Phase II Trial of pTVG-HP DNA Vaccine with or without pTVG-AR DNA Vaccine and Pembrolizumab in Patients with Castration-Resistant, Metastatic Prostate Cancer

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UW18037

INVESTIGATIONAL AGENT:

Referencing:

BB IND 18543 – Combination of pTVG-HP DNA encoding human prostatic acid phosphatase and pTVG-AR DNA encoding androgen receptor ligand-binding domain (AR-LBD); and Pembrolizumab (Keytruda ®)

STUDY SPONSOR / PROTOCOL PRINCIPAL INVESTIGATOR:

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STUDY SUPPORTER:

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Prostate Cancer Foundation (MSN 207843)
Prostate Cancer Foundation (MSN 196431)

STUDY SITE INFORMATION

Lead Study Site: University of Wisconsin Carbone Cancer Center
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Madison, WI 53705

Participating Study Site: Washington University Siteman Cancer Center
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Actual Start Date:	November 25, 2019
Actual End of Accrual Date:	May 24, 2024
Projected End of Study Date:	May 31, 2031

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SYNOPSIS:

Primary Objective:

To determine the 6-month progression-free survival rate in patients with metastatic, castration-resistant prostate cancer (mCRPC) treated with pembrolizumab in combination with one versus two DNA vaccines as T-cell activating agents

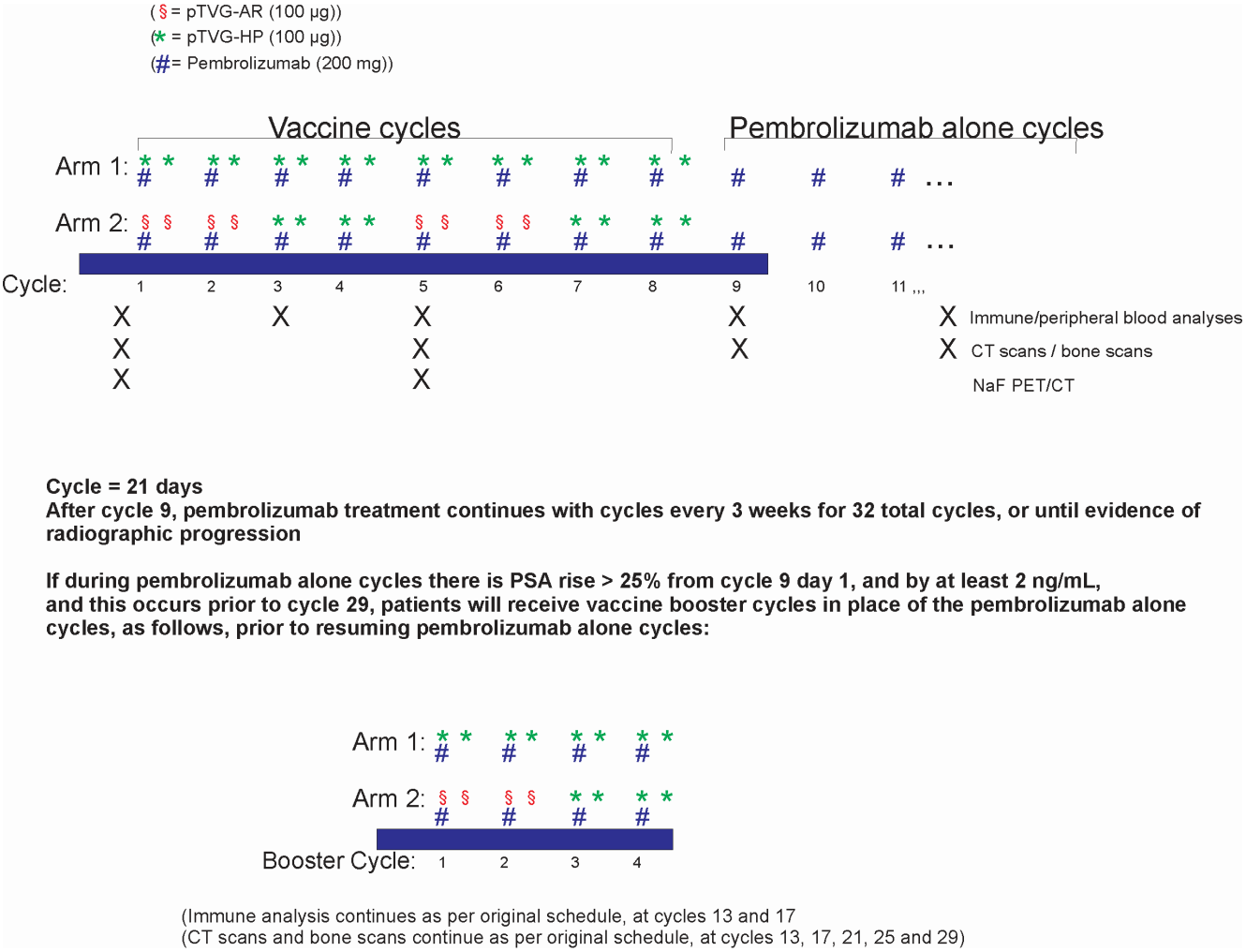
Secondary Objectives:

1. To determine the overall objective response rate (using Prostate Cancer Working Group 3 (PCWG3) criteria)
2. To determine the PSA response rate (decline $\geq$ 50%)
3. To determine the median radiographic progression-free survival rate
4. To determine median duration of PSA and/or objective response in responding individuals
5. To determine the overall survival
6. To determine whether the development of antigen-specific Th1 immunity elicited with treatment to either antigen (PAP or AR) is associated with PSA response
7. To evaluate the safety and tolerability of pTVG-HP DNA vaccine with or without pTVG-AR DNA vaccine and pembrolizumab

Exploratory Objectives:

1. To evaluate PAP-specific and AR-specific antibody responses following treatment with pembrolizumab and DNA vaccine(s)
2. To determine whether treatment with either sequence elicits immunologic antigen spread to other prostate associated antigens
3. To determine whether pre-existing antigen-specific immunity is predictive of immunological or objective clinical response
4. To determine, in a subset of patients, whether early changes in bone observed by NaF PET/CT are associated with immunological or objective clinical response, or pathological changes associated with immune-cell infiltration
5. To determine second PSA response rates in patients treated in follow-on courses
6. To determine whether treatment elicits changes in the gut microbiota composition
7. To determine whether changes in gut microbiota are associated with clinical response (PSA decline $\geq$ 50%)

Study Scheme:



Note: Patients may undergo more than one series of booster cycles.

Trial Summary:

Abbreviated Title	PAP vs. PAP + AR vaccines with pembrolizumab for treatment of mCRPC
Trial Phase	*II*
Clinical Indication	Prostate cancer, metastatic castration-resistant
Trial Type	Randomized, open-label, multi-institution
Type of control	N/A – one versus two vaccines
Route of administration	Pembrolizumab – intravenous DNA vaccines – intradermal
Trial Blinding	N/A
Treatment Groups	Arm 1: 100 µg pTVG-HP i.d. days 1, 8 + 200 mg pembrolizumab IV day 1 of 21-day cycles x 8 cycles Arm 2: 100 µg pTVG-AR i.d days 1, 8 + 200 mg pembrolizumab IV day 1 of q 21-day cycles, for cycles 1, 2, 5 and 6 alternating with 100 µg pTVG-HP i.d. days 1, 8 + 200 mg pembrolizumab IV day 1 in 21-day cycles, for cycles 3, 4, 7 and 8 Following cycle 8, subsequent 21-days cycles for both Arm 1 and Arm2: Pembrolizumab 200 mg IV day 1 of 21-day cycles. In the event of PSA rise (25% increase over cycle 9 day 1, minimum of 2 ng/ml), and no evidence of radiographic progression, patients will receive 4 additional vaccine booster cycles (subjects may have more than 1 round of booster cycles): Arm 1: 100 µg pTVG-HP i.d. days 1, 8 + 200 mg pembrolizumab IV day 1 of 21-day cycles x 4 cycles Arm 2: 100 µg pTVG-AR i.d days 1, 8 + 200 mg pembrolizumab IV day 1 of q 21-day cycles, for cycles 1 and 2 followed by 100 µg pTVG-HP i.d. days 1, 8 + 200 mg pembrolizumab IV day 1 in 21-day cycles, for cycles 3 and 4
Number of evaluable trial participants	60
Estimated enrollment period	*30 months*
Estimated duration of trial	*114 months (30 months of accrual + two years of treatment (32 cycles) and five years of follow up per patient)*
Duration of Participation	84 months per patient (including 5 years of telephone follow up)
Estimated average length of treatment per patient	12 months

Plan of Treatment:

ARM 1: (pTVG-HP + pembrolizumab)

Treatment Visit:	Screening		Vaccine Cycles (Cycles 1-8)[s]		Cycle 9 (21 +/- 3 days from cycle 8 day 1)	Pembro Alone Cycles[st]	Vaccine Booster Cycles[s,t,^]		EOT (21 +/- 3 days after cycle 32 day 1)	28-day follow up (28 +/- 7 days after off-treatment date, if prior to cycle 32)[y]	Long-term follow up
	Within 6 weeks of day 1	Within 4 weeks of day 1	Day 1 (21 +/- 3 days from previous cycle day 1)	Day 8 (7 +/- 2 days from day 1)	Cycle 10 + (21 +/- 3 days from previous cycle)		Day 1 (21 +/- 3 days from previous cycle day 1)	Day 8 (7 +/- 2 days from day 1)			
History and Physical Exam											
History	X										X
Consent	X										
Physical exam[x], ECOG PS		X	X		X	X	X		X	X	
Tox assessment[a]		X	X		X	X	X		X	X	
Lab Tests											
CBC	X		X		X	X	X		X	X	
Chemistry panel[f z]	X		X		X	X	X		X	X	
PT/INR, PTT[k b]		X	X								
Serum testosterone	X										
PSA and PAP	X		X		X	X	X		X	X	
Procedures											
CT abdomen/pelvis[r]	X[j]		X[g]		X[h]	X[o]	X[o]		X[jh]	X[i]	
Bone scan[l]	X		X[g]		X[h]	X[o]	X[o]		X[h]	X[i]	
NaF PET/CT[b w]		X[bc]	X[bg]								
Tissue biopsy[b w]		X[bd]	X[bgm]								
Tetanus immunization[w]		X[e]									
Blood for immune analyses[w]		X	X[v]		X	X[p]	X[p]		X	X[q]	
Stool sample for microbiome analysis[w]		X	X[g]								
Treatments											
DNA Immunization (pTVG-HP)[n]			X	X			X	X			
Pembrolizumab infusion			X		X	X	X				

[a] Study coordinator or research nurse review adverse events and concomitant medications
[b] Bone biopsy for subset of up to 12 patients at UWCCC lead site that will be selected to have biopsies and investigational NaF PET/CT performed.
[c] NaF PET/CT scan – within 4 weeks 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 baseline immune blood draw and at least two days 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, total bilirubin, alkaline phosphatase, amylase, creatine kinase (CK), TSH (thyroid stimulating hormone), LDH and cortisol (cortisol only required on treatment days and not at screening, EOT or 28 day follow up))
[g] Procedures for cycle 5 day 1 ONLY - can be +/- 7 days of this visit, NaF PET/CT scans to be done prior to biopsy for patients receiving biopsy
[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 and PTT only required for those subjects who will be undergoing a biopsy and only required at biopsy timepoints (screening and C5D1). May be performed up to 7 days prior to biopsy, required same day if subject is on coumadin. PT and/or INR may be done.

[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] Biopsy can be performed +/- 14 days of this visit
[n] For Arm 1, Subjects will receive pTVG-HP vaccine on days 1 and 8 of each cycle for Cycles 1 – 8 and for Cycles 1 – 4 of the vaccine booster cycles (if applicable) .
[o] CT of abdomen and pelvis (+/- chest as clinically indicated) and bone scintigraphy continue every ~12 weeks, +/- 7 days from visits at day 1 of cycles 13, 17, 21, 25, and 29
[p] 100 mL green top tubes and 10 mL red top tubes collected at day 1 of cycles 13 and 17.
[q] Blood for immune analysis only collected if not previously collected within 60 days
[r] MRI imaging may substitute for CT, but the same type of imaging must be used to determine eligibility and progression
[s] Treatment cycles are defined as 21 days.
[t] Following cycle 8, subjects will continue to receive pembrolizumab (Pembro) at 3-week intervals, up to a total of 2 years (32 cycles) of treatment, or until evidence of radiographic progression, or criteria are met to receive vaccine booster cycles (once booster cycles are complete, subject will resume Pembro at 3-week intervals, up to a total of 2 years (32 cycles) of treatment, or until evidence of radiographic progression).
[u] In the event of PSA rise (25% increase over cycle 9 day 1 (6 months), minimum of 2 ng/ml), and no evidence of radiographic progression, patients will receive 4 vaccine booster cycles occurring in succession but following the overall cycle schedule (i.e If Vaccine booster cycle 1 falls on cycle 10 of the study then booster cycle 2 will fall on cycle 11 of the study, and so on). Subjects will then resume Pembro alone cycles once booster cycles are complete (i.e. if booster cycle 1 occurred on cycle 10, then the Pembro alone cycles would resume on cycle 14 of the study).
[v] Blood for immune analyses only collected on C3D1 and C5D1 (+/-3 days). If subject had blood collected under study XP02832 "Evaluation of Immunity to Malignancy" and the collection date falls within the requirements for these visits, that blood and/or results from the XP02832 study may be used for this study's evaluation.
[w] Correlative studies (biopsies, NaF PET/CT, blood for immune analyses, tetanus, and stool sample for microbiome analysis) will only be performed at UWCCC lead site. Other participating sites will not perform these procedures.
[x] Physical Exam to include vital signs (temperature, blood pressure, heart rate, and respiration rate).
[y] 28 day follow-up visit should be 28 days +/- 7 days from the off treatment date which is defined as the date the decision was made to take the subject off study treatment.
[z] Cortisol only due on treatment. Not due at screening, EOT or 28d FU visits.
[^] Subjects may undergo more than one series of booster cycles if the booster cycle criteria is met again (ex. PSA rise is >25% at C9D1 again, there is no evidence of radiographic progression, etc.).

ARM 2: (pTVG-AR + pTVG-HP + pembrolizumab)

Treatment Visit:	Screening		Vaccine Cycles (Cycles 1-8)[s]		Cycle 9 (21 +/- 3 days from cycle 8 day 1)	Pembro Alone Cycles[st]	Vaccine Booster Cycles[s,t,^]		EOT (21 +/- 3 days after cycle 32 day 1)	28-day follow up (28 +/- 7 days after off-treatment date, if prior to cycle 32)[y]	Long-term follow up
	Within 6 weeks of day 1	Within 4 weeks of day 1	Day 1 (21 +/- 3 days from previous cycle day 1)	Day 8 (7 +/- 2 days from day 1)		Cycle 10 + (21 +/- 3 days from previous cycle)	Day 1 (21 +/- 3 days from previous cycle day 1)	Day 8 (7 +/- 2 days from day 1)			
History and Physical Exam											
History	X										X
Consent	X										
Physical exam[x], ECOG PS		X	X		X	X	X		X	X	
Tox assessment[a]		X	X		X	X	X		X	X	
Lab Tests											
CBC	X		X		X	X	X		X	X	
Chemistry panel[f z]	X		X		X	X	X		X	X	
PT/INR, PTT[k b]		X	X								
Serum testosterone	X										
PSA and PAP	X		X		X	X	X		X	X	
Procedures											
CT abdomen/pelvis[r]	X[j]		X[g]		X[h]	X[o]	X[o]		X[jh]	X[i]	
Bone scan[l]	X		X[g]		X[h]	X[o]	X[o]		X[h]	X[i]	
NaF PET/CT[b w]		X[bc]	X[bg]								
Tissue biopsy[b w]		X[bd]	X[bgm]								
Tetanus immunization[w]		X[e]									
Blood for immune analyses[w]		X	X[v]		X	X[p]	X[p]		X	X[q]	
Stool sample for microbiome analysis[w]		X	X[g]								
Treatments											
DNA Immunization (pTVG-AR)[n]			X (cycles 1, 2, 5, 6)	X (cycles 1, 2, 5, 6)			X (cycles 1, 2)	X (cycles 1, 2)			
DNA Immunization (pTVG-HP)[n]			X (cycles 3, 4, 7, 8)	X (cycles 3, 4, 7, 8)			X (cycles 3, 4)	X (cycles 3, 4)			
Pembrolizumab infusion			X		X	X	X				

[a] Study coordinator or research nurse review adverse events and concomitant medications
[b] Bone biopsy for subset of up to 12 patients at UWCCC lead site that will be selected to have biopsies and investigational NaF PET/CT performed
[c] NaF PET/CT scan – within 4 weeks of day 1 – to provide baseline study for comparison prior to treatment
[d] Biopsy within 2 weeks prior to day 1
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[f] Where chemistry labs indicated, the following serum tests are to be performed: Chem 7 (sodium, potassium, bicarbonate, BUN, creatinine, glucose), ALT, AST, total bilirubin, alkaline phosphatase, amylase, creatine kinase (CK), TSH (thyroid stimulating hormone), LDH and cortisol (cortisol only required on treatment days and not at screening, EOT or 28 day follow up)
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[m] Biopsy can be performed +/- 14 days of this visit
[n] For Arm 2, Subjects will receive pTVG-AR vaccine on days 1 and 8 of Cycles 1, 2, 5, and 6 and days 1 and 8 of Cycles 1 and 2 of the vaccine booster cycles (if applicable) . Subjects will receive pTVG-HP vaccine on days 1 and 8 of Cycles 3, 4, 7, and 8 and days 1 and 8 of Cycles 3 and 4 of the vaccine booster cycles (if applicable) .
[o] CT of abdomen and pelvis (+/- chest as clinically indicated) and bone scintigraphy continue every ~12 weeks, +/- 7 days from visits at day 1 of cycles 13, 17, 21, 25, and 29
[p] 100 mL green top tubes and 10 mL red top tubes collected at day 1 of cycles 13 and 17.
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[^] Subjects may undergo more than one series of booster cycles if the booster cycle criteria is met again (ex. PSA rise is >25% at C9D1 again, there is no evidence of radiographic progression, etc.).

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 26,000 U.S. men are estimated to die as a result of prostate cancer in 2017 [1]. Treatment with surgery and radiation remain effective for presumed organ-confined disease, however approximately one third of these patients will have progressive or metastatic disease at 10 years [2]. 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]. 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 published studies we identified in a murine model using a DNA vaccine targeting a tumor antigen that immunization led to the generation of CD8+ T cells with cytolytic activity, and that these cells express PD-1 either transiently, with T-cell activation, or over many weeks, depending on the binding affinity of the vaccine-presented epitope for MHC class I [21, 22]. Moreover, immunization led 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 [21]. 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. These findings suggested that anti-tumor DNA vaccines might best be employed with PD-1 checkpoint inhibitors at the time of immunization, when PD-1 expression is upregulated with T-cell activation.

The evaluation of PD-1 blockade with DNA vaccination has been recently evaluated in a pilot clinical trial (NCT02499835) [23]. 26 patients with castration-resistant, metastatic prostate cancer were randomized to receive the pTVG-HP DNA vaccine, administered every 2 weeks x 6, followed by pembrolizumab administered every 3 weeks x 4 over 24 weeks, or to receive these agents concurrently on the same schedule over 12 weeks. As shown in Figure 1, PSA declines were observed almost exclusively in patients treated with the concurrent schedule. This is consistent with findings in animal models, as described above [21, 22]. PSA declines were observed in approximately half of individuals treated with the concurrent schedule. Given these findings, the current trial aims to expand on these findings, and determine if the number of responding individuals can be increased by broadening the population of T cells activated with immunization. Specifically, this trial will evaluate the use of one versus two DNA vaccines, delivered concurrently with PD-1 blockade using pembrolizumab. ***The hypothesis to be tested is that delivering two vaccines with PD-1 blockade will elicit a greater frequency and magnitude of tumor-directed CD8+ T cells, and thereby increase the percentage of patients experiencing objective anti-tumor effect as measured by prolonged time to disease progression, PSA declines, and/or objective radiographic responses.***

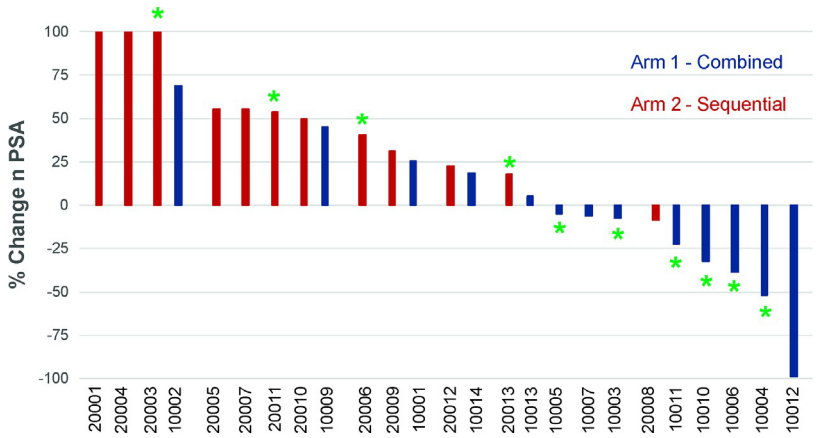

Figure 1: Changes in serum PSA – patients treated with pTVG-HP and pembrolizumab. 26 patients were randomized to treatment with pTVG-HP delivered 6 times over 12 weeks either concurrently with pembrolizumab (blue, delivered 4 times over 12 weeks), or sequentially (red, DNA vaccination over 12 weeks followed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

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 [24, 25]. 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 [26]. 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* [27, 28]. 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 [29]. 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 [30, 31, 32, 33]. 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 [34]. In rodent studies, we have similarly demonstrated that PAP can be immunologically targeted using genetic vaccines, and a plasmid DNA vaccine in particular [31, 32]. We have previously reported the results of two early phase trials conducted in patients with early, PSA-recurrent (non-metastatic) prostate cancer using this same DNA vaccine (pTVG-HP) evaluating the dose and schedule of vaccination [35, 36]. In 38 patients treated, no significant adverse events were observed. 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 [35, 36]. 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 [37]. 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 [36]. 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 EpCam+ circulating epithelial cells (CEC) with PD-L1 expression, analogous to our findings in murine models [38]. These finding further suggested 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.

B. The androgen receptor (AR) is a rational target for anti-tumor vaccines for prostate cancer

The AR is a steroid hormone receptor that plays a crucial role in the development of the normal prostate gland, as well as in the progression of prostate cancer. Patients with metastatic disease are initially treated with androgen deprivation therapy, and androgen deprivation typically is continued indefinitely once a patient has metastatic prostate cancer. While the importance of the AR in the development and progression of cancer has led it to be the central molecular target for patients with recurrent disease for more than half a century, it also suggests that the AR may be an attractive vaccine target antigen. In addition to its critical role in oncogenicity, the AR also has several other characteristics of an ideal target antigen, including its intracellular expression and its elevated frequency of expression (both within an individual tumor as well as frequency of patients). Furthermore, while the expression of the AR is not strictly limited to prostate tissue, the expression of AR is actually more restricted to prostate than the expression of several other common tumor antigen targets, including HER-2/neu or even PSMA, both of which have been safely targeted by immunization approaches [39, 40, 41, 42]. In fact, while prostate cancer vaccines have largely focused on targeting antigens whose expression is restricted to the prostate, this is contrary to the approach commonly taken with other solid tumors, which focus on vaccines targeting functionally-important antigens such as HER-2/Neu [41, 43, 44], CEA [45, 46, 47], MUC1 [47, 48, 49], and EGFR vIII [50, 51, 52] for a variety of solid tumors. To date, unusual autoimmune diseases have not been observed in patients treated on vaccine trials targeting these antigens.

We have previously studied the immunogenicity of the AR, showing that patients with prostate cancer have an increased frequency of AR-specific antibody responses (irrespective of patients' disease stage), and that patients with these antibody responses have a higher level of AR-specific cellular immune responses [53]. Furthermore, we were able to routinely culture peptide-specific cytotoxic T cells from prostate cancer patients (all fifteen patients screened had peptide-specific T cells to at least one of the AR peptides identified [54]). This work also identified two HLA-A2 epitopes (AR805 and AR811) that are naturally presented on the surface of prostate tumor cells, and found that T cells specific for these epitopes had multi-functional cytokine expression and could lyse prostate tumor cells [54]. Taken together, these data show that the AR is also an immunogenic antigen, and thus may be a logical vaccine target antigen.

We have previously evaluated the ligand-binding domain of the AR (AR LBD) as a vaccine target in murine models. We have reported that a DNA vaccine encoding the AR LBD was able to elicit AR-specific CD8+ T cells in immune competent, male mice [55]. Moreover, immunization of prostate tumor-bearing transgenic mice led to an increase in their survival [55]. These findings, and the safety of this approach was confirmed in an IND-enabling study [56]. Given these

findings, a multi-institution phase I clinical trial evaluating the safety and immunological efficacy of this vaccine (pTVG-AR) is nearly complete, having been evaluated in patients with newly metastatic prostate cancer (NCT02411786). To date there have been no serious adverse events related to treatment in 40 patients treated. Preliminarily, that study has demonstrated that immune response to the AR target can be elicited, and that response to AR is associated with a longer time to progression. Given this, and that the pTVG-AR uses the same vector backbone as the pTVG-HP vaccine, the current trial will evaluate these vaccines together, versus pTVG-HP alone, in combination with pembrolizumab.

C. PD-1 blockade and cancer treatment

The PD-1 receptor-ligand interaction is a major pathway hijacked by tumors to suppress immune control. The normal function of PD-1, expressed on the cell surface of activated T-cells under healthy conditions, is to down-modulate unwanted or excessive immune responses, including autoimmune reactions. PD-1 (encoded by the gene *Pdcd1*) is an immunoglobulin (Ig) superfamily member related to cluster of differentiation 28 (CD28) and cytotoxic T-lymphocyte-associated protein 4 (CTLA-4) that has been shown to negatively regulate antigen receptor signaling upon engagement of its ligands (PD-L1 and/or PD-L2) [57, 58].

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 [59]. In addition, early phase clinical trials using PD-1 or PD-L1 inhibitors have identified that the expression of at least one of the ligands for PD-1 (PD-L1) on the target tumor cell by biopsy is associated with clinical response to therapy [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.

The structure of murine PD-1 has been resolved [60]. PD-1 and its family members are type I transmembrane glycoproteins containing an Ig-variable–type (IgV-type) domain responsible for ligand binding and a cytoplasmic tail responsible for the binding of signaling molecules. The cytoplasmic tail of PD-1 contains 2 tyrosine-based signaling motifs, an immunoreceptor tyrosine-based inhibition motif, and an immunoreceptor tyrosine-based switch motif. Following T-cell stimulation, PD-1 recruits the tyrosine phosphatases, SHP-1 and SHP-2, to the immunoreceptor

tyrosine-based switch motif within its cytoplasmic tail, leading to the dephosphorylation of effector molecules such as CD3 zeta (CD3ζ), protein kinase C-theta (PKCθ), and zeta-chain-associated protein kinase (ZAP70), which are involved in the CD3 T-cell signaling cascade [58, 61, 62, 63]. The mechanism by which PD-1 down-modulates T-cell responses is similar to, but distinct from, that of CTLA-4, because both molecules regulate an overlapping set of signaling proteins [64, 65]. As a consequence, the PD-1/PD-L1 pathway is an attractive target for therapeutic intervention in multiple types of cancer, including prostate cancer.

Pembrolizumab is a potent humanized immunoglobulin G4 (IgG4) monoclonal antibody (mAb) with high specificity of binding to the programmed cell death 1 (PD-1) receptor, thus inhibiting its interaction with programmed cell death ligand 1 (PD-L1) and programmed cell death ligand 2 (PD-L2). Based on preclinical in vitro data, pembrolizumab has high affinity and potent receptor blocking activity for PD-1. Pembrolizumab has an acceptable preclinical safety profile and is in clinical development as an intravenous (IV) immunotherapy for advanced malignancies. Keytruda® (pembrolizumab) is indicated for the treatment of patients across a number of indications because of its mechanism of action to bind the PD-1 receptor on the T cell. For more details on specific indications refer to the Investigator brochure.

D. 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 [66, 67, 68], 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 [32, 69]. 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 [70] and CD8+ T-cells specific for the targeted antigen [66, 71, 72, 73, 74]. In animal models, the use of an intradermal route of vaccine administration, in particular, tends to promote this Th1/CTL-biased immune response [70, 75, 76]. 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 [77, 78]. 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.

As described above, we have previously reported the results of two clinical trials using a DNA vaccine encoding human PAP (pTVG-HP). In the first phase I/II clinical trial, subjects with non-castrate, non-metastatic prostate cancer were immunized six times at two-week intervals with this 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 [79]. 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 [35]. 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 [37].

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 [36]. Favorable changes in PSA doubling time were again observed, and tended to be greatest in patients with evidence of long-term immunity [36]. 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 responses in murine models and a pilot human clinical trial

Given our findings in human trials that some patients did not develop evidence of immune response, and even those that did still showed evidence of disease progression, we have sought to evaluate methods to increase the efficacy of DNA vaccines and evaluate potential mechanisms of tumor escape. As described above, we have identified that immunization elicits CD8+ T cells, which, upon secretion of IFNγ, leads to increased PD-L1 expression in tumors [21]. Moreover, efforts to increase the immunogenicity of vaccination by encoding epitopes with greater affinity for MHC class I led to an inferior anti-tumor response that was mediated by increased and prolonged expression of PD-1 on antigen-specific CD8+ T cells [21, 22]. The anti-tumor efficacy of vaccination could be increased by blocking the PD-1/PD-L1 interaction at the time of immunization [21, 22]. The importance of these findings for human tumor immunotherapy has recently been demonstrated in a pilot clinical trial (NCT02499835) [23]. The goal of this trial was to determine if it was better to first elicit a PD-1-regulated T cell response with vaccination, and then block PD-1 (by using vaccine followed by PD-1 blockade), or to block PD-1 at the time T

cells are activated with vaccination prior to them being able to be deregulated in the PD-L1-expressing tumor environment (by using vaccine delivered concurrently with PD-1 blockade). 26 patients with castration-resistant, metastatic prostate cancer were randomized to receive the pTVG-HP DNA vaccine, administered every 2 weeks x 6, followed by pembrolizumab administered every 3 weeks x 4 over 24 weeks, or to receive these agents concurrently on the same schedule over 12 weeks. As shown in Figure 1, PSA declines were observed almost exclusively in patients treated with the concurrent schedule, and declines were most observed in patients who developed Th1-biased immunity to the PAP target antigen. PSA declines were observed in approximately half of individuals treated with the concurrent schedule.

F. Treatments can affect the composition of the gut microbiome, and the composition of the gut microbiome can affect responses to cancer treatments

It has been well documented that the intestinal microbiota composition can affect the efficacy of chemotherapy agents as well as T-cell checkpoint blockade therapies [59, 60, 61, 62]. Moreover, the response of tumors to T-cell checkpoint blockade can be transferred to "avatar" mice using fecal microbiota transplant from patients who were classified as clinical responders, whereas feces from non-responder patients failed to do so [63]. This approach has led to the identification of several bacterial species that favor anti-cancer immunosurveillance and response to T-cell checkpoint therapies. For example, fecal microbiota transplantation was performed using feces from patients with non-small cell lung cancer to recolonize germ-free or antibiotic-treated mice. Feces from patients with clinical responses conveyed a stronger immune response against murine tumors treated with PD-1 blockade. By restoring "immunogenic" bacteria, such as *Akkermansia muciniphila*, by oral gavage to non-responding mice, PD-1 anti-tumor activity was restored and associated with increased CCR9+CD4+ T cells trafficking to mesenteric lymph nodes [63].

It has been previously demonstrated that the gut microbiota can be altered by androgen-targeted therapies used in patients with prostate cancer [64, 65]. Given that patients with advanced prostate cancer are all treated with androgen deprivation, it is conceivable that this confers some resistance to immune checkpoint blockade therapies, agents that have traditionally had little efficacy as single agents for advanced prostate cancer. Hence understanding the composition of the gut microbiota in these patients, and whether the composition has been associated with lack of response to T cell checkpoint therapies for other diseases, could provide understanding about the best application of these therapies for advanced prostate cancer. Moreover, it is unknown whether changes to the gut microbiota might occur after anti-tumor vaccination, and whether this may be associated with tumor response.

G. Summary

Given all of these findings, the current protocol aims to determine if the efficacy of anti-tumor vaccination can be improved by broadening the population of T cells activated with immunization, and delivering these with concurrent PD-1 blockade. Specifically, this trial will evaluate the use of one versus two DNA vaccines, delivered concurrently with PD-1 blockade using pembrolizumab, and delivered over a prolonged period of time (to maximum response or 2 years (32 cycles of treatment). The hypothesis to be tested is that delivering two vaccines with PD-1 blockade will elicit a greater frequency and magnitude of tumor-directed CD8+ T cells, and thereby increase the percentage of patients experiencing objective anti-tumor effect as measured by PSA declines and/or objective radiographic responses. In the previous pilot trial, patients

received vaccines at every 2-week intervals and pembrolizumab at every 3-week intervals. Emerging data from animal studies has suggested that "density" of vaccination (every week rather than every 2 weeks) is preferable for initiating an immune response, but periods of time between booster immunization may be important for establishing immune memory. In order to accommodate these observations into a workable schedule, the current trial will aim for two vaccinations per 3-week cycle with pembrolizumab, and using two such cycles consecutively prior to alternating vaccine. In addition, because most patients with metastatic prostate cancer have bone metastases, a site that is difficult to characterize objectively, a subset of patients treated at the University of Wisconsin Carbone Cancer Center will undergo NaF PET/CT with site-directed biopsies to determine if this modality can identify early metabolic signals associated with immune infiltration as an early treatment biomarker. An exploratory aim will be to determine whether treatment elicits changes in the gut microbiome, and whether clinical response is associated with changes in the composition of the gut microbiome. Based on published studies and our own prior experience, the median time to disease progression in this stage of disease is approximately 3 months. Hence the use of 6-month progression-free survival provides a clinically relevant endpoint, and a basis for comparing the two treatment arms. Patients without progression at 6-months will continue with pembrolizumab alone until PSA rise, at which point vaccine courses will be restarted. This approach will permit evaluation of second courses of treatment in responding individual. Overall, identification of PSA responses (defined as PSA decline $\geq$ 50%), objective radiographic responses, as well as delays in the time to disease progression, will serve as evidence of clinical activity to compare the treatment arms and be used for planning future confirmatory clinical trials.

3. OBJECTIVES

H. Primary Objective

To determine the 6-month progression-free survival rate in patients with metastatic, castration-resistant prostate cancer (mCRPC) treated with pembrolizumab in combination with one versus two DNA vaccines as T-cell activating agents

I. Secondary Objectives:

1. To determine the overall objective response rate (using Prostate Cancer Working Group 3 (PCWG3) criteria)

2. To determine the PSA response rate (decline $\geq$ 50%)

3. To determine the median radiographic progression-free survival rate

4. To determine median duration of PSA and/or objective response in responding individuals

5. To determine the overall survival

6. To determine whether the development of antigen-specific Th1 immunity elicited with treatment to either antigen (PAP or AR) is associated with PSA response

7. To evaluate the safety and tolerability of pTVG-HP DNA vaccine with or without pTVG-AR DNA vaccine and pembrolizumab

J. Laboratory and Exploratory Objectives:

1. To evaluate PAP-specific and AR-specific antibody responses following treatment with pembrolizumab and DNA vaccine(s)
2. To determine whether treatment with either sequence elicits immunologic antigen spread to other prostate associated antigens

3. To determine whether pre-existing antigen-specific immunity is predictive of immunological or objective clinical response

4. To determine, in a subset of patients, whether early changes in bone observed by NaF PET/CT are associated with immunological or objective clinical response, or pathological changes associated with immune-cell infiltration

5. To determine second PSA response rates in patients treated in follow-on courses

6. To determine whether treatment elicits changes in the gut microbiota composition

7. To determine whether changes in gut microbiota are associated with clinical response (PSA decline $\geq$ 50%)

4. PRODUCT INFORMATION

K. 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 multiple different types of cancer. While this agent is commercially available, 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, the dose of pembrolizumab for this study is 200 mg every 3 weeks (Q3W). Based on the totality of data generated in the Keytruda development program, 200 mg Q3W is the appropriate dose of pembrolizumab for adults across all indications and regardless of tumor type. As outlined below, this dose is justified by:

- Clinical data from 8 randomized studies demonstrating flat dose- and exposure-efficacy relationships from 2 mg/kg Q3W to 10 mg/kg every 2 weeks (Q2W),
- Clinical data showing meaningful improvement in benefit-risk including overall survival at 200 mg Q3W across multiple indications, and
- Pharmacology data showing full target saturation in both systemic circulation (inferred from pharmacokinetic [PK] data) and tumor (inferred from physiologically-based PK [PBPK] analysis) at 200 mg Q3W

Among the 8 randomized dose-comparison studies, a total of 2262 participants were enrolled with melanoma and non-small cell lung cancer (NSCLC), covering different disease settings (treatment naïve, previously treated, PD-L1 enriched, and all-comers) and different treatment settings (monotherapy and in combination with chemotherapy). Five studies compared 2 mg/kg Q3W versus 10 mg/kg Q2W (KN001 Cohort B2, KN001 Cohort D, KN002, KN010, and KN021), and 3 studies compared 10 mg/kg Q3W versus 10 mg/kg Q2W (KN001 Cohort B3, KN001 Cohort F2 and KN006). All of these studies demonstrated flat dose- and exposure-response relationships across the doses studied representing an approximate 5- to 7.5-fold difference in exposure. The 2 mg/kg (or 200 mg fixed-dose) Q3W provided similar responses to the highest doses studied. Subsequently, flat dose-exposure-response relationships were also observed in other tumor types including head and neck cancer, bladder cancer, gastric cancer and classical Hodgkin Lymphoma, confirming 200 mg Q3W as the appropriate dose independent of the tumor type. These findings are consistent with the mechanism of action of pembrolizumab, which acts by interaction with immune cells, and not via direct binding to cancer cells.

Additionally, pharmacology data clearly show target saturation at 200 mg Q3W. First, PK data in KN001 evaluating target-mediated drug disposition (TMDD) conclusively demonstrated saturation of PD-1 in systemic circulation at doses much lower than 200 mg Q3W. Second, a PBPK analysis was conducted to predict tumor PD-1 saturation over a wide range of tumor penetration and PD-1 expression. This evaluation concluded that pembrolizumab at 200 mg Q3W achieves full PD-1 saturation in both blood and tumor.

Finally, population PK analysis of pembrolizumab, which characterized the influence of body weight and other participant covariates on exposure, has shown that the fixed-dosing provides similar control of PK variability as weight based dosing, with considerable overlap in the distribution of exposures from the 200 mg Q3W fixed dose and 2 mg/kg Q3W dose. Supported by these PK characteristics, and given that fixed-dose has advantages of reduced dosing complexity and reduced potential of dosing errors, the 200 mg Q3W fixed-dose was selected for evaluation across all pembrolizumab protocols.

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.

L. Plasmid DNA vaccines

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.

pTVG-AR (pTVG4 vector encoding cDNA for the AR LBD)

The DNA plasmid was constructed using the same vector as for the pTVG-HP plasmid, and the sequence of the cDNA insert has been confirmed by standard DNA sequencing to confirm its identity. A bacterial strain was transformed with this plasmid and transferred to the Waisman Clinical BioManufacturing Facility (WCBF) at the University of Wisconsin from which a master cell bank was prepared. Complete sequencing of the plasmid has been performed. The bacteria will be grown and amplified in culture under kanamycin selection, and plasmid DNA will be purified under GMP conditions for this trial. Lot(s) will be tested for appearance, plasmid homogeneity, DNA identity by restriction endonuclease evaluation, protein contamination, RNA contamination, genomic DNA contamination, sterility, endotoxin, and pH. Once passing all of these criteria, the vaccine will be supplied by the WCBF in single-use vials containing 0.6 mL of 0.2 mg/mL pTVG-AR in phosphate-buffered saline. Vials will be stored at <-60°C until the day of use.

GM-CSF

GM-CSF (Leukine®, Sargramostim) has been previously used as a vaccine adjuvant, in particular in previous trials with these DNA vaccines. However, a trial using GM-CSF in combination with pTVG-AR was completed in 2020 which demonstrated little benefit with the addition of GM-CSF. Specifically, that trial randomized patients to receive vaccine on one of two different schedules, and with or without GM-CSF as a vaccine adjuvant. Immune responses were elicited irrespective of the use of GM-CSF, although differences in immune response were observed with respect to vaccine treatment schedule. In addition, in an ongoing trial using pTVG-HP with nivolumab (UW18008), in which patients receive GM-CSF only if there is not a PSA decline within the first month of starting treatment, nearly half of patients have experienced a PSA decline and have not had GM-CSF added to their treatment. Hence, GM-CSF will no longer be used with either vaccine in future trials to avoid potential unnecessary toxicity related to this agent. The current trial was initiated using GM-CSF (200 mcg dose co-administered i.d. with each vaccination), however was amended in 2021 after the accrual of ~20 patients, based on these data, to eliminate GM-CSF administration from subsequent subjects. With the protocol amendment in 2021, existing subjects will not continue to receive GM-CSF (i.e. discontinued from all treated subjects at that time).

DNA vaccine preparation and administration

Vials will be thawed on the day of administration. Specifically:

For each of the DNA immunizations: Vials consist of 0.6 mL of 0.2 mg/mL pTVG-HP or pTVG-AR. 0.25 mL will then be drawn into each of two tuberculin syringes. This effectively provides a 100-µg dose of DNA.

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. The same site will be used for subsequent vaccinations.

Labeling, packaging, storage and return of pembrolizumab and DNA vaccines

Pembrolizumab will be provided by Merck as follows:

Product Name & Potency	**Dosage Form**
Pembrolizumab 100 mg/ 4mL	Solution for Injection

pTVG-HP and pTVG-AR will be provided in single-use vials

Supplies will be labeled in accordance with regulatory requirements.

This trial is open-label; therefore, the participant, the trial site personnel, the Sponsor and/or designee are not blinded to treatment. Drug identity (name, strength) is included in the label text; random code/disclosure envelopes or lists are not provided.

Clinical supplies must be stored in a secure, limited-access location under the storage conditions specified on the label. Receipt and dispensing of trial medication must be recorded by an authorized person at the trial site. Clinical supplies may not be used for any purpose other than that stated in the protocol.

The site investigator is responsible for keeping accurate records of the clinical supplies received from Merck or designee, the amount dispensed to and returned by the participants and the amount remaining at the conclusion of the trial. Upon completion or termination of the study, all unused and/or partially used investigational product will be destroyed at the site per institutional policy. It is the Investigator's responsibility to arrange for disposal of all empty containers, provided that procedures for proper disposal have been established according to applicable federal, state, local and institutional guidelines and procedures, and provided that appropriate records of disposal are kept.

5. PATIENT SELECTION

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 (i.e. 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 28 days before day 1 (for flutamide, apalutamide, enzalutamide, or other 2nd generation AR antagonists) or 6 weeks (for bicalutamide or nilutamide) prior to registration. Moreover, subjects who demonstrate an anti-androgen withdrawal response, defined as a $\geq$ 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 3 (PCWG3) bone scan criteria [86] 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 15 mm 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 weaned to a daily corticosteroid dose equivalent of no more than 5 mg prednisone daily for at least 28 days prior to day 1.
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, liver, and coagulation function as evidenced by the following within 6 weeks of day 1:

WBC	$\geq$ 2000 / mm^3
ANC	$\geq$ 1500 / mm^3
HgB	$\geq$ 9.0 gm/dL independent of transfusion (Subjects must not have received a blood transfusion within 14 days)
Platelets	$\geq$ 100,000 / mm^3
Creatinine	$\leq$ 1.5 x institutional ULN
Total bilirubin	$\leq$ 1.5 x institutional ULN OR direct bilirubin $\leq$ ULN for participants with total bilirubin levels > 1.5 x ULN
AST, ALT	$\leq$ 2.5 x institutional upper limit of normal
PT or INR	$\leq$ 1.5 x ULN unless participant is receiving anticoagulant therapy and PT is within therapeutic range of intended use of anticoagulant (only required for patients receiving biopsy)

PTT ≤ 1.5 x ULN unless participant is receiving anticoagulant therapy and aPTT is within therapeutic range of intended use of anticoagulant (only required for patients receiving biopsy)

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. A subset of patients (6 patients per treatment arm) treated at the lead UW site 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 9 for more details.
13. A subset of patients (6 patients per treatment arm) treated at the lead UW site must be willing to undergo NaF PET/CT scans for the investigational component of this trial. See section 9 for more details.
14. For those patients who are sexually active, they must be willing to use barrier contraceptive methods, and refrain from donating sperm, during the period of treatment on this trial and for four weeks after the last DNA immunization treatment
15. 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

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 day 1, or while on study, is prohibited:
    - Systemic corticosteroids (at doses over the equivalent of 5 mg prednisone daily); inhaled, intranasal or topical corticosteroids are acceptable
    - PC-SPES
    - 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
    - Apalutamide

- Radium 223 (Xofigo®)
- Any other hormonal agent or supplement being used with the intent of cancer treatment must be reviewed by the PI for eligibility

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. Patients must have recovered from all radiation-related toxicities and not have had radiation pneumonitis.
7. Major surgery within 4 weeks of registration is prohibited
8. Prior cytotoxic chemotherapy (for example, but not limited to, docetaxel, mitoxantrone, cabazitaxel) within 28 days of registration is prohibited
9. Prior treatment with an anti-PD-1, anti-PD-L1, or anti-PD-L2 agent, or with any agent directed to another T-cell stimulatory or inhibitory receptor (e.g. CTLA-4, OX-40, CD137).
10. Patients with a history of life-threatening autoimmune disease
11. Patients with a history of non-infectious pneumonitis that required corticosteroid treatment, or has current pneumonitis
12. Patients with a history of allergic reactions to the tetanus vaccine
13. Patients who have undergone splenectomy or who have a diagnosis of immunodeficiency
14. Patients must not have other active malignancies other than non-melanoma skin cancers or superficial (non-muscle-invasive) carcinoma of the bladder. Subjects with a history of other cancers who have been adequately treated and have been recurrence-free for $\geq$ 3 years are eligible.
15. Patients with known brain metastases and/or carcinomatous meningitis
16. Patients who have received a live vaccine within 30 days prior to the first dose of study drug. Examples of live vaccines include, but are not limited to, the following: measles, mumps, rubella, varicella/zoster (chicken pox), yellow fever, rabies, Bacillus Calmette–Guérin (BCG), and typhoid vaccine. Seasonal influenza vaccines for injection are generally killed virus vaccines and are allowed; however, intranasal influenza vaccines (eg, FluMist®) are live attenuated vaccines and are not allowed.
17. Any antibiotic therapy for documented infection within 1 month of day 1, or anticipated need for antibiotic therapy for documented infection 1 month after beginning treatment
18. Patients with active autoimmune disease that has required systemic treatment in the past 2 years (i.e. with use of disease modifying agents, corticosteroids or immunosuppressive drugs). Replacement therapy (eg., thyroxine, insulin, or physiologic corticosteroid replacement therapy for adrenal or pituitary insufficiency, etc.) is not considered a form of systemic treatment.
19. 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 confound results of the study, over the treatment period.
20. Any known psychiatric or substance abuse disorders that would interfere with cooperation with the requirement of the trial.
21. Patients who have concurrent enrollment on other phase I, II, or III investigational treatment studies cannot be actively receiving treatment and the last dose cannot be within 4 weeks of day 1. They must be in the follow up phase of the study.

6. EXPERIMENTAL DESIGN

This will be a 1:1 randomized, open-label, multi-institution phase II trial designed to evaluate the immunological and clinical effect of one versus two DNA vaccines, used as T-cell activating therapies, and given concurrently with pembrolizumab. Study arms will be defined as follows:

Course 1:

Arm 1: 100 µg pTVG-HP administered intradermally (i.d.) days 1, 8 + 200 mg pembrolizumab IV day 1 of 21-day cycles x 8 cycles

Arm 2: 100 µg pTVG-AR i.d days 1, 8 + 200 mg pembrolizumab IV day 1 of 21-day cycles, for cycles 1, 2, 5 and 6 alternating with
100 µg pTVG-HP i.d. days 1, 8 + 200 mg pembrolizumab IV day 1 of 21-day cycles, for cycles 3, 4, 7 and 8

Patients who have not come off trial at the end of cycle 8 (month 6) will continue to receive pembrolizumab at 3-week intervals, up to a total of 2 years of treatment (32 cycles), or until evidence of radiographic progression. If prior to 2 years, and in the absence of radiographic progression, patients with rise in PSA (25% over month 6 value and with minimum rise of 2 ng/mL) will resume vaccination in follow-on courses:

Arm 1: 100 µg pTVG-HP i.d. days 1, 8 + 200 mg pembrolizumab IV day 1 of 21-day cycles x 4 cycles

Arm 2: 100 µg pTVG-AR i.d days 1, 8 + 200 mg pembrolizumab IV day 1 of q 21-day cycles, for two cycles followed by
100 µg pTVG-HP i.d. days 1, 8 + 200 mg pembrolizumab IV day 1 in 21-day cycles, for two cycles

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. Given the absence of significant adverse events observed in previous clinical trials with pTVG-HP and pTVG-AR, no adverse events > grade 2 are anticipated related to those agents. 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 the reference safety dataset (RSD), a locked safety analysis set of 2799 individuals that supports the current labeled safety profile for pembrolizumab (in subjects with melanoma or non-small cell lung cancer), a total of 1020 (36.4%) subjects experienced a grade 3 AE and 336 (12%) experienced a grade 4 AE. Of these, immune-related grade 3 adverse events of specific interest (AEOSIs) were observed in 139 (5%) individuals and grade 4 AEOSIs were observed in 16 (0.6%) individuals. It is conceivable that combining vaccine with pembrolizumab could result in a higher frequency of adverse events related to the target of vaccination. To date this has not been observed in over 40 patients treated on a pilot trial with pTVG-HP and pembrolizumab. However, in the current trial patients treated on Arm 2 will

receive two vaccines, and hence there is a theoretical possibility of increased toxicity in that arm. Therefore 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. In addition, each of the first 6 patients randomized to Arm 2 will be staggered to start at least 3 weeks after the preceding patient assigned to that study arm. In this way, toxicity will be able to be monitored over at least 18 weeks in a smaller number of patients. If there are no adverse events > grade 2 that are not attributed to pembrolizumab alone, subsequent enrollment will proceed without staggering accrual. In addition, given that over 40 patients have been treated with the single pTVG-HP vaccine and pembrolizumab in a separate trial, and there have been no adverse events > grade 2 that were not attributed to pembrolizumab alone, there will be no staggering of accrual for patients assigned to Arm 1. Given the AE profile for pembrolizumab, a total toxicity rate of 50% for Grade ≥ 3 events (or 20% for Grade ≥ 4 events) with 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. To monitor for particular toxicities that might arise from combination therapies, an adverse event rate of 10% for Grade ≥ 3 or 5% ≥ Grade 4 unusual or specific immune-related AEOSIs with an attribution of at least possibly related to study treatment will also be considered excessive.

Continuous toxicity monitoring based on repeated significance testing with a Pocock boundary will be applied after the first 5 patients have been accrued and evaluated for toxicities within an arm. The toxicity monitoring will be conducted within each of the two study arms. A toxicity rate p0 of at most 15% of Grade ≥ 3 toxicity events will be considered as acceptable while a toxicity rate p1=35% or more will be considered as unacceptably high. Assuming a boundary probability of 0.1 under p0, the stopping boundaries of the repeated significance testing procedure within an arm are as follows: 2/5, 3/9, 4/13, 5/17, 6/22, 7/26, or 8/30. If the number of Grade ≥ 3 toxicities exceeds the boundaries, i.e., ,if at least 3 out of the first 5 patients, at least 4 out of the first 6-9 patients, at least 5 out of the first 10-12 patients, at least 6 out of the first 13-17 patients, at least 7 out of the first 18-22 patients, at least 8 out of the first 23-26 patients, at least 9 out of the first 27 to 30 patients, at least 10 out of the first 31-35 patients or at least 11 out of the first 36 to 40 patients have been accrued and evaluated for toxicities within an arm.

Evidence that the toxicity rate of unusual or specific immune-related AEOSI within an arm excessive will be considered as sufficient if during any stage of the trial (after the accrual of the first 5 patients) the lower limit of the 90% one-sided confidence interval for the estimate of the true toxicity rate exceeds 10% for Grade ≥ 3 or 5% for Grade ≥ 4 toxicities within an 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.

Endpoints

Patients may come off study treatment at the time of radiographic (CT and/or bone scintigraphy) disease progression, with progression (requiring patients to discontinue treatment) being defined only AFTER the first 3-month staging evaluation (to account for possible delayed radiographic responses). Patients will come off study treatment 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 PET/CT scans used for the biopsy cohort will be investigational and will not be used for making individual subject treatment decisions with respect to progression necessitating continuing or discontinuing protocol treatment. Furthermore, no findings or results from the investigational NaF PET/CT scans will be shared with the subjects. Such findings on investigational NaF 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 after discussion with the sponsor PI. Some subjects may receive NaF PET/CT scans as a standard alternative to bone scintigraphy, in which case these will be used to determine response/progression. 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. In particular, evaluations performed at 6-month time point should be performed even if off-study prior to that time, if feasible. At the end of 6 months, patients without progression will continue to receive pembrolizumab every 3 weeks, up to 2 years (32 cycles) maximum, or until radiographic disease progression. Patients on pembrolizumab alone who have PSA rise (25% increase over month 6 value, and minimum of 2 ng/mL rise) will resume a 3-month (4-cycle) follow-on course of vaccine treatment. The goal here is to minimize treatment but maintain a prolonged time to radiographic progression. All subjects who have not come off study earlier for reasons of adverse events, physician/patient discretion or radiographic progression will come off study at the end of two years of total treatment.

The primary endpoint of the trial will be the 6-month progression-free survival rate. Secondary clinical endpoints include objective response, PSA response, progression-free and overall survival. 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). NaF PET/CT may be used as an alternative to bone scintigraphy if that is the institution's standard, however either bone scintigraphy or NaF PET/CT must be used for evaluation at each study time point for comparability. MRI imaging may substitute for CT, but the same type of imaging must be used to determine eligibility and progression. Radiographic disease progression and response will be defined using PCWG2 published criteria [87], 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 requiring study discontinuation. These criteria, in addition to being more standard for contemporary clinical trials in advanced prostate cancer to account for bone flair occasionally seen following active treatment by bone scans, 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.

7. MEASUREMENT OF EFFECT (PCWG3 RECOMMENDATIONS)

A. Malignant Disease Evaluation

To assess objective response, it is necessary to estimate the overall tumor burden at baseline 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, 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, 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, PCWG3 criteria will be used to identify "measurable" lesions at each of the baseline (pre-treatment and 3 month time points), and RECIST 1.1 and iRECIST criteria will be used to monitor for change over time (consistent with PCWG3 criteria) [86].

NOTE: Although NaF PET/CT can be substituted for standard bone scintigraphy if it is available as a standard of care test, all of the pre-treatment and post-treatment evaluations must use the same test (either NaF PET/CT or bone scintigraphy).

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 ≥ 15 mm in short axis when assessed by 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 baseline measurements, or the appearance of one or more unequivocal new measurable 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 (<0.2 ng/mL). 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 ≥ 2 new lesions, with symptoms, will constitute disease progression. Progression must be confirmed with repeat bone scintigraphy (or NaF PET/CT) at least 6 weeks later demonstrating ≥ 2 new lesions. In this case, the date of progression would be the date that the first two new lesions were detected. This is to eliminate the possibility of flair responses seen on bone imaging scans. PCWG3 criteria also specifically highlight that bone scan progression is defined from the first on-treatment scan, rather than the baseline scan to account for possible flare phenomena.

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 an endpoint, 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 pre-treatment (study entry) 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.

d. **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.

e. **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.

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

g. **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.

h. **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 **100%** increase in PSA over the nadir PSA, and ≥ 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.

5. 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.

6. 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

A. First Documentation of Response

The time between initiation of therapy and first documentation of PR or CR.

B. 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.

C. 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.

1. 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.

2. Duration of Stable Disease

A measurement from baseline 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.

D. Overall Survival

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

E. Progression Free-Survival

Progression-free survival (PFS) will be defined 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.

F. iRECIST Guidelines

Due to the use of immunotherapies, the trial will adopt iRECIST guidelines for assessments of radiographic responses and time to progression for this trial. This section gives a summary of iRECIST guidelines and the application to this trial. Particular attention should be paid to these criteria for the 12-week response assessment.

The following table, adapted from the iRECIST guidelines, is a snapshot of changes between RECIST 1.1 and iRECIST guidelines.

	RECIST 1.1	iRECIST
Measurable and non-measurable disease, number and site of target disease	Measurable: >10mm for visceral disease, >15mm for LN lesions, Maximum 5 lesions (2 per organ) All other disease non-target (>10mm for LN disease)	Measurability as per RECIST 1.1 New lesions assessed per RECIST 1.1 but recorded separately for target vs nontarget
CR, PR, or SD	Cannot have met criteria for PD before CR, PR, or SD	Can have had iUPD (one or more) but not iCPD, before iCR, iPR, or iSD
Confirmation of CR or PR	Not required for randomized trials	As per RECIST 1.1
Confirmation of SD	Not required	As per RECIST 1.1
After initial progression (iUPD) but then iCR, iPR, or iSD	Any PD precludes later CR, PR or SD	iCR/iPR/iSD can be achieved after initial iUPD. Sum of target lesion diameters at time of iCR/iPR/iSD should be noted and status should be reset. Subsequent progression after iCR/iPR/iSD should be another iUPD, until confirmation iCPD
New Lesions	Result in PD; recorded but not measured	Results in iUPD at first occurrence iCPD only assigned on basis of new lesions if at next assessment additional new lesions appear or an increase in size of new lesions seen (>5mm of sum of new lesion target or any increase in new lesion non-target)
Confirmation of progression	Not required unless equivocal	Required
Consideration of clinical status	Not included in assessment	Clinical stability is considered when deciding whether treatment should continue after iUPD
CR: Complete Response, **iCR:** Immune Complete Response, **iPR**: Immune Partial Response, **iUPD**: Immune Unconfirmed Progressive Disease, **iCPD:** Immune Confirmed Progressive Disease, **iSD:** Immune Stable Disease, **LN:** Lymph Node, **PD**: Progression of disease, **PR:** Partial Response**, SD:** Stable Disease		

In general, iRECIST guidelines help define progression in the contest of immune therapy. New lesions should be assessed and subcategorized as target lesions and measured for further follow up, or non-target lesions. At the time of initial iUPD, patients must be clinically stable by investigator assessment in order to continue immunotherapy-based treatment. iRECIST guidelines also recommend that confirmation of progression be obtained between 4 to 8 weeks of the initial iUPD scan date.

Investigators will need to assess clinical stability to continue treatments at time of iUPD. Clinical stability to continue treatment in light of iUPD is defined as follows. Absence of any of the following: worsening of performance status, clinically relevant increase in disease-related symptoms such as pain that are associated with disease progression, and increased management/treatments of disease-related symptoms (including increased analgesic agents, radiation, or other palliative treatments)

Please refer to full iRECIST guidelines for further guidance on assessing response/progression. In particular, Table 2 (replicated below) of the guidelines give specific recommendations of timepoint response assignment.

	Timepoint response with no previous iUPD in any category	Timepoint response with previous iUPD in any category*
Target lesions: iCR; non-target lesions: iCR; new lesions: no	iCR	iCR
Target lesions: iCR; non-target lesions: non-iCR/non-iUPD; new lesions: no	iPR	iPR
Target lesions: iPR; non-target lesions: non-iCR/non-iUPD; new lesions: no	iPR	iPR
Target lesions: iSD; non-target lesions: non-iCR/non-iUPD; new lesions: no	iSD	iSD
Target lesions: iUPD with no change, or with a decrease from last timepoint; non-target lesions: iUPD with no change, or decrease from last timepoint; new lesions: yes	Not applicable	New lesions confirm iCPD if new lesions were previously identified and they have increased in size (≥5 mm in sum of measures for new lesion target or any increase for new lesion non-target) or number; if no change is seen in new lesions (size or number) from last timepoint, assignment remains iUPD
Target lesions: iSD, iPR, iCR; non-target lesions: iUPD; new lesions: no	iUPD	Remains iUPD unless iCPD is confirmed on the basis of a further increase in the size of non-target disease (does not need to meet RECIST 1.1 criteria for unequivocal progression)
Target lesions: iUPD; non-target lesions: non-iCR/non-iUPD, or iCR; new lesions: no	iUPD	Remains iUPD unless iCPD is confirmed on the basis of a further increase in sum of measures ≥5 mm; otherwise, assignment remains iUPD
Target lesions: iUPD; non-target lesions: iUPD; new lesions: no	iUPD	Remains iUPD unless iCPD is confirmed based on a further increase in previously identified target lesion iUPD in sum of measures ≥5 mm or non-target lesion iUPD (previous assessment need not have shown unequivocal progression)
Target lesions: iUPD; non-target lesions: iUPD; new lesions: yes	iUPD	Remains iUPD unless iCPD is confirmed on the basis of a further increase in previously identified target lesion iUPD sum of measures ≥5 mm, previously identified non-target lesion iUPD (does not need to be unequivocal), or an increase in the size or number of new lesions previously identified
Target lesions: non-iUPD or progression; non-target lesions: non-iUPD or progression; new lesions: yes	iUPD	Remains iUPD unless iCPD is confirmed on the basis of an increase in the size or number of new lesions previously identified

Abbreviations:

CR: complete response
iCR: immune complete response
iPR: immune partial response
iUPD: immune unconfirmed progressive disease
iCPD: immune confirmed progressive disease
iSD: immune stable disease
LN: lymph node
PD: progression of disease
PR: partial response
SD: stable disease

NOTE: Both RECIST and iRECIST will be reviewed to judge response, but ultimately iRECIST will be used to make final response assessments.

G. 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.

3. CT or MRI

CT or magnetic resonance imaging (MRI) are the best currently available and most reproducible methods for measuring target lesions. Conventional CT or 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.

4. 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.

H. 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), 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). 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 or pTVG-AR 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 the adverse event is considered immune-related, both pembrolizumab and pTVG-AR (and/or pTVG-HP) 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 to be at least possibly due to pembrolizumab, but unlikely related (or unrelated) to pTVG-HP or pTVG-AR vaccine, no further pembrolizumab treatments will be given. The patient will continue with DNA vaccine administration only as per protocol, if the adverse event is not immune-related. If immune-realted, both the pembrolizumab and vaccine(s) will be discontinued. However, if a patient develops a Grade 3 toxicity with an attribution of at least possibly related to pTVG-HP or

pTVG-AR vaccine, both vaccine(s) 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 or pTVG-AR vaccine, no further treatments will be given. Patients will not have any dose modifications (or change in dose or schedule) of pembrolizumab in this study except as noted in section 8.1. If the toxicity does not resolve, or the criteria for resuming treatment (to Grade 1 or less as in the table below) are not met, the patient will discontinue pembrolizumab (and continuing vaccinations, if the adverse event was not believed to be immune-mediated), or discontinue both study medications (vaccine and pembrolizumab) if the adverse event was considered immune-mediated and the vaccinations were already being held. 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 non-immune related toxicity from pembrolizumab could still potentially benefit from continued DNA vaccination. While rare overall, autoimmune events observed following pembrolizumab treatment of patients with melanoma typically resolved and did not necessarily lead to discontinuation of treatment.

If a subject has to hold treatment for a visit, confirm with the sponsor that the subject should skip the current visit and resume treatment at the next timepoint (i.e. if a subject has to hold drug at C7D1 due to an AE, the patient would skip C7D1, be reevaluated at C7D8 and if necessary to continuing holding, come back and resume treatment at C8D1, if allowable per protocol).

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

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

General instructions:

1. Severe and life-threatening irAEs should be treated with IV corticosteroids followed by oral steroids. Other immunosuppressive treatment should begin if the irAEs are not controlled by corticosteroids.
2. Study intervention must be permanently discontinued if the irAE does not resolve or the corticosteroid dose is not ≤10 mg/day within 12 weeks of the last study intervention treatment.
3. The corticosteroid taper should begin when the irAE is ≤ Grade 1 and continue at least 4 weeks.
4. If study intervention has been withheld, study intervention may resume after the irAE decreased to ≤ Grade 1 after corticosteroid taper.

irAEs	Toxicity Grade (CTCAE v5.0)	Action With Pembrolizumab	Corticosteroid and/or Other Therapies	Monitoring and Follow-up
Pneumonitis	Grade 2	Withhold	• Administer corticosteroids (initial dose of 1 to 2 mg/kg prednisone or equivalent) followed by taper • Add prophylactic antibiotics for opportunistic infections	• Monitor participants for signs and symptoms of pneumonitis • Evaluate participants with suspected pneumonitis with radiographic imaging and initiate corticosteroid treatment
	Recurrent Grade 2, Grade 3 or 4	Permanently discontinue		
Diarrhea/Colitis	Grade 2 or 3	Withhold	• Administer corticosteroids (initial dose of 1 to 2 mg/kg prednisone or equivalent) followed by taper	• Monitor participants for signs and symptoms of enterocolitis (ie, diarrhea, abdominal pain, blood or mucus in stool with or without fever) and of bowel perforation (ie, peritoneal signs and ileus) • Participants with ≥Grade 2 diarrhea suspecting colitis should consider GI consultation and performing endoscopy to rule out colitis • Participants with diarrhea/colitis should be advised to drink liberal quantities of clear fluids. If sufficient oral fluid intake is not feasible, fluid and electrolytes should be substituted via IV infusion
	Recurrent Grade 3 or Grade 4	Permanently discontinue		
AST or ALT Elevation or Increased Bilirubin	Grade 2 [a]	Withhold	• Administer corticosteroids (initial dose of 0.5 to 1 mg/kg prednisone or	• Monitor with liver function tests (consider weekly or more frequently until liver enzyme

irAEs	Toxicity Grade (CTCAE v5.0)	Action With Pembrolizumab	Corticosteroid and/or Other Therapies	Monitoring and Follow-up
			equivalent) followed by taper	value returned to baseline or is stable)
	Grade 3 [b] or 4 [c]	Permanently discontinue	• Administer corticosteroids (initial dose of 1 to 2 mg/kg prednisone or equivalent) followed by taper	
T1DM or Hyperglycemia	New onset T1DM or Grade 3 or 4 hyperglycemia associated with evidence of β-cell failure	Withhold [d]	• Initiate insulin replacement therapy for participants with T1DM • Administer antihyperglycemic in participants with hyperglycemia	• Monitor participants for hyperglycemia or other signs and symptoms of diabetes
Hypophysitis	Grade 2	Withhold	• Administer corticosteroids and initiate hormonal replacements as clinically indicated	• Monitor for signs and symptoms of hypophysitis (including hypopituitarism and adrenal insufficiency)
	Grade 3 or 4	Withhold or permanently discontinue [d]		
Hyperthyroidism	Grade 2	Continue	• Treat with nonselective beta-blockers (eg, propranolol) or thionamides as appropriate	• Monitor for signs and symptoms of thyroid disorders
	Grade 3 or 4	Withhold or permanently discontinue [d]		
Hypothyroidism	Grade 2, 3 or 4	Continue	• Initiate thyroid replacement hormones (eg, levothyroxine or	• Monitor for signs and symptoms of thyroid disorders

irAEs	Toxicity Grade (CTCAE v5.0)	Action With Pembrolizumab	Corticosteroid and/or Other Therapies	Monitoring and Follow-up
			liothyronine) per standard of care	
Nephritis: grading according to increased creatinine or acute kidney injury	Grade 2	Withhold	• Administer corticosteroids (prednisone 1 to 2 mg/kg or equivalent) followed by taper	• Monitor changes of renal function
	Grade 3 or 4	Permanently discontinue		
Neurological Toxicities	Grade 2	Withhold	• Based on severity of AE administer corticosteroids	• Ensure adequate evaluation to confirm etiology and/or exclude other causes
	Grade 3 or 4	Permanently discontinue		
Myocarditis	Grade 1	Withhold	• Based on severity of AE administer corticosteroids	• Ensure adequate evaluation to confirm etiology and/or exclude other causes
	Grade 2, 3 or 4	Permanently discontinue		
Exfoliative Dermatologic Conditions	Suspected SJS, TEN, or DRESS	Withhold	• Based on severity of AE administer corticosteroids	• Ensure adequate evaluation to confirm etiology or exclude other causes
	Confirmed SJS, TEN, or DRESS	Permanently discontinue		
All Other irAEs	Persistent Grade 2	Withhold	• Based on severity of AE administer corticosteroids	• Ensure adequate evaluation to confirm etiology or exclude other causes
	Grade 3	Withhold or discontinue based on the event [e]		
	Recurrent Grade 3 or Grade 4	Permanently discontinue		

AE(s)=adverse event(s); ALT= alanine aminotransferase; AST=aspartate aminotransferase; CTCAE=Common Terminology Criteria for Adverse Events; DRESS=Drug Rash with Eosinophilia and Systemic Symptom; GI=gastrointestinal; IO=immuno-oncology; ir=immune related; IV=intravenous; SJS=Stevens-Johnson Syndrome; T1DM=type 1 diabetes mellitus; TEN=Toxic Epidermal Necrolysis; ULN=upper limit of normal.

Note: Non-irAE will be managed as appropriate, following clinical practice recommendations.

[a] AST/ALT: >3.0 to5.0 x ULN if baseline normal; >3.0 to 5.0 x baseline, if baseline abnormal; bilirubin:>1.5 to 3.0 x ULN if baseline normal; >1.5 to 3.0 x baseline if baseline abnormal

irAEs	Toxicity Grade (CTCAE v5.0)	Action With Pembrolizumab	Corticosteroid and/or Other Therapies	Monitoring and Follow-up

[b] AST/ALT: >5.0 to 20.0 x ULN, if baseline normal; >5.0 to 20.0 x baseline, if baseline abnormal; bilirubin:>3.0 to 10.0 x ULN if baseline normal; >3.0 to 10.0 x baseline if baseline abnormal

[c] AST/ALT: >20.0 x ULN, if baseline normal; >20.0 x baseline, if baseline abnormal; bilirubin: >10.0 x ULN if baseline normal; >10.0 x baseline if baseline abnormal

[d] The decision to withhold or permanently discontinue pembrolizumab is at the discretion of the investigator or treating physician. If control achieved or ≤ Grade 2, pembrolizumab may be resumed.

[e] Events that require discontinuation include, but are not limited to: encephalitis and other clinically important irAEs (eg, vasculitis and sclerosing cholangitis).

Patient should receive appropriate supportive care measures as deemed necessary by the treating physician. Suggested supportive care measures for the management of AEs with potential immunologic etiology are outlined along with the dose modification guidelines in the table above. Where appropriate, these guidelines include the use of oral or IV treatment with corticosteroids, as well as additional anti-inflammatory agents if symptoms do not improve with administration of corticosteroids. Note that several courses of steroid tapering may be necessary as symptoms may worsen when the steroid dose is decreased. For each disorder, attempts should be made to rule out other causes such as metastatic disease or bacterial or viral infection, which might require additional supportive care. The treatment guidelines are intended to be applied when the Investigator determines the events to be related to pembrolizumab.

Note: If after the evaluation of the event, it is determined not to be related to pembrolizumab, the Investigator does not need to follow the treatment guidance. Refer to the table above for guidelines regarding dose modification and supportive care. It may be necessary to perform conditional procedures such as bronchoscopy, endoscopy, or skin photography as part of evaluation of the event.

Note: For grade 1 or grade 2 amylase and/or lipase, in the absence of symptomatic pancreatitis, patients will continue with treatment. Treatment with pembrolizumab will be withheld for grade 3 amylase and/or lipase changes, or if symptoms of pancreatitis with grade 2 amylase and/or lipase. Pembrolizumab may be resumed if asymptomatic and laboratory changes are ≤ grade 2.

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

NCI CTCAE Grade	Treatment	Premedication at Subsequent Dosing
Grade 1 Mild reaction; infusion interruption not indicated; intervention not indicated	Increase monitoring of vital signs as medically indicated until the participant is deemed medically stable in the opinion of the investigator.	None
Grade 2 Requires therapy or infusion interruption but responds promptly to symptomatic treatment (e.g., antihistamines, NSAIDs, narcotics, IV fluids); prophylactic medications indicated for ≤24 hrs	**Stop Infusion.** Additional appropriate medical therapy may include but is not limited to: IV fluids Antihistamines NSAIDs Acetaminophen Narcotics Increase monitoring of vital signs as medically indicated until the participant is deemed medically stable in the opinion of the investigator. If symptoms resolve within 1 hour of stopping drug infusion, the infusion may be restarted at 50% of the original infusion rate (e.g. from 100 mL/hr to 50 mL/hr). Otherwise dosing will be held until symptoms resolve and the participant should be premedicated for the next scheduled dose. **Participants who develop Grade 2 toxicity despite adequate premedication should be permanently discontinued from further study drug treatment**	Participant may be premedicated 1.5h (± 30 minutes) prior to infusion of pembrolizumab with: Diphenhydramine 50 mg po (or equivalent dose of antihistamine). Acetaminophen 500-1000 mg po (or equivalent dose of analgesic).
Grades 3 or 4 Grade 3: Prolonged (i.e., not rapidly responsive to symptomatic medication and/or brief interruption of infusion); recurrence of symptoms following initial improvement; hospitalization indicated for other clinical sequelae (e.g., renal impairment, pulmonary infiltrates) Grade 4: Life-threatening; pressor or ventilatory support indicated	**Stop Infusion.** Additional appropriate medical therapy may include but is not limited to: Epinephrine** IV fluids Antihistamines NSAIDs Acetaminophen Narcotics Oxygen Pressors Corticosteroids Increase monitoring of vital signs as medically indicated until the participant is deemed medically stable in the opinion of the investigator. Hospitalization may be indicated. **In cases of anaphylaxis, epinephrine should be used immediately. **Participant is permanently discontinued from further pembrolizumab treatment.**	No subsequent dosing
Appropriate resuscitation equipment should be available at the bedside and a physician readily available during the period of drug administration. For further information, please refer to the Common Terminology Criteria for Adverse Events v. 4.0 (CTCAE) at http://ctep.cancer.gov		

8.1 Dosing Delay (reasons other than treatment related AEs):
Pembrolizumab may be interrupted for situations other than treatment-related AEs such as medical / surgical events or logistical reasons not related to study therapy. Participants should be placed back on study therapy within 3 weeks of the scheduled interruption, unless otherwise discussed with the Sponsor. The reason for interruption should be documented in the patient's study record. When the subject resumes treatment with pembrolizumab they should skip the missed cycle and resume at the next cycle (i.e. skip C7 and resume at C8D1).

If a vaccine only visit needs to be delayed/missed for reasons other than treatment related AEs, reach out to Sponsor Investigator for further guidance.

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, total bilirubin, alkaline phosphatase, amylase, creatine kinase (CK), thyroid stimulating hormone (TSH), LDH and cortisol. (Note: Cortisol will only be collected when on treatment, therefore cortisol is not due at screening, EOT or 28d Follow Up visits.) Whenever a CBC is indicated, this will include differential and platelet count. This plan is summarized in the Schema.

Because one biomarker endpoint (taking place at UWCCC lead site only) is to evaluate metastatic tissue prior to and after treatment, up to 12 patients (ideally 6 per study arm) with disease amenable to biopsy will initially be requested to undergo one pretreatment biopsy and up to two post-treatment biopsies roughly three months after the start of treatment. It is our intent to have a minimum of 6 patients per study arm (up to 12 patients total) with evaluable pre-treatment and post-treatment biopsies, with post-treatment biopsies obtained from the same site as the pre-treatment biopsy and a second post-treatment biopsy from a clinically responding (or non-responding, if the initial site is a responding lesion) lesion if scans suggest a mixed response. If this is reached, subsequent patients with viable lesions amenable to biopsy will 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.

TREATMENT:

NOTE: Correlative studies (biopsies, NaF PET/CT, blood for immune analyses, tetanus, and stool sample for microbiome analysis) will only be performed at UWCCC lead site. Other participating sites will not perform these procedures

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 (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.

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

1. Physical examination, including vital signs, symptoms and medications assessment, and ECOG performance score
2. 200-ml peripheral blood (green-top heparinized tubes) and 10 mL peripheral blood (red-top tubes) for sera to evaluate baseline immune responses
3. Tetanus immunization (to take place after baseline blood draw for immunological analysis and prior to day 1). Note: If the Tetanus/Diptheria is not available at the enrolling site at the time of a subject's enrollment, the Tetanus/Diptheria may be omitted (Please notify sponsor-investigator and document the reason for omission. Additionally, document whether the subject received tetanus as part of standard of care in the last year.)
4.
5. Stool sample for microbiome analysis
6. Investigational NaF PET/CT scan – to occur within 4 weeks prior to day 1 (minimum of 6 patients at UWCCC site only, only for patients with known bone metastatic disease)
7. Blood for PT/INR and PTT only for patients undergoing biopsy (must be within 7 days of biopsy for patients not taking Coumadin)
8. Tissue biopsy – metastatic site – to take place within 2 weeks prior to day 1 (selected patients only, minimum of 6 patients at UWCCC site only)

INITIAL IMMUNIZATION CYCLES (CYCLES 1-8):

c. Day 1 (21 +/- 3 days after last cycle day 1)

1. Physical examination, including vital signs, symptoms and medications assessment, and ECOG performance score
2. Blood draw for CBC, differential and platelets, chemistry panel, PSA and PAP (can be performed up to 3 days prior to this visit)
3. Subjects will receive pTVG-HP DNA (Arm 1, or cycles 3, 4, 7 and 8 for Arm 2) or pTVG-AR DNA (cycles 1, 2, 5 and 6 for Arm 2) 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 200 mg dose, administered intravenously over 30 minutes
6. Subjects will be observed for additional 30 minutes for unanticipated adverse events.
7. NOTE: For Cycle 3, Day 1:
    1. 100-ml peripheral blood (green-top heparinized tubes) and 10 mL peripheral blood (red-top tubes) for sera to evaluate immune responses
8. NOTE: For Cycle 5, Day 1:
    1. 100-ml peripheral blood (green-top heparinized tubes) and 10 mL peripheral blood (red-top tubes) for sera to evaluate immune responses
    2. Blood for PT/INR and PTT only for patients undergoing biopsy (may be 7 days up to day of biopsy for patients not taking Coumadin)
    3. Stool sample for microbiome analysis

4. CT scan of abdomen and pelvis (CT of chest as clinically indicated), bone scintigraphy (can be performed +/- 7 days of this visit)
5. NaF PET/CT scan for those patients with baseline scan (can be performed +/- 7 days of this visit)
6. Tissue biopsy of same metastatic lesion as in pre-treatment period, and from second site of differentially responding lesion (if mixed response on NaF PET/CT imaging) (can be performed +/- 14 days of this visit) *If the pre-treatment site is not appropriate for the Week 12 biopsy, a new site may be selected. Archived tissue corresponding to the selected site may be obtained (if available) in order to provide a matched set for comparison.*

d. Day 8 (7 +/- 2 days after day 1 of each cycle)

1. Subjects will receive pTVG-HP DNA (Arm 1, or cycles 3, 4, 7 and 8 for Arm 2) or pTVG-AR (cycles 1, 2, 5 and 6 for Arm 2) 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.

PEMBROLIZUMAB ALONE CYCLES:

e. Cycle 9 (21 +/- 3 days after Cycle 8, day 1)

1. Physical examination, including vital signs, symptoms and medications 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) – NOTE: PSA from this visit used as baseline to determine whether subsequent courses of treatment will be administered
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 (can be performed +/- 7 days of this visit)
4. Pembrolizumab 200 mg dose, administered intravenously over 30 minutes
5. CT scan of abdomen and pelvis (CT of chest as clinically indicated), bone scintigraphy (can be performed +/- 7 days of this visit)

f. Cycles 10 and following (21 +/- 3 days after Cycle 9, day 1)

1. Physical examination, including vital signs, symptoms and medications 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. Pembrolizumab 200 mg dose, administered intravenously over 30 minutes
4. NOTE: Every 4 cycles (i.e. continuing every 12 weeks) at cycles 13, 17, 21, 25, and 29): CT scan of abdomen and pelvis (CT of chest as clinically indicated), bone scintigraphy. Scans can be performed +/- 7 days from day of visit.

5. NOTE: For cycles 13 and 17: 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 (can be performed +/- 7 days of this visit)

If patients are observed in the course of the pembrolizumab alone cycles to have:

1) **absence of radiographic progression**
2) **evidence of PSA rise (25% increase over value obtained at cycle 9 day 1, and minimum of 2 ng/mL increase)**
3) **this occurs prior to cycle 29**

THEN Subjects will undergo 4 vaccine booster cycles in place of the pembrolizumab cycles above, and then resume pembrolizumab alone cycles as above. Immune blood draws (cycles 13 and 17) and CT scans/bone scintigraphy (cycles 13, 17, 21, 25, 29) continue as above.

VACCINE BOOSTER CYCLES

g. Day 1 (21 +/- 3 days after last cycle day 1)

1. Physical examination, including vital signs, symptoms and medications 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 (Arm 1, or cycles 3 or 4 for Arm 2) or pTVG-AR DNA (cycles 1 or 2 for Arm 2) 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 200 mg dose, administered intravenously over 30 minutes
6. Subjects will be observed for additional 30 minutes for unanticipated adverse events.

h. Day 8 (7 +/- 2 days after day 1)

1. Subjects will receive pTVG-HP DNA (Arm 1, or cycles 3 or 4 for Arm 2) or pTVG-AR (cycles 1 or 2 for Arm 2) 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.

Following these 4 vaccine booster cycles, patients resume pembrolizumab alone cycles to complete 32 total cycles. If criteria are met for additional vaccine booster cycles, these can occur prior to study cycle 29.

i. END OF TREATMENT (EOT) VISIT (21 +/- 3 days after cycle 32 day 1)

1. Physical examination, including vital signs, symptoms and medications 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 (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)

j. 28-Day Follow Up Visit (28 +/- 7 days after Off-treatment date, if prior to cycle 32)

Off treatment date is defined as the date the decision was made to take the subject off study treatment.

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 EOT evaluation after cycle 32, however for patients coming off study prior to cycle 32, 28-day follow up visit will include the following:

1. Physical examination, including vital signs, symptoms and medication 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

k. Long-term Follow-Up

1. Subjects will be followed for a total of 5 years. Subjects will be contacted by telephone (if not already being seen in clinic) annually for 2 years following the last study treatment 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. Subjects will be followed for an additional three years (annually for years 3-5) to collect survival status. The specific information to be collected for years 1-2 annually will include:
    - Date of contact
    - Current medications for prostate cancer
    - 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

Years 3-5 will only collect survival status.

l. Participant Withdrawal / Discontinuation Criteria

Participants may discontinue study treatment at any time for any reason or be dropped from the study treatment at the discretion of the investigator should any untoward effect occur. In addition, a participant may be discontinued from study treatment by the investigator or the Sponsor if study treatment is inappropriate, the trial plan is violated, or for administrative and/or other safety reasons. Specific details regarding procedures to be performed at study treatment discontinuation are provided in Section 9 above.

A participant must be discontinued from study treatment but continue to be monitored in the study for any of the following reasons:

- The participant or participant's legally acceptable representative requests to discontinue study treatment
- Confirmed radiographic disease progression as outlined in Section 7
- Any progression or recurrence of any malignancy, or any occurrence of another malignancy that requires active treatment
- Unacceptable adverse experiences as described in Section 8.
- The participant has a medical condition or personal circumstance which, in the opinion of the investigator and/or sponsor, placed the participant at unnecessary risk from continued administration of study treatment.
- Noncompliance with study treatment or procedure requirements
- Recurrent Grade 2 pneumonitis
- Discontinuation of treatment may be considered for participants who have attained a confirmed complete response (CR) and have been treated for at least 8 cycles (at least 24 weeks). These participants may be eligible to resume treatment as per study protocol within the two-year study period.
- The participant is lost to follow-up
- Completion of 35 treatments (approximately 2 years) with pembrolizumab (32 treatments for this particular protocol)
- Administrative reasons

10. RESPONSE MONITORING

m. 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 version 4.0 of the NCI common terminology criteria (CTCAE).

n. Immunological Monitoring

Blood will be collected by peripheral blood draw (up to 210 mL) pre-immunization, and at weeks 6, 12, 24, 36, 48, and 96, 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 will be the primary method of analysis and antigen-specific T-cell proliferation, flow cytometric assays of antigen-specific cytokine secretion, and ELISA tests for antigen-specific antibodies, may be included as other methods of analysis. Some of the samples will be batch shipped to Dr. Zitvogel's laboratory for additional immune analysis as it relates to the gut microbiome. The primary antigens tested will be PAP (experimental), AR LBD (experimental), PSA (negative control), thyroglobulin (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.

i. Quantitative assessment of antigen-specific CD8+ T-cell effector immunity

Antigen-specific (PAP and/or AR LBD) 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 [37]. IFNγ and granzyme B will be preferred analytes evaluated, as these are specifically associated with inflammatory/tissue-destructive (Th1-type, cytolytic) immune responses. Specifically, fresh or 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 peptide library (15-mers spanning the amino acid sequence of the antigen, and overlapping by 11 amino acids), 2 µg/ml AR LBD peptide library, 2 µg/ml PSA peptide library, 2 µg/mL thyroglobulin, 250 ng/ml tetanus toxoid, or 2.5 µg/ml PHA (positive mitogenic control) for 24-48 hours. Plates will then be washed with PBS containing 0.05% Tween-20 and incubated for 2.5 hours at room temperature with 50 µl/well PBS containing 5 µg/ml biotinylated detection antibodies for either IFNγ or granzyme B. After incubation, wells will be washed with PBS, and further incubated with 100 µl/well streptavidin-labeled alkaline phosphatase (BioRad, Hercules, CA) and then developed with 100 µl/well BCIP/NBT colorimetric substrate (BioRad). The colorimetric reaction will be stopped by rinsing the plates under cool tap water, and wells will be allowed to dry completely before spots are enumerated with an ELISPOT automatic plate reader. Fluorometric assays of detection may be substituted for those described above.

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 [36]. 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 and/or AR-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.

ii. Assessment of antigen-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 may be assessed by a PKH26 dye dilution assay as previously reported [36]. 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 pools of peptides spanning the amino acid sequence of PAP, PSA, and AR LBD, 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 [36]. 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 and/or AR LBD-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.

iii. Assessment of antigen-specific T cells by intracellular cytokine staining

PAP-specific and/or AR LBD-specific T-cell cytokine expression by intracellular cytokine staining: As another method of evaluating antigen-specific T cell responses, intracellular cytokine staining may 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 pool of overlapping 15-mer peptides, PSA, AR LBD, 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 and/or AR LBD-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 [36]. 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 antigen-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.

iv. Assessment of antigen-specific antibody immunity

Enzyme-linked immunosorbent assay (ELISA) for the detection of antibodies responses to PAP or AR LBD: The presence of a coexisting humoral immune response to PAP, AR LBD, or other antigens will be evaluated by ELISA using an indirect method similar to that described previously [27]. Specifically, Immulon-4 ELISA plates (Dynex Technologies Inc.) will be coated with 2 µg/ml purified PAP or AR LBD 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 [27]. Other methods, including Luminex bead-based assays, may substitute for these ELISA methods.

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

v. 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 [88, 89, 90]. IgG specific for 126 antigens, including 29 cancer-testis antigens [91, 92] and 97 prostate antigens frequently immunologically recognized [93, 94, 95, 96] will be identified by screening a high-density phage array expressing these individual antigens, as we have previously reported [88, 90, 91]. 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 [88, 97], 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.

o. Histopathology Evaluation

Tissue biopsies will be obtained from metastatic bone lesions from a small subset of subjects (the same lesion per patient) prior to treatment and at week 12. Bone biopsies are required due to their correlation with the NaF PET/CT imaging. If the pre-treatment site is not appropriate for the week 12 biopsy, a new site (bone) may be selected. Archived tissue corresponding to the selected site may be obtained (if available) in order to provide a matched set for comparison. 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. Other stains may be performed as well. Staining and quantification will be reviewed and may include evaluations including: number of 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 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. For patients with evidence of a mixed radiographic response at week 12, two post-treatment biopsies will be performed (from the same pre-treatment site and from a corresponding responding/non-responding site). 2-6 biopsy samples total will be obtained from the selected lesion(s), 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.

If archived tissue is needed, a block should be requested. If a block cannot be obtained, 5 unstained slides (with 5 μm diameter) should be requested.

p. Gut Microbiome Composition Analysis

Stool samples will be collected by patients, according to the IMHS guidelines, before treatment and at week 12. These samples will be collected by patients directly into 4 tubes containing stabilizer mediums, one tube with RNAlater for metagenomics analysis, and three tubes with transport medium for culture and metabolomics studies. Stool samples will be collected within 24 hours of the study visit, and will be stored at -80°C within 24 hours of collection. These will be stored and batch shipped to Dr. Zitvogel's laboratory for analysis along with some of the blood samples collected for research on this study. Laurence Zitvogel's team at the Gustave Roussy Cancer Center (GRCC)-France will perform genomic analysis of microbial DNA extracted from microbiota communities in these stool samples. Metagenomics is a culture-independent technology that allows studying structure and function of all gut microbial genes. They will use a standardized and automated workflow that has been shown previously to improve the quality of assembly, gene prediction and redundancy removal. Bioinformatics experts in this field will

process the data to individualize bacterial, viral and archeal species to identify driver microbes, and identify changes in microbial populations from pretreatment to posttreatment. If samples permit, culturomics methods provide the opportunity to use live bacteria in experiments, such as fecal microbial transplantation (FMT). Moreover, if samples permit, metabolomics analyses will be performed in order to understand the role and influence of bacterial bio-products on the immunomodulators activity. All analysis will be considered exploratory, and conducted in the laboratory of Dr. Zitvogel.

q. NaF PET/CT Imaging

Patients accrued at the UWCCC lead site with biopsiable bone disease will be assessed at baseline and week 12 by NaF PET/CT. Metastatic prostate cancer lesions in bone will first be localized and identified based on functional NaF PET uptake, assisted with the anatomical information provided by CT scans. Segmentation will be performed using an automatic segmentation method (e.g. a fixed SUV threshold), and adjusted with physician guidance. Scans from different time points will be registered to one another using an articulated registration technique employing a rigid registration of skeletal elements (bones) from CT followed by registration optimization by combining with deformable registration of bones and lesions from NaF PET/CT. The lesions between pre-treatment and follow-up scans will be matched to establish longitudinal correspondence of the lesions. For each patient, comprehensive treatment response metrics will be calculated, consisting of SUV_{total} (total disease burden), SUV_{max} (maximum intensity lesion), SUV_{mean} (average intensity), the number of lesions, and total volume of bone lesions. In addition, imaging response metrics will be calculated for each individual lesion. This methodology will specifically be used to assess the growth rate of bone metastatic disease by evaluating changes from baseline to week 12.

NaF-PET/CT IMAGING PROCEDURES: To ensure uniformity as well as optimal data interpretation, a standardized image acquisition protocol will be utilized and the overall imaging analyses will be conducted under the supervision of Dr. Robert Jeraj, PhD (co-investigator).

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 up to 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.

11. STATISTICAL CONSIDERATIONS

r. Overview

This is a 1:1 randomized, open-label, multi-institution phase II trial designed to evaluate the immunological and clinical effect of one versus two DNA vaccines, used as T-cell activating therapies, and given concurrently with pembrolizumab. The rationale for conducting this trial is summarized in Section 2.

s. Objectives

Primary Objective:

To determine the 6-month progression-free survival rate in patients with metastatic, castration-resistant prostate cancer (mCRPC) treated with pembrolizumab in combination with one versus two DNA vaccines as T-cell activating agents

Secondary Objectives:

1. To determine the overall objective response rate (using Prostate Cancer Working Group 3 (PCWG3) criteria)
2. To determine the PSA response rate (decline $\geq$ 50%)
3. To determine the median progression-free survival rate
4. To determine median duration of PSA and/or objective response in responding individuals
5. To determine the overall survival
6. To determine whether the development of antigen-specific Th1 immunity elicited with treatment to either antigen (PAP or AR) is associated with PSA response
7. To evaluate the safety and tolerability of pTVG-HP DNA vaccine with or without pTVG-AR DNA vaccine and pembrolizumab

Exploratory Objectives:

1. To evaluate PAP-specific and AR-specific antibody responses following treatment with pembrolizumab and DNA vaccine(s)
2. To determine whether treatment with either sequence elicits immunologic antigen spread to other prostate associated antigens
3. To determine whether pre-existing antigen-specific immunity is predictive of immunological or objective clinical response
4. To determine, in a subset of patients, whether early changes in bone observed by NaF PET/CT are associated with immunological or objective clinical response, or pathological changes associated with immune-cell infiltration
5. To determine second PSA response rates in patients treated in follow-on courses
6. To determine whether treatment elicits changes in the gut microbiota composition
7. To determine whether changes in gut microbiota are associated with clinical response (PSA decline $\geq$ 50%)

t. Study Design

The study is designed as a randomized, two-arm, open-label, multi-institution phase 2 trial. Study arms will be defined as follows:

Course 1:

Arm 1: 100 µg pTVG-HP administered intradermally (i.d.) days 1, 8 + 200 mg pembrolizumab IV day 1 of 21-day cycles x 8 cycles

Arm 2: 100 µg pTVG-AR i.d days 1, 8 + 200 mg pembrolizumab IV day 1 of 21-day cycles, for cycles 1, 2, 5 and 6 alternating with
100 µg pTVG-HP i.d. days 1, 8 + 200 mg pembrolizumab IV day 1 of 21-day cycles, for cycles 3, 4, 7 and 8

Patients who have not come off trial at the end of cycle 8 (month 6) will continue to receive pembrolizumab at 3-week intervals, up to a total of 2 years (32 cycles) of treatment, or until evidence of radiographic progression. If prior to 2 years, and in the absence of radiographic progression, patients with rise in PSA (25% over month 6 value and with minimum rise of 2 ng/mL) will resume vaccination in follow-on courses:

Arm 1: 100 µg pTVG-HP i.d. days 1, 8 + 200 mg pembrolizumab IV day 1 of 21-day cycles x 4 cycles

Arm 2: 100 µg pTVG-AR i.d days 1, 8 + 200 mg pembrolizumab IV day 1 of q 21-day cycles, for two cycles followed by
100 µg pTVG-HP i.d. days 1, 8 + 200 mg pembrolizumab IV day 1 in 21-day cycles, for two cycles

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

u. Randomization and Stratification

Eligible patients will be randomized to the two study arms with an allocation ratio of 1:1. The randomization will be stratified by institution and prior treatment (or not) with prior second generation AR targeting agents. Treatment arm assignment will be made by biostatics/investigational pharmacy after a patient has been registered.

v. Sample Size and Power Calculation

The expected 6-months PFS rate in Arm 1 (pTVG-HP DNA vaccine and Pembrolizumab) in this patient population is 20-30%. It is hypothesized that adding pTVG-AR DNA vaccine (Arm 2) will increase the 6-months PFS rate to at least 55%. The following table shows the required sample sizes for detecting various differences in the 6-months PFS rates between the two study arms, assuming 80% power and a one-sided 0.10 significance level. As this is a phase II study, a less stringent significance level of 0.10 rather than the traditional 0.05 level will be used for the primary efficacy evaluation.

Required sample sizes to detect a difference of 20-30% vs. 50-60% in the 6-months PFS rates between arms			
	6-months PFS rate in Arm 2		
6-months PFS rate in Arm 1	0.50	0.55	0.60
0.20	54	42	32
0.25	80	58	44
0.30	124	82	58

A sample size of 60 evaluable patients (30 per arm) is proposed for this study. The following table shows the attainable power levels for detecting various differences in the 6-months PFS rates between arms with a sample size of 30 patients per arm at the one-sided 0.10 significance level.

Attainable power levels for detecting various differences in the 6-months PFS rates between arms with the proposed sample size of 30 patients per arm			
	6-months PFS rate in Arm 2		
6-months PFS rate in Arm 1	0.50	0.55	0.60
0.20	88.8%	94.8%	98.0%
0.25	77.1%	87.4%	94.1%
0.30	62.0%	75.8%	86.6%

Hence, the proposed sample size of 60 evaluable patients will provide 77-98% power to detect an anticipated difference in the 6-months PFS rates of 20-25% in Arm 1 vs. 50-60% in Arm 2. Analogously, the sample size will provide between 62-94% power to detect a difference of 25-30% in Arm 1 vs. 50-60% in Arm 2. PASS software (NCSS, LLC) version 12 was used to conduct the samples size calculations.

w. Analysis

i. 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 9.4 or greater.

There are many comparisons made between the two trial arms for multiple measurements, both clinical and laboratory. However, since this is an early phase efficacy trail, no adjustment will be made for multiple comparisons.

ii. 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 with pTVG-HP DNA vaccine alone or in combination with pTVG-AR DNA vaccine and Pembrolizumab to at least week 12). The safety population consists of all subjects who received at least one dose of pTVG-HP DNA vaccine with or without pTVG-AR DNA vaccine and 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.

To attain 60 evaluable participants, the trial may randomize more than 60 participants if any are deemed unevaluable.

iii. 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 a chi-square or Fisher's exact test.

iv. Analysis of Primary Endpoint

The 6-month PFS rate will be calculated for each study arm along with the corresponding two-sided 95% confidence interval which will be constructed using the Wilson score method. Patients who withdraw from the study without a progression or death event before the 6-month assessment will be excluded from this analysis. The stratified (by randomization strata) Mantel-Haenszel test will be used to compare the 6-months PFS rates between study arms.

v. Analysis of Secondary Endpoints

F.5.1. Overall Objective Response Rate and PSA Response Rates

The number of responses will be summarized in tabular format, stratified by study arm. Of note, objective response rate using radiographic criteria will apply only to subjects with RECIST-measurable disease (i.e. not subjects with bone-only metastatic disease). Response rates (overall objective response and PSA response) will be calculated for each study arm along with the corresponding two-sided 95% confidence interval which will be constructed using the Wilson score method. The stratified (by randomization strata) Mantel-Haenszel test will be used to compare the overall objective response rates between study arms.

F.5.2. Progression-Free Survival and Overall Survival

Progression-Free and overall survival will be analyzed using the Kaplan-Meier method. Median PFS and OS will be calculated for each study arm and reported along with the corresponding 95% confidence intervals will which will constructed using the nonparametric Brookmeyer and Crowley method. The stratified (by randomization strata) log-rank test will be used to compare PFS and OS between study arms.

F.5.3. Antigen-specific Th1 immune Response

The number and frequencies of antigen-specific Th1 immune responses will be summarized in tabular format for each study arm and both study arms combined. A generalized linear model with a logit link function will be used to evaluate whether antigen-specific Th1 immunity elicited with treatment to either antigen (PAP or AR) is associated with PSA response. The interaction term between treatment arm and antigen-specific Th1 immune will be included in this model.

F.5.4. 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.

vi. Exploratory Analyses

Generalized linear models with a logit link function will be used to evaluate PAP-specific and AR-specific antibody responses following treatment with pembrolizumab and DNA vaccine(s), and to determine whether treatment with either sequence elicits immunologic antigen spread to other prostate associated antigens. Logistic regression analysis will be utilized to determine whether pre-existing antigen-specific immunity is predictive of immunological or objective clinical response. The interaction term between pre-existing antigen-specific immunity status and treatment arm will be included in this model and evaluated. Analogously, a generalized linear model with subject specific random effects will be used to determine whether early changes in bone observed by NaF PET/CT are associated with immunological or objective clinical response, or pathological changes associated with immune-cell infiltration. The number and frequencies of second PSA responses in patients treated in follow-on courses will be summarized in tabular format. The stratified (by randomization strata) Mantel-Haenszel test will be used to compare second PSA response rates between study arms. Bacterial, viral and archeal species loads identified from the bioinformatics analysis of the microbiome samples will be compared between pre- vs. post-treatment assessment using a paired t-test. The false discovery rate will be controlled using the Benjamini-Hochberg False Discovery Rate (FDR) method. Logistic analyses will be conducted to determine whether changes in gut microbiota are associated with clinical response.

12. ADMINISTRATIVE CONSIDERATIONS

x. 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. Coded stool samples will be initially stored at -80°C in a research laboratory and shipped to Dr. Zitvogel's laboratory for analysis. No patient identifiers, only coded information, will be transmitted to Dr. Zitvogel's laboratory.

y. 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.

z. 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

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

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

5. Any clinical significant laboratory abnormality.

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

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

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

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

Note: In addition to the above criteria, adverse events meeting any of the below criteria, although not serious per ICH definition, are reportable to the Merck in the same timeframe as SAEs to meet certain local requirements. Therefore, these events are considered serious by Merck for collection purposes.
- Is another important medical event
- Is a new cancer (that is not a condition of the study)
- Is associated with an overdose.

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

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

All adverse events will be recorded from time of treatment initiation through 30 days after the final dose of study treatment, as well as any time after these 30 days for events that are believed to be at least possibly related to study treatment. Please note: SAEs,

regardless of relationship, occurring from the time of consent until 90 days after the final dose of pembrolizumab will be collected and reported as per section E.1.

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

B. Disease Oriented Team Meetings

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

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

The review of Protocol Summary Reports (PSRs) enables the UWCCC DSMC to assess whether the study should continue, continue with modifications, be suspended, or be closed. Following their review, the UWCCC DSMC will notify the sponsor-investigator of their recommendation and the authority for continuing, modifying, suspending, or closing the protocol is the responsibility of the applicable sponsor-investigator, Principal Investigator, IRB, FDA or other regulatory authority associated with the protocol.
Based on the risk level of this study, as determined by the UWCCC Protocol Review and Monitoring Committee, PSRs must be submitted to the UWCCC DSMC by the UWCCC site on a quarterly basis.

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

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

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

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

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

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

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

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

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

- Other investigators involved with the study at the UWCCC
- IRB of record for the study conducted at the UWCCC, per the IRB's reporting requirements
- UWCCC DSMC (Refer to section D)
- Sponsor-Investigator
- UW Institutional Biosafety Officer [Andrea Ladd: Andrea.Ladd@wisc.edu] (NOTE: IBC is notified only if there is an unexpected potential biohazard exposure)

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

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

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

D. Serious Adverse Event Reporting

Serious Adverse Events Requiring 24-Hour Reporting

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

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

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

E. Sponsor-Investigator Responsibilities for SAE Review

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

- FDA
- All participating investigators on the study, and the external global sponsor (if applicable).
- Other oversight committees (NIH OSP)
- Study supporters **within 1 day** (For additional details, see section E.1):
    - Merck Co.
      Attn: Merck Global Safety, Worldwide Product Safety
      Fax: 215-661-6229

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

- FDA
- All participating investigators on the study, and the external global sponsor (if applicable)
- Other oversight committees (NIH OSP)
- Study supporters **within 1 day** (For additional details, see section E.1):
    - Merck Co.

Attn: Merck Global Safety, Worldwide Product Safety
Fax: 215-661-6229

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

Merck
For purposes of this study, an overdose of pembrolizumab will be defined as any dose of 1,000 mg or greater (≥5 times the indicated dose). No specific information is available on the treatment of overdose of pembrolizumab. In the event of overdose, the participant should be observed closely for signs of toxicity. Appropriate supportive treatment should be provided if clinically indicated.

If an adverse event(s) is associated with ("results from") the overdose of a Merck product, the adverse event(s) is reported as a serious adverse event, even if no other seriousness criteria are met.

If a dose of Merck's product meeting the protocol definition of overdose is taken without any associated clinical symptoms or abnormal laboratory results, the overdose is reported as a non-serious Event of Clinical Interest (ECI), using the terminology "accidental or intentional overdose without adverse effect."

All reports of overdose with and without an adverse event must be reported within 24 hours to the Sponsor-Investigator and within 2 working days hours to Merck Global Safety. (Attn: Worldwide Product Safety; FAX 215-661-6229)

For the time period beginning when the study drug is initiated until treatment allocation/randomization, any serious adverse event, or follow up to a serious adverse event, including death due to any cause that occurs to any participant must be reported within 24 hours to the Sponsor-Investigator and within 2 working days to Merck Global Safety if it causes the participant to be excluded from the trial, or is the result of a protocol-specified intervention, including but not limited to washout or discontinuation of usual therapy, diet, placebo treatment or a procedure.

For the time period beginning at treatment allocation/randomization through 90 days following cessation of treatment, or 30 days following cessation of treatment if the participant initiates new anticancer therapy, whichever is earlier, any serious adverse event, or follow up to a serious adverse event, including death due to any cause, whether or not related to the Merck product (pembrolizumab), must be reported within 24 hours to the Sponsor-Investigator and within 2 working days to Merck Global Safety.

Additionally, any serious adverse event, considered by an investigator who is a qualified physician to be related to Merck product that is brought to the attention of the investigator at any time following consent through the end of the specified safety follow-up period specified in the paragraph above, or at any time outside of the time period specified in the previous paragraph also must be reported immediately to Merck Global Safety.

All participants with serious adverse events must be followed to outcome.

SAE reports and any other relevant safety information are to be forwarded to the Merck Global Safety facsimile number: +1-215-661-6229

A copy of all 7 and 15 Day Reports and Annual Progress Reports is submitted as required by FDA, European Union (EU), Pharmaceutical and Medical Devices agency (PMDA) or other local regulators. Investigators will cross reference this submission according to local regulations to the Merck Investigational Compound Number (IND, CSA, etc.) at the time of submission. Additionally, investigators will submit a copy of these reports to Merck & Co., Inc. (Attn: Worldwide Product Safety; FAX 215-661-6229) at the time of submission to FDA.

Events of Clinical interest

Selected non-serious and serious adverse events are also known as Events of Clinical Interest (ECI) and must be reported within 2 working days to Merck Global Safety. (Attn: Worldwide Product Safety; FAX 215-661-6229).

For the time period beginning when the consent form is signed until treatment allocation/randomization, any ECI, or follow up to an ECI, that occurs to any participant must be reported within 2 working days to Merck Global Safety if it causes the participant to be excluded from the trial, or is the result of a protocol-specified intervention, including but not limited to washout or discontinuation of usual therapy, diet, placebo treatment or a procedure.

For the time period beginning at treatment allocation/randomization through 90 days following cessation of treatment, or 30 days following cessation of treatment if the participant initiates new anticancer therapy, whichever is earlier, any ECI, or follow up to an ECI, whether or not related to Merck product, must be reported within 2 working days to Merck Global Safety.

Events of clinical interest for this trial include:

1. An overdose of Merck product, as defined above, that is not associated with clinical symptoms or abnormal laboratory results.
2. An elevated AST or ALT lab value that is greater than or equal to 3X the upper limit of normal and an elevated total bilirubin lab value that is greater than or equal to 2X the upper limit of normal and, at the same time, an alkaline phosphatase lab value that is less than 2X the upper limit of normal, as determined by way of protocol-specified laboratory testing or unscheduled laboratory testing.*

*Note: These criteria are based upon available regulatory guidance documents. The purpose of the criteria is to specify a threshold of abnormal hepatic tests that may require an additional evaluation for an underlying etiology.

14. POTENTIAL RISKS AND BENEFITS, AND PROCEDURES TO MINIMIZE RISK

aa. Potential Risks

i. From immunization with DNA plasmid encoding PAP or AR LBD

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.

With respect to those subjects in Arm 2 also receiving immunization targeting the androgen receptor (AR), there is a theoretical risk of unwanted immune reactions to tissues expressing the AR. The androgen receptor is expressed at highest levels in prostate tissue, but is also expressed in other tissues, notably bladder, skeletal muscle, liver, testis, and brain. Thus it remains theoretically possible that autoimmune reactions could occur to these tissues. Of note, we have previously identified that patients with prostate cancer can have existing immune responses specific for the AR LBD, and do not have evidence of autoimmune toxicity [53]. Thus it is also conceivable that immune responses could be elicited without evidence of toxicity. We have also immunized tumor-bearing mice for up to one year, and observed an increase in survival without evidence of toxicity. However, in order to evaluate 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, will be evaluated at regular intervals, as outlined above. The serum creatine kinase level will be used as a serum marker to monitor for evidence of subclinical muscle toxicity, and serum measures for evidence of liver dysfunction will also be monitored. Subjects with evidence of autoimmune disease believed to be secondary to the immunizations will be taken off study and treated with corticosteroids (if required), as described above.

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 [98]. 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.

ii. 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, hypoparathyroidism 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.

iii. 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

iv. From tetanus immunization

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

v. 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 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.

vi. From NaF PET/CT

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

vii. 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.

bb. Potential Benefits

No benefits are guaranteed. It is hoped that individual patients treated with a DNA vaccine 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, AR, 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

cc. 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.

dd. 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 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.

ee. 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. Future research will be cancer specific and may involve investigations such as genetic analysis. 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. All samples and data that are shared outside of the University of Wisconsin will be coded and all personal identifiers removed. Only Dr. McNeel and the study team will have access to this information. Subjects wishing to withdraw their banked samples will be required to contact the study team with their request, otherwise their samples may be used indefinitely for cancer research. All research samples and data derived from these specimens will be maintained indefinitely in the laboratory of Dr. McNeel. All information with patient identifiers or medical history information will be kept in locked cabinets or secured databases available only to the study personnel to maintain patient confidentiality.

ff. 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.

gg. 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 physician INDs for the pTVG-HP and pTVG-AR DNA vaccines.

UW Principal Investigator:

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

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