performed even if off-study prior to that time, if feasible. All subjects who have not come off study earlier for these reasons will come off study at the end of one year.

The primary endpoints of the trial will be clinical endpoints (safety, 6 month PFS, and objective response rates). 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 and response will be defined using PCWG2 published criteria [65], and 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. These criteria, in addition to being more standard for contemporary clinical trials in advanced prostate cancer, were also specifically designed to capture late radiographic objective responses that have been observed with immune-based therapies, including pembrolizumab and other T-cell checkpoint inhibitors [80]. Time to progression will be determined from registration for all subjects. 6-month progression-free survival will be calculated for each treatment arm, as well as the median progression-free survival.

The primary endpoint for the extended treatment Arms 3 and 4 will be time to radiographic progression.

7. Measurement of Effect (PCWG2 Recommendations)

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 ~ day 85 (3 months). Objective progression at that time point (Month 3, day 85) 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. For all of these measurements, PCWG2 criteria will be used to identify "measurable" lesions at each of the baseline (pre-treatment and 3 month time points), and RECIST 1.1 criteria will be used to monitor for change over time (consistent with PCWG2 criteria).

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 $\leq$ 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 (or NaF PET/CT), 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 (or NaF PET/CT) at least 6 weeks later demonstrating $\geq$ 2 new lesions. This is to eliminate the possibility of flair responses seen on bone imaging 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 treatment prolongs the time to progression using a delayed baseline evaluation after the treatment has been given time to elicit an immunologically-mediated 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), or a specific Grade 2 event as described below that affects agent dosing, with an attribution of at least possibly related to the study or treatment procedures, and occurring between the pre-study visit and within one month of the final study vaccine treatment or within 90 days of the last treatment with pembrolizumab (whichever is later). In general, if a patient develops Grade 3 toxicity with an attribution of at least possibly related to treatment (vaccine and/or pembrolizumab), the treatment schedule will be held until the toxicity resolves to Grade 1 or less. There will be no dose reductions due to adverse events. If a patient develops a second Grade 3 event both believed at least possibly due to treatment, or any Grade 4 event, no further treatments will be given. The patient will remain on study, however, with collection of radiographic and laboratory data as per protocol. The rationale for this is that patients could continue to benefit in terms of anti-tumor response and this could in fact be related to the development of other autoimmune toxicities. If the adverse event occurs during the initial 12 weeks (Arm 1) or 24 weeks (Arm 2) of treatment, a treatment delay of up to one week will be permitted, with the expectation that the event resolve to < grade 2 for retreatment, and that subsequent treatment will resume at the original study intervals. A delay > 1 week will result in a skipped dose/treatment. While rare overall, autoimmune events observed following pembrolizumab treatment of patients with melanoma typically resolved and did not necessarily lead to discontinuation of treatment.

For patients specifically treated in Arms 3 or 4, the following management rules will apply: If a patient develops Grade 3 toxicity with an attribution of at least possibly related to pembrolizumab and unlikely related (or unrelated) to pTVG-HP vaccine, the pembrolizumab treatment will be held until the toxicity resolves to Grade 1 or less, with the vaccine schedule continuing as per protocol. If a patient develops a second Grade 3 event (or any Grade 4 event) believed to be at least possibly due to pembrolizumab, but unlikely related (or unrelated) to pTVG-HP vaccine, no further pembrolizumab treatments will be given. The patient will continue with pTVG-HP administration only as per protocol. However, if a patient develops a Grade 3 toxicity with an attribution of at least possibly related to pTVG-HP vaccine, both pTVG-HP and pembrolizumab treatment will be

held until the toxicity resolves to Grade 1 or less. If a patient develops a second Grade 3 event (or any Grade 4 event) believed at least possibly related to pTVG-HP vaccine, no further treatments will be given. The patient will remain on study, however, with collection of radiographic and laboratory data as per protocol. The rationale for these modifications is that patients could continue to benefit in terms of anti-tumor response and this could in fact be related to the development of other autoimmune toxicities, and patients who have toxicity from pembrolizumab could still potentially benefit from continued pTVG-HP vaccination.

Specific symptoms and potential adverse events that have been identified in previous trials with pembrolizumab are described below. Management for these specific conditions will be conducted as described, consistent with what has been previously recommended in the FDA prescribing information "package insert":

Infusion-related reactions:

Treatment with pembrolizumab will be permanently discontinued for grade 3 or 4 infusion-related reaction.

Constitutional symptoms:

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.

Fatigue was reported in 47% (and 7% grade 3 fatigue) of 89 patients with melanoma who received pembrolizumab in the trial leading to its approval. As this is a potentially expected event, and not life-threatening, treatment will not be held for fatigue grade 3 or lower.

Hematologic:

Anemia was reported in 14% (and 5% grade 3 anemia) of 89 patients with melanoma who received pembrolizumab in the trial leading to its approval. Anemia is also common in patients with advanced prostate cancer. In the absence of findings to suggest this is an immune-mediated event, treatment will not be held for anemia grade 3 or lower.

Pulmonary adverse events:

Pneumonitis occurred in 12 (2.9%) of 411 melanoma patients receiving pembrolizumab, including 8 (1.9%) with grade 3 and 1 (0.2%) with grade 4 events. The median time to development of symptoms was 4.9 months. Patients will be monitored for signs and symptoms of pneumonitis, with radiographic imaging (chest X-ray or CT) and specialist referral as clinically indicated. Specifically, a CT of the chest will be performed at baseline as a basis for comparison, and then subsequently as clinically indicated. Pembrolizumab will be held for grade 2 pneumonitis, and permanently discontinued for grade 3 or higher events. Symptomatic patients will be treated with high-dose systemic corticosteroids ($\geq$ 40 mg prednisone or equivalent per day) for at least one

week followed by taper, or as clinically indicated. If retreatment is possible, treatment with pembrolizumab will continue to be held until the toxicity resolves to < grade 2.

Immune-mediated colitis adverse events:

Colitis (including microscopic colitis) occurred in 4 (1%) of 411 melanoma patients receiving pembrolizumab, including 1 patient with grade 2 and 2 patients with grade 3 events. The median time to development of symptoms was 6.5 months. Patients will be monitored for signs and symptoms of colitis (abdominal pain +/- diarrhea), with radiographic imaging and/or colonoscopy as clinically indicated. Pembrolizumab will be held for grade 2 or grade 3 colitis, and permanently discontinued for grade 4 events. Symptomatic patients will be treated with high-dose systemic corticosteroids (≥ 40 mg prednisone or equivalent per day) for one week followed by taper, or as clinically indicated. If retreatment is possible, treatment with pembrolizumab will continue to be held until the toxicity resolves to < grade 2.

Immune-mediated hepatitis adverse events:

Hepatitis (including liver-associated pain and/or elevation of serum liver function tests) occurred in 2 (0.5%) of 411 melanoma patients receiving pembrolizumab, including 1 patient with a grade 4 event. The time to development of symptoms was 22 days. Patients will be monitored by laboratory tests for changes in liver function. Pembrolizumab will be held for grade 2 or higher liver function test abnormalities, and permanently discontinued for grade 3 or 4 events. Patients with grade 2 or higher events will be treated with high-dose systemic corticosteroids (≥ 40 mg prednisone or equivalent per day) for at least one week followed by taper, or as clinically indicated. If retreatment is possible, treatment with pembrolizumab will continue to be held until the toxicity resolves to < grade 2.

Immune-mediated hypophysitis adverse events:

Hypophysitis occurred in 2 (0.5%) of 411 melanoma patients receiving pembrolizumab, including 1 patient with grade 2 and 1 patient with grade 4 events. The median time to development of symptoms was 1.5 months. Patients will be monitored for signs and symptoms of hypophysitis, which could include headache, visual disturbances, polyuria, and polydipsia; lab tests will be performed as clinically indicated. Pembrolizumab will be held for grade 2 or higher hypophysitis, and permanently discontinued for grade 4 events. Patients with grade 2 or higher events will be treated with high-dose systemic corticosteroids (≥ 40 mg prednisone or equivalent per day) for at least one week followed by taper, or as clinically indicated. If retreatment is possible, treatment with pembrolizumab will continue to be held until the toxicity resolves to < grade 2.

Immune-mediated nephritis adverse events:

Nephritis occurred in 3 (0.7%) of 411 melanoma patients receiving pembrolizumab, including 1 patient each with grade 2, 3, or 4 events. Two of these patients developed acute interstitial nephritis. The time to development of symptoms was 11 months, and 5 months after the last dose in at least one patient. Patients will be monitored for changes in renal function, with other lab tests as clinically indicated. Pembrolizumab will be held for grade 2 or higher nephritis, and permanently discontinued for grade 3 or higher events. Patients with grade 2 or higher events will

be treated with high-dose systemic corticosteroids (≥ 40 mg prednisone or equivalent per day) for at least one week followed by taper, or as clinically indicated. If retreatment is possible, treatment with pembrolizumab will continue to be held until the toxicity resolves to < grade 2.

Immune-mediated thyroid function adverse events:

Hyperthyroidism occurred in 5 (1.2%) of 411 melanoma patients receiving pembrolizumab, including 2 patients with grade 2 events and 1 patient with a grade 3 event. The median time to development of symptoms was 1.5 months. Patients will be monitored for serum changes in thyroid function, and subsequently with other lab tests as clinically indicated. Pembrolizumab will be held for grade 3 or higher hyperthyroidism, and permanently discontinued for grade 4 events. Patients with grade 3 or higher events will be treated with high-dose systemic corticosteroids (≥ 40 mg prednisone or equivalent per day) for at least one week followed by taper, or as clinically indicated. If retreatment is possible, treatment with pembrolizumab will continue to be held until the toxicity resolves to < grade 2.

Hypothyroidism occurred in 34 (8.3%) of 411 melanoma patients receiving pembrolizumab, including 1 patient with a grade 3 event. The median time to development of hypothyroidism was 3.5 months. Patients will be monitored for serum changes in thyroid function, and subsequently with other lab tests if clinically indicated. Isolated hypothyroidism will be managed with thyroid hormone replacement therapy without treatment interruption and without corticosteroids.

Other immune-mediated adverse events:

Other clinically significant, immune-mediated adverse reactions have been observed in <1% of patients treated with pembrolizumab, including exfoliative dermatitis, uveitis, arthritis, myositis, pancreatitis, hemolytic anemia, partial seizures, adrenal insufficiency, myasthenic syndrome, optic neuritis, and rhabdomyolysis. For suspected immune-mediated adverse events apart from those previously listed, efforts should be made to confirm the etiology and exclude other causes. Based on the severity of symptoms, and as directed by the treating physician, pembrolizumab should be either discontinued or held until improvement in symptoms to < grade 2. Corticosteroids may be administered if clinically indicated, again as directed by the treating physician.

9. Plan of Treatment

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

Because one biomarker endpoint is to evaluate metastatic tissue prior to and after treatment, patients with disease amenable to biopsy will initially be required to undergo one pretreatment biopsy and one post-treatment biopsy roughly three months after the start of treatment. It is our intent to have at least 6 patients per study arm with evaluable pre-treatment and post-treatment

biopsies. If this is reached, subsequent patients with viable lesions amenable to biopsy may not be required to undergo these procedures. It is expected that some patients may have a baseline biopsy and not be able to undergo a repeat biopsy, and some patients may not have viable disease to biopsy, and these instances will not affect study participation. Similarly, up to 6 subjects per arm will be required to complete the FLT PET scan at baseline and again at month 3 (Week 12). If there are subjects who complete the first but not the second scan, more than 6 subjects in an arm may, after approval from the Study PI, undergo the FLT PET scans in order to have at least 6 subjects who complete both scans. A maximum of 10 evaluable subjects between Arms 3 and 4 will be required to undergo the NaF PET scans. Selected subjects in Arm #3 will undergo either the FLT PET or the NaF PET based on the area of their disease (soft tissue vs bone). Subjects in Arm 4 will not receive additional biopsies but may undergo NaF PET.

FIRST COHORT: STUDY ARMS 1 and 2:

A. Prescreen / Eligibility (performed within 6 weeks of Day 1)

1. Confirm potential eligibility by history, pathology, diagnosis; no exclusions by history
2. CT scan of abdomen and pelvis, bone scan
3. Sign consent form
4. Evaluation of CBC, chemistry panel, serum prostate specific antigen (PSA), serum PAP, and serum testosterone.

B. Pre-Treatment Evaluations (within 4 weeks of Day 1; can coincide with prescreen evaluation)

1. Physical examination, symptoms assessment, and ECOG performance score
2. Randomization – treatment arm assignment
3. Baseline research leukapheresis (50-100 mL approximate total volume, for immunological monitoring) – to take place within 4 weeks prior to day 1.
   NOTE: For patients unable to undergo leukapheresis at this time point, a 200-cc blood draw may be substituted (twenty 10-mL green-top heparinized tubes).
4. Tetanus immunization (to take place after baseline leukapheresis and prior to day 1)
5. 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
6. FLT PET/CT scan (to occur within 4 weeks prior to day 1).
7. Tissue biopsy – metastatic site – to take place within 2 weeks prior to day 1

C. Immunization #1 (Day 1)

1. Physical examination, symptoms assessment, and ECOG performance score
2. Blood draw for CBC, differential and platelets, chemistry panel, PSA and PAP
3. 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.

4. Following vaccinations, the subjects will be observed for 60 minutes for unanticipated adverse events.

D. ARM 1 SUBJECTS ONLY: Anti-PD-1 #1 (Day 1, 1-3 hours after day 1 immunization)

1. Pembrolizumab 2 mg/kg administered intravenously over 30 minutes
2. Subjects will be observed for additional 30 minutes for unanticipated adverse events. Vital signs (temperature, heart rate and blood pressure) will be assessed and recorded by the staff prior to, and at the end of, the treatment period.

E. Immunization #2 - Day 15 (+/- 3 days)

1. 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.
2. Following vaccinations, the subjects will be observed for 60 minutes for unanticipated adverse events.

F. ARM 1 SUBJECTS ONLY: Anti-PD-1 #2 - Day 22 (7 +/- 3 days after day 15 visit)

1. Physical examination, symptoms assessment, and ECOG performance score
2. Blood draw for CBC, differential and platelets, chemistry panel, PSA and PAP
3. Pembrolizumab 2 mg/kg administered intravenously over 30 minutes
4. Subjects will be observed for additional 30 minutes for unanticipated adverse events. Vital signs (temperature, heart rate and blood pressure) will be assessed and recorded by the staff prior to, and at the end of, the treatment period.

G. Immunization #3 - Day 29 (14 +/- 3 days after day 15 visit)

1. 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.
2. Following vaccinations, the subjects will be observed for 60 minutes for unanticipated adverse events.

H. Immunization #4 - Day 43 (14 +/- 3 days after day 29 visit)

1. Physical examination, symptoms assessment, and ECOG performance score
2. Blood draw for CBC, 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
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.

I. ARM 1 SUBJECTS ONLY: Anti-PD-1 #3 (Day 43, 1-3 hours after day 43 immunization)

1. Pembrolizumab 2 mg/kg administered intravenously over 30 minutes
2. Subjects will be observed for additional 30 minutes for unanticipated adverse events. Vital signs (temperature, heart rate and blood pressure) will be assessed and recorded by the staff prior to, and at the end of, the treatment period.

J. Immunization #5 - Day 57 (14 +/- 3 days after day 43 visit)

1. 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.
2. Following vaccinations, the subjects will be observed for 60 minutes for unanticipated adverse events.

K. ARM 1 SUBJECTS ONLY: Anti-PD-1 #4 – Day 64 (7 +/- 3 days after day 57 visit)

1. Physical examination, symptoms assessment, and ECOG performance score
2. Blood draw for CBC, differential and platelets, chemistry panel, PSA and PAP
3. Pembrolizumab 2 mg/kg administered intravenously over 30 minutes
4. Subjects will be observed for an additional 30 minutes for unanticipated adverse events. Vital signs (temperature, heart rate and blood pressure) will be assessed and recorded by the staff prior to, and at the end of, the treatment period.

L. Immunization #6 - Day 71 (14 +/- 3 days after day 57 visit)

1. 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.
2. Following vaccinations, the subjects will be observed for 60 minutes for unanticipated adverse events.

M. 3-Month Follow-up - Day 85 (14 +/- 3 days after day 71 visit)

1. Physical examination, symptoms assessment, and ECOG performance score.
2. Blood draw for CBC, chemistry panel, serum PSA and PAP (can be performed +/- 7 days of this visit)
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 (can be performed +/- 7 days of this visit)
4. CT scan of abdomen and pelvis, bone scintigraphy (can be performed +/- 7 days of this visit, but prior to pembrolizumab treatment for patients in Arm 2)
5. FLT PET/CT scan (can be performed +/- 7 days of this visit, but prior to pembrolizumab treatment for patients in Arm 2)
6. Tissue biopsy of same metastatic lesion as in pre-treatment period (can be performed +/- 7 days of this visit, but prior to pembrolizumab treatment for patients in Arm 2)

ARM 2 SUBJECTS ONLY – Anti-PD-1 #1:

7. Pembrolizumab 2 mg/kg administered intravenously over 30 minutes
8. Subjects will be observed for an additional 30 minutes for unanticipated adverse events. Vital signs (temperature, heart rate and blood pressure) will be assessed and recorded by the staff prior to, and at the end of, the treatment period.

N. DAY 106 – (21 +/- 3 days after day 85 visit):

1. Physical examination, symptoms assessment, and ECOG performance score.
2. Blood draw for CBC, chemistry panel, serum PSA and PAP

ARM 2 SUBJECTS ONLY – Anti-PD-1 #2:

3. Pembrolizumab 2 mg/kg administered intravenously over 30 minutes
4. Subjects will be observed for an additional 30 minutes for unanticipated adverse events. Vital signs (temperature, heart rate and blood pressure) will be assessed and recorded by the staff prior to, and at the end of, the treatment period.

O. DAY 127 – (21 +/- 3 days after day 106 visit):

1. Physical examination, symptoms assessment, and ECOG performance score.
2. Blood draw for CBC, chemistry panel, serum PSA and PAP

ARM 2 SUBJECTS ONLY – Anti-PD-1 #3:

3. Pembrolizumab 2 mg/kg administered intravenously over 30 minutes
4. Subjects will be observed for an additional 30 minutes for unanticipated adverse events. Vital signs (temperature, heart rate and blood pressure) will be assessed and recorded by the staff prior to, and at the end of, the treatment period.

P. DAY 148 – (21 +/- 3 days after day 127 visit):

1. Physical examination, symptoms assessment, and ECOG performance score.
2. Blood draw for CBC, chemistry panel, serum PSA and PAP

ARM 2 SUBJECTS ONLY – Anti-PD-1 #4:

3. Pembrolizumab 2 mg/kg administered intravenously over 30 minutes
4. Subjects will be observed for an additional 30 minutes for unanticipated adverse events. Vital signs (temperature, heart rate and blood pressure) will be assessed and recorded by the staff prior to, and at the end of, the treatment period.

Q. 6-month Follow-up – Day 169 (21 days +/- 3 days after day 148 visit)

1. Physical examination, symptoms assessment, and ECOG performance score
2. Blood draw for CBC, chemistry panel, serum PSA and PAP

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)
   NOTE: For patients unable to undergo leukapheresis at this time point, a 200-cc blood draw may be substituted (twenty 10-mL green-top heparinized tubes).

R. 9-Month Follow-up (84 days +/- 10 days following 6-month study visit)

1. Physical examination, symptoms assessment, and ECOG performance score.
2. Blood draw for CBC, 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)

S. 12-Month Follow-up (84 days +/- 10 days following 9-month study visit)
OFF-STUDY Visit

1. Physical examination, symptoms assessment, and ECOG performance score.
2. Blood draw for CBC, 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)

T. Long-term Follow-Up

1. Subjects will be contacted by telephone (if not already being seen in clinic) annually for 2 years following the last DNA vaccination to collect clinical information to identify any potential long-term risks. This information has been requested by FDA for all gene delivery trials to assess potential long-term risks. The specific information to be collected annually will include:
   - Date of contact
   - Current medications
   - Hospitalizations (dates and reasons for hospital admission)
   - Stage of prostate cancer, treatments for prostate cancer, and recent serum PSA level
   - New cancer diagnoses
   - New autoimmune disorders
   - New hematologic or neurologic disorders
   - Other new medical diagnoses

- Date of death if patient deceased

EXTENDED TREATMENT COHORT – ARM 3:

U. Prescreen / Eligibility (performed within 6 weeks of Day 1)

1. Confirm potential eligibility by history, pathology, diagnosis; no exclusions by history
2. CT scan of abdomen and pelvis (CT of chest as clinically indicated), bone scan
3. Sign consent form
4. Evaluation of CBC, chemistry panel, serum prostate specific antigen (PSA), serum PAP, and serum testosterone.

V. Pre-Treatment Evaluations (within 4 weeks of Day 1; can coincide with prescreen evaluation)

1. Physical examination, symptoms assessment, and ECOG performance score
2. Baseline research leukapheresis (50-100 mL approximate total volume, for immunological monitoring) – to take place within 4 weeks prior to day 1.
   NOTE: For patients unable to undergo leukapheresis at this time point, a 200-cc blood draw may be substituted (twenty 10-mL green-top heparinized tubes).
3. Tetanus immunization (to take place after baseline leukapheresis and prior to day 1)
4. 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
5. FLT PET/CT scan – to occur within 4 weeks prior to day 1 (up to 6 evaluable patients per cohort)
6. NaF PET/CT scan – to occur within 4 weeks prior to day 1 (max of 10 evaluable patients)
7. Blood for PT/INR for patients undergoing biopsy (may be 30 days up to day of biopsy for patients not taking Coumadin)
8. Tissue biopsy – metastatic site – to take place within 2 weeks prior to day 1 (minimum of 6 patients per cohort)

W. Immunization #1 (Day 1)

1. Physical examination, symptoms assessment, and ECOG performance score
2. Blood draw for CBC, differential and platelets, chemistry panel, PSA and PAP
3. 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.
3. Following vaccinations, the subjects will be observed for 60 minutes for unanticipated adverse events.
4. Pembrolizumab 2 mg/kg, maximum 200 mg dose, administered intravenously over 30 minutes
5. Subjects will be observed for additional 30 minutes for unanticipated adverse events.

X. Immunization #2 - Week 3 (21 +/- 3 days after Day 1 visit)
Immunization #4 - Week 9 (21 +/- 3 days after Week 6 visit)
Immunization #6 - Week 15 (21 +/- 3 days after Week 12 visit)
Immunization #8 - Week 21 (21 +/- 3 days after Week 18 visit)
Immunization #10 - Week 27 (21 +/- 3 days after Week 24 visit)
Immunization #12 - Week 33 (21 +/- 3 days after Week 30 visit)
Immunization #14 - Week 39 (21 +/- 3 days after Week 36 visit)
Immunization #16 - Week 45 (21 +/- 3 days after Week 42 visit)

1. Symptoms assessment
2. Blood draw for CBC, differential and platelets, and chemistry panel (can be performed +/- 3 days of this visit).
3. Blood draw for PSA and PAP (can be performed +/- 3 days of this visit)
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.
6. Pembrolizumab 2 mg/kg, maximum 200 mg dose, administered intravenously over 30 minutes
7. Subjects will be observed for additional 30 minutes for unanticipated adverse events.

Y. Immunization #3 – Week 6 (21 +/- 3 days after Week 3 visit)

8. Physical examination, symptoms assessment, and ECOG performance score
9. Blood draw for CBC, differential and platelets, chemistry panel, PSA and PAP
10. 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 (can be performed +/- 7 days of this visit)
11. 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.
12. Following vaccinations, the subjects will be observed for 60 minutes for unanticipated adverse events.
13. Pembrolizumab 2 mg/kg, maximum 200 mg dose, administered intravenously over 30 minutes
14. Subjects will be observed for additional 30 minutes for unanticipated adverse events.
15. NaF PET/CT scan –+/- 7 days from this visit (only if baseline NaF PET/CT obtained)

Z. Immunization #5 – Week 12 (21 +/- 3 days after Week 9 visit)

1. Physical examination, symptoms assessment, and ECOG performance score.
2. Blood draw for CBC, chemistry panel, serum PSA and PAP (can be performed up to 3 days prior to this visit)

3. Blood for PT/INR for patients undergoing biopsy (may be 30 days up to day of biopsy for patients not taking Coumadin)
4. 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 (can be performed +/- 7 days of this visit)
5. 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.
6. Following vaccinations, the subjects will be observed for 60 minutes for unanticipated adverse events.
7. Pembrolizumab 2 mg/kg, maximum dose 200 mg, administered intravenously over 30 minutes
8. Subjects will be observed for additional 30 minutes for unanticipated adverse events.
9. CT scan of abdomen and pelvis (CT of chest as clinically indicated), bone scintigraphy (can be performed +/- 7 days of this visit)
10. FLT PET/CT scan for those patients with baseline scan (can be performed +/- 7 days of this visit)
11. NaF PET/CT scan –+/- 7 days from this visit (only if baseline NaF PET/CT obtained)

12. Tissue biopsy of same metastatic lesion as in pre-treatment period (can be performed +/- 7 days of this visit)

AA. <u>Immunization #7 – Week 18 (21 +/- 3 days after Week 15 visit)</u>
Immunization #11 – Week 30 (21 +/- 3 days after Week 27 visit)
Immunization #15 – Week 42 (21 +/- 3 days after Week 39 visit)

1. Physical examination, symptoms assessment, and ECOG performance score.
2. Blood draw for CBC, chemistry panel, serum PSA and PAP (can be performed up to 3 days prior to this visit)
3. 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.
4. Following vaccinations, the subjects will be observed for 60 minutes for unanticipated adverse events.
5. Pembrolizumab 2 mg/kg, maximum 200 mg dose, administered intravenously over 30 minutes
6. Subjects will be observed for additional 30 minutes for unanticipated adverse events.

BB. <u>Immunization #9 – Week 24 (21 +/- 3 days after Week 21 visit):</u>

1. Physical examination, symptoms assessment, and ECOG performance score.
2. Blood draw for CBC, chemistry panel, serum PSA and PAP (can be performed up to 3 days prior to this visit)
3. Leukapheresis (~1.0 blood volume, 100 mL collection) for T-cell response evaluation (can be performed +/- 7 days of this study visit)
   NOTE: For patients unable to undergo leukapheresis at this time point, a 200-cc blood draw may be substituted (twenty 10-mL green-top heparinized tubes).

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. 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.
6. Following vaccinations, the subjects will be observed for 60 minutes for unanticipated adverse events.
7. Pembrolizumab 2 mg/kg, maximum 200 mg dose, administered intravenously over 30 minutes
8. Subjects will be observed for additional 30 minutes for unanticipated adverse events.
9. CT scan of abdomen and pelvis (CT of chest as clinically indicated), bone scintigraphy (can be performed +/- 7 days of this visit)

CC. Immunization #13 – Week 36 (21 +/- 3 days after Week 33 visit):

1. Physical examination, symptoms assessment, and ECOG performance score.
2. Blood draw for CBC, chemistry panel, serum PSA and PAP (can be performed up to 3 days prior to this visit)
3. 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.
4. Following vaccinations, the subjects will be observed for 60 minutes for unanticipated adverse events.
5. Pembrolizumab 2 mg/kg, maximum 200 mg dose, administered intravenously over 30 minutes
6. Subjects will be observed for additional 30 minutes for unanticipated adverse events.
7. CT scan of abdomen and pelvis (CT of chest as clinically indicated), bone scintigraphy (can be performed +/- 7 days of this visit)

DD. Week 48 Visit (End of Study) (21 +/- 3 days after Week 45 visit):

1. Physical examination, symptoms assessment, and ECOG performance score.
2. Blood draw for CBC, chemistry panel, serum PSA and PAP (can be performed up to 3 days prior to this visit)
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 (can be performed +/- 7 days of this visit)
4. CT scan of abdomen and pelvis (CT of chest as clinically indicated), bone scintigraphy (can be performed +/- 7 days of this visit)

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

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

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

FF. Long-term Follow-Up

1. Subjects will be contacted by telephone (if not already being seen in clinic) annually for 2 years following the last DNA vaccination to collect clinical information to identify any potential long-term risks. This information has been requested by FDA for all gene delivery trials to assess potential long-term risks. The specific information to be collected annually will include:
    - Date of contact
    - Current medications
    - Hospitalizations (dates and reasons for hospital admission)
    - Stage of prostate cancer, treatments for prostate cancer, and recent serum PSA level
    - New cancer diagnoses
    - New autoimmune disorders
    - New hematologic or neurologic disorders
    - Other new medical diagnoses
    - Date of death if patient deceased

EXTENDED TREATMENT COHORT – ARM 4:

GG. Prescreen / Eligibility (performed within 6 weeks of Day 1)

1. Confirm potential eligibility by history, pathology, diagnosis; no exclusions by history
2. CT scan of abdomen and pelvis (CT of chest as clinically indicated), bone scan
3. Sign consent form
4. Evaluation of CBC, chemistry panel, serum prostate specific antigen (PSA), serum PAP, and serum testosterone.

HH. Pre-Treatment Evaluations (within 4 weeks of Day 1; can coincide with prescreen evaluation)

1. Physical examination, symptoms assessment, and ECOG performance score
2. Baseline research leukapheresis (50-100 mL approximate total volume, for immunological monitoring) – to take place within 4 weeks prior to day 1.
   NOTE: For patients unable to undergo leukapheresis at this time point, a 200-cc blood draw may be substituted (twenty 10-mL green-top heparinized tubes).
3. Tetanus immunization (to take place after baseline leukapheresis and prior to day 1)
4. 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

5. NaF PET/CT scan – to occur within 4 weeks prior to day 1 (max of 10 evaluable patients)

II. Immunization #1 (Day 1)

1. Physical examination, symptoms assessment, and ECOG performance score
2. Blood draw for CBC, differential and platelets, chemistry panel, PSA and PAP
3. 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.
6. Following vaccinations, the subject will be observed for 60 minutes for unanticipated adverse events.
7. Pembrolizumab 2 mg/kg, maximum 200 mg dose, administered intravenously over 30 minutes
8. Subjects will be observed for additional 30 minutes for unanticipated adverse events.

JJ. Immunization #2 - Week 2 (14 +/- 3 days after Day 1 visit)
Immunization #6 - Week 10 (14 +/- 3 days after Week 8 visit)
Immunization #8 - Week 14 (14 +/- 3 days after Week 12 visit)
Immunization #10 - Week 18 (14 +/- 3 days after Week 16 visit)
Immunization #12 - Week 22 (14 +/- 3 days after Week 20 visit)
Immunization #14 - Week 26 (14 +/- 3 days after Week 24 visit)
Immunization #16 - Week 30 (14 +/- 3 days after Week 28 visit)
Immunization #18 - Week 34 (14 +/- 3 days after Week 32 visit)
Immunization #20 - Week 38 (14 +/- 3 days after Week 36 visit)
Immunization #22 - Week 42 (14 +/- 3 days after Week 40 visit)
Immunization #24 - Week 46 (14 +/- 3 days after Week 44 visit)

1. Symptoms assessment
2. 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.
3. Following vaccinations, the subject will be observed for 60 minutes for unanticipated adverse events.

KK. Immunization #4 – Week 6 (14 +/- 3 days after Week 4 visit)

1. Symptoms assessment
2. 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 (can be performed +/- 7 days of this visit)
3. 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.
4. Following vaccinations, the subject will be observed for 60 minutes for unanticipated adverse events.
5. NaF PET/CT scan –+/- 7 days from this visit (only if baseline NaF PET/CT obtained)

LL. Immunization #3 - Week 4 (14 +/- 3 days after Week 2 visit)
Immunization #5 - Week 8 (14 +/- 3 days after Week 6 visit)
Immunization #9 - Week 16 (14 +/- 3 days after Week 14 visit)
Immunization #11 - Week 20 (14 +/- 3 days after Week 18 visit)
Immunization #15 - Week 28 (14 +/- 3 days after Week 26 visit)
Immunization #17 - Week 32 (14 +/- 3 days after Week 30 visit)
Immunization #21 - Week 40 (14 +/- 3 days after Week 38 visit)
Immunization #23 - Week 44 (14 +/- 3 days after Week 42 visit)

1. Physical examination, symptoms assessment, and ECOG performance score.
2. Blood draw for CBC, chemistry panel, serum PSA and PAP (can be performed up to 3 days prior to this visit)
3. 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.
4. Following vaccinations, the subject will be observed for 60 minutes for unanticipated adverse events.
5. Pembrolizumab 2 mg/kg, maximum dose 200 mg, administered intravenously over 30 minutes
6. Subjects will be observed for additional 30 minutes for unanticipated adverse events.

MM. Immunization #7 - Week 12 (14 +/- 3 days after Week 10 visit)
Immunization #19 - Week 36 (14 +/- 3 days after Week 34 visit)

1. Physical examination, symptoms assessment, and ECOG performance score.
2. Blood draw for CBC, chemistry panel, serum PSA and PAP (can be performed up to 3 days prior to this visit)
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 (can be performed +/- 7 days of this visit)
4. CT scan of abdomen and pelvis (CT of chest as clinically indicated), bone scintigraphy (can be performed +/- 7 days of this visit)
5. 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.
6. Following vaccinations, the subject will be observed for 60 minutes for unanticipated adverse events.
7. Pembrolizumab 2 mg/kg, maximum 200 mg dose, administered intravenously over 30 minutes

8. Subjects will be observed for additional 30 minutes for unanticipated adverse events.

9. AT WEEK 12 ONLY (NOT week 36): NaF PET/CT scan –+/- 7 days from this visit (only if baseline NaF PET/CT obtained)

NN. Immunization #13 – Week 24 (14 +/- 3 days after Week 22 visit):

1. Physical examination, symptoms assessment, and ECOG performance score.
2. Blood draw for CBC, chemistry panel, serum PSA and PAP (can be performed up to 3 days prior to this visit)
3. Leukapheresis (~1.0 blood volume, 100 mL collection) for T-cell response evaluation (can be performed +/- 7 days of this study visit)
   NOTE: For patients unable to undergo leukapheresis at this time point, a 200-cc blood draw may be substituted (twenty 10-mL green-top heparinized tubes).
4. 10 ml peripheral blood (red-top tubes) for sera to evaluate antibody responses
5. CT scan of abdomen and pelvis (CT of chest as clinically indicated), bone scintigraphy (can be performed +/- 7 days of this visit)
6. 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.
7. Following vaccinations, the subject will be observed for 60 minutes for unanticipated adverse events.
8. Pembrolizumab 2 mg/kg, maximum 200 mg dose, administered intravenously over 30 minutes
9. Subjects will be observed for additional 30 minutes for unanticipated adverse events.

OO. Week 48 Visit (End of Treatment) (14 +/- 3 days after Week 46 visit):

1. Physical examination, symptoms assessment, and ECOG performance score.
2. Blood draw for CBC, chemistry panel, serum PSA and PAP (can be performed up to 3 days prior to this visit)
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 (can be performed +/- 7 days of this visit)
4. CT scan of abdomen and pelvis (CT of chest as clinically indicated), bone scintigraphy (can be performed +/- 7 days of this visit)

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

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

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

blood (green-top heparinized tubes) for T-cell response evaluation and CTC analysis

4. Patients to remain in telephone contact to report new symptoms up to 90 days after treatment with last dose of pembrolizumab

QQ. Long-term Follow-Up

1. Subjects will be contacted by telephone (if not already being seen in clinic) annually for 2 years following the last DNA vaccination to collect clinical information to identify any potential long-term risks. This information has been requested by FDA for all gene delivery trials to assess potential long-term risks. The specific information to be collected annually will include:
    - Date of contact
    - Current medications
    - Hospitalizations (dates and reasons for hospital admission)
    - Stage of prostate cancer, treatments for prostate cancer, and recent serum PSA level
    - New cancer diagnoses
    - New autoimmune disorders
    - New hematologic or neurologic disorders
    - Other new medical diagnoses
    - Date of death if patient deceased

10. Response Monitoring

A. Safety

All subjects will be assessed at regular intervals, as per the study calendar, for evidence of adverse events by exam, symptoms, and clinical laboratory findings. For these assessments, we will use the NCI common terminology criteria, version 4 (http://ctep.cancer.gov/protocoldevelopment/electronic_applications/docs/ctcaev4.pdf).

B. Immunological Monitoring

Blood will be collected by either peripheral blood draw (up to 120 mL), leukapheresis (50-100 mL), or leukapheresis replacement draw (200 mL) pre-immunization, at weeks 6, 12 and 24 of treatment, and at months 9 and 12 (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.

B.1. Quantitative assessment of PAP-specific CD8+ T-cell effector immunity

PAP-specific IFNγ- and granzyme B-secreting T-cell precursor frequency quantification by ELISPOT: ELISPOT will be used as the preferred methodology, as it permits analysis of low-frequency events (LOD ~ 1:100,000 cells) and also permits simultaneous analysis of cryopreserved batched specimens [30]. IFNγ and granzyme B will be preferred analytes evaluated, as these are specifically associated with inflammatory/tissue-destructive (Th1-type, cytolytic) immune responses. 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), 2 µg/ml of peptide libraries specific for PAP or 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 previously reported as the mean (+/- standard deviation) number of spot-forming-units (sfu) per 10^6 cells (frequency), calculated by subtracting the mean number of spots obtained from the no antigen control wells from the mean number obtained in the experimental wells, normalized to 10^6 starting PBMC, from 8-well replicate assays [31]. 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.

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

Ability of T-cell lines to proliferate in response to antigenic stimulation: T-cell proliferation in response to antigen stimulation as a measure of memory T-cell responses will be assessed by a PKH26 dye dilution assay as previously reported [31]. Specifically, PBMC will be labeled *in vitro* with PKH26 dye (Sigma, St. Louis, MO) according to the 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 2 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 37ºC/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. Other cell surface markers may be assessed, other antigens evaluated, and/or other methods of antigen-specific T-cell proliferation.

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, as previously reported [31]. 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.

B.3. Assessment of PAP-specific T cells by intracellular cytokine staining

PAP-specific T-cell cytokine expression by intracellular cytokine staining: As another method of evaluating AR LBD-specific T cell responses, intracellular cytokine staining will be used. Specifically, fresh or cryopreserved PBMC from subjects at the various time points will be rapidly thawed, cultured in 96-well microtiter plates in the presence of test antigens (PAP (protein or pool of overlapping 15-mer peptides), PSA, tetanus, PHA, or media only) for 4-24 hours, and then will be analyzed for intracellular cytokine expression. Specifically, stimulated cells will be treated with monensin for 4-8 hours at 37ºC/5%CO_2. Cells will then be washed with PBS/3%FCS, followed by staining for surface molecules (including CD3, CD4, and CD8 to identify T cell subsets, as well as CCR7 and CD45RO to identify memory populations, and potentially other cell surface markers). Cells will then be fixed, permeabilized, and then stained for intracellular expression of a panel of cytokines (including, but not limited to, IFNγ, TNFα, IL-2, IL-17, and IL-10), or control IgG labeled with the appropriate fluorochromes. Cells will be analyzed on a flow cytometer, and results will be determined by identifying the frequency of T cells (either total T cells and/or T cell subsets, including CD4+ and CD8+ T cells, as well as various memory

populations or regulatory populations) expressing each individual cytokine, or cells expressing multiple cytokines.

REPORTING AND RESPONSE DEFINITION: Data will be reported as a mean and standard deviation of antigen-specific (PAP-specific) CD4+ or CD8+ T cells expressing one or more cytokines per 10^6 PBMC using triplicate assessments for each antigen-stimulation condition, as previously reported [31]. 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 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.

B.4. 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 (or other antigens) will be evaluated by ELISA using an indirect method similar to that described previously [34]. Specifically, Immulon-4 ELISA plates (Dynex Technologies Inc.) will be coated with 2 µg/ml purified PAP protein (Research Diagnostics, Inc., or other antigens or commercial sources) in 0.1 M $NaHCO_3/Na_2CO_3$ buffer (pH 9.6) overnight at 4ºC. After blocking with PBS/1% BSA for 1 hour at room temperature, wells will be washed with PBS + 0.05% Tween-20 (PBS-Tween) and then incubated for 1 hour with human sera diluted 1:25, 1:50, 1:100 and 1:200. After washing, plates will then be sequentially incubated with a peroxidase-conjugated anti-human IgG detection antibody (Amersham), followed by peroxidase enzyme TMB substrate (Kierkegaard and Perry Laboratories). The color reaction will be stopped with 1N H_2SO_4 and the optical density measured at 450 nm. Antibody titers for PAP-specific IgG antibodies will be determined as previously described [34].

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).

B.5. 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. Other antigens may be evaluated as well. Antigen–driven swelling will be determined as previously described [66]. 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), 1 μg of mouse anti-human CTLA-4 monoclonal Ab (clone AS32, Ab Solutions, Mountain View, CA), or 1 μg of mouse anti-human PD-1 monoclonal Ab (or potentially other antibodies to other regulatory molecules), 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 [67]. 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. The identification of PD-1 (or CTLA-4) regulated responses will be defined as the identification of an increase ($>15 \times 10^{-4}$ inches) in a PAP-specific response in the presence of anti-PD-1 (or anti-CTLA-4) versus IgG control, as in Figure 6.

B.6. Assessment of antigen-spread to other prostate-associated antigens

High-throughput immunoblot (HTI): An exploratory objective of the study will be to determine if patients treated develop "off-target" prostate cancer antigen-specific immune responses as evidence of antigen spread. Future studies may evaluate T-cell responses to non-targeted antigens. However, the primary evaluation will be to evaluate IgG responses to a panel of prostate-associated antigens as we have previously reported in patients treated with vaccines or other immune-modulating agents [68, 69, 70]. IgG specific for 126 antigens, including 29 cancer-testis antigens [71, 72] and 97 prostate antigens frequently immunologically recognized [73, 74, 75, 76] will be identified by screening a high-density phage array expressing these individual antigens, as we have previously reported [68, 70, 71]. An alternative to this approach may include the evaluation of responses to panels of protein antigens fixed to support membranes provided by commercial vendors. The primary analysis will be conducted using sera obtained at 6 months, and compared with IgG responses identified at baseline, to determine whether IgG specific for individual antigens are elicited over time. Other time points will be assessed to determine the durability and kinetics of immune response development, and confirmatory ELISA studies will be performed where feasible.

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

C. Circulating Tumor Cell (CTC) Evaluation

CTC will be enumerated and characterized at the same time points as for immune evaluation (pre-treatment, after 6 weeks, and at quarterly intervals for up to one year) using flow cytometry. Specifically, as in Figure 7, PBMC obtained at these time points will be stained with fluorochrome-labeled antibodies specific for, and including at least, CD45, EpCAM, PD-1, CTLA-4, and DAPI. CTC will be defined as CD45-EpCAM+DAPI- cells, and the percentage of these events among all live cellular events will be determined at the different time points. The % of CTC expressing PD-1 or CTLA-4 will also be determined. Results will be reported in tabular format from the different time points, and general trends will be assessed. These results will not be strictly quantitative, as multiple replicates to determine standard deviation are not feasible. Other methods of CTC capture and enumeration may also be used. Further investigational studies may be conducted with CTC collected by these methods.

D. Histopathology Evaluation

Tissue biopsies will be obtained from metastatic lesions (the same lesion per patient) prior to treatment and at week 12. The purpose of these studies is to determine whether immunization affects PD-L1 expression in the tumor (likely by eliciting tumor antigen-specific T cells secreting IFNγ), whether concurrent treatment with anti-PD-1 mAb leads to an increase in infiltration of CD8+ T cells, and whether treatment increases expression of other T-cell regulatory ligands on T cells (PD-1, CTLA-4, TIM-3, BTLA, LAG-3) or tumors (e.g. HVEM, phosphatidyl serine, PD-L2). Consequently, biopsy specimens obtained pre-treatment and after 12 weeks will be stained with antibodies specific for CD3, CD4, CD8, FoxP3, PD-1, CTLA-4, TIM3, BTLA, LAG-3, PD-L1, PD-L2, phosphatidyl serine, HVEM and potentially other markers. Staining and quantification will be reviewed by a pathologist blinded to the treatment groups to determine CD8+ T cells per field, CD4+FoxP3+ (Treg):CD8+ T cell ratio, PD-L1 expression, and whether these or the expression of CD8+ T cells expressing one regulatory receptors (or tumor cells expressing one or more regulatory ligands) change from pre-treatment to the 12 week time point.

Logistically, patients will be scheduled for a CT-guided biopsy of a bone or soft tissue abnormality consistent with prostate cancer metastasis. This site of biopsy will be determined by the interventional radiology staff in conjunction with the local PI after review of available scans (bone scans, CT scans, MRI), with preference given to lesions meeting the following factors: (1) size of lesion (preference towards the largest lesion, given the need for biopsy and re-biopsy after 12 weeks); (2) safety of biopsy (with obvious preference to lesions with the least risk to the patient); and (3) for bone lesions with the greatest intensity activity noted on bone scintigraphy. Coordination and acquisition of biopsy material will be performed by the UWCCC TSB (Translational Science Biocore). Standard laboratory tests to assess bleeding and coagulation will also be completed prior to the procedure. Conscious sedation will be used as necessary (typically by using intravenous fentanyl and midazolam) and with appropriate nursing support. In order to obtain adequate tumor sample, and as long as it is deemed safe, and based on accessibility, target lesion size, specimen integrity, specimen appearance, and intraprocedural bleeding, an attempt will be made to obtain 2-6 biopsy samples. Biopsies will be performed using sterile technique and lidocaine local anesthetic. For bone lesions, at least an 11-13 gauge bone-cutting needle, with or without a co-axial trocar, will be used. 2-6 biopsy samples will be obtained of the selected lesion, with multiple samples obtained by: 1) sequential advancement of needle passes through a fixed

trocar, such that each needle pass progresses more deeply into the bone lesion; or 2) re-insertion and re-direction of the needle and/or trocar through another region of the bone lesion. At the completion of the procedure, CT imaging will be repeated to assess for immediate complications. The patient will be observed for 2-4 hours before discharge.

Samples will be transported to the UWCCC TRIP (Translational Research Initiatives in Pathology) lab for formalin fixation, paraffin embedding, sectioning, H&E staining, and ultimately for IHC analysis as described above. Other types of gene expression profiling may be used as well to evaluate gene expression changes, and changes in T cell subtypes infiltrating tumors prior to and after treatment.

E. FLT PET/CT Imaging

3'-Deoxy-3'-[^{18}F]Fluorothymidine (FLT) positron emission tomography (PET)/computed tomography (CT) uses a thymidine analogue that accumulates in proliferating tissue. Proliferating tissue could include malignant lesions or immune cells. Following vaccination we have observed a proliferation of CD4+ and CD8+ T cells specific for PAP, at least as assessed *in vitro* following reencounter with the PAP antigen [29, 31]. In a study of patients with melanoma treated intranodally with a dendritic cell vaccine, FLT PET/CT could detect uptake in treated lymph nodes compared to control lymph nodes following vaccination, uptake that persisted for up to 3 weeks [78]. Consequently, FLT PET/CT may be useful as a molecular imaging tool to detect immune activation after immunization, at the same time as detecting decreases in proliferating tumor masses as a result of treatment. We hypothesize that FLT PET/CT will be able to detect increased uptake in left axillary draining lymph nodes (draining site of intradermal immunization) at 12 weeks compared with pre-treatment studies, and that a decrease in uptake will be observed in soft tissue metastatic deposits. FLT PET/CT will be performed at baseline (within 4 weeks of starting treatment) and at week 12, thus enabling comparative evaluation with other imaging obtained at that time and biopsy results.

FLT PET/CT IMAGING PROCEDURES: To ensure uniformity as well as optimal data interpretation, analyses will be conducted under the supervision of Dr. Robert Jeraj, PhD (co-investigator) at the University of Wisconsin Image Analysis Core (IMAC) facility, with all radiographic analysis by Dr. Jeraj and designees blinded to study treatment arm.

- At the beginning of each imaging session, a CT scan will be obtained on the combined PET/CT scanner. The CT scan is an integral part of the PET/CT acquisition and serves for attenuation correction calculations and better PET/CT image co-registration. It will enable better delineation of the patient anatomy, which will be important when comparing different scans. An FLT-PET scan will follow. The patients will be injected up to 15 mCi (555 MBq) of ^{18}F-FLT.

- Dynamic imaging (15 cm field of view) will be performed first to obtain arterial input function and post injection kinetics of the tracer. The field of view will be positioned so that either a tumor mass or axillary draining nodes will be in the field of view. If applicable, the region that will allow at least a partial heart view will be selected. The dynamic scan will be performed for 30 minutes. The arterial blood decay curve of ^{18}F-FLT will be estimated from the time-dependent

activity in the left ventricular blood pool. The arterio-venous difference will be quantitatively assayed from the time-dependent activity in the right cardiac chambers.

• After the initial dynamic imaging of the heart region, a static whole body scan will be initiated. The whole body scan will be initiated at approximately 60 minutes after the injection. To minimize errors of the relative change assessment, the subsequent whole body scans will be initiated within 5 minutes of the initiation time of the baseline scan. The whole body scan will be performed with up to 10 minutes/scanning position, lasting up to 60 minutes. The patient will be scanned "head first". Together with the dynamic imaging, the total body scanning time will be up to 90 minutes (30 minutes dynamic plus up to 60 minutes static whole body scan).

• All the scans will be acquired in a 3D mode to increase spatial resolution of the imaging data.

FLT-PET/CT image reconstruction:

• For both the dynamic and whole body scans, two PET image reconstructions will be used – optimal qualitative and optimal quantitative reconstructions. The optimal quantitative reconstruction will be used for quantitative evaluation; the optimal qualitative reconstruction will be used for clinical read of the FLT PET/CT scans.

FLT-PET/CT image analysis:

• All the PET image data will be coregistered to the baseline (pretreatment) image data to enable comparison of the spatially dependent changes of the investigated tumor and metastasis parameters during the therapy. The scans will be coregistered based on the CT data. Each of the metastases will be coregistered locally, segmented and treated individually. Up to five metastases will be used in this analysis. Both the CT data, as well as the corresponding FLT PET data, will be analyzed for spatial correlation. The correlation coefficients, as well as joint histograms, will be used to evaluate similarity of the distributions.

• The CT data will be analyzed to establish anatomical changes in tumor size. Evaluation will be based on the change of the volume of the metastatic mass, as segmented from a CT scan: $\Delta V = V_{follow\text{-}up} - V_{baseline}$.

• The dynamic FLT-PET/CT imaging data will be used to perform kinetic analysis of the imaging data, thus allowing increase correlation to the biological parameters (cell proliferation index).

• The static total body FLT-PET/CT imaging data will be used to identify tumor masses. Each of the metastasis will be treated individually. Standardized uptake values (SUV) will be used to assess the FLT PET uptake. SUVmax, SUVmean and SUVtotal will be recorded and analyzed. In addition, the spatial distribution of the change will be determined, in case of heterogeneous tumor uptake distribution.

F. NaF PET/CT Imaging

One of the best PET imaging agents for detection of bone metastasis is ^{18}F-Sodium fluoride (NaF). NaF uptake is characterized by high and rapid bone uptake accompanied by very rapid blood clearance, which results in a high bone-to-background ratio in a short time. The retention and clearance mechanisms of NaF are well understood (16). NaF retention is a 2-phase process (17). NaF is rapidly taken up by the bone, which leads to rapid clearance from the plasma. It is cleared from plasma in a biexponential manner -- the first phase has a half-life of 0.4 h, and the second phase has a half-life of 2.6 h (18). Retention of NaF in the bone is almost perfect -- essentially all the NaF that is delivered to bone by the blood is retained in the bone (19), illustrated by the fact that one hour after administration of NaF, only about 10% of the injected dose remains in the blood (20). In the first phase, the $^{18}F_2$ ion exchanges for an OH_2 ion on the surface of the hydroxyapatite matrix of bone. In the second phase, the $^{18}F_2$ ion migrates into the crystalline matrix of bone, where it is retained until the bone is remodeled; therefore its retention directly reflects active bone metabolism. As a result, its accumulation is proportional to both blood flow and osteoblastic (metabolic) activity; thus, it represents an excellent PET tracer for imaging of bone metastasis (21, 22). NaF PET has been validated clinically as a diagnostic imaging modality. In this context, NaF PET has been shown to be superior to both FDG PET and ^{99m}Tc-MDP SPECT in detection of bone metastases (16, 25). NaF is a FDA approved imaging tracer for use with PET/CT. We hypothesize that NaF PET/CT will be able to detect early changes in number of bone metastases, as well as changes in osteoblastic activity that may reflect inflammatory effects. Three NaF PET/CT will be obtained in 6 - 10 patients with known osteoblastic metastases (baseline, at week 6 (+/- 1 wk), and again at week 12 (+/- 1 wk)).

Sodium Fluoride F 18 (NaF) Injection

NaF is commercially available. Ordering, Dosimetry, Quality Assurance, Quality Control, and Storage will be per usual protocols.

"Sodium Fluoride F 18 Injection is provided as a ready-to-use, isotonic, sterile, pyrogen-free, clear and colorless solution. Each mL of the solution contains 10–400 mCi fluoride ^{18}F at the end of synthesis (EOS) reference time in 0.9% aqueous sodium chloride. Fluoride ^{18}F ions decay by positron emission with a half-life of 109.7 minutes. The drug product complies with the United States Pharmacopeia (USP) monograph for Sodium Fluoride F-18 Injection, and is manufactured following procedures that conform to the radiopharmaceuticals for PET compounding standards (USP <823>)" (Investigator's Brochure. Edition 1, July 2008. p. 10). Following the standard dose, patients will be injected with 5 mCi of NaF.

For full details regarding Physical, Chemical, and Pharmaceutical Properties and Formulation; Nonclinical Studies; and Previous Human Experience, please refer to the commercial package insert for Sodium Fluoride F-18 injection.

Imaging Procedures:

To ensure uniformity, the established UW Nuclear Medicine image acquisition and reconstruction procedures for NaF PET/CT will be utilized. Please refer to the UW Nuclear Medicine Standard Operating Procedure for image acquisition and other image-related details.

Image Analysis:

Image analysis will be performed in Nuclear Medicine and led by Dr. Perlman (co-investigator). Changes in number of lesions, SUVtotal/SUVmax/SUVmean will be calculated for individual lesions using established PERCIST criteria [81].

11. Statistical Considerations

A. Overview

This is a pilot trial to evaluate the immunological and clinical effect of combining a DNA vaccine encoding PAP (and using a rhGM-CSF adjuvant) with a monoclonal antibody (pembrolizumab) specific for PD-1. The rationale for conducting this trial is summarized in Section 2 (E).

B. Objectives

The primary objectives are to:

1. To evaluate the safety of pembrolizumab in combination with pTVG-HP in patients with castration-resistant, metastatic prostate cancer
2. To determine the 6-month progression-free survival and median time to radiographic progression in patients with castration-resistant metastatic prostate cancer treated with pembrolizumab in combination with pTVG-HP
3. To evaluate the anti-tumor response rates (objective response rate and PSA response rate, using PCWG2 criteria) in patients with castration-resistant metastatic prostate cancer treated with pembrolizumab in combination with pTVG-HP

The secondary objectives are to:

1. To determine whether either treatment sequence, or PAP-specific immune response, is associated with prolonged (6-month) radiographic progression-free survival
2. To evaluate effects of schedule (concurrent versus delayed administration of pembrolizumab) on the magnitude of PAP-specific T-cell responses, PD-1 expression on circulating T cells, and PD-L1 expression on circulating epithelial cells (CEC) and on tumor biopsies
3. To determine the median time to radiographic progression using concurrent administration schedules

The exploratory objectives are to:

1. To evaluate effects of treatment on the number of circulating tumor cells
2. To evaluate PAP-specific antibody responses following treatment with pembrolizumab and pTVG-HP DNA vaccine
3. To determine whether treatment with either sequence elicits immunologic antigen spread to other prostate associated antigens

4. To determine whether pre-existing or vaccine-induced PD-L1 expression on CEC or tumor biopsies is predictive of objective clinical response
5. To determine whether treatment elicits expression of other regulatory molecules on tumor-specific T cells (e.g. TIM3, BTLA, and LAG3) or tumor cells (e.g. HVEM, phosphatidyl serine, PD-L2)
6. To determine whether PD-1-regulated antigen-specific T-cells identified by *trans vivo* DTH testing can identify patients who develop objective clinical responses with PD-1 blockade therapy in combination with pTVG-HP.
7. To determine whether changes in lymph nodes and soft tissue lesions are observed by FLT PET/CT after treatment with vaccine with or without pembrolizumab
8. To determine if PD-1 inhibitor therapy in combination with pTVG-HP will change number and activity (SUV) in osteoblastic metastases as measured by NaF PET/CT.

C. 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: pTVG-HP (100 µg) with rhGM-CSF (208 µg) administered intradermally (i.d.) biweekly 6 times beginning at day 1 pembrolizumab 2 mg/kg, administered intravenously every 3 weeks 4 times, beginning at day 1

Arm 2: pTVG-HP (100 µg) with rhGM-CSF (208 µg) administered intradermally (i.d.) biweekly 6 times beginning at day 1 pembrolizumab 2 mg/kg, administered intravenously biweekly 6 times, the first dose administered two weeks after the last pTVG-HP vaccination

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

During accrual to these 2 study arms, clinical observations suggested it could be advantageous to further investigate the combined treatment arm (Arm 2), but over a longer period of time. Specifically, objective responses and PSA declines were observed during the 3 months of treatment, but progression occurred after discontinuing therapy. Hence a third, non-randomized arm was proposed after 12 evaluable subjects were accrued to Arms 1 and 2 to further investigate concurrent treatment, and specifically timed with vaccine administration.

Extended treatment Arm 3:

pTVG-HP (100 µg) with rhGM-CSF (208 µg) administered intradermally (i.d.) every 3 weeks, for a maximum of 16 doses

Pembrolizumab 2 mg/kg, with a maximum dose of 200 mg, administered intravenously every 3 weeks, for a maximum of 16 doses, beginning on day 1 after the first pTVG-HP vaccination

During accrual to Arm 3, laboratory observations suggested it may be preferable to use vaccine at every 2-week intervals rather than every 3-week intervals. In order to evaluate this schedule with concurrent pembrolizumab, but ideally reduce the potential toxicity from pembrolizumab by

decreasing the frequency of administration, a separate extended treatment arm (Arm 4) is proposed after completion of accrual to Arm 3.

Extended treatment Arm 4:

pTVG-HP (100 µg) with rhGM-CSF (208 µg) administered intradermally (i.d.) every 2 weeks, for a maximum of 24 doses

Pembrolizumab 2 mg/kg, with a maximum dose of 200 mg, administered intravenously every 4 weeks, for a maximum of 12 doses, beginning on day 1 after the first pTVG-HP vaccination,

D. Randomization and Stratification

Eligible patients will be randomized to the first two study arms with an allocation ratio of 1:1. The randomization will be based on permutated blocks of size 2-4. Treatment arm assignment will be made by biostatics/investigational pharmacy after a patient has been registered.

E. Sample Size and Power Calculation

The expected 6-months progression-free survival rate in Arm 2 (pembrolizumab is administered sequentially after pTVG-HP) is 25%. It is hypothesized that the treatment of pTVG-HP, when administered concurrently with pembrolizumab (Arm 1), will result in a substantial increase in the 6-month progression-free survival rate. Specifically, it is anticipated that the 6-month progression-free survival rate in Arm 1 will be at least 65-75%. The following table shows the required sample sizes for detecting various differences in the 6-month progression-free survival rates between the two study arms, assuming 80% power and a one-sided 0.10 significance level. Since this is a pilot study, a less stringent significance level of 0.10 rather the traditional 0.05 level will be used for the primary efficacy evaluation.

	6-month progression free survival rate Arm 1			
6-month progression free survival rate Arm 2	0.65	0.70	0.75	0.80
0.20	15	11	10	9
0.25	17	15	11	10

A sample size of 15 patients per arm is proposed. This sample size will provide 80% power to detect an anticipated absolute difference of at least 45% (e.g., 25% vs. 70% or 20% vs. 65%) in the 6-months progression free survival rates between the two arms. These calculations are based on Fisher's exact test. Furthermore, the proposed sample size of 15 patients per arm is also sufficient to provide accuracy in estimating toxicity rates. Specifically, the toxicity rates (i.e., of grade ≥ 2, grade ≥ 3, grade 4 toxicities) for each arm will be estimated with a standard error of less than 15% and the 95% confidence interval of the toxicity rates will be no wider than 45%. The probability of observing at least one event of a serious adverse event within a study arm is >95% if the adverse event rate is 20%. Analogously, the probability of observing at least one event of serious adverse event is at least 79% if the adverse event rate is only 10%.

In order to account for unevaluable (for efficacy evaluation) patients, 32 eligible patients (16 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 32 patients will be accrued within 18 months.

A total sample size of 20 evaluable patients is proposed for the extended treatment Arm 3 to evaluate the median time to radiographic progression. The proposed concurrent administration schedule will be considered as ineffective if it results in a median time to radiographic progression of 3 months or less. Hence, the null hypothesis that the median time to radiographic progression is at most 3 months will be tested against the alternative hypothesis that the median is greater than 3 months. A median time to radiographic progression of 6 months or more, on the other hand, will be considered as promising to warrant further clinical evaluation of the proposed concurrent administration schedule. With the proposed sample size of 20 evaluable patients for the extended treatment Arm 3, a median time to radiographic progression of 6 months will be detected with 90% power at the one-sided 0.05 significance level, assuming an accrual period of 12 months with a uniform accrual pattern and a minimum follow-up period of 12 months. Furthermore, a total sample size of 20 evaluable patients is also proposed for the extended treatment Arm 4, to evaluate the median time to radiographic progression for this study arm. The null hypothesis that the median time to radiographic progression is at most 3 months will be tested against the alternative hypothesis that the median is greater than 3 months. With the proposed sample size of 20 evaluable patients for the extended treatment Arm 4, a median time to radiographic progression of 6 months will be detected with 90% power at the one-sided 0.05 significance level, assuming an accrual period of 12 months with a uniform accrual pattern and a minimum follow-up period of 12 months. Furthermore, the proposed sample size of 20 patients for both Arm 3 and Arm 4 is adequate to estimate toxicity rates within each arm with sufficient accuracy. Specifically, toxicity rates will be estimated with a standard error of less than 12% and the corresponding two-sided 95% confidence intervals will be no wider than 40%.

NaF PET/CT will be obtained in 6 - 10 patients with known osteoblastic metastases at baseline, week 6 and week 12. This sample size will provide at least 80% power to detect moderate to large effect sizes ranging from 1.0-2.0 for the differences in SUV metrics between time points at the two-sided 0.05 significance level.

F. Analysis

F.1. General

This section outlines the statistical analysis strategy and procedures for the study. Prior to the analysis of the final study data, a detailed Statistical Analysis Plan (SAP) will be written describing all analyses that will be performed.

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 9.3 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. A two-sided test at a significance level of 0.05 will be used for all comparisons, unless noted otherwise.

F.2. Analysis Populations

The ITT population consists of all patients who are randomized. The per-protocol population consists of all evaluable patients, i.e., patients who have been treated according to protocol (all inclusion/exclusion criteria are satisfied, and adequate treatment compliance (complete treatment to at least day 43). The safety population consists of all subjects who received at least one dose of pTVG HP alone or in combination with pembrolizumab. All primary, secondary and correlative outcomes will be analyzed for both the IIT and per-protocol population. The IIT population will be the primary analysis population. Safety will be evaluated using the safety population.

F.3. Baseline Comparability

All measurements (variables) collected at baseline will be summarized and compared between study arms. These include demographic variables, ECOG PS, lab parameters (CBC, chemistry panel, serum testosterone, PSA and PAP) medical history information and CTCs. 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.

F.4. Analysis of Primary Endpoints

F.4.1. Analysis of Toxicity Rates and Serum Chemistry Parameters

Subjects will be evaluated at each visit by a review of systems based on the most recent version of the NCI common toxicity criteria. Furthermore, serum chemistries, including renal function tests, blood counts, liver function tests, and serum amylase, will be evaluated at 6-12 week intervals. Toxicities will be summarized by type and severity in tabular format. Toxicity rates (grade 2, grade 3, grade 4, grade ≥ 2, grade ≥ 3, etc.) will be calculated for each study arm and reported along the corresponding 95% confidence intervals. The 95% confidence intervals will be constructed using the Wilson score method. Fisher's exact test will be used to compare toxicity rates between study arms. Serum chemistry and amylase parameters will be summarized using standard descriptive statistics. Changes from the baseline assessment will be evaluated using a paired t-test.

F.4.2. 6-Month Progression Free Survival Rate and Median Time to Radiographic Progression

The 6-month progression-free survival rate, along with the corresponding two-sided 95% confidence intervals, will be reported for each arm, and for the overall combined study. The Wilson

score method will be used to construct the confidence intervals. Fisher's exact test will be used to compare the 6-month progression-free survival rates between study arms.

Median time to radiographic progression will be estimated for each arm using the Kaplan-Meier method. The median time to radiographic progression will be reported for each of the four arms along with the corresponding 95% confidence intervals which will be constructed using the Brookmeyer-Crowley method. The log-rank test will be used to perform the comparison of time to radiographic progression between Arm 1 versus Arm 2. A one-sided 0.10 significance level will be used to conduct the comparison of the 6-month progression-free survival rate and time to radiographic progression between study arms. Within Arm 3 and Arm 4, the null hypothesis that the median time to radiographic progression is at most 3 months will be tested against the alternative hypothesis that the median time to radiographic progression is greater than 3 months.

In order to evaluate the 6-month progression-free rate and median time to radiographic progression 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.

F.4.3. Objective Response Rate and PSA Response Rate

The objective response rate and PSA response rate will be calculated for each study arm and for both arms combined along with the corresponding 95% confidence intervals. The Wilson score method will be used to construct the confidence intervals. Fisher's exact test will be used to compare the objective response and PSA response rates between study arms. Duration of response will be analyzed using the Kaplan-Meier method.

F.5. Analysis of Secondary Endpoints

F.5.1. PAP-Specific Immune Response

The number and frequencies of PAP-specific immune responses will be summarized in tabular format for each study arm and both study arms combined. A log-linear model will be used to evaluate whether PAP-specific immune response predicts the 6-months progression-free survival rate. The interaction between treatment (pembrolizumab administered sequentially versus delayed) and PAP-specific immune response will be included in the model. Analogously, a Cox proportional hazard regression model will be used to examine the association between PAP-specific immune response and the time to radiographic progression.

F.5.2. PAP-Specific T-Cell Response, PD-1 Expression, PD-L1 Expression

PD-1 and PD-L1 expression levels will be summarized in terms of means, standard deviations and ranges for each study arm separately and for both arms combined. The number and frequencies of PAP-specific T-cell responses will be summarized in tabular format. Fisher's exact test will be used to compare the PAP-specific T-cell response rates between study arms. A linear regression model or a negative binomial regression model will be utilized to evaluate the effects of schedule (concurrent versus delayed administration of pembrolizumab) on PD-1 expression on the number of circulating T cells, and PD-L1 expression on the number of circulating epithelial cells. The

choice of the model will be dependent on the distribution of the outcome variables (number of circulating T-cells and number of circulating epithelial cells).

F.6. Exploratory Analyses

F.6.1. Evaluation of Treatment Effects on the Number of Circulating Tumor Cells

The number of circulating tumor cells will be summarized in terms of means and standard deviations. Within each arm, and for both arms combined, changes in the number of circulating tumor cells from the pre-treatment (date of screening/randomization) to the post-treatment assessments (day 43, day 85, day 169, month 9, and off-study) assessments will be evaluated using a paired t-test. A two-sample t-test will be used to compare the number of circulating tumor cells at each time point, and the changes in the number of circulating tumor cells from the pre-treatment assessment, between study arms. In order to evaluate longitudinal changes in the number of circulating tumor cells, linear mixed effects modeling with subject specific random effects will be conducted.

F.6.2. Evaluating of Treatment Effects on PAP-specific Antibody Responses

The number and frequency of PAP-specific antibody responses will be summarized in tabular format, stratified by study arm and for both arms combined. A generalized linear mixed effects model with subject specific random effects will be used to evaluate the treatment effect of the proposed pTVG-HP DNA vaccine and pembrolizumab (sequentially versus concurrently) combination on PAP-specific antibody response.

F.6.3. Evaluation whether Treatment with either Sequence 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. Furthermore, linear regression analysis will be conducted to evaluate whether treatment with either sequence (pembrolizumab administered sequentially or concurrently) elicits immunologic antigen spread to other prostate associated antigens.

F.6.4. Evaluation of Pre-Existing or Vaccine Induced PD-L1 Expression on CEC or Tumor Biopsies as a Predictor for Clinical Response

Logistic regression analysis will be conducted to examine whether pre-existing or vaccine induced PD-L1 expression on CEC or tumor biopsies predict clinical response. This analysis will be conducted for both arms combined. The results will be summarized in terms of odds ratios along with the corresponding 95% confidence intervals.

F.6.5. Evaluation whether Treatment with either Sequence Elicits other Regulatory Molecules on Tumor-Specific T cells or Tumor Cells

Linear regression analysis will be conducted to determine whether treatment with either sequence elicits other regulatory molecules on tumor-specific T cells or tumor cells.

F.6.6. Evaluation whether PD-1-Regulated Antigen-specific T-cells Identified by *trans vivo* DTH Testing Can Identify Patients who Develop Objective Clinical Responses with PD-1 Blockade Therapy in Combination with pTVG-HP

The number and frequencies of PD-1-regulated antigen specific T-cell responses will be summarized in tabular format. Logistic regression analysis will be conducted to examine whether PD-1 regulated antigen-specific T-cells predict clinical response. The sensitivity, specificity, negative predictive value and positive predictive value of PD-1 regulated antigen specific T-cell responses for predicting clinical response will be calculated and reported along with the corresponding 95% confidence intervals.

F.6.7. Evaluation of changes in FLT uptake by FLT PET/CT after Treatment with pTVG-HP with or without Pembrolizumab

Standard PET parameters, including SUV_{total}, SUV_{max}, SUV_{mean}, and the number of lesions, in terms of soft tissue lesions and major lymph node groups, will be obtained. Parameters will be summarized in terms of means, standard deviations and ranges. Within each arm and for both arms combined, changes in parameters will be evaluated using a paired t-test (for SUV) or nonparametric Wilcoxon signed rank test (number of lesions). Since the distribution of SUV values is generally skewed, a log-transformation will be utilized before conducting the comparisons. A two-sample t-test or nonparametric Wilcoxon rank sum test will be used to compare changes in parameters between study arms.

F.6.8 Evaluation of changes in NaF uptake by NaF PET/CT after treatment with pTVG-HP with or without Pembrolizumab

Standard PET parameters, including SUV_{total}, SUV_{max}, SUV_{mean}, and the number of lesions, in terms of osteoblastic lesions, will be obtained using established PERCIST criteria. To ensure uniformity, the established UW Nuclear Medicine image acquisition and reconstruction procedures for NaF PET/CT will be utilized. Please refer to the UW Nuclear Medicine Standard Operating Procedure for image acquisition and other image-related details. Image analysis will be performed in Nuclear Medicine and led by Dr. Perlman (co-investigator).

12. 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, and specimens may be listed with unique codes for individual subjects and obtained at the different time points. Lymphocytes collected will be stored in liquid nitrogen, and sera will be aliquoted and stored at –80°C in the research laboratory of the protocol sponsor (Dr. McNeel) for immune analyses.

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 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.

13. Data and Safety Monitoring Plan

A. Definitions

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

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

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

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 for events that are believed to be at least possibly related to study treatment.

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

B. Oversight and Monitoring Plan

The UWCCC Data and Safety Monitoring Committee (DSMC) is responsible for the regular review and monitoring of all ongoing clinical research in the UWCCC. A summary of DSMC activities are as follows:

- Reviews all clinical trials conducted at the UWCCC for subject safety, protocol compliance, and data integrity.
- Reviews all Serious Adverse Events (SAE) requiring expedited reporting, as defined in the protocol, for all clinical trials conducted at the UWCCC, and studies conducted at external sites for which UWCCC acts as an oversight body.
- Reviews all reports generated through the UWCCC DSMS elements (Internal Audits, Quality Assurance Reviews, Response Reviews, Compliance Reviews, and Protocol Summary Reports) described in Section II of this document.
- Notifies the Local Principal Investigator of DSMC decisions and, if applicable, any requirements for corrective action related to data or safety issues.
- Notifies the CRC of DSMC decisions and any correspondence from the DSMC to the Local Principal Investigator.
- Works in conjunction with the UW Health Sciences IRB in the review of relevant safety information as well as protocol deviations, non-compliance, and unanticipated problems reported by the UWCCC research staff.
- Ensures that notification is of SAEs requiring expedited reporting is provided to external sites participating in multi-institutional clinical trials coordinated by the UWCCC.

C. Monitoring and Reporting Guidelines

Phase I/II and Phase II Trials

UWCCC quality assurance and monitoring activities are determined by study sponsorship and risk level of the protocol as determined by the UWCCC protocol review and monitoring committee (PRMC). All protocols (including Intervention Trials, Non-Intervention Trials, Behavioral and Nutritional Studies, and trials conducted under a Training Grant) are evaluated by the PRMC at the time of committee review. UWCCC monitoring requirements for trials without an acceptable external DSMB are as follows:

a) Intensive Monitoring

Protocols subject to intensive monitoring generally include UW Institutional Phase I and Institutional Trials of any phase involving recombinant DNA/gene transfer. These protocols undergo continuous review of data and subject safety at weekly Phase I/Disease Oriented Working Group (DOWG) meetings where the results of each subject's treatment are discussed and the discussion is documented in the DOWG meeting minutes. The discussion includes the number of subjects enrolled, significant toxicities, dose adjustments, and responses observed. Protocol Summary Reports are submitted on a quarterly basis by the study team for review by the DSMC**.**

D. Review and Oversight Requirements

Serious Adverse Event – Reported Within 24 Hours

Serious Adverse Events requiring reporting within 24 hours (as described 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. The OnCore SAE Details Report must be submitted along with other report materials as appropriate (NCI AdEERS form or FDA Medwatch Form #3500 and/or any other documentation available at that time of initial reporting). The DSMC Chair will review the information and determine if immediate action is required. Within 10 working days, all available subsequent SAE documentation must be submitted electronically along with a 24 hour follow-up SAE Details Report to saenotify@uwcarbone.wisc.edu. All information is entered and tracked in the UWCCC database.

The Protocol Principal Investigator notifies all investigators involved with the study at the UWCCC, the IRB, the sponsor, and the funding agency and provides documentation of these notifications to the DSMC.

If the SAE occurs on a clinical trial in which the UW PI serves as the sponsor-investigator, the PI reviews the event to determine whether the SAE requires reporting to the FDA and other participating investigators.

For a multiple-institutional clinical trial the Protocol Principal Investigator (Investigator sponsor) is responsible for ensuring SAEs are reported to the FDA as well as to all participating investigators

See Section E.1 for detailed instructions on SAE reporting.

Serious Adverse Event – Reported within 10 Days

Serious Adverse Events requiring reporting within 10 days (as described in the protocol) must also be reported to the Data and Safety Monitoring Committee (DSMC) Chair via an email to saenotify@uwcarbone.wisc.edu. The OnCore SAE Details Report must be submitted along with

other report materials as appropriate (NCI AdEERS form or FDA Medwatch Form #3500 and/or any other documentation available at that time of initial reporting). The DSMC Chair will review the information and determine if further action is required. All information is entered and tracked in the UWCCC database.

The Protocol Principal Investigator notifies all investigators involved with the study at the UWCCC, the IRB, the sponsor, and the funding agency and provides documentation of these notifications to the DSMC.

If the SAE occurs on a clinical trial in which the UW PI serves as the sponsor-investigator, the PI reviews the event to determine whether the SAE requires reporting to the FDA and other participating investigators.

For a multiple-institutional clinical trial the Protocol PI (Investigator sponsor) is responsible for ensuring SAEs are reported to the FDA as well as to all participating investigators.

See Section E.2 for detailed instructions on SAE reporting.

Sponsor-Investigator Responsibilities for SAE Review

In the event the UWCCC Principal Investigator is acting as the Sponsor-Investigator (i.e., the PI holds the IND), the PI assumes responsibilities of the study sponsor in accordance with FDA 21 CFR 312.32. In this capacity, the UWCCC PI reviews all reports of serious adverse events occurring on the study at the UWCCC and participating external sites and makes a determination of 1) suspectedness (i.e., whether there is a reasonable possibility that the drug caused the AE); and 2) unexpectedness (the event is not listed in the Investigator's Brochure or is not listed at the specificity or severity that has been observed) in the context of this study. SAE with suspected causality to study drug and deemed unexpected are reported as IND Safety Reports by the UWCCC PI to the FDA, all participating investigators on the study, and the external global sponsor (if applicable) within 15 calendar days. All fatal or life-threatening SAE that are unexpected and have suspected causality to the study drug will be reported by the UWCCC PI to the FDA, all participating investigators on the study, and the external global sponsor (if applicable) within 7 calendar days.

Study Progress Review

Protocol Summary Reports (PSR) are required to be submitted to the DSMC in the timeframe determined by the risk level of the study (quarterly; semi-annually; or annually). The PSR provides a cumulative report of SAEs, as well as instances of non-compliance, protocol deviations, and unanticipated problems, toxicities and responses that have occurred on the protocol in the timeframe specified. PSRs for those protocols scheduled for review are reviewed at each DSMC meeting. Cumulative reports will also be due annually to FDA (pertaining to this IND), and to the NIH OBA (pertaining to this OBA reference number).

Protocol Summary Reports enable DSMC committee members to assess whether significant benefits or risks are occurring that would warrant study suspension or closure. This information is evaluated by the DSMC in conjunction with other reports of quality assurance activities (e.g., reports from Internal Audits, Quality Assurance Reviews, etc.) occurring since the prior review of the protocol by the DSMC. Additionally, the DSMC requires the study team to submit external

DSMB or DSMC reports, external monitoring findings for industry-sponsored studies, and any other pertinent 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 and ensure the appropriate action is taken for the protocol (e.g., suspension or closure). The DSMC ensures that the PI reports any temporary or permanent suspension of a clinical trial to the sponsor (e.g., NCI Program Director, Industry Sponsor Medical Monitor, Cooperative Group Study Chair, etc.) and other appropriate agencies. DSMC findings and requirements for follow-up action are submitted to the CRC.

E. 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.

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

a. To the FDA and NIH OBA*:*

Report the SAE to the FDA using the current FDA Med Watch form (cuttently available at http://www.fda.gov/downloads/Safety/MedWatch/HowToReport/DownloadForms/UCM082728.pdf) or another comparable form such as the National Institute of Health Office of Biotechnology Activities form listed below.
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 current reporting form (currently located at http://oba.od.nih.gov/oba/rac/Adverse_Event_Template.pdf). 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 (30 days after the last treatment administration) must be reported to Madison Vaccines Inc. within 24 hours of being made aware of the SAE. Notification can be made via phone or telefacsimile a copy of the FDA Med Watch form to:

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

Fax: To be provided

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

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

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

a. To the FDA and NIH OBA

Report the SAE using the current FDA Med Watch form (currently available at http://www.fda.gov/downloads/Safety/MedWatch/HowToReport/DownloadForms/UCM082728.pdf) or another comparable form such as the National Institute of Health Office of Biotechnology Activities reporting form listed below.

Print the completed Med Watch form and fax it to (800) 332-0178.

Report the SAE to the National Institute of Health Office of Biotechnology Activities using their current reporting form (currently located at http://oba.od.nih.gov/oba/rac/Adverse_Event_Template.pdf). 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 (30 days after the last treatment administration) must be reported to Madison Vaccines Inc. within 24

hours of being made aware of the SAE. Notification can be made via phone or telefacsimile a copy of the FDA Med Watch form to:

Madison Vaccines Inc.
Attn: Safety Manager
Phone: (608) 467-5269
Fax: To be provided

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

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 CRF Part 312)

NOTE: Investigators MUST immediately report to the PI and any other parties outlined in the protocol 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).**

ALL SERIOUS adverse events that meet the above criteria* MUST be immediately reported to the UWCCC within the timeframes detailed in the table below:

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

Expedited AE reporting timelines are defined as:

- **24-Hour; 5 Calendar Days** – The AE must initially be reported within 24 hours of learning of the AE, followed by a complete expedited report within 5 calendar days of the initial 24-hour report.
- **10 Calendar Days** – A complete expedited report on the AE must be submitted within 10 calendar days of learning of the AE

[1] Serious adverse events that occur more than 30 days after the last administration of investigational agent/intervention (or 90 days for pembrolizumab) 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 3, 4, and 5 AEs

Expedited 10 calendar day reports for:

- Grade 2 AEs resulting in hospitalization or prolongation of hospitalization

[2] For studies using PET or SPECT IND agents, the AE reporting period is limited to 10 radioactive half-lives, rounded UP to the nearest whole day, after the agent/intervention was last administered. Footnote "1" above applies after this reporting period.

14. 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 remain for 60 minutes following each treatment to evaluate the skin site of treatment and for any immediate allergic events. Subjects will also be asked to keep a record of unusual site or other reactions for two days after immunization. To date, in studies using pTVG-HP only without anti-PD-1, allergic reactions have been rare (grade 3 angioedema observed in 1 of over 50 treated subjects, <5% individuals).

With respect to eliciting unwanted immunological reactions, PAP, a protein whose expression is essentially restricted to the prostate, does share homology with other tissue phosphatases, notably lysosomal acid phosphatase (LAP), most prevalent in pancreatic tissue. No such toxicity has been reported in clinical trials targeting PAP by means of a dendritic cell vaccine, and the generation of

PAP-specific CTL in rat models similarly did not elicit detectable evidence of autoimmune disease in non-prostate tissues. Moreover, this was not seen in previous clinical trials with this DNA vaccine with over 50 subjects. This could theoretically be potentiated, however, with the anti-PD-1 therapy. Consequently, in order to further evaluate this potential toxicity in humans, subjects will be evaluated at each visit by a review of systems based on the NCI common toxicity criteria. In addition, subjects will be examined and serum chemistries, including renal function tests, blood counts, liver function tests, and serum amylase, will be evaluated at 6-12 week intervals, as outlined above. The serum amylase will be used as a serum marker to monitor for evidence of subclinical pancreatic inflammation. Again, while no autoimmune treatment-limiting adverse events have been observed to date in other trials, it is conceivable that by using a checkpoint inhibitor such as anti-PD-1 with an immunization approach targeting the PAP antigen that more autoimmune events could be observed than with either agent alone. For this reason, these blood tests will be continued to be monitored in this trial.

While there has been a suggestion that the plasmid DNA could insert into the host chromosomal DNA, this has not been documented in any other study, and several laboratory investigations have suggested the possibility of this occurring is less than the spontaneous mutation rate, and therefore not a real risk [79]. 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 [63]. 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 treatment with pembrolizumab

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

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

A.4. From blood tests

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

A.5. 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.6. From tetanus immunization

A tetanus booster vaccine (commercially available vaccine delivered as an intramuscular injection) will be delivered prior to receiving the DNA vaccine 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.7. From tissue biopsies

Standard laboratory tests will be done to assess bleeding risk before the procedure is done. The sample will be taken from an area of reduced risk of complications. Possible side effects of a biopsy include bleeding, infection, bruising, pain or discomfort at the biopsy site and possible side effects from the local anesthetic (pain or bruising at the site where anesthetic is given). The main discomfort associated with this test is pain when the bone or tissue is being withdrawn. In order to make the procedure more comfortable, subjects will get a local anesthetic to numb the area. A mild sedative may also be given. While sedated, subjects will be able to respond to commands. It is recommended that subjects be observed for 2-4 hours after the biopsy.

CT scans for biopsies: At the time of biopsy, a CT scan may be done to determine where the biopsy will be done. After the biopsy, a CT scan may be done over the biopsy site to look for any immediate complications. These scans will be performed in addition to the scans as part of normal cancer care. The scans involve exposure to radiation in the form of x-rays. The level of radiation used is kept to a minimum to prevent damage to body cells. Under some rare circumstances of prolonged, high-dose exposure, x-rays can cause adverse health effects, such as skin reddening (erythema), skin tissue injury, hair loss, and cataracts. The amount of radiation from the additional scans in this study is not known to be associated with any serious health risks. The exposure related to this CT scan equals approximately one year of standard environmental background radiation.

A.8. From FLT PET/CT and NaF PET/CT

The FLT PET/CT and NaF PET/CT scans will expose subjects to a small amount of radiation, approximately equal to one bone scan.. The amount of radiation subjects will receive from this study is within the limits of the federal government's rules and regulations and is thought to be safe. Risks generally associated with insertion of a catheter (for the administration of the tracer) include pain, bruising, lightheadedness, and on rare occasions, infection.

Reproductive risks of the FLT PET/CT and NaF PET/CT scans: The effects of the FLT and NaF tracers on the developing fetus are unknown. Subjects should discuss the use of reliable methods of birth control like condoms and spermicidal foam or sexual abstinence with their study doctor(s). Procreative sex should be avoided prior to study entry and for the duration of study participation. If a subject's partner is of child-bearing potential, the subject must be surgically sterile or use an acceptable method of birth control. If a subject's partner becomes pregnant while the subject is participating in this study, she should inform her treating physician immediately.

A.9. Confidentiality

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

B. Potential Benefits

No benefits are guaranteed. It is hoped that individual patients treated with the DNA vaccine either together with, or in sequence with, pembrolizumab will derive a clinical response as suggested by an objective disease response, decline in serum PSA, and/or a prolonged 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, and could suggest means by which anti-PD-1 therapies could be used in prostate cancer, as these agents have demonstrated substantial benefit for patients with other types of malignancies other than prostate cancer. Thus, it is hoped that study participants and future patients will benefit from research participation in the current study.

15. 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 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 internal database. 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 the database. The system will prompt the user to the forms that are required based upon the patient's enrollment and treatment dates.

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

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

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

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

C. Description of Procedures to Maintain Confidentiality of Research Specimens

All specimens obtained for the immunological evaluation of this trial (blood specimens) will be delivered to the laboratory of Dr. McNeel. Specimens received by Dr. McNeel's lab will only be handled by laboratory personnel who have undergone HIPAA training and annual UWHC-mandated blood-borne pathogen safety training. Receipt of specimens will be entered into a database that will provide a unique code for each specimen. All stored specimens (sera, peripheral blood mononuclear cells) will be labeled with this unique code and the date of preparation. Thus, all patient identifiers will be removed from the final stored samples, and any data generated will contain only the unique code as identifier. A database will be maintained to link individual coded specimens with an individual subject (name, hospital medical record number, date/time point) in

order to be able to compare information from samples obtained at different time points from a specific individual, and ultimately for transfer of research data to the clinical trials database, if necessary. This research database will only be available to the study investigators, not other laboratory personnel, to maintain confidentiality. Subject sera will be maintained in Dr. McNeel's laboratory, stored in aliquots at –20°C to –80°C. Peripheral blood mononuclear cells will be stored in Dr. McNeel's laboratory, stored in aliquots in liquid nitrogen. If warranted, samples may be sent to an external institution or a contract research organization for the purpose of collaboration and /or analysis.

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

D. Modifications of Protocol and Deviations from Protocol

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

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

E. Withdrawal from Study Protocol

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

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

16. Roles and Responsibilities of Specific Study Personnel at UWCCC

Protocol sponsor:

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

UW 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 serve as principal investigator and in this capacity will be both a clinical co-investigator for this study, involved in the recruitment, treatment and review of individual treated subjects, and will also oversee all UW/IRB aspects related to direct patient care issues.

17. References:

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