Abbreviated Title: Docetaxel & PROSTVAC in mCSPC
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Title: Docetaxel and PROSTVAC for Metastatic Castration Sensitive Prostate Cancer

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Investigational Agents:

Drug Name	PROSTVAC-V/F	Docetaxel
IND Number	15455	15455
Sponsor	Center for Cancer Research, NCI	Center for Cancer Research, NCI
Manufacturer	Bavarian Nordic, Inc.	Generic
Supplier	Bavarian Nordic, Inc.	CC Pharmacy

PRÉCIS

Background:

- A phase III trial demonstrated that combining docetaxel and androgen deprivation therapy (ADT) significantly improved survival (57.6 vs 44.0 months (HR=0.56, (0.44-0.70), p <0.0001) for men with metastatic castration sensitive prostate cancer (mCSPC).
- PROSTVAC (developed by the National Cancer Institute [NCI] and licensed to Bavarian Nordic Immunotherapeutics, Mountain View, CA) is a therapeutic cancer vaccine for prostate cancer.

- Preclinical and clinical studies support the potential synergy in the combination of docetaxel and PROSTVAC. The potential to combine docetaxel with vaccine in mCSPC could improve upon the survival advantage that has been previously seen.

Objectives:

Primary

- To determine if PROSTVAC combined with docetaxel is able to induce greater antigen spreading (i.e., a broader immune response) with greater associated response score compared to docetaxel alone after 19 weeks.

Key Eligibility Criteria:

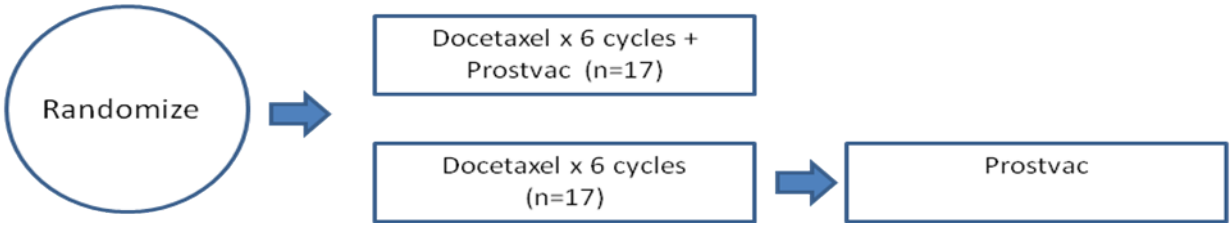
- Must have castrate sensitive prostate cancer (rising PSA and testosterone over 100) or is within 134 days of starting ADT (Arm A or B) or within 28 days of start ADT (Arm C)
- Histopathological confirmation of prostate cancer
- Patients must have metastatic disease
- Patients must have a performance status of 0 to 2 according to the ECOG criteria
- Patients must have adequate bone marrow, hepatic, and renal function

Design

- This is a randomized trial of ADT followed by simultaneous docetaxel 75 mg/m2 q3 weeks x 6 cycles + PROSTVAC q3 weeks x 6 cycles versus ADT followed by sequential docetaxel 75 mg/m2 q3 weeks x 6 cycles followed by PROSTVAC q3 weeks x 6 cycles in men with newly diagnosed mCSPC.
- Patients who have not started ADT or who have been on ADT 28 days or fewer will be assigned to treatment with PROSTVAC for 4 -6 injections followed by docetaxel 75 mg/m2 q3 weeks x 6 cycles.

SCHEMA

<u>Arm A and Arm B</u>



Arm C

Patients who have not started ADT or who have been on ADT for less than 28 days will be assigned to treatment with PROSTVAC for 6 injections followed by docetaxel 75 mg/m2 q3 weeks x 6 cycles. Investigator may use clinical discretion to start docetaxel after 4 vaccines (e.g., 1 dose of vaccinia-PROSTVAC, followed by 3 doses of fowlpox-PROSTVAC).

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STATEMENT OF COMPLIANCE

The trial will be carried out in accordance with International Council for Harmonisation Good Clinical Practice (ICH GCP) and the following:

- United States (US) Code of Federal Regulations (CFR) applicable to clinical studies (45 CFR Part 46, 21 CFR Part 50, 21 CFR Part 56, 21 CFR Part 312, and/or 21 CFR Part 812)

National Institutes of Health (NIH)-funded investigators and clinical trial site staff who are responsible for the conduct, management, or oversight of NIH-funded clinical trials have completed Human Subjects Protection and ICH GCP Training.

The protocol, informed consent form(s), recruitment materials, and all participant materials will be submitted to the Institutional Review Board (IRB) for review and approval. Approval of both the protocol and the consent form must be obtained before any participant is enrolled. Any amendment to the protocol will require review and approval by the IRB before the changes are implemented to the study. In addition, all changes to the consent form will be IRB-approved; an IRB determination will be made regarding whether a new consent needs to be obtained from participants who provided consent, using a previously approved consent form.

1 INTRODUCTION

1.1 STUDY OBJECTIVES

1.1.1 Primary Objectives:

- To determine if PROSTVAC combined with docetaxel is able to induce greater antigen spreading (i.e., a broader immune response) with greater associated response score as defined in section **1.2.5** compared to docetaxel alone after 19 weeks.

1.1.2 Secondary Objectives:

- Evaluate immunologic response among immune subsets (flow cytometry).
- Evaluate antigen-specific immune responses and response scores at 39 weeks in both groups and 1 year in both groups.
- Compare antigen-specific immune responses and response scores at 19 weeks in the combination arm (docetaxel and PROSTVAC) and compared to 39 weeks in the sequence arm (docetaxel followed by PROSTVAC).
- In addition, patients getting PROSTVAC prior to chemotherapy will be evaluated for antigen specific responses after completing vaccine followed by 6 cycles of chemotherapy.
- Evaluate radiographic and biochemical time to progression in all groups.
- Evaluate proportion of patients with PSA >0.2 ng/ml at 6 and 12 months.
- Evaluate changes in the tumor microenvironment with biopsies pre and post (2 cycles of vaccine therapy; alone or combination) when feasible (Biopsies could include primary tumor.)
- Evaluate pharmacogenomic studies to evaluate drug metabolism and transporters.

- Evaluate overall survival.
- The scores from the subset consisting of previously untreated patients on the superior randomized arm will be tested against the patients on the new PROSTVAC then docetaxel arm (Arm C).

1.2 BACKGROUND AND RATIONALE

Among the approximately 220,000 men diagnosed each year with prostate cancer in the United States, approximately 10% of them will be diagnosed with metastatic disease. [1] In addition, for the approximately 90,000 men who cannot be cured of their prostate cancer, many of them will also develop metastatic disease before the initiation of androgen deprivation therapy (ADT). [2] Until recently, ADT was the only standard of care for patients with metastatic castration prostate cancer (mCSPC).

ECOG 3805 was designed to evaluate docetaxel (for six 3-week cycles) with ADT in patients with mCSPC. (Patients were allowed to have started ADT within 120 days of starting Docetaxel.) The results of the study indicated that docetaxel extended overall survival 57.6 vs. 44.0 months (HR=0.61 (0.47-0.80), p=0.0003). In addition, patients treated with ADT+docetaxel for six 3-week cycles there was a delay in progression (development of metastatic castration resistant prostate cancer) of 20.7 vs. 14.7 months (HR=0.56, (0.44-0.70), p <0.0001). [3] Recently, a second similar study has supported these findings demonstrating an improvement in survival when a comparable population of patients received six cycles of docetaxel plus standard of care compared to standard of care alone (65 vs. 43 months; HR=0.73(0.59-0.89), p=0.002). [4] Based on the findings of this study, six cycles of docetaxel + ADT is now a standard of care for patients with mCSPC.

With this new available standard of care option, there is interest to build on these findings. Furthermore, there is a strong rationale (generated from preclinical and clinical studies done here at the NCI within GMB and LTIB) that the benefits of docetaxel could be enhanced by immunotherapy combinations.

1.2.1 Immunotherapy in Prostate Cancer

The goal of therapeutic cancer vaccines is to generate a targeted immune response leading to immune-mediated anti-tumor activity. Sipuleucel-T is a therapeutic cancer vaccine generated from peripheral blood mononuclear cells obtained from individual patients via leukapheresis. This vaccine is generated after a patient's peripheral immune cells are collected via leukapheresis, transported to a regional processing center where they are exposed in vitro to a PAP/GM-CSF fusion protein. At the end of this process, the activated cellular product is re-infused into the patient. A full course of therapy repeats this process 3 times every 2 weeks for 1 month.[5] A phase III trial (n = 512) demonstrated an overall survival benefit for the vaccine (25.8 months vs. 21.7 months; P = 0.032).[6] Based on these overall survival findings, the FDA

approved sipuleucel-T for the treatment of asymptomatic or minimally symptomatic mCRPC, making it the first FDA-approved therapeutic cancer vaccine for the treatment of any malignancy.

PROSTVAC

PROSTVAC (PROSTVAC™; developed by the National Cancer Institute [NCI] and licensed to Bavarian Nordic, Mountain View, CA), an off-the-shelf therapeutic cancer vaccine, offers an alternative strategy to sipuleucel-T. [7, 8] (The LTIB and Bavarian Nordic have an ongoing CRADA for the preclinical and clinical development of PROSTVAC.) To target prostate-specific antigen (PSA), PROSTVAC vaccine employs genetically altered poxviruses to generate an immune response. Administered subcutaneously, the poxviruses deliver the transgenes for the tumor associated antigens (PSA) to antigen presenting cells through cellular infection. Once these pox viruses are within the cellular cytoplasm, the transgenes are processed. The end result is an antigen presenting cell expressing a PSA peptide within the major histocompatibility complex, resulting in PSA-specific cytolytic T lymphocytes activation. [8] (**Figure 1**) This approach does not require expensive, labor-intensive *ex vivo* preparation of patients' peripheral blood. PROSTVAC is thus potentially more logistically and financially feasible over the long-term than sipuleucel-T. [7]

PROSTVAC is very well-tolerated, with common side effects of grade 1 injection-site reactions or flu-like symptoms. [9] PROSTVAC has been investigated in 2 phase II trials in mCRPC, both of which administered the vaccine at monthly intervals until disease progression. An industry-sponsored, placebo-controlled, multicenter trial in 125 mCRPC patients randomized them 2:1 in favor of PROSTVAC; the placebo was an empty poxviral vector containing no transgenes. As was seen in the sipuleucel-T studies, patients receiving vaccine showed no change in TTP, yet had an overall survival benefit (25.1 months with PROSTVAC vs. 16.6 months with placebo; $P = 0.0061$). [6] (**Figure 2**) A second phase II study of PROSTVAC of 32 mCRPC patients at the NCI demonstrated that the vaccine was able to generate a T-cell specific immune response and patients with the greatest magnitude of this response had superior outcomes. [10]. A phase III trial (NCT01322490) of Prostvac monotherapy, however, was reported to have not met its primary endpoint of overall survival in September 2017. Nonetheless, the combination of Prostvac and docetaxel remains scientifically rational and this trial will continue. Furthermore, at this point neither this trial nor the phase III trial have suggested a safety concern for Prostvac.

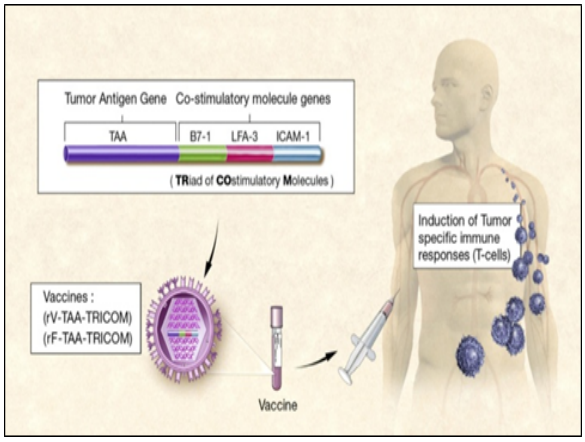

Figure 1. Poxviral vaccine strategy. **Modified poxvirus contains transgenes for the tumor-associated antigen PSA and 3 T-cell costimulatory molecules (TRICOM).**

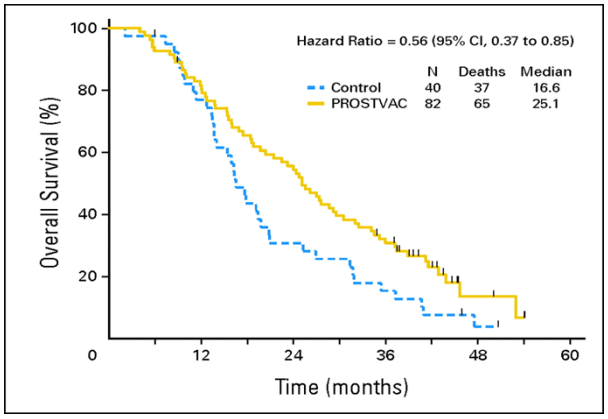

Figure 2. PROSTVAC improved survival in mCRP patients in a randomized multi center phase II.

1.2.2 Rationale for Combining Vaccine with Docetaxel

Preclinical data

There is important preclinical data that has been cultivated from studies in the LTIB supporting the combination of docetaxel and vaccine. Docetaxel has been shown to induce immunogenic modulation resulting in changes in the surface antigen expression of cancer cells. The resulting up-regulation of cell surface molecules like ICAM-1, MUC-1, and MHC class 1 molecules potentially enhancing immune recognition and immune mediated cell lysis by vaccine-activated immune cells. [11] Even non-lethal doses of docetaxel increased sensitivity to antigen-specific cytotoxic T cell killing and a broadening of the immune response resulting in the targeting of multiple antigens (antigen-cascade/spreading). [11, 12]

Clinical Data

The clinical experience in prostate cancer utilizing the combination of chemotherapy and vaccines was previously investigated in an NCI clinical study. The phase II trial randomized 28 patients to docetaxel plus viral-vector based vaccine (vaccinia and fowlpox virus expressing PSA gene and the co-stimulatory gene B7.1) vs. vaccine alone. The combination was deemed safe; immune responses, as measured by antigen-specific-T cell activation against PSA antigen were equivalent in both arms suggesting that antigen specific T-cell activation at was unaffected by weekly docetaxel and the accompanying dexamethsone comedication. [9]

Additional data from a study involving breast cancer may have particular relevance in prostate cancer due to the use of docetaxel in combination with PANVAC, a pox-viral vaccine, targeting MUC-1 and CEA and encoding three co-stimulatory molecules (TRICOM), similar to PROSTVAC. [13] This study randomized 48 patients to either docetaxel alone or docetaxel plus the PANVAC vaccine. The final data suggested increased clinical benefit based on progression free survival (PFS) when vaccine was combined with docetaxel vs. docetaxel alone (PFS of 7.9 vs. 3.9 months, HR = 0.65, one-sided p=0.09). [14] (**Figure 3**)

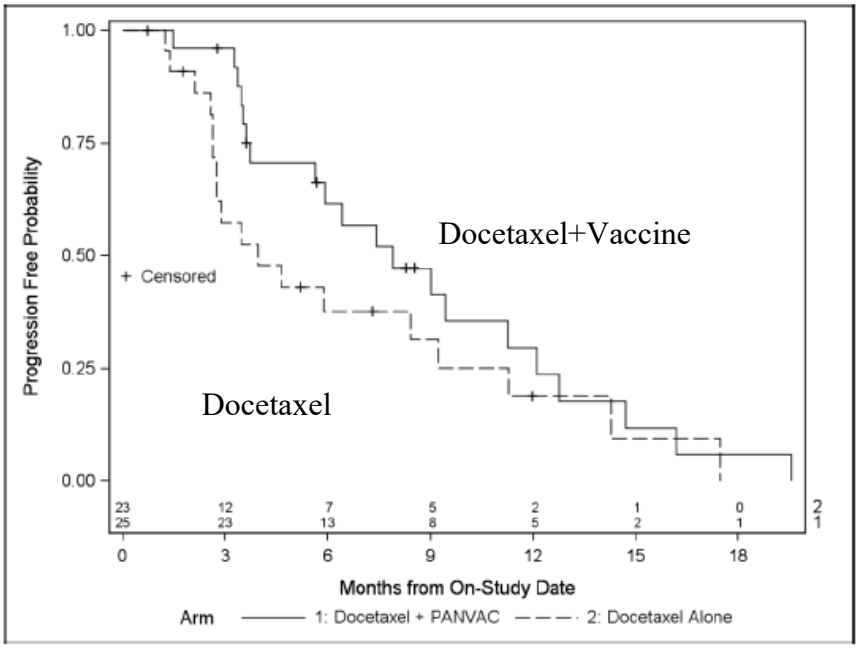

Figure 3. Comparison of progression-free survival (PFS). **Medians: Docetaxel plus PANVAC vaccine 7.9 months versus docetaxel alone 3.9 months median PFS (p = 0.09, one-sided, meets predefined study statistical goal), HR = 0.65 (95% CI: 0.34 – 1.14). [14]**

The combination of preclinical and clinical studies performed by our group here at the NCI provide a strong foundation for combining docetaxel and PROSTVAC in metastatic castration sensitive prostate cancer

1.2.3 Initiating Therapies with Vaccines Earlier May Enhance Clinical Outcomes

Retrospective data from several studies suggests that therapeutic cancer vaccines may slow the growth rate of tumors and this can have important implications. Using a mathematical equation to calculate tumor growth, survival can be predicted in several cancers including using disease-specific metrics, such as M-spike in multiple myeloma, tumor size in renal cell cancer and PSA in prostate cancer. (Tumor growth rates are based on tumor growth and regression kinetics and have been modeled using several relevant disease parameters. [15, 16] Using this equation, an analysis of 5 prostate cancer studies conducted at the NCI, including one using the vaccine PROSTVAC, evaluated the impact of the various treatments on tumor growth rates and survival which can be calculated based on these growth rates. [17] When the 4 NCI trials that used cytotoxic were evaluated, the results were consistent with other cytoreductive therapies in other tumor types. Chemotherapy-based treatment temporarily altered or decreased the growth rate while patients were on the therapy, but once the therapy became less effective or was discontinued, the growth rate reverted to pretreatment rates and death was predictable based on the mathematical equation and off-study growth kinetics. [17] The vaccine trial involving PROSTVAC, however, was not consistent with this previously demonstrated model. In this trial, no change in tumor growth rate was seen while patients were on-study, consistent with that the trial had a median disease progression by conventional measures at 3 months. [10] However, unlike patients on chemotherapy, these patients' mortality could not be predicted by their post-treatment, off-study growth rates. Instead, these patients' survival times were more prolonged than would have been anticipated by their off-study growth rates. A separate analysis of this population indicated that subsequent therapies did not differentially improve survival for patients with better vs. poor outcomes, suggesting that benefit from ensuing treatment does not account for this apparent disconnect between off-study growth rate and a far better survival time than would have been predicted using this model.

One hypothesis regarding this outcome appears to be supported by data from other trials. Perhaps the tumor growth rate, which was not significantly altered in the short term, was impacted in the time after treatment to such a degree that it improved survival. [18] While this would be an unrealistic outcome with chemotherapy or hormonal therapy alone, given their transient clinical impact, it is plausible with immune therapies, which can initiate a persistent immune response that may evolve through antigen spreading well beyond the treatment period. Other vaccine trials in prostate cancer have supported this hypothesis.

In addition, larger clinical trials may support the hypothesis that some modern immune therapies alter tumor growth rate without altering short-term disease progression, as evaluated by

conventional measures such as PSA or RECIST-based imaging assessments. Indeed, multiple phase III trials of sipuleucel-T in mCRPC, a phase III trial of ipilimumab in metastatic melanoma, and the phase II trial of PROSTVAC in mCRPC all reported improved survival without changes in median disease progression frees survival. [6, 19] These findings are consistent with the phenomenon of altered tumor growth kinetics following immune-based treatment, which affect long-term survival in the absence of any short-term decrease in tumor burden.

If it is possible for therapeutic cancer vaccines to induce a dynamic and sustained immune response that indeed slows the growth rate of tumors, then it would appear rationale that initiating such an immune response earlier would have a greater impact than initiating that immune response later in the disease process.

This is one aspect of the new CHAARTED data that is so appealing. Six cycles of docetaxel with ADT improved survival by large magnitudes (greater than a year) over a 4-5 year timeline, providing the potential to enhance survival with immunotherapy by impacting growth rate and having more impact on survival than has been seen in previous studies, where anticipated survival is shorter.

1.2.4 Antigen Spreading/Antigen Cascade as an Endpoint

Perhaps the greatest aspect of immunotherapy is its potential to induce an immune response that has the ability to evolve *in vivo*.[20] When immune cells lyse cancer cells in the presence of an activated immune response, it is possible that additional antigens will be taken up and processed by the immune system. These secondary antigen targets may be more relevant to the overall anti-tumor response as they have the ability to be recognized by the immune system and will likely be present across multiple clonal populations of cancer cells. It is then possible that when these secondary antigens are targeted and a subsequent population of cancer cells is killed, additional antigens could be targeted in a similar manner.[21] This phenomenon known as antigen spreading or antigen cascade has been demonstrated in multiple studies and in multiple tumor types. Some studies have associated the greater breadth of the immune response (i.e. more antigens being targeted) with improved clinical outcomes.[22-24] Based on these data, it is likely that initial immune target is not as important as the ability of initial immune response to lead to antigen cascade *in vivo* resulting in a broader, adaptable, and personalized anti-tumor immune effect. As immunotherapies become more refined and immune monitoring improves, it would seem that the breadth of immune response should be an important focus in the development of future immune strategies.

It remains unclear whether vaccines combined with an immune stimulatory chemotherapy such as docetaxel enhances the immune response more than sequential use. The use of vaccine after chemotherapy when the tumor has been cytoreduced and the volume associated

immunosuppressive effects of the tumor have been diminished may also be a sound treatment strategy. [25] These two treatment strategies have yet to be compared in prospective randomized trials and could inform future clinical trials with vaccines and other immunotherapies.

1.2.5 Evaluating Immune Response

Our group in the LTIB has previously evaluated immunologic parameters in clinical trials with PROSTVAC among several immunotherapy studies. A previous trial in mCRPC patients with PROSTVAC alone suggested that patients with greatest magnitude of T-cell-specific response against PSA had favorable clinical outcomes. [10] That same trial also suggested that changes in regulatory T-cell function were also associated with improved clinical outcomes. [26] While thethese findings are not surrogate markers of response, they have improved our knowledge of a vaccine-generated immune response and provided a better understanding of what factors are potentially important in mounting a sufficient anti-tumor immune response that could be associated with improved clinical outcomes. These and other data have allowed us to optimize vaccines in subsequent clinical trials. Few groups are as well positioned and have the experience as the LTIB to conduct this rigorous immune testing from clinical samples of patients treated with PROSTVAC.

PSA-Specific Immune Responses

While many clinical trials have been done in biochemically recurrent prostate cancer, there is little reported on the impact of those therapies on the immunologic response that is near the depth and scope that is proposed in this trial. The proposed immunologic studies will be done on all patients by the Laboratory of Tumor Immunology and Biology at the NCI Clinical Center under the direction of Dr. Jeffrey Schlom.

Unlike previous studies involving PROSTVAC, which evaluated immune responses to a single 9 amino acid section of the 244 amino acid PSA protein, the immune analysis in this trial will evaluate overlapping 15 amino acid sequences of the entire PSA protein. (**Figure 4**). This more extensive analysis will provide a more thorough understanding of the immune response initiated by PROSTVAC and a greater opportunity for clinical correlations. This type of analysis has not been previously prospectively performed in biochemically recurrent prostate cancer patients. Advantages of this approach include the ability to assess both CD4 and CD8 T cells, identification of multifunctional T cells (those producing ≥2 cytokines), and identification of T lymphocytes with lytic potential (CD107a expression). (**Table 1**).

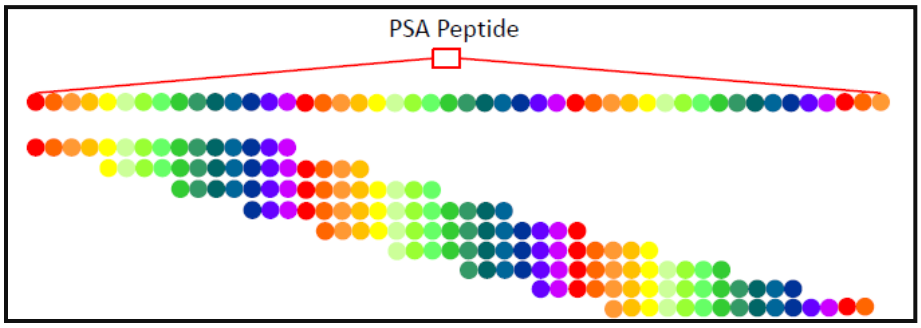

Figure 4. Overlapping PSA Peptide Assessment. **This innovative approach to evaluating immune response allows for assessment of immune response to the entire PSA protein using overlapping 15 amino acid sequences. (Previous techniques have focused on assessing just one of these peptides.)**

		Immune Responses to PSA							
		CD4				CD8			
	PT	CD107a	IFNg	IL2	TNF	CD107a	IFNg	IL2	TNF
Cohort 1 – No Vaccine	11								
	13								
	20								
	22					1427			274
	25								630
	3								
	5								
	10								
Cohort 2 with Prostvac	2		786		374	5269	453		323
	8		345			633			
	12								
	18				402				
	21								
	24					1242			
	14	821							
	16								
	26	815							
	27								

Table 1. Preliminary Data from a Trial of PROSTVAC in mCRPC**. This table shows how this multi peptide approach can be used in this proposal. In this previous (unpublished) trial, 2 cohorts of patients were evaluated using the proposed methods. (Responses are listed as absolute # of CD4 or CD8 T Lymphocytes Producing Cytokine or Positive for CD107a per $1x10^6$ cells plated.) This readout shows the breadth of this analysis as both CD4 and CD8 tell cells are evaluated using multiple parameters (cytokine production signifying activation – IFN-γ, IL-2 and TNF-α) and lytic potential as measured by CD107a expression. Had only one peptide been used to analyze this immune response it is likely that many of these immune responders would not have been identified. (unpublished)**

Defining a Response and the Response Score

A positive "response" will be defined as increased CD107a expression or increased intracellular cytokine production for given antigen for CD4 or CD8 cells. For example, Patient #2 in Table 1 will be scored has having 5 responses (CD4: INFg, TNF and CD8: CD107a, INFg and TNF).

In order to give added weight to the spread antigens (MUC1 and Brachyury) they will be weighted 150% higher than PSA antigens. Thus the scoring will be as follows:

For PSA:
1 point each of the four CD4 parameters (CD107a, IFN-γ, IL-2 and TNF-α) – max total of 4
1 point each of the four CD8 parameters (CD107a, IFN-γ, IL-2 and TNF-α) – max total of 4
Total possible score for PSA response is 8

For MUC1:
1.5 points each of the four CD4 parameters (CD107a, IFN-γ, IL-2 and TNF-α) – max total of 6
1.5 points each of the four CD8 parameters (CD107a, IFN-γ, IL-2 and TNF-α) – max total of 6
Total possible score for PSA response is 12.

For Brachyury:
1.5 points each of the four CD4 parameters (CD107a, IFN-γ, IL-2 and TNF-α) – max total of 6
1.5 points each of the four CD8 parameters (CD107a, IFN-γ, IL-2 and TNF-α) – max total of 6
Total possible score for PSA response is 12.

The primary comparison between the 2 arms will be at 19 weeks, with additional comparisons at 39 weeks and 1 year. In addition, a comparison will be made when combination therapy is complete in the docetaxel + PROSTVAC arm (19 weeks) and when sequential therapy is stopped in the docetaxel followed by PROSTVAC arm (39 weeks).

1.2.6 Flow Cytometry Analysis of Immune subsets (Biomarker Development)

As part of the extensive immune interrogation, peripheral immune cells will be evaluated by 30 markers to assess 127 immune subsets. (**Table 2**). This methodology has been previously described (13). This added analysis will provide preliminary data to develop a Peripheral Immunoscore based on the frequency of specific pre-determined immune cell subsets in the blood of patients prior to therapy. The score is calculated from the changes in frequency of certain immune cell subsets after vaccine treatment. The resultant panel of markers would reflect immune response to the vaccine.

Table 2. Analysis of Peripheral Immune Cells

1. **CD4**: Helper T lymphocytes (32 subsets)
2. **CD8**: Cytotoxic T lymphocytes (29 subsets)
    - **Markers of PD-1 pathway and T cell activation (in CD4 and CD8):**
        - **EOMES:** activation
        - **TCR-αβ:** activation
        - **Tbet:** activation
        - **BATF:** activation/exhaustion
    - **Maturation status of T lymphocytes (in CD4 and CD8)**:
        - **Naïve**: $CD45RA^{+}$ $CCR7^{+}$
        - **Effector Memory**: $CD45RA^{-}$ $CCR7^{-}$
        - **Terminal (EMRA)**: $CD45RA^{+}$ $CCR7^{-}$
    - **T lymphocyte markers (in CD4 and CD8)**:

  - **CTLA-4:** inhibition
  - **PD-1:** activation/inhibition
  - **PD-L1:** activation/cross-inhibition
  - **TIM-3:** inhibition
  - **ICOS**: activation (only on CD4)

3. **Tregs**: Regulatory T lymphocytes ($CD4^+$ $CD25^+$ $FoxP3^+$ $CD127^-$) (7 subsets)
   - **CD45RA**: Tregs highly expandable *in vitro*
   - **CTLA-4**: Treg suppression
   - **CD49d:** "contaminating" effector lymphocytes (non-Tregs)
   - **ICOS**: Treg suppression
   - **PD-1:** activation/inhibition
   - **PD-L1:** cross-inhibition
4. **B lymphocytes**: $CD19^+$ (5 subsets)
   - **CTLA-4:** inhibition
   - **TIM-3:** inhibition
   - **PD-1:** activation/inhibition
   - **PD-L1:** cross-inhibition
5. **NK**: Natural killer cells ($CD56^+$ $CD3^-$) (20 subsets)
   - **$CD16^+$ $CD56^{br}$**: Functional intermediate, lytic and cytokine production
   - **$CD16^+$ $CD56^{dim}$**: Mature NK, cytokine production
   - **$CD16^-$ $CD56^{br}$:** Immature, abundant in human placenta
   - **$CD16^-$ $CD56^{dim}$**: non-lytic, non-cytokine production
   - **TIM-3:** activation
   - **PD-1:** activation/inhibition
   - **PD-L1:** cross-inhibition
6. **NK-T**: $CD56^+$ $CD3^+$ (4 subsets)
   - **TIM-3:** activation
   - **PD-1:** activation/inhibition
   - **PD-L1:** cross-inhibition
7. **cDCs (Conventional DCs):** $CD3^-CD56^-CD1c^+CD303^-$ (5 subsets)
8. **pDCs (plasmacytoid DCs):** $CD3^-CD56^-CD1c^-CD303^+$ (5 subsets)
   - **Markers of DC activation**
     - **CD83:** activation
     - **TIM-3:** inhibition
     - **PD-1:** activation/inhibition
     - **PD-L1:** cross-inhibition
9. **MDSCs**: Myeloid-derived suppressor cells ($CD11b^+$ $HLA\text{-}DR^{low/-}$ $CD33^+$) (20 subsets)
   - **CD14**: Common Myeloid Marker (high in monocytes, dim in granulocytes)
   - **CD15**: Granulocyte marker
   - **CD16**: most immature monocytic MDSCs
   - **PD-1:** activation/inhibition
   - **PD-L1:** cross-inhibition

1.2.7 Pharmacogenomic Studies

One blood sample per patient will be collected in a purple top tube for pharmacogenetic studies to analyze the genomic DNA and assess genotype of the most relevant drug metabolizing enzymes and transporters (DMET). DNA will be analyzed on a DMET Plus (Affymatrix) genotyping platform that tests for 1,936 genetic variations in 225 drug disposition genes, including 47 CYP (phase I metabolism) genes, 13 non-CYP (phase I metabolism) genes, 78 phase II metabolizing genes (including UGTs), 63 transporters, 4 genes involved in facilitation of drug transporters, 9 genes involved in global regulation of drug metabolizing/transporting proteins, 4 drug binding proteins, and 4 drug targets.

Of specific interest to DTX are polymorphisms in CYP3A4/5 (metabolism), ABCB1 (transport), and OATP1B3 (transport), all of which are included in the DMET analysis. OATP1B3 genotype is particularly interesting based on a previous study that demonstrated a polymorphism that is predictive of response to ADT therapy failure. [27] Men with at least one copy of the wild-type allele (T) at position 334 have a shorter time (p=0.048) from ADT to AI (1.2 yrs) versus men with two copies of the mutant (G) allele (1.57 yrs), consistent with *in vitro* data that the wild-type *SLCO1B3* is more functional at importing testosterone vs the mutant (G-allele) [28].

1.2.8 Experimental Imaging May Help improve our understanding of castration sensitive disease progressing to castration resistant prostate cancer

Conventional imaging in prostate cancer primarily consists of computed tomography (CT) scans and *technetium*-99m bone scans, which have been used to evaluate prostate cancer for nearly 3 decades, but the field has not benefited from newer technology that is rapidly advancing the field of radiology. In collaboration with the NCI's Molecular Imaging Program under the direction of Dr. Peter Choyke, several experimental and emerging imaging platforms will be evaluated with the ultimate goal of prospectively evaluating these tools both individually and in combination as a means to assess castration sensitive disease and its ultimate progression to castration resistant disease. Each platform has a sound rationale for its use in this population and are detailed below.

Endorectal MRI: For local recurrence or locally untreated prostate cancer, the preferred method is multiparametric MRI typically with an endorectal coil at 3T. MRI has the ability to localize small deposits of disease within the prostate (in the case of prior radiation therapy) or in the prostate bed (in the case of radical prostatectomy). Typically, the latter are found around the urethra, often near the anastomosis. These are detected with high resolution MRI particularly employing T2 weighted and dynamic contrast enhanced (DCE) MRI. Recurrences typically enhance early and more profoundly than surrounding tissue. Because MRI does not involve radiation it is highly amenable to repeated scanning for therapeutic monitoring.

Sodium Fluoride (NaF) PET: For advanced disease, sodium fluoride PET is the most sensitive method of detecting prostate cancer osteoblastic bone metastases.[29, 30] (**Figure 5**) Sodium

fluoride is incorporated into the bone matrix. Thus during osteoblastic bone formation, there is avid uptake of the radiolabeled agent. Very small doses (3mCi) of activity are needed for satisfactory imaging. Sodium fluoride PET/CT has proven to be substantially more sensitive than conventional Tc-99m bone scans. In addition to being substantially faster than conventional bone scan (can be performed within 1 hour of injection) sodium fluoride PET/CT has the added virtue of providing a registered CT scan to the PET image. Many lesions identified on the PET scan can be correlated with degenerative changes on CT, eliminating them further consideration. Software is now available to automatically detect true positive sodium fluoride images, which can then be monitored over time. Increases in standardized uptake value (SUV) are associated with progressive disease. Thus, sodium fluoride PET/CT is the most sensitive method to detect prostate cancer bone metastases. One limitation of its use in monitoring bone metastases is that there can be a "lagging indicator" of response. Despite response to therapy, some bone metastases continue to show activity on sodium fluoride PET/CT due to the ongoing osteoblastic remodeling. For this reason, substantial changes in SUV are required before one can conclude that disease is progressing or regressing on NaF scans.

1.2.9 Rationale for Arm C

The primary goal of this clinical trial is to evaluate the optimal timing of immunotherapy with chemotherapy as this is largely unknown in medical oncology. The trial was originally designed to answer the question of concurrent vs. sequential (with vaccine second). This was largely due to the fact that we did not anticipate having patients contact us who recently started ADT. In fact, the majority of patients enrolled on this study started ADT under our care or direction. Thus, since the standard therapy for this regimen is to initiate ADT and then subsequently start chemotherapy within 4 months, it provided an opportunity to initiate vaccine prior to chemotherapy and determine if that would be an optimal sequence. Certainly an activated immune response prior to cytotoxic therapy could synergize with the chemotherapy as supported by preclinical data above (section **1.2.2**), but we do not have clinical/immunologic data on this from human studies. This study can provide to address this scientific question,

Given that Arm A and B have nearly completed accrual, we are unable to randomize patients among all 3 Arms. Thus Arm C will be for patients who have started ADT within 1 month of enrollment. The statistical design compensates for the lack of random assignment as much as possible and that is why more patients are required (26 vs. 17). Regardless, this comparison would Arm C to Arm A and B will provide valuable insight into optimal sequencing of immunotherapy with chemotherapy. Furthermore, since the primary endpoints are immunologic and will be assessed at the end of the study, they cannot be influenced by provider knowledge of a non-randomized arm. It is possible that patient variations may exist between Arm A or B vs. Arm C, but that is something that will need to be taken into account with any analysis done. Regardless, the nature of this study would require a future confirmatory study.

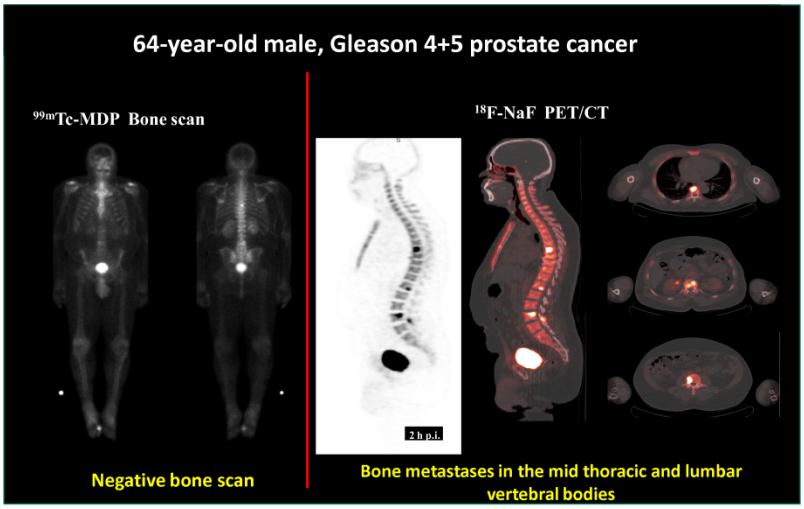

Figure 5. Increase Sensitivity of ^{18}F-NaF PET/CT. **This case illustrates the increased sensitivity of ^{18}F-NaF PET/CT in a patient whose bone scan was negative but PET/CT was positive. The prospective value of such findings in biochemically recurrent prostate cancer remains undefined. Patients evaluated as part of this proposal will be followed prospectively to see if conventional imaging confirms findings such as these after longer follow-up. In addition, the use of multiple radiographic platforms will allow for a composite assessment that could improve the specificity of such findings. This proposal will prospectively**

2 ELIGIBILITY ASSESSMENT AND ENROLLMENT

2.1 ELIGIBILITY CRITERIA

2.1.1 Inclusion Criteria

2.1.1.1 Documented histopathological confirmation of prostate cancer from a CLIA-certified laboratory.

2.1.1.2 Patients must have metastatic disease, defined as at least one lesion on bone scan or at least one lesion that are measurable per RECIST 1.1 (see section **6.3.2**). (Patients who have metastatic disease by these criteria prior to ADT, but then have changes after ADT that diminish the size of these lesions or changes on bone scan are still eligible.)

2.1.1.3 Patients must have a performance status of 0 to 2 according to the ECOG criteria (see **Appendix B**)

2.1.1.4 Patients must have adequate bone marrow, hepatic, and renal function with:

- ANC ≥ 1500/μL, without CSF support
- Platelets ≥ 100,000/μL
- AST(SGOT) ≤ 2.5 x upper limit of normal (ULN);
- ALT(SGPT) ≤ 2.5 x upper limit of normal (ULN);
- Total serum bilirubin ≤ 1.5 x upper limit of normal (ULN), OR in patients with Gilbert's syndrome, a total bilirubin ≤ 3.0)
- Serum albumin ≥ 2.8 g/dL
- Lipase < 2.0 x the upper limit of normal and no radiologic or clinical evidence of pancreatitis

- Creatinine ≤ 1.5 X institutional upper limits of normal

  OR

  Creatinine clearance of ≥ 50 ml/min/1.73 m2 for patients with creatinine levels above institutional normal by 24-hour urine.

2.1.1.5 Willing to travel to the NIH for follow-up visits

2.1.1.6 18 years of age or older.

2.1.1.7 Able to understand and sign informed consent.

2.1.1.8 May have had up to 24 months of ADT (testosterone suppression therapy) in the nonmetastatic setting and are at least 12 months removed from treatment.

2.1.1.9 Men treated or enrolled on this protocol must also agree to use adequate contraception, prior to the study, for the duration of study participation, and 4 months after completion. Sexually active subjects and their female partners must agree to use medically accepted barrier methods of contraception (e.g., male or female condom) during the course of the study and for 4 months after the last dose of study drug(s), even if oral contraceptives are also used. All subjects of reproductive potential must also agree to use both a barrier method and a second method of birth control during the course on the study and for 4 months after the last dose of study drug(s).Should a woman become pregnant or suspect she is pregnant while her partner is participating in this study, she should inform her treating physician immediately.

2.1.1.10 Must have started ADT for metastatic disease within 134 days (for Arm A and B) or within 30 days (for Arm C).

2.1.2 Exclusion Criteria

2.1.2.1 Immunocompromised status due to:

- Human immunodeficiency virus (HIV) positivity.
- Active autoimmune diseases such as Addison's disease, Hashimoto's thyroiditis, systemic lupus erythematosus, Sjögren syndrome, scleroderma, myasthenia gravis, Goodpasture syndrome or active Grave's disease. Patients with a history of autoimmunity that has not required systemic immunosuppressive therapy or does not threaten vital organ function including CNS, heart, lungs, kidneys, skin, and GI tract will be allowed.
- Other immunodeficiency diseases

2.1.2.2 Chronic administration (defined as daily or every other day for continued use > 14 days) of corticosteroids deemed systemic by investigator within 28 days before the first planned dose of PROSTVAC. Use of inhaled steroids, nasal sprays, and topical creams for small body areas is allowed.

2.1.2.3 Evidence of rising PSA on ADT

2.1.2.4 Serious intercurrent medical illness that, in the judgment of the investigator, would interfere with patient's ability to carry out the treatment program.

2.1.2.5 Other medications used for urinary symptoms including 5-alpha reductase inhibitors (finasteride and dutasteride) and alternative medications known to alter PSA (e.g. phytoestrogens and saw palmetto)

2.1.2.6 History of allergic reactions attributed to compounds of similar chemical or biologic composition to poxviral vaccines (e.g., vaccinia vaccine)

2.1.2.7 Known allergy to eggs, egg products, aminoglycoside antibiotics (for example, gentamicin or tobramycin).

2.1.2.8 History of atopic dermatitis or active skin condition (acute, chronic, exfoliative) that disrupts the epidermis

2.1.2.9 Previous serious adverse reactions to smallpox vaccination

2.1.2.10 Unable to avoid close contact or household contact with the following high-risk individuals for three weeks after the Day 1 vaccination: (a) children ≤ 3 years of age, (b) pregnant or nursing women, (c) individuals with prior or concurrent extensive eczema or other eczemoid skin disorders, or (d) immunocompromised individuals, such as those with HIV.

2.1.2.11 Receipt of an investigational agent within 28 days (or 60 days for an antibody-based therapy) before the first planned dose of study drugs.

2.1.2.12 Patients who test positive for HBV or HCV

2.1.2.13 Uncontrolled hypertension (SBP>170/ DBP>105)

2.1.2.14 Patients who have had prior chemotherapy for prostate cancer.

2.1.2.15 The subject has had evidence within 2 years of the start of study treatment of another malignancy which required systemic treatment (with the exception of nonmelanoma skin cancers or carcinoma in situ of the bladder).

2.1.2.16 The subject has active brain metastases or epidural disease.

2.1.2.17 Patients with greater than or equal to grade 2 peripheral neuropathy at baseline.

2.1.2.18 Patients with history of splenectomy.

2.1.3 Recruitment Strategies

This study will be listed on available websites (www.clinicaltrials.gov, https://ccr.cancer.gov/clinical-trials-search-start) and participants will be recruited from the current patient population at NIH.

2.2 SCREENING EVALUATION

A. Pathological confirmation of diagnosis by a CLIA certified laboratory may be obtained any time prior to enrollment.

B. The following parameters will be obtained within 8 weeks prior to start of treatment:

- HIV test
- Hepatitis B and C
- Tc-99 whole-body scintigraphy
- CT (or MRI may be substituted at investigator's discretion) of chest, abdomen and pelvis
- History and physical examination including vital signs
- ECOG performance status
- Complete blood count plus differential and platelet count
- Hepatic (alkaline phosphatase, AST, ALT, total bilirubin, direct bilirubin), Mineral (albumin, calcium, magnesium, phosphorus) and Acute Care (sodium, potassium, chloride, bicarbonate, creatinine, glucose, BUN, eGFR) Panels
- Serum lipase
- Serum PSA
- Testosterone level

2.3 BASELINE EVALUATION

To be performed within 16 days prior start of treatment (these tests will not need to be repeated if they were done at screening within the appropriate timeframe):

- History and physical exam including weight and vital signs
- ECOG performance status
- CBC with differential and platelet count, prothrombin time/INR, activated partial thromboplastin time
- Urinalysis (may be omitted in patients with incontinence who cannot produce a clean sample)
- Serum chemistries (Na+, K+, Cl-, CO2, BUN, creatinine, glucose, AST/ALT, bilirubin, calcium, phosphorous, albumin, magnesium, alkaline phosphatase, LDH, ionized calcium, amylase, lipase, total protein, GGT, uric acid)
- Serum Testosterone level
- Serum PSA
- Lymphocyte phenotyping CD3/CD4/CD8
- Baseline electrocardiogram 12 lead ECG (EKG) on all patients, and appropriate cardiologic evaluation, as clinically indicated, to provide baseline function and identify any patients who should be monitored closely for cardiac risks associated with vaccinia vaccination
- HLA-A, B, C and HLA-A*02 and A*03 subtype may be collected at any time during the study if not previously collected. This test does not need to be repeated prior to enrollment if previously collected at NIH.
- Biopsy (optional)
- Endorectal MRI (optional)

2.4 PARTICIPANT REGISTRATION AND STATUS UPDATE PROCEDURES

Registration and status updates (e.g., when a participant is taken off protocol therapy and when a participant is taken off-study) will take place per CCR SOP ADCR-2, CCR Participant Registration & Status Updates found here.

2.4.1 Treatment Assignment and Randomization/StratificationProcedures

Cohorts

Number	Name	Description
1	ADT established	Patients who have started ADT for metastatic prostate cancer no more than 134 days prior and no fewer than 29 days prior to enrollment
2	ADT not yet established	Patients who have not started ADT or who have been on ADT for 28 days or fewer

Arms

Letter	Name	Description
A	Sequential docetaxel followed by PROSTVAC	6 cycles of docetaxel followed by PROSTVAC
B	Combined docetaxel with PROSTVAC	6 cycles of docetaxel concurrently administered with PROSTVAC
C	PROSTVAC prior to docetaxel	4-6 injections of PROSTVAC followed by 6 cycles docetaxel

Stratifications

Name	Distinct Options	Notes
Disease Volume	*High* *Low*	Done at time of randomization to minimize differences n disease volumes between arms

Randomization and Arm Assignment

Subjects in Cohort 1 will be stratified and randomized on a 1:1 basis between Arm A and Arm B**.** The computerized randomization will be performed by the Central Registration Office.

Subjects in cohort 2 will be directly assigned to Arm C.

CRO staff will send a secured e-mail to Principal Investigator and research nurse with the Verification of Registration of the patient and treatment arm patient is randomized. This process will be completed within 15–30 minutes of faxing the eligibility checklist. CRO will keep track of all randomization data in Clinical Data Registry (CDR) of CRO.

3 STUDY IMPLEMENTATION

3.1 STUDY DESIGN

This is a randomized trial of ADT followed by simultaneous docetaxel 75 mg/m2 q3 weeks x 6 cycles + PROSTVAC q3 weeks x 6 cycles (Arm B) versus ADT followed by sequential docetaxel 75 mg/m2 q3 weeks x 6 cycles followed by PROSTVAC q3 weeks x 6 cycles (Arm A) in men with newly diagnosed mCSPC.

Arm C will evaluate patients who have not started ADT or who have been on ADT for no more than 28 days. For these patients, vaccinia-PROSTVAC $2x10^8$ infectious units subcutaneously will be initiated within 28 days of starting ADT. This will be followed by fowlpox-PROSTVAC booster $1x10^9$ infectious units subcutaneously on weeks 3, 6, 9, 12 and 15. Vaccine may be discontinued after week 9 or with confirmed rise in PSA based on investigator discretion. After vaccine discontinuation, docetaxel will be initiated within 28 days of last vaccine but not beyond 134 days of initiating ADT. Docetaxel will be given alone at 75 mg/m^2 intravenously every three weeks for a total of 6 doses.

3.1.1 Protocol Stopping Rules

- This study will utilize the Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 for grading systemic toxicity
- One occurrence of grade 5 toxicity by the NCI-CTCAE version 4.0 attributable to the treatment regimen.
- Two occurrences of grade 4 toxicity by the NCI-CTCAE version 4.0 attributable to the treatment regimen

3.2 DRUG ADMINISTRATION

3.2.1 ADT

Standard ADT will be used based on standard dosing scheduling. GnRH agonists and GnRH antagonist are acceptable as long as patients maintain castrate levels of testosterone. Orchiectomy would also be an option if that is desired by the patient.

3.2.2 PROSTVAC Vaccine

All vaccines in this trial should be handled according to the guidelines outlined by each department. Study staff administering the vaccine or assessing the vaccine site should wear personal protection consistent with the standard of practice outlined by each department or unit.

Patients receiving PROSTVAC-V should be isolated just prior to the administration of vaccine and can be removed from isolation after the vaccine is administered and bandage is secured over the injection site. **Appendix C** defines individuals at risk for vaccinia exposure.

3.2.2.1 Arm A – sequential docetaxel + PROSTVAC

PROSTVAC-V (vaccinia) will be administered subcutaneously in week 19 at a dose of $2x10^8$ infectious units.

PROSTVAC-F (fowlpox) will be administered subcutaneously at a dose of $1x10^9$ infectious units starting in week 21. Administration on day 1 of each cycle will continue for 6 cycles. (Weeks 21, 24, 27, 30, 33 and 36)

3.2.2.2 Arm B – concurrent docetaxel + PROSTVAC

PROSTVAC-V (vaccinia) will be administered subcutaneously in week -2 at a dose of $2x10^8$ infectious units.

PROSTVAC-F (fowlpox) will be administered subcutaneously at a dose of $1x10^9$ infectious units starting on day 1, week 1. Administration on day 1 of each cycle will continue for 6 cycles. (Weeks 1, 4, 7, 10, 13 and 16)

3.2.2.3 Arm C - PROSTVAC prior to Docetaxel

PROSTVAC-V (vaccinia) will be administered subcutaneously in week 1 at a dose of $2x10^8$ infectious units.

PROSTVAC-F (fowlpox) will be administered subcutaneously at a dose of $1x10^9$ infectious units starting on week 3. Administration on day 1 of each cycle will continue for 5 total cycles. (including Weeks 6, 9, 12, 15). Vaccine may be discontinued after week 9 or with confirmed rise in PSA based on investigator discretion at which time docetaxel could begin within 28 days.

One dose of vaccinia-PROSTVAC and 3 doses of fowlpox-PROSTVAC may be adequate to generate an immune response based on previous studies. The investigator may use clinical discretion to forgo dose 4 and 5 of fowlpox-PROSTVAC and initiate chemotherapy.[10]

In addition, for patients who may have a rise in PSA while on vaccine alone, vaccine can be discontinued early at the discretion of the investigator and docetaxel can be initiated with confirmed rise in PSA.

3.2.2.4 Dosing Delay

- Patients must have recovered to ≤ grade 1 toxicity related to vaccine for the parameters used to assess levels of organ function required for eligibility (see Section **2.1**) after each vaccination in order to receive a subsequent vaccination.
- If ≥ grade 3 nonautoimmune toxicity attributable to the vaccine persists for > 42 days, the patient will not receive further vaccine inoculations and will be removed from the protocol treatment and followed for resolution of toxicity and immune/survival endpoints.

- Patients who develop grade 3 injection site reactions will have their vaccine held until injection site reaction resolves to grade 2 or less.
- Patients who develop ≥ a grade 2 allergic or autoimmune disease that threatens vital organ function or any ≥ grade 3 autoimmunity, not related to a therapeutic response, will be removed from the protocol treatment and followed for resolution of toxicity and immune/survival endpoints.
- Patients who develop any grade 4 toxicity attributable to vaccine will be removed from the protocol treatment and followed for resolution of toxicity and immune/survival endpoints.
- If a scheduled vaccine or docetaxel dose is missed due to scheduling or logistical issues, the vaccine or docetaxel may be given within 7 days of the appointed time.

3.2.2.5 Dose modifications

No dose modifications are allowed with this vaccine.

3.2.3 Docetaxel

Treatment will be administered primarily on an outpatient basis. Reported adverse events and potential risks are described. Appropriate dose modifications are described. No investigational or commercial agents or therapies other than those described may be administered with the intent to treat the patient's malignancy.

Agent	Premedications	Dose	Route	Schedule	Cycle length
Docetaxel	8 mg of oral dexamethasone 12 hours, 3 hours, and 1 hour before infusion **OR** 12 mg IV 15-30 minutes prior to infusion	75 mg/m2	IV over 1 hour	Day 1 for Arm A Day 2 or Day 3 for Arm B	21 days (3 weeks)

Docetaxel 75mg/m^2 will be administered intravenously as described in **Appendix D** every 21 days (i.e., a 3-week cycle) for up to 6 cycles. All patients will receive 8 mg of dexamethasone orally 12 hours, 3 hours and 1 hour prior to docetaxel infusion OR may receive dexamethasone 12 mg intravenously prior to docetaxel.

In the event of a hypersensitivity reaction, infusion times may be longer. Hypersensitivity reactions may be managed clinically according to PI discretion. Please see **Appendix D** for suggested management guidelines (optional).

To minimize patient exposure to phthalate plasticizers (e.g., DEHP), which may be leached from PVC containers and administration sets, administer docetaxel only through polyethylene-lined administration sets.

3.2.3.1 Dose Reduction

Dose Level	Docetaxel (mg/m^2)
Level 0	75 mg/m^2
Level - 1	65 mg/m^2
Level - 2	55 mg/m^2

3.2.3.2 Docetaxel Dose Modifications

Patients should have an ANC ≥1500 cells/mm^3, a platelet count ≥ 75,000 cells/mm^3 (grade 1 hematologic toxicity) and resolution of any grade 3 or higher non-hematologic toxicity to ≤ grade 1 or baseline in order to initiate another treatment cycle of docetaxel.

For febrile neutropenia developed within a prior cycle, docetaxel can be continued during future cycles at a dose reduction d and if indicated, with the addition of filgrastim support. For grade 4 neutropenia lasting greater than 5 days docetaxel will be held and patients will be treated with filgrastim support. Docetaxel will be resumed at the same dose once neutropenia has resolved to ≤ grade 1 or baseline.

Docetaxel Dose Modifications for Neutropenia without Fever:

When Neutrophils fall to	**for a Duration of**	**Recommended Course**
<1000 but ≥ 500/mcL (Grade 3)	Any length of time	No dose reduction for future cycles.
<500/mcL (Grade 4)	≤ 5days	No dose reduction for future cycles.
<500/mcL (Grade 4)	> 5 days	No dose reduction. DOCETAXEL will be held and patients will be treated with filgrastim support. DOCETAXEL will be resumed at the same dose once neutropenia has resolved to ≤ grade 1 or baseline.

For grade 3 constipation or grade 3 fatigue, treatment can resume with a dose reduction after resolution of toxicity to ≤ grade 1 or baseline. Study Calendar See

Appendix A.

3.3 COST AND COMPENSATION

3.3.1 Costs

NIH does not bill health insurance companies or participants for any research or related clinical care that participants receive at the NIH Clinical Center. If some tests and procedures performed outside the NIH Clinical Center, participants may have to pay for these costs if they are not covered by insurance company. Medicines that are not part of the study treatment will not generally be provided or paid for by the NIH Clinical Center.

3.3.2 Compensation

Participants will not be compensated on this study.

3.3.3 Reimbursement

The NCI will cover the costs of some expenses associated with protocol participation. Some of these costs may be paid directly by the NIH and some may be reimbursed to the participant/guardian as appropriate. The amount and form of these payments are determined by the NCI Travel and Lodging Reimbursement Policy.

3.4 CRITERIA FOR REMOVAL FROM PROTOCOL THERAPY AND OFF STUDY CRITERIA

Prior to documenting removal from study, effort must be made to have all subjects complete a safety visit within 30 days following the last dose of study therapy (see section **3.5**).

3.4.1 Criteria for removal from protocol therapy

Patients will be removed from treatment for the following:

- Clinical or radiographic progression of disease.
- Serological progression, as defined in protocol section **6.3.6**, may remain on treatment at the discretion of the PI.
- Grade 2 or greater autoimmune disease that threatens vital organs (Patients may be given the option to continue the study with standard of care docetaxel as clinically indicated with no further vaccine).
- Grade 3 or greater autoimmune response that is not related to a therapeutic response (Patients may be given the option to continue the study with standard of care docetaxel as clinically indicated with no further vaccine).
- Any Grade 4 non-heme toxicity that is possibly, probably or definitely related to the protocol treatment will require a patient to be off-treatment.
- Intercurrent illness or medical circumstances. If at any time the constraints of this protocol are detrimental to the patient's health, the patient may be removed from protocol therapy and reasons for withdrawal will be documented.
- Any grade of seizure will require a patient to be off-treatment.
- Completion of protocol therapy.

3.4.2 Off-Study Criteria

- Patient is off-treatment and/or has agreed to be followed on a long term therapy protocol as outlined in section **3.6**.
- Patient requests to be taken off study. Reasons for withdrawal will be documented.
- Noncompliance with protocol guidelines (patient removed at discretion of Principal Investigator).
- Death
- Screen failure

3.5 FOLLOW-UP EVALUATIONS

After subjects have stopped taking the study medication for any of the reasons listed in Section **3.4**, they will be seen at NIH for a safety visit within 30 days of drug discontinuation. The safety assessments may be performed by a local physician and laboratory if patients are unable to return to the NIH Clinical Center at this time. The investigator will contact the local physician to discuss the safety assessments outlined below and request that medical records be sent to the NIH.

Patients who completed protocol therapy including the safety visit without disease progression will be followed every 12 weeks +/- 6 weeks until disease progression. Disease progression is defined in Section **6.3**.

The following assessments will be performed during the follow up and safety visit:

- History and Physical Examination
- CBC with differential and platelet count, prothrombin time/INR, activated partial thromboplastin time
- Serum chemistries (Na+, K+, Cl-, CO2, BUN, creatinine, glucose, AST/ALT, bilirubin, calcium, phosphorous, albumin, magnesium, alkaline phosphatase, LDH, ionized calcium, amylase, lipase, total protein, GGT, uric acid)
- Serum PSA level
- Adverse event reporting as needed as defined in section **7.**

During the safety visit, if there are no unresolved grade 3 or higher AEs, we may, when feasible ask the patient to enroll on the long term therapy protocol as outlined in section **3.6** to find out how they are doing and to determine survival status. If there are unresolved grade 3 – 4 AEs, patients will be followed either at the NIH Clinical Center or by their local physician. In the latter case, we will obtain the physician's record of AEs.

Any scans performed outside of the NIH will also be obtained when possible.

3.6 POST-STUDY EVALUATION

The Biologic Response Modifiers Advisory Committee has recommended that long-term follow-up extend over a period of 15 years. Information regarding the findings will be reported to the FDA. Patients assigned to receive PROSTVAC will be offered enrollment in the 04-C-0274 "Follow-Up Study of Subjects Previously Enrolled in Immunotherapy Studies Utilizing Gene Transfer or other immunotherapeutic agents" once off study.

4 CONCOMITANT MEDICATIONS/MEASURES

Subjects must inform the investigators of the current or planned use or all other medications during the study (including prescription medications, vitamins, herbal and nutritional supplements, and over-the-counter medications).

For the administration of PROSTVAC, antiemetics, stool softeners and antidiarrheal agents may be administered as required, but are not anticipated to be needed and should not be used prophylactically on the first cycle. The selection of the specific antiemetic regimen is at the discretion of the treating physician. Antiemetic regimens will not include steroids.

Other supportive care with blood components, antibiotics, analgesics, general medical therapy, etc., will be delivered as required. Any patients taking antibiotics for any reason must complete that course of therapy and be free of evidence of further infection before receiving any dose of vaccine.

Concurrent systemic corticosteroid use (daily or every other day for continued use > 14 days) should be avoided within 28 days before the first planned dose of PROSTVAC. Use of inhaled steroids, nasal sprays, and topical creams for small body areas is allowed.

Symptomatic anemia should be treated with appropriate red blood cell or erythropoietin support.

Thrombocytopenia should be treated conservatively. In the absence of bleeding or a planned invasive procedure, platelet transfusions should be given for a platelet count below 10,000/mm3. If invasive procedures are planned or the patient develops bleeding, platelet transfusions should be administered in accordance with the standard of practice, usually maintaining a platelet count of > 50,000/mm3.

Any evidence of disseminated intravascular coagulation (DIC), hemolytic uremic syndrome (HUS), or thrombotic thrombocytopenic purpura (TTP) including thrombocytopenia, hemolytic anemia, renal failure, fever or neurologic changes should be thoroughly evaluated and closely monitored and supported as clinically indicated.

4.1 CONCURRENT MEDICATIONS/INTERVENTIONS

4.1.1 Anticancer Therapy

If a subject requires additional systemic anticancer treatment, study treatment must be discontinued. Local intervention is discouraged unless medically unavoidable. Subjects receiving local intervention (*e.g.*, palliative radiation) are allowed to continue to receive study treatment at the investigator's discretion.

4.1.2 Other Medications

Subjects must be instructed to inform the investigators of the current or planned use or all other medications during the study (including prescription medications, over-the-counter medications, vitamins and herbal and nutritional supplements). It is the responsibility of the investigator to ensure that details regarding all medications are documented.

Bisphosphonates started prior to screening activities or initiated during the course of the study to control bone pain may be used with caution.

Colony stimulating factors (*e.g.*, erythropoietin and granulocyte colony-stimulating factors) administered as dictated by safety purposes are acceptable while the subject is enrolled on study. Pain medications administered as dictated by standard practice are acceptable while the patient is enrolled on the study.

No concurrent investigational agents are permitted

4.1.3 Neutropenia

Growth factors may be used as medically indicated if patient develops fever and neutropenia. Dose reduction or interruption of docetaxel is also recommended as described.

4.2 TREATMENT OF VACCINIA VACCINATION COMPLICATIONS

4.2.1 Vaccinia Immune Globulin (VIG)

First-line treatment of some of the complications of vaccinia caused by dissemination of vaccinia virus (severe cases of inadvertent inoculation involving extensive lesions or if comorbid conditions exist, severe cases of generalized vaccinia in patients that are systemically ill and whose condition might be toxic or who have serious underlying immunosuppressive illnesses, eczema vaccinatum, and progressive vaccinia) is with VIG. VIG is contraindicated, however, for the treatment of isolated vaccinial keratitis. VIG is a sterile solution of the immunoglobulin fraction of pooled plasma from individuals inoculated with vaccinia vaccine. VIG is only available through the CDC's Strategic National Pharmaceutical Stockpile by contacting the CDC's Clinician Information Line at 1-877-554-4625 or the Director's Emergency Operations Center (DEOC) at 770-488-7100. Upon receipt of a call from a patient or upon direct observation of a patient, or contact, who manifests signs and symptoms of any of the above conditions, the investigator should place a call to the CDC as soon as possible:

1) to initiate review of the clinical case,

2) to seek consultation on the appropriateness of VIG therapy,

3) to determine the appropriate VIG dose and dosing method for administration, if VIG therapy is required, and

4) to determine how to access and have the appropriate doses of VIG delivered.

Early institution of VIG therapy is advised following recognition of clinical symptoms compatible with some vaccinia complications (eczema vaccinatum, severe generalized vaccinia, progressive vaccinia, and some cases of inadvertent inoculation). The effectiveness of VIG therapy appears to be time dependent. VIG has not proven to be of benefit in the treatment of post-vaccinial encephalitis, and is contraindicated for treatment of isolated vaccinial keratitis due to the increased risk of corneal scarring. A new IV formulation of VIG that has a lower level of aggregated protein allowing it to be used by either the IM or IV route is available through the CDC. This formulation will most likely be preferred for administration and the CDC will instruct investigators regarding appropriate dosing and method of administration based on the formulation and availability. There is no guarantee that VIG will successfully treat complications. At present, there are no other anti-viral therapies of proven benefit for the treatment of vaccinia-related complications.

4.2.2 Cidofovir (Vistide®, Gilead Sciences)

Cidofovir is an FDA-approved antiviral drug for the treatment of CMV retinitis among patients with AIDS. Cell-based *in vitro* studies and animal model studies have demonstrated this agent's antiviral activity against certain orthopoxviruses. Currently, its efficacy in the treatment of vaccinia-related complications in humans is unknown. According to the CDC, VIG is recommended as first line of therapy. Cidofovir may be considered as a secondary treatment, and will only be used when VIG therapy is not effective [Medical Management of Smallpox (Vaccinia) Vaccine Adverse Reactions: Vaccinia Immune Globulin and Cidofovir. Last updated September 28, 2009. Available at: http://www.bt.cdc.gov/agent/smallpox/vaccination/mgmt-adv-reactions.asp].

5 BIOSPECIMEN COLLECTION

Please note that:

- Tubes and media specified below may be substituted based on availability with the permission of the PI or laboratory investigator.
- The location of specimen processing or analysis may be adjusted with the permission of the PI or laboratory investigator

5.1 CORRELATIVE STUDIES FOR RESEARCH/PHARMACOKINETIC STUDIES

All patients enrolling will have blood samples drawn for PK studies and samples collected for the other studies as described below.

The plasma concentration-time data will be analyzed using WinNonlin software (Pharsight, Mountain View, CA). The maximum concentration, time to maximum concentration, the area under the curve extrapolated to infinity, and clearance will be calculated.

5.1.1 Collection of Specimens

One 6mL sodium heparin tube (BD, Franklin Lakes, NJ) is collected from each patient on Cycle 1 -at the following time points: before docetaxel administration, at approximately 5 minutes prior to the end of docetaxel infusion, and at 15 minutes, 30 minutes, 3h, 6h, 12h and 24h after the end of docetaxel infusion.

5.1.2 Handling and Processing of Specimen(s)

The samples will be placed immediately on wet ice and refrigerated. The date and exact time of each blood draw should be recorded on the sample tube and the PK sheet.

Please e-mail NCIBloodcore@mail.nih.govat least 24 hours before transporting samples (the Friday before is preferred).

For sample pickup, page 102-11964.

For immediate help, call 240-760-6180 (main BPC number) or, if no answer, 240-760-6190 (main clinical pharmacology lab number).

For questions regarding sample processing, contact NCIBloodcore@mail.nih.gov.

Upon arrival in the Biospecimen Processing Core, samples will be centrifuged, and the plasma transferred into cryovials for storage at -80C until the time of analysis. In addition, samples will be barcoded as described in section **5.3.**

The pharmacokinetic analysis will be performed on a research basis in the Biospecimen Processing Core.

5.2 BIOMARKER STUDIES

5.2.1 Genetic Biomarkers

5.2.1.1 Collection of Specimens

One 6ml EDTA tube (BD, Franklin Lakes, NJ) will be collected from patients at baseline (after consent, prior to docetaxel or vaccinia initiation).

5.2.1.2 Handling and Processing of Specimens

Immediately after collection, invert the blood tube 8-10 times. Place the tube on wet ice and then store at 4˚C in the refrigerator. The date and exact time of each blood draw should be recorded on the tube.

Please e-mail NCIBloodcore@mail.nih.gov at least 24 hours before transporting samples (the Friday before is preferred).

For sample pickup, page 102-11964.

For immediate help, call 240-760-6180 (main BPC number) or, if no answer, 240-760-6190 (main clinical pharmacology lab number).

For questions regarding sample processing, contact NCIBloodcore@mail.nih.gov.

Upon arrival in the BPC, genomic DNA will be extracted and analyzed for genetic polymorphisms.

The genotyping will be performed using DMET (Drug Metabolizing Enzymes and Transporters) array on a research basis in the Molecular Pharmacology Section.

5.2.2 Circulating Tumor Cells

5.2.2.1 Collection of Specimens for Veridex Analysis

CTC enumeration: Two 7cc lavender top tubes for flow-based CTC analysis will be collected before start of treatment (pre-docetaxel for Arm A, pre-vaccinia for Arm B & C), week 13 for Arm A & B, and week 9 for Arm C. Up to nine patients may have peripheral blood collected at the same time points in one 7.5cc CellSave Preservative Tube for Veridex CTC analysis.

5.2.2.2 Handling and Processing of Specimens for Veridex Analysis

CTC enumeration: As soon as possible after the patient is scheduled please send email notification to DTB Clinical Translation Unit-designated laboratory: Min-Jung Lee at

leemin@mail.nih.gov that the sample is scheduled. After the sample is drawn please call 240-760-6330 to communicate that the sample is ready. Keep the sample on the unit at room temperature. The sample will be picked up by the lab and processed for CTC enumeration.

The flow cytometric analyses will be performed by the DTB Clinical Translation Unit-designated laboratory. DTB Clinical Translation Unit will deliver CellSave tubes to the research nursing staff and arrange for sending the tubes for Veridex analysis.

5.2.2.3 Collection of Specimens for Epic Analysis

Arm C only: One 10ml Streck Cell-Free DNA (brown/black tube) will be collected from patients at baseline (pre-treatment), prior to docetaxel, and at completion of treatment.

5.2.2.3.1 Rationale of investigation for Epic Analysis

- Methods are in development for the purification and analysis of circulating tumor cells (CTC).
- One of these novel CTC technologies, developed by Epic Sciences, is summarized as follows: whole blood is aliquoted onto slides, nucleated peripheral blood cells are attached to slides and examined, cytokeratin-positive/CD45-negative cells with an intact nucleus and a malignancy-consistent morphology are identified as CTCs, and their exact positions on the slides recorded. This technology has the advantage of being able to identify cells that may be CTC but cytokeratin negative. Since positive or negative selection is not needed, all circulating cells are captured and analyzed via proprietary technology.
- Multiplex analysis technologies have been developed to examine the levels and activities of androgen receptor.

The evaluation of CTCs may give valuable insight into how treatment affects changes in CTC phenotype, such as AR splice variants, and how such changes are associated with clinical outcomes.

5.2.2.3.2 Handling and Processing of Specimens for Epic Analysis

The Biospecimen Processing Core will be paged at 102-11964 for tube pick up and shipping via FedEx Priority Overnight to Epic Sciences. These samples will be sent in ambient shippers provided by Epic Sciences to keep samples at room temperature. Alternatively, PBMCs collected and stored at -80^{o}C for immunology assays may also be shipped to Epic Sciences for CTC analysis. Samples will be shipped to the following address:

Epic Sciences
Attn: Ryan Dittamore
9381 Judicial Drive, Ste. 200
San Diego, CA 92121
858-356-6610, ext. 124

5.2.3 Immunologic Parameters

- Antibodies to PSA, vaccinia, fowlpox may be tested

- Leukocyte CD3, CD4, CD8 subsets; CD4:CD8 ratio will be drawn at baseline and at each visit when on treatment and then at each follow-up visit while the patient remains on trial.
- Additional studies will include but are not limited to quantitative and qualitative assessments of regulatory T-cells, Natural Killers cells, Myeloid Derived Suppressor Cells, anti-glycan antibodies and Naïve T-cell/new thymic emigrants.
- Assessment of levels of cytokines.
- Immunologic studies will be repeated more frequently if clinically indicated, and any abnormalities potentially related to treatment will be followed until they have resolved, or have been determined not to be treatment-related.
- Peripheral blood mononuclear cells (PBMC):
  Intracellular cytokine staining assays for Brachyury-specific T lymphocytes using PSA, MUC-1 and Brachyury-specific peptides CD3, CD4, CD8, and CD 19 subsets, NK markers and CD4:CD8 ratio.
- Phenotypic and functional analysis of immune cell subsets by flow cytometry.

5.2.3.1 Collection of Specimens

6 (10mL) green top sodium heparin tubes for PBMC; 2 (8mL) SST tubes for serum sample will be collected as follows:

- Arm A:
  - Baseline (pre-treatment), post 3 and 6 cycles of docetaxel (week 10 and 19), post 1st dose of PROSTVAC-F (week 24), at completion of treatment, and every 6 months until progression.
- Arm B:
  - Baseline (pre-treatment), post 1st dose of PROSTVAC-F (week 4), at completion of treatment (week 19), and every 6 months until progression.
- Arm C:
  - Baseline (pre-treatment), post 1st dose of PROSTVAC-F (week 6), pre-docetaxel, post 3 cycles of docetaxel (chemo week 10), at completion of treatment, and every 6 months until progression.

5.2.3.2 Handling and Processing of Specimen(s)

The samples will be processed through:

Clinical Services Program
NCI Frederick Cancer Research and Development Center
PO Box B
Frederick, MD 21702
301-846-1000

On days samples are drawn, CSP should be notified (phone: [301] 846-5893; fax [301] 846-6222). She will arrange same-day courier delivery of the specimens.

The research samples will contain labels on the blood tubes that have the patient's initials, date of birth, the assigned protocol, and the date the sample was drawn. The transmittal forms accompanying the samples also contain the same information.

Once a patient's treatment schedule has been determined, it should be faxed to the Laboratory of Tumor Immunology and Biology/ NIH (Fax: [301] 496-2756; phone: [301] 496-9573) for planning purposes.

5.2.3.3 Immunologic Assays

5.2.3.3.1 Sera Antibody Analysis:

Serum will be stored at -80 degrees Celsius and there will be planned analysis for generation of antibodies to PSA, BCG, PAP, PSMA, PSCA, and/or MUC-1.

5.2.3.4 Natural Killer (NK) CELLS

The number and phenotype of NK cells will be determined by phenotypic analysis of PMBCs stained for CD56, CD3, CD8, and CD16 by flow cytometry.

5.2.3.5 Immune Subsets

Subsets of immune cells will also be followed in response to treatment.

5.2.3.6 Regulatory T Cells

Regulatory T cells have been shown to inhibit the activation and function of T cells that participate in antigen-specific immune responses. Higher levels of regulatory T-cells have been reported in the peripheral blood mononuclear cells of patients with several types of tumors. The number and phenotype of regulatory T-cells in peripheral blood mononuclear cells from patients in this study will be determined by 7-color flow cytometry analysis. Cells will be resuspended in staining buffer (phosphate-buffered saline containing 3% fetal bovine serum) and stained for CD4, CD25, CD127, FoxP3, CTLA-4, CD45RA, and CD8. The ratios between regulatory T-cells and CD4 effector cells and the ratios between regulatory T-cells and CD8 Effector cells will also be analyzed.

5.2.3.7 Additional Assays

Blood samples may be used for additional research studies, which may include one of the following - phenotypic and functional analysis of immune-cell subsets and analyses for cytokines (IFN-γ, IL-10, IL-12, IL-2, IL-4, etc.), chemokines, antibodies related to the tumor or vaccine, tumor associated antigens related to the tumor or the vaccine, and/or other immune response markers.

5.2.3.8 Biopsy Analysis

When logistically feasible, consenting patients will have a biopsy prior to treatments and after 2 months of vaccine. Biopsy findings will assess immune responses in the tumor microenvironment using immune histochemistry as the primary analysis and could include tissue microarrays.

Biopsy sites will vary based on patient and the location of disease. Sites could include intraprostatic tumors or metastatic sites. Biopsies will be limited to 1 site (although a biopsy within the prostate itself may have up to 3 or 4 lesions to be biopsied).

5.2.3.8.1 *Immunohistochemistry*

The Laboratory of Pathology and the Warren G. Magnuson Clinical Center will perform Immunohistochemistry on biopsied tissue, if the patient elects to have the procedure. Immunohistochemistry of these tissue specimens will be obtained for CD4, CD8, and FOX-P3. In addition, phenotypic analysis of infiltrating immune cells will be performed.

Immunohistologic grading schema of the lesions

Score	%positive cells of each subtype
0	0
1	1-25%
2	26-50%
3	>50%

All staining will be categorized as being membrane or nuclear

5.3 SAMPLE STORAGE, TRACKING AND DISPOSITION

Samples will be ordered in CRIS and tracked through the Clinical Trial Data Management System. Should a CRIS screen not be available, the CRIS downtime procedures will be followed. Samples will not be sent outside NIH without appropriate approvals and/or agreements, if required.

5.3.1 Biospecimen Processing Core - Storage

All samples sent to the Biospecimen Processing Core (BPC) will be barcoded, with data entered and stored in the Labmatrix utilized by the BPC. This is a secure program, with access to Labmatrix limited to defined BPC personnel, who are issued individual user accounts. Installation of Labmatrix is limited to specified computers specified. These computers all have a password restricted login screen.

Labmatrix creates a unique barcode ID for every sample and sample box, which cannot be traced back to patients without Labmatrix access. The data recorded for each sample includes the patient ID, name, trial name/protocol number, time drawn, cycle time point, dose, material type, as well as box and freezer location. Patient demographics associated with the clinical center patient number are provided in the system. For each sample, there are notes associated with the processing method (delay in sample processing, storage conditions on the ward, etc.).

Barcoded samples are stored in barcoded boxes in a locked freezer at either -20 or -80°C according to stability requirements. These freezers are located onsite in the BPC and offsite at NCI Frederick Central Repository Services in Frederick, MD. Visitors to the laboratory are required to be accompanied by laboratory staff at all times.

Access to stored clinical samples is restricted. Samples will be stored until requested by a researcher named on the protocol. All requests are monitored and tracked in Labmatrix. All researchers are required to sign a form stating that the samples are only to be used for research purposes associated with this trial (as per the IRB approved protocol) and that any unused

samples must be returned to the BPC. It is the responsibility of the NCI Principal Investigator to ensure that the samples requested are being used in a manner consistent with IRB approval.

Following completion of this study, samples will remain in storage as detailed above. Access to these samples will only be granted following IRB approval of an additional protocol, granting the rights to use the material.

If, at any time, a patient withdraws from the study and does not wish for their existing samples to be utilized, the individual must provide a written request. Following receipt of this request, the samples will be destroyed (or returned to the patient, if so requested). The PI will record any loss or unanticipated destruction of samples as a deviation. Reporting will be per the requirements of section **7.2**.

Sample barcodes are linked to patient demographics and limited clinical information. This information will only be provided to investigators listed on this protocol, via registered use of the Labmatrix. It is critical that the sample remains linked to patient information such as race, age, dates of diagnosis and death, and histological information about the tumor, in order to correlate genotype with these variables.

5.3.2 Handling and Storage for Research Samples Managed by DTB Clinical Translation Unit

Using a secure laboratory computerized database and a backup hardcopy process, all specimen collection and processing steps are documented and the specific location of each specimen is tracked. Each new specimen collected is assigned a unique 2D barcode identifier that can be linked to the original specimen collected and other relevant information within the inventory system. To ensure patient confidentiality, only containers used for the initial specimen collections will be labeled with patient identifiers. Labels without identifiers will be applied to all subsequent specimen containers. When specimens are processed and aliquoted, no patient information will be included on the new containers. Original specimen containers will be discarded. Only de-linked specimens will be stored.

The inventory process contains other security provisions sufficient to safeguard patient privacy and confidentiality. Access to the inventory system and associated documents is restricted to appropriate personnel only. SOPs ensure that any changes in the informed consent made by a patient and relayed to the PI will be reflected in the inventory system to ensure that specimens are destroyed as appropriate. All laboratory personnel are trained to adhere to SOPs and are monitored for high-quality performance.

5.3.3 Samples Sent to Clinical Services Program (CSP)

All data associated with patient samples are protected by a secure database. All samples drawn at the NIH Clinical Center will be transported to the NCI Frederick Central Repository by Leidos couriers.

Samples will be tracked and managed by the Central Repository database. All samples will be stored in liquid nitrogen. These freezers are located at NCI Frederick Central Repository in Frederick, Maryland.

American Type Culture Collection (ATCC) manages the NCI-Frederick Central Repositories under subcontract to Leidos Biomedical Research, Inc. NCI-Frederick Central Repositories store,

among other things, biological specimens in support of NIH clinical studies. All specimens are stored in secure, limited access facilities with sufficient security, back up and emergency support capability and monitoring to ensure long-term integrity of the specimens for research.

ATCC role is limited to clinical research databases and repositories containing patient specimens. ATCC does not conduct nor has any vested interest in research on human subjects, but does provide services and support the efforts of its customers, many of which are involved in research on human subjects.

It is the intent and purpose of ATCC to accept only coded samples and sample information. To the limit of our ability, every effort will be made to ensure that protected information is not sent electronically or by hard copy or on vial labels.

Sample data is stored in the BioSpecimen Inventory System II (BSI). This inventory tracking system is used to manage the storage and retrieval of specimens as well as maintain specimen data. BSI is designed for controlled, concurrent access. It provides a real-time, multi-user environment for tracking millions of specimens. The system controls how and in what order database updates and searches are performed. This control prevents deadlocks and race conditions. For security, BSI has user password access, three types of user access levels, and 36 user permissions (levels of access) that can be set to control access to the system functions. BSI provides audit tracking for processes that are done to specimens including shipping, returning to inventory, aliquoting, thawing, additives, and other processes. BSI tracks the ancestry of specimens as they are aliquoted, as well as discrepancies and discrepancy resolution for specimens received by the repository. If a specimen goes out of the inventory, the system maintains data associated with the withdraw request. Vials are labeled with a unique BSI ID which is printed in both eye readable and bar coded format. No patient specific information is encoded in this ID.

Investigators are granted view, input and withdraw authority only for their specimens. They may not view specimen data or access specimens for which they have not been authorized. Access to specimen storage is confined to repository staff. Visitors to the repositories are escorted by repository staff at all times.

5.3.4 Protocol Completion/Sample Destruction

Once primary research objectives for the protocol are achieved, intramural researchers can request access to remaining samples, provided they have an IRB-approved protocol and patient consent.

Samples and associated data will be stored permanently unless the patient withdraws consent. If the patient withdraws consent the participant's data will be excluded from future distributions, but data that have already been distributed for approved research use will not be able to be retrieved. The PI will record any loss or unanticipated destruction of samples as a deviation. Reporting will be per the requirements of section **7.2**.

6 DATA COLLECTION AND EVALUATION

6.1 DATA COLLECTION

Eligible patients must be confirmed and checklist completed. Consent form must be signed prior to registration with Central Registration Office.

Data will be secured in a 21 CFR Part 11-compliant data capture system provided by the NCI CCR. Data will be collected using protocol-specific case report forms, and verified for accuracy and completeness. Hard copies of data will be stored in locked secured areas and data will be entered onto a secured electronic data base. The following protocol-specific study forms will be complete and stored: eligibility checklist (developed by Central Registration Office, CRO). A copy of all serious AE forms will be kept in the research record.

Treatment is given according to protocol (dated notes about doses given, complications, and clinical outcomes).

Toxicity is assessed according to protocol (laboratory report slips, etc.)

Response is assessed according to protocol (X-ray, scan, lab reports, and date noted on clinical assessment, as appropriate).

Drug Accountability Records are kept for each patient.

For Arm A patients, who have not yet received PROSTVAC, we will only collect adverse events that are serious and/or unexpected. Once patients begin the PROSTVAC injection, adverse events will be collected as outlined in **Section 7**.

The PI will be responsible for overseeing entry of data into a 21 CFR Part 11-compliant data capture system provided by the NCI CCR and ensuring data accuracy, consistency and timeliness. The principal investigator, associate investigators/research nurses and/or a contracted data manager will assist with the data management efforts. Primary and final analyzed data will have identifiers so that research data can be attributed to an individual human subject participant.

All adverse events, including clinically significant abnormal findings on laboratory evaluations, regardless of severity, will be followed until return to baseline or stabilization of event. Patients will be followed for adverse events for 30 days after removal from study treatment or until off-study, whichever comes first.

End of study procedures: Data will be stored according to HHS, FDA regulations, and NIH Intramural Records Retention Schedule as applicable.

Loss or destruction of data: Should we become aware that a major breach in our plan to protect subject confidentiality and trial data has occurred, this will be reported expeditiously per requirements in section **7.2.1**.

6.2 DATA SHARING PLANS

6.2.1 Human Data Sharing Plan

What data will be shared?

I will share human data generated in this research for future research as follows:

☒ Coded, linked data in an NIH-funded or approved public repository.

☒ Coded, linked data in BTRIS (automatic for activities in the Clinical Center)

How and where will the data be shared?

Data will be shared through

☒ An NIH-funded or approved public repository. Insert name or names: clinicaltrials.gov.

☒ BTRIS (automatic for activities in the Clinical Center)

☒ Publication and/or public presentations.

When will the data be shared?

☒ Before publication.

☒ At the time of publication or shortly thereafter.

6.3 RESPONSE CRITERIA

Response and progression will be evaluated in this study using the new international criteria proposed by the revised Response Evaluation Criteria in Solid Tumors (RECIST) guideline (version 1.1) [31] and Prostate Cancer Clinical Trials Working Group criteria (PCWG2) [32]. Changes in the largest diameter (unidimensional measurement) of the tumor lesions and the shortest diameter in the case of malignant lymph nodes are used in the RECIST criteria.

6.3.1 Definitions

Evaluable for toxicity: All patients will be evaluable for toxicity from the time of their first treatment

Evaluable for objective response: Only those patients who have measurable disease present at baseline, have received at least one cycle of therapy, and have had their disease re-evaluated will be considered evaluable for response. These patients will have their response classified according to the definitions stated below. (Note: Patients who exhibit objective disease progression prior to the end of cycle 1 will also be considered evaluable.)

Evaluable Non-Target Disease Response: Patients who have lesions present at baseline that are evaluable but do not meet the definitions of measurable disease, have received at least one cycle of therapy, and have had their disease re-evaluated will be considered evaluable for non-target disease. The response assessment is based on the presence, absence, or unequivocal progression of the lesions.

6.3.2 Disease Parameters

Measurable disease: Measurable lesions are defined as those that can be accurately measured in at least one dimension (longest diameter to be recorded :

- By chest x-ray: ≥20 mm;
- By CT scan:
    - Scan slice thickness 5 mm or under: as ≥10 mm
    - Scan slice thickness >5 mm: double the slice thickness
- With calipers on clinical exam: ≥10 mm.

All tumor measurements must be recorded in millimeters (or decimal fractions of centimeters).

Malignant lymph nodes. To be considered pathologically enlarged and measurable, a lymph node must be ≥15 mm in short axis when assessed by CT scan (CT scan slice thickness recommended to be no greater than 5 mm). At baseline and in follow-up, only the short axis will be measured and followed.

Non-measurable disease. All other lesions (or sites of disease), including small lesions (longest diameter <10 mm or pathological lymph nodes with ≥10 to <15 mm short axis), are considered non-measurable disease. Bone lesions, leptomeningeal disease, ascites, pleural/pericardial effusions, lymphangitis cutis/pulmonitis, inflammatory breast disease, and abdominal masses (not followed by CT or MRI), are considered as non-measurable.

Note: Cystic lesions that meet the criteria for radiographically defined simple cysts should not be considered as malignant lesions (neither measurable nor non-measurable) since they are, by definition, simple cysts.

'Cystic lesions' thought to represent cystic metastases can be considered as measurable lesions, if they meet the definition of measurability described above. However, if non-cystic lesions are present in the same patient, these are preferred for selection as target lesions.

Target lesions. All measurable lesions up to a maximum of 2 lesions per organ and 5 lesions in total, representative of all involved organs, should be identified as **target lesions** and recorded and measured at baseline. Target lesions should be selected on the basis of their size (lesions with the longest diameter), be representative of all involved organs, but in addition should be those that lend themselves to reproducible repeated measurements. It may be the case that, on occasion, the largest lesion does not lend itself to reproducible measurement in which circumstance the next largest lesion which can be measured reproducibly should be selected. A sum of the diameters (longest for non-nodal lesions, short axis for nodal lesions) for all target lesions will be calculated and reported as the baseline sum diameters. If lymph nodes are to be included in the sum, then only the short axis is added into the sum. The baseline sum diameters will be used as reference to further characterize any objective tumor regression in the measurable dimension of the disease.

Non-target lesions. All other lesions (or sites of disease) including any measurable lesions over and above the 5 target lesions should be identified as **non-target lesions** and should also be recorded at baseline. Measurements of these lesions are not required, but the presence, absence, or in rare cases unequivocal progression of each should be noted throughout follow-up.

6.3.3 Methods for Evaluation of Measurable Disease

All measurements should be taken and recorded in metric notation using a ruler or calipers. All baseline evaluations should be performed as closely as possible to the beginning of treatment and never more than 8 weeks before the beginning of the treatment.

The same method of assessment and the same technique should be used to characterize each identified and reported lesion at baseline and during follow-up. Imaging-based evaluation is preferred to evaluation by clinical examination unless the lesion(s) being followed cannot be imaged but are assessable by clinical exam.

Clinical lesions: Clinical lesions will only be considered measurable when they are superficial (e.g., skin nodules and palpable lymph nodes) and ≥10 mm diameter as assessed using calipers (e.g., skin nodules). In the case of skin lesions, documentation by color photography, including a ruler to estimate the size of the lesion, is recommended.

Chest x-ray: Lesions on chest x-ray are acceptable as measurable lesions when they are clearly defined and surrounded by aerated lung. However, CT is preferable.

Conventional CT and MRI: This guideline has defined measurability of lesions on CT scan based on the assumption that CT slice thickness is 5 mm or less. If CT scans have slice thickness greater than 5 mm, the minimum size for a measurable lesion should be twice the slice thickness. MRI is also acceptable in certain situations (e.g. for body scans).

Use of MRI remains a complex issue. MRI has excellent contrast, spatial, and temporal resolution; however, there are many image acquisition variables involved in MRI, which greatly impact image quality, lesion conspicuity, and measurement. Furthermore, the availability of MRI is variable globally. As with CT, if an MRI is performed, the technical specifications of the scanning sequences used should be optimized for the evaluation of the type and site of disease. Furthermore, as with CT, the modality used at follow-up should be the same as was used at baseline and the lesions should be measured/assessed on the same pulse sequence. It is beyond the scope of the RECIST guidelines to prescribe specific MRI pulse sequence parameters for all scanners, body parts, and diseases. Ideally, the same type of scanner should be used and the image acquisition protocol should be followed as closely as possible to prior scans. Body scans should be performed with breath-hold scanning techniques, if possible.

PET-CT: At present, the low dose or attenuation correction CT portion of a combined PET-CT is not always of optimal diagnostic CT quality for use with RECIST measurements. However, if the site can document that the CT performed as part of a PET-CT is of identical diagnostic quality to a diagnostic CT (with IV and oral contrast), then the CT portion of the PET-CT can be used for RECIST measurements and can be used interchangeably with conventional CT in accurately measuring cancer lesions over time. Note, however, that the PET portion of the CT introduces additional data which may bias an investigator if it is not routinely or serially performed.

Ultrasound: Ultrasound is not useful in assessment of lesion size and should not be used as a method of measurement. Ultrasound examinations cannot be reproduced in their entirety for independent review at a later date and, because they are operator dependent, it cannot be guaranteed that the same technique and measurements will be taken from one assessment to the next. If new lesions are identified by ultrasound in the course of the study, confirmation by CT

or MRI is advised. If there is concern about radiation exposure at CT, MRI may be used instead of CT in selected instances.

Endoscopy, Laparoscopy: The utilization of these techniques for objective tumor evaluation is not advised. However, such techniques may be useful to confirm complete pathological response when biopsies are obtained or to determine relapse in trials where recurrence following complete response (CR) or surgical resection is an endpoint.

Tumor markers: Tumor markers alone cannot be used to assess response. If markers are initially above the upper normal limit, they must normalize for a patient to be considered in complete clinical response. Specific guidelines for both CA-125 response (in recurrent ovarian cancer) and PSA response (in recurrent prostate cancer) have been published [32-34]. In addition, the Gynecologic Cancer Intergroup has developed CA-125 progression criteria which are to be integrated with objective tumor assessment for use in first-line trials in ovarian cancer [35].

Cytology, Histology: These techniques can be used to differentiate between partial responses (PR) and complete responses (CR) in rare cases (e.g., residual lesions in tumor types, such as germ cell tumors, where known residual benign tumors can remain).

The cytological confirmation of the neoplastic origin of any effusion that appears or worsens during treatment when the measurable tumor has met criteria for response or stable disease is mandatory to differentiate between response or stable disease (an effusion may be a side effect of the treatment) and progressive disease.

FDG-PET: While FDG-PET response assessments need additional study, it is sometimes reasonable to incorporate the use of FDG-PET scanning to complement CT scanning in assessment of progression (particularly possible 'new' disease). New lesions on the basis of FDG-PET imaging can be identified according to the following algorithm:

a. Negative FDG-PET at baseline, with a positive FDG-PET at follow-up is a sign of PD based on a new lesion.
b. No FDG-PET at baseline and a positive FDG-PET at follow-up: If the positive FDG-PET at follow-up corresponds to a new site of disease confirmed by CT, this is PD. If the positive FDG-PET at follow-up is not confirmed as a new site of disease on CT, additional follow-up CT scans are needed to determine if there is truly progression occurring at that site (if so, the date of PD will be the date of the initial abnormal FDG-PET scan). If the positive FDG-PET at follow-up corresponds to a pre-existing site of disease on CT that is not progressing on the basis of the anatomic images, this is not PD.
c. FDG-PET may be used to upgrade a response to a CR in a manner similar to a biopsy in cases where a residual radiographic abnormality is thought to represent fibrosis or scarring. The use of FDG-PET in this circumstance should be prospectively described in the protocol and supported by disease-specific medical literature for the indication. However, it must be acknowledged that both approaches may lead to false positive CR due to limitations of FDG-PET and biopsy resolution/sensitivity.

Note: A ‘positive’ FDG-PET scan lesion means one which is FDG avid with an uptake greater than twice that of the surrounding tissue on the attenuation corrected image.

6.3.4 Response Criteria

6.3.4.1 Evaluation of Target Lesions

Complete Response (CR): Disappearance of all target lesions. Any pathological lymph nodes (whether target or non-target) must have reduction in short axis to <10 mm.

Partial Response (PR): At least a 30% decrease in the sum of the diameters of target lesions, taking as reference the baseline sum of diameters.

Progressive Disease (PD): At least a 20% increase in the sum of the diameters of target lesions, taking as reference the smallest sum on study (this includes the baseline sum if that is the smallest on study). In addition to the relative increase of 20%, the sum must also demonstrate an absolute increase of at least 5 mm. (Note: the appearance of one or more new lesions is also considered progressions).

Stable Disease (SD): Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum of diameters while on study.

6.3.4.2 Evaluation of Non-Target Lesions

Complete Response (CR): Disappearance of all non-target lesions and normalization of tumor marker level. All lymph nodes must be non-pathological in size (<10 mm short axis).

Note: If tumor markers are initially above the upper normal limit, they must normalize for a patient to be considered in complete clinical response.

Non-CR/Non-PD: Persistence of one or more non-target lesion(s) and/or maintenance of tumor marker level above the normal limits.

Progressive Disease (PD): Appearance of one or more new lesions and/or *unequivocal progression* of existing non-target lesions. *Unequivocal progression* should not normally trump target lesion status. It must be representative of overall disease status change, not a single lesion increase.

Although a clear progression of "non-target" lesions only is exceptional, the opinion of the treating physician should prevail in such circumstances, and the progression status should be confirmed at a later time by the review panel (or Principal Investigator).

6.3.4.3 Metastatic Bone Lesions

The Prostate Cancer Working Group 2 (PCWG2) criteria published guidelines on measuring treatment effects on bone metastases using bone scan and controlling for tumor flare.[36] PCWG2 defines disease progression as the appearance of two or more lesions on bone scan. However, new lesions seen on the first post-treatment bone scan may represent disease that was not detected on the pre-study scan or a "tumor flare" phenomenon. Thus, PCWG2 recommends that a repeat scan performed 6 or more weeks later should be performed before declaring disease progression by bone scan.[36] Confirmatory scans may not be necessary in the presence of progressive soft tissue disease or the appearance of multiple new bone lesions that in the opinion of the investigator are consistent with a progressive overall clinical course.

For this study, if a patient has new lesions on the first post-treatment bone scan, they may remain on-study with the first post-treatment bone scan now becoming the new baseline for future evaluations. All subsequent scans can be conducted as scheduled in the protocol or as clinically indicated and compared to the new baseline (first post-treatment scan) in determining disease progression by bone scan. Should the subsequent scans show 2 or more new lesions relative to the new baseline, the patient will then meet progression criteria. The date of disease progression is recorded as the date of the first scan when the new lesions are first detected.[36]

6.3.4.4 Evaluation of Best Overall Response

The best overall response is the best response recorded from the start of the treatment until disease progression/recurrence (taking as reference for progressive disease the smallest measurements recorded since the treatment started). The patient's best response assignment will depend on the achievement of both measurement and confirmation criteria.

For Patients with Measurable Disease (i.e., Target Disease)

Target Lesions	Non-Target Lesions	New Lesions	Overall Response	Best Overall Response when Confirmation is Required*
CR	CR	No	CR	≥4 wks. Confirmation**
CR	Non-CR/Non-PD	No	PR	≥4 wks. Confirmation**
CR	Not evaluated	No	PR	
PR	Non-CR/Non-PD/not evaluated	No	PR	
SD	Non-CR/Non-PD/not evaluated	No	SD	Documented at least once ≥4 wks. from baseline**
PD	Any	Yes or No	PD	no prior SD, PR or CR
Any	PD***	Yes or No	PD	
Any	Any	Yes	PD	
* See RECIST 1.1 manuscript for further details on what is evidence of a				

	new lesion.
**	Only for non-randomized trials with response as primary endpoint.
***	In exceptional circumstances, unequivocal progression in non-target lesions may be accepted as disease progression.
Note:	Patients with a global deterioration of health status requiring discontinuation of treatment without objective evidence of disease progression at that time should be reported as "*symptomatic deterioration."* Every effort should be made to document the objective progression even after discontinuation of treatment.

For Patients with Non-Measurable Disease (i.e., Non-Target Disease)

Non-Target Lesions	New Lesions	Overall Response
CR	No	CR
Non-CR/non-PD	No	Non-CR/non-PD*
Not all evaluated	No	not evaluated
Unequivocal PD	Yes or No	PD
Any	Yes	PD
* 'Non-CR/non-PD' is preferred over 'stable disease' for non-target disease since SD is increasingly used as an endpoint for assessment of efficacy in some trials so to assign this category when no lesions can be measured is not advised		

6.3.5 Duration of Response

Duration of overall response: The duration of overall response is measured from the time measurement criteria are met for CR or PR (whichever is first recorded) until the first date that recurrent or progressive disease is objectively documented (taking as reference for progressive disease the smallest measurements recorded since the treatment started).

The duration of overall CR is measured from the time measurement criteria are first met for CR until the first date that progressive disease is objectively documented.

Duration of stable disease: Stable disease is measured from the start of the treatment until the criteria for progression are met, taking as reference the smallest measurements recorded since the treatment started, including the baseline measurements.

6.3.6 Serologic Response:

PSA will be assessed at each visit during treatment and every 12 weeks thereafter. Patients will be assigned response based on the following criteria.

Complete Serological Response: PSA level less than 0.2 ng/ml measured for 2 consecutive measurements at least 4 weeks apart.

Serological Partial Response: decline of PSA at least 50% measured for 2 consecutive measurements at least 4 weeks apart.

Serological Progression:

- PSA has risen above 4 ng/ml or an increase in PSA of more than 50% above nadir (lowest PSA on ADT) or confirmed rise in PSA.
- Values must be measured for 2 consecutive measurements at least 2 weeks apart.
- The date of the first increase will be recorded as progression.

Time to Serologic Progression will be defined as interval between initiation of ADT and date of PSA progression or death from any cause.

6.4 TOXICITY CRITERIA

The following adverse event management guidelines are intended to ensure the safety of each patient while on the study. The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 will be utilized for AE reporting. All appropriate treatment areas should have access to a copy of the CTCAE version 4.0. A copy of the CTCAE version 4.0 can be downloaded from the CTEP web site (http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm#ctc_40).

6.5 ANTIGEN SPREAD SCORING

As described in section 1.2.5 of the background, antigen spread will be evaluated using CD4 and CD8 analysis of CD107a expression and IFN-γ, IL-2 and TNF-α intracellular cytokine staining. In order to give added weight to the spread antigens not targeted specifically by the vaccine (MUC1 and Brachyury) they will be weighted 150% of the PSA responses. The scoring parameters are below with a possible maximum score of 30.

For PSA:

1 point each of the four CD4 parameters (CD107a, IFN-γ, IL-2 and TNF-α) – max total of 4

1 point each of the four CD8 parameters (CD107a, IFN-γ, IL-2 and TNF-α) – max total of 4

Total possible score for PSA response is 8

For MUC1:

1.5 points each of the four CD4 parameters (CD107a, IFN-γ, IL-2 and TNF-α) – max total of 6

1.5 points each of the four CD8 parameters (CD107a, IFN-γ, IL-2 and TNF-α) – max total of 6

Total possible score for PSA response is 12.

For Brachyury:

1.5 points each of the four CD4 parameters (CD107a, IFN-γ, IL-2 and TNF-α) – max total of 6

1.5 points each of the four CD8 parameters (CD107a, IFN-γ, IL-2 and TNF-α) – max total of 6

Total possible score for PSA response is 12.

7 NIH REPORTING REQUIREMENTS/Data and Safety Monitoring Plan

7.1 DEFINITIONS

Please refer to definitions provided in Policy 801: Reporting Research Events found https://irbo.nih.gov/hrpp-policy-guidelines/.

7.2 OHSRP OFFICE OF COMPLIANCE AND TRAINING/IRB REPORTING

7.2.1 Expedited Reporting

Please refer to the reporting requirements in Policy 801: Reporting Research Events and Policy 802 Non-Compliance Human Subjects Research found https://irbo.nih.gov/hrpp-policy-guidelines/. Note: Only IND Safety Reports that meet the definition of an unanticipated problem or present new information that might affect the willingness of participants to enroll or remain on the study will need to be reported per these policies.

7.2.2 IRB Requirements for PI Reporting at Continuing Review

Please refer to the reporting requirements in Policy 801: Reporting Research Events found https://irbo.nih.gov/hrpp-policy-guidelines/.

7.3 NCI CLINICAL DIRECTOR REPORTING

Problems expeditiously reported to the OHSRP in the NIH eIRB system will also be reported to the NCI Clinical Director/designee; therefore, a separate submission for these reports is not necessary.

In addition to those reports, all deaths that occur within 30 days after receiving a research intervention **s**hould be reported via email unless they are due to progressive disease.

To report these deaths, please send an email describing the circumstances of the death to the NCICCRQA@mail.nih.gov within one business day of learning of the death.

7.4 INSTITUTIONAL BIOSAFETY COMMITTEE (IBC) REPORTING CRITERIA

7.4.1 Serious Adverse Event Reports to IBC

The Principal Investigator (or delegate) will notify IBC of any unexpected fatal or life-threatening experience associated with the use of PROSTVAC as soon as possible but in no event later than 7 calendar days of initial receipt of the information. Serious adverse events that are unexpected and associated with the use of the PROSTVAC, but are not fatal or life-threatening, must be reported to the NIH IBC as soon as possible, but not later than 15 calendar

days after the investigator's initial receipt of the information. Adverse events may be reported by using the FDA Form 3500a.

7.4.2 Annual Reports to IBC

Within 60 days after the one-year anniversary of the date on which the IBC approved the initial protocol, and after each subsequent anniversary until the trial is completed, the Principal Investigator (or delegate) shall submit the information described below. Alternatively, the IRB Continuing Review report can be sent to the IBC in lieu of a separate report. Please include the IBC protocol number on the report.

7.4.2.1 Clinical Trial Information

A brief summary of the status of the trial in progress or completed during the previous year. The summary is required to include the following information:

- the title and purpose of the trial
- clinical site
- the Principal Investigator
- clinical protocol identifiers
- participant population (such as disease indication and general age group, e.g., adult or pediatric);
- the total number of participants planned for inclusion in the trial; the number entered into the trial to date whose participation in the trial was completed; and the number who dropped out of the trial with a brief description of the reasons
- the status of the trial, e.g., open to accrual of subjects, closed but data collection ongoing, or fully completed,
- if the trial has been completed, a brief description of any study results.

7.4.2.2 Progress Report and Data Analysis

Information obtained during the previous year's clinical and non-clinical investigations, including:

- a narrative or tabular summary showing the most frequent and most serious adverse experiences by body system
- a summary of all serious adverse events submitted during the past year
- a summary of serious adverse events that were expected or considered to have causes not associated with the use of the gene transfer product such as disease progression or concurrent medications
- if any deaths have occurred, the number of participants who died during participation in the investigation and causes of death

- a brief description of any information obtained that is pertinent to an understanding of the gene transfer product's actions, including, for example, information about dose-response, information from controlled trials, and information about bioavailability.

7.5 NIH Required Data and Safety Monitoring Plan

7.5.1 Principal Investigator/Research Team

The clinical research team will meet on a regular basis weekly when patients are being actively treated on the trial to discuss each patient. Decisions about dose level enrollment and dose escalation if applicable will be made based on the toxicity data from prior patients.

All data will be collected in a timely manner and reviewed by the principal investigator or a lead associate investigator. Events meeting requirements for expedited reporting as described in section **7.2.1** will be submitted within the appropriate timelines.

The principal investigator will review adverse event and response data on each participant to ensure safety and data accuracy. The principal investigator will personally conduct or supervise the investigation and provide appropriate delegation of responsibilities to other members of the research staff.

7.5.2 Safety Monitoring Committee (SMC)

This protocol will require oversight from the Safety Monitoring Committee (SMC). Initial review will occur as soon as possible after the annual NIH-IRB continuing review date. Subsequently, each protocol will be reviewed as close to annually as the quarterly meeting schedule permits or more frequently as may be required by the SMC. For initial and subsequent reviews, protocols will not be reviewed if there is no accrual within the review period. Written outcome letters will be generated in response to the monitoring activities and submitted to the Principal investigator and Clinical Director or Deputy Clinical Director, CCR, NCI.

8 SPONSOR PROTOCOL/SAFETY REPORTING

8.1 Definitions

8.1.1 Adverse Event

Any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and which does not necessarily have a causal relationship with this treatment. An adverse event (AE) can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not related to the medicinal (investigational) product (ICH E6 (R2)).

8.1.2 Serious Adverse Event (SAE)

An adverse event or suspected adverse reaction is considered serious if in the view of the investigator or the sponsor, it results in any of the following:

- Death,
- A life-threatening adverse event (see section **8.1.3**)

- Inpatient hospitalization or prolongation of existing hospitalization
    - A hospitalization/admission that is pre-planned (i.e., elective or scheduled surgery arranged prior to the start of the study), a planned hospitalization for pre-existing condition, or a procedure required by the protocol, without a serious deterioration in health, is not considered a serious adverse event.
    - A hospitalization/admission that is solely driven by non-medical reasons (e.g., hospitalization for patient or subject convenience) is not considered a serious adverse event.
    - Emergency room visits or stays in observation units that do not result in admission to the hospital would not be considered a serious adverse event. The reason for seeking medical care should be evaluated for meeting one of the other serious criteria.
- Persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- A congenital anomaly/birth defect
- Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered a serious adverse drug experience when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

8.1.3 Life-threatening

An adverse event or suspected adverse reaction is considered "life-threatening" if, in the view of either the investigator or sponsor, its occurrence places the patient or subject at immediate risk of death. It does not include an adverse event or suspected adverse reaction that, had it occurred in a more severe form, might have caused death. (21CFR312.32)

8.1.4 Severity

The severity of each Adverse Event will be assessed utilizing the CTCAE version 4.0.

8.1.5 Relationship to Study Product

All AEs will have their relationship to study product assessed using the terms: related or not related.

- Related – There is a reasonable possibility that the study product caused the adverse event. Reasonable possibility means that there is evidence to suggest a causal relationship between the study product and the adverse event.

- Not Related – There is not a reasonable possibility that the administration of the study product caused the event.

8.2 ASSESSMENT OF SAFETY EVENTS

AE information collected will include event description, date of onset, assessment of severity and relationship to study product and alternate etiology (if not related to study product), date of resolution of the event, seriousness and outcome. The assessment of severity and relationship to the study product will be done only by those with the training and authority to make a diagnosis

and listed on the Form FDA 1572 as the site principal investigator or sub-investigator. AEs occurring during the collection and reporting period will be documented appropriately regardless of relationship. AEs will be followed through resolution.

SAEs will be:

- Assessed for severity and relationship to study product and alternate etiology (if not related to study product) by a licensed study physician listed on the Form FDA 1572 as the site principal investigator or sub-investigator.
- Recorded on the appropriate SAE report form, the medical record and captured in the clinical database.
- Followed through resolution by a licensed study physician listed on the Form FDA 1572 as the site principal investigator or sub-investigator.

For timeframe of recording adverse events, please refer to section **6.1**. All serious adverse events recorded from the time of first investigational product administration must be reported to the sponsor.

8.3 Reporting of Serious Adverse Events

Any AE that meets protocol-defined serious criteria must be submitted immediately (within 24 hours of awareness) to OSRO Safety.

All SAE reporting must include the elements described in section **8.2**.

SAE reports will be submitted via an electronic SAE reporting system (e.g. HiLIT). In the event of system downtime or issues, SAE reports will be submitted using the CCR SAE Report form to the sponsor at: OSROSafety@mail.nih.gov. CCR SAE report form and instructions can be found at: https://nih.sharepoint.com/:u:/r/sites/NCI-CCR-OCD-Communications/SitePages/Forms-and-Instructions.aspx?csf=1&web=1&e=uWBXtI

Following the assessment of the SAE by OSRO, other supporting documentation of the event may be requested by the OSRO Safety and should be provided as soon as possible.

8.4 Safety Reporting Criteria to the Pharmaceutical Collaborators

All events must be reported in the timelines defined in section **8.3** to OSROSafety@mail.nih.gov.

The CCR Office of Regulatory Affairs will send all reports to the manufacturer as described below.

Reporting to Bavarian Nordic, Inc.

The investigator should submit all safety reports that are sent to the FDA using the Medwatch 3500 Form to:

Bavarian Nordic, Inc.
Email: pharmacovigilance@bavarian-nordic.com;
Fax number for pharmacovigilance at BN: 888-465-1219

Attention: Karen Latina

8.5 REPORTING PREGNANCY

All required pregnancy reports/follow-up to OSRO will be submitted to: OSROSafety@mail.nih.gov and to the CCR PI and study coordinator. Forms and instructions can be found here:

https://nih.sharepoint.com/:u:/r/sites/NCI-CCR-OCD-Communications/SitePages/Forms-and-Instructions.aspx?csf=1&web=1&e=uWBXtI

8.5.1 Paternal exposure

Male participants should refrain from fathering a child or donating sperm during the study and for 4 months after the last dose of PROSTVAC.

Pregnancy of the participant's partner is not considered to be an AE. However, the outcome of all pregnancies (spontaneous miscarriage, elective termination, ectopic pregnancy, normal birth, or congenital abnormality) occurring from the date of the first dose until 4 months after the last dose should, if possible, be followed up and documented.

8.6 REGULATORY REPORTING FOR STUDIES CONDUCTED UNDER CCR-SPONSORED IND

Following notification from the investigator, CCR, the IND sponsor, will report any suspected adverse reaction that is both serious and unexpected. CCR will report an AE as a suspected adverse reaction only if there is evidence to suggest a causal relationship between the study product and the adverse event. CCR will notify FDA and all participating investigators (i.e., all investigators to whom the sponsor is providing drug under its INDs or under any investigator's IND) in an IND safety report of potential serious risks from clinical trials or any other source, as soon as possible, in accordance to 21 CFR Part 312.32.

All serious events will be reported to the FDA at least annually in a summary format.

8.7 SPONSOR PROTOCOL DEVIATION REPORTING

A Protocol Deviation is defined as any non-compliance with the clinical trial Protocol, Manual of Operational Procedures (MOP) and other Sponsor approved study related documents, GCP, or protocol-specific procedural requirements on the part of the participant, the Investigator, or the study site staff inclusive of site personnel performing procedures or providing services in support of the clinical trial.

It is the responsibility of the study Staff to document any protocol deviation identified by the Staff or the site Monitor in the CCR Protocol Deviation Tracking System (PDTS) online application. The entries into the PDTS online application should be timely, complete, and maintained per CCR PDTS user requirements.

In addition, any deviation to the protocol should be documented in the participant's source records and reported to the reviewing IRB per their guidelines. OSRO required protocol deviation reporting is consistent with E6(R2) GCP: Integrated Addendum to ICH E6(R1): 4.5

Compliance with Protocol; 5.18.3 (a), and 5.20 Noncompliance; and ICH E3 16.2.2 Protocol deviations.

9 CLINICAL MONITORING

Clinical site monitoring is conducted to ensure:

- that the rights of the participants are protected;
- that the study is implemented per the approved protocol, Good Clinical Practice and standard operating procedures; and,
- the quality and integrity of study data and data collection methods are maintained.

Monitoring for this study will be performed by NCI CCR Office of Sponsor and Regulatory Oversight (OSRO) Sponsor and Regulatory Oversight Support (SROS) Services contractor. Clinical site monitoring activities will be based on OSRO standards, FDA Guidance E6(R2) Good Clinical Practice: Integrated Addendum to ICH E6(R1) March 2018, and applicable regulatory requirements.

Details of clinical site monitoring will be documented in a Clinical Monitoring Plan (CMP) developed by OSRO. CMPs will be protocol-specific, risk-based and tailored to address human subject protections and integrity of the study data. OSRO will determine the intensity and frequency of monitoring based on several factors, including study type, phase, risk, complexity, expected enrollment rate, and any unique attributes of the study and the site. The Sponsor will conduct a periodic review of the CMP to confirm the plan's continued appropriateness. A change to the protocol, significant or pervasive non-compliance with GCP, or the protocol may trigger CMP updates.

OSRO SROS Monitoring visits and related activities will be conducted throughout the life cycle of each protocol. The first activity is before the study starts to conduct a Site Assessment Visit (SAV) (as warranted), followed by a Site Initiation Visit (SIV), Interim Monitoring Visit(s) (IMVs), and a study Close-Out Visit (COV).

Some monitoring activities may be performed remotely, while others will occur at the study site(s). Monitoring visit reports will describe visit activities, observations, and associated action items or follow-up required for resolution of any issues, discrepancies, or deviations. Monitoring reports will be distributed to the study PI, NCI CCR QA, CCR Protocol Support Office, coordinating center (if applicable), and the Sponsor regulatory file.

The site Monitor will inform the study team of any deviations observed during monitoring visits. If unresolved, the Monitor will request that the site Staff enter the deviations in the CCR Protocol Deviation Tracking System (PDTS) for deviation reporting to the Sponsor and as applicable per institutional and IRB guidance.

10 STATISTICAL CONSIDERATIONS

10.1 ARMS A AND B

The primary objective of this trial is to determine if PROSTVAC when combined with docetaxel can induce antigen spreading or a greater breadth of immune response compared to docetaxel followed by PROSTVAC. Patients enrolled onto this trial will be randomized to either ADT

followed by simultaneous docetaxel + PROSTVAC (combination) or ADT followed by sequential docetaxel then PROSTVAC. The main endpoint will be the mean over all patients, by arm, of the sum of the individual antigens considered positive (from 0 to 30 as described in section **1.2.5** and **6.5**), and called the response score, determined for each patient at 19 weeks following treatment initiation. It is anticipated that combining PROSTVAC with docetaxel (combination) may induce a much greater response than docetaxel alone; thus, the objective will be to determine if a large difference in results may be observed. Each patient will have a response score from 0 to 30 determined (see section **1.2.5** and **6.5**). To minimize differences in disease volume between the arms, patients will be stratified for low vs. high volume disease at randomization (definition: Low volume disease is defined as any metastatic disease that is not extensive. High volume disease is defined as: Visceral Metastases (extranodal) and /or Bone Metastases, at least 4 or more bone lesions, one of which must be outside of the vertebral column and pelvis.) With 17 evaluable patients per arm, there would be 81% power to detect a 1.0 SD difference (effect size=1.0) between the mean of the response scores on the two arms using a two-tailed 0.05 significance level two group t-test. In practice, if the response scores on either arm do not follow a normal distribution (Shapiro-Wilks test $p<0.05$), then the two arms will be compared by a Wilcoxon rank sum test.

In addition, as a secondary evaluation, patients' response scores will be compared between the two arms at 39 weeks and 1 year. Another secondary objective will be to compare in the same fashion patients after completing combination or sequence therapy; thus response scores after 19 weeks in the docetaxel + PROSTVAC arm will be compared to 39 weeks in the docetaxel followed by PROSTVAC arm. As these comparisons will be considered exploratory, the results will be presented without correction for the comparison made at the primary time points.

Other secondary objectives are to evaluate immunologic response including antigen-specific T-cell responses, T cell proliferation, Antibody Response, Natural Killer (NK) Cells, Regulatory T Cells; to evaluate changes in the tumor microenvironment with biopsies pre and post (2 cycles of therapy) when feasible (biopsies could include primary tumor); and to evaluate time to progression on PET imaging (NaF PET). These other secondary objectives will each be analyzed using appropriate nonparametric methods when applicable and the results from these analyses will be presented without formal adjustment for multiple comparisons, but in the context of the number of such evaluations undertaken. Time to progression will be estimated using a Kaplan-Meier curve.

10.2 ARM C

Following amendment D, patients who have not been treated with 28 days of ADT will be enrolled onto a single arm (Arm C) with PROSTVAC upfront followed by docetaxel. The objective for these subsequent previously untreated patients will be to obtain the same 0-30 response score and to compare these scores to those obtained from the arm with superior scores from among the two randomized arms. In order to do so, 26 evaluable patients without prior treatment will be enrolled onto the new arm. Assuming that there are 17 evaluable patients on the better of the two randomized arms, there will be 80% power to detect a 1.0 SD (effect size=1.0) difference between the means of the scores on the two arms using a 0.025 significance level two group t-test. In practice, if the response scores on either arm do not follow a normal distribution (Shapiro-Wilks test $p<0.05$), then the two arms will be compared by a Wilcoxon rank sum test.

The significance level is set at 0.025 for the second comparison since it is being added as a new primary comparison, and to be conservative, but the original comparison will remain at the 0.05 level since its interpretation should not be diminished by the subsequent addition of a second primary objective. Although the comparison is not based on two concurrently randomized arms, the results should at least offer a suggestion of whether there is a difference or not with respect to the score evaluated; if appropriate, analyses can be done to determine the similarity of traits for patients being compared with respect to factors that might be shown to be associated with the magnitude of the score.

As a secondary objective, the scores from the subset consisting of previously untreated patients on the superior randomized arm will be tested against the patients on the new PROSTVAC then docetaxel arm (Arm C). This comparison may be made with low power since the number of previously untreated patients on the randomized arm is expected to be limited. As this will also be a non-randomized comparison, it will be interpreted cautiously in that context.

10.3 ACCRUAL

Among the initial 34 randomized patients, 8 were inevaluable. Thus, additional patients will be added in amendment D in order to allow for the number inevaluable to date and in the future. In order to allow for the possibility of a small number of inevaluable patients in Arm C as well, the protocol ceiling will be set at 34 + 26 + 14 (allowing for up to 12 inevaluable in arms A and B together, and 2 in arm C) = 74. Based on accrual rates in the first 8 months the trial has been open, approximately 2 to 3 patients per month are expected to enroll onto the trial. As a result, including the amendment, the study is expected to complete enrollment within 2 to 3 years.

11 COLLABORATIVE AGREEMENTS

11.1 AGREEMENT TYPE

11.1.1 Cooperative Research and Development Agreement (CRADA):

This study is conducted under a CRADA with Bavarian Nordic, Inc. (CRADA # 02377)

12 HUMAN SUBJECTS PROTECTIONS

12.1 RATIONALE FOR SUBJECT SELECTION

Subjects treated on this study, will be individuals with newly diagnosed metastatic castrate sensitive prostate cancer. Eligibility assessment will be based solely on the patient's medical status. Recruitment of patients onto this study will be through standard CCR mechanisms. No special recruitment efforts will be conducted.

12.2 INCLUSION OF WOMEN AND MINORITIES

Men of all races and ethnic groups are eligible for this trial. Women are excluded as prostate cancer does not exist in this population.

12.3 Participation of Children

Men under the age of 18 will not be eligible for participation in this study based on the fact that patients under 18 are unlikely to have this disease and there are unknown toxicities in pediatric patients.

12.4 Participation of Subjects Unable to Give Consent

Adults unable to give consent are excluded from enrolling in the protocol. However re-consent may be necessary and there is a possibility, though unlikely, that subjects could become decisionally impaired. For this reason and because there is a prospect of direct benefit from research participation (section **12.6**), all subjects will be offered the opportunity to fill in their wishes for research and care, and assign a substitute decision maker on the "NIH Advance Directive for Health Care and Medical Research Participation" form so that another person can make decisions about their medical care in the event that they become incapacitated or cognitively impaired during the course of the study. Note: The PI or AI will contact the NIH Ability to Consent Assessment Team (ACAT) for evaluation as needed for the following: an independent assessment of whether an individual has the capacity to provide consent; assistance in identifying and assessing an appropriate surrogate when indicated; and/or an assessment of the capacity to appoint a surrogate.

Please see section **0** for consent procedure.

12.5 Evaluation of Benefits and Risks/Discomforts

Potential risks of vaccine in this patient population include the range of side effects outlined in section **13**. PROSTVAC has been well tolerated in previous large trials.

12.5.1 Alternative Approaches or Treatments

Patients will be advised verbally and in writing regarding the risks and benefits of this trial, treatment requirements, and alternative approaches to entering the trial. Written consents will be obtained.

12.5.2 Procedures to Eliminate or Minimize Potential Risks

This study may involve unforeseeable risks for patients, such as side effects whose exact nature and severity are unpredictable. Scrupulous care will be taken to minimize such side effects. All patients will be given blood tests, physical examinations, and scans, as described in the monitoring schedule **(Appendix A**: Study Calendar), and must have a local physician to provide long-term care and monitoring for complications. Immediate medical treatment is available at the Clinical Center, NCI, Bethesda, Maryland, for any patients who suffer physical injury as a result of participation in this study. No compensation is available, but any injury will be evaluated and treated in keeping with the benefits or care to which patients are entitled under applicable regulations.

12.5.3 Provisions for Monitoring Data Collection to Ensure Subject Safety

As information is gathered from this trial, clinical results will be shared with patients. Laboratory and clinical data will be frequently gathered and any new significant finding(s) found

during the course of the research, which may affect a patient's willingness to participate further, will be explained.

Confidentiality of information concerning participants will be maintained, including in all publications and presentations resulting from this study. Names of participants or material identifying participants will not be released without permission, except as such release is required by law. Records at the National Cancer Institute are maintained according to current legal requirements, and are made available for review, as required by the Food and Drug Administration or other authorized users, only under the guidelines established by the Federal Privacy Act.

12.5.4 Optional Biopsies

All care will be taken to minimize risks that may be incurred by tumor sampling. However, there are procedure-related risks (such as bleeding, infection and visceral injury) that will be explained fully during informed consent. If patients suffer any physical injury as a result of the biopsies, immediate medical treatment is available at the NCI's Clinical Center in Bethesda, Maryland. Although no compensation is available, any injury will be fully evaluated and treated in keeping with the benefits or care to which patients are entitled under applicable regulations.

12.5.5 Research Radiation Risks

After screening, which is performed on a separate protocol, this research study may involve exposure to radiation from 2 low dose CT with Sodium Fluoride PET (NaF PET) scans, up to 3 TC99 bone scans, and up to 3 CT CAP scans. This will result in an exposure of up to 5.27 rems per year which is associated with an increased risk of cancer. Due to the known radiosensitivity of reproductive organs, patients will be asked to use birth control and will be monitored after the last injection to observe the effects, if any, of radiation.

12.5.6 Risks from Contrast

CT scans often use a contrast agent. There is a small risk of having a reaction to the contrast and most often include nausea, pain in the vein where the contrast is given, headache, metallic and/or bitter taste in the mouth and a warm, flushing feeling. Rarely, some people have some severe allergic reactions to the contrast which may include skin rashes, shortness of breath, wheezing or low blood pressure.

12.5.7 Risks from Other Study Procedures

Side effects of blood draws include pain and bruising in the area where the needle is inserted, lightheadedness, and rarely, fainting.

There are minimal risks associated with electrocardiogram as this is a relatively safe procedure.

12.6 RISKS/BENEFITS ANALYSIS

This study involves clinical research with an experimental vaccine designed to generate an immune response against antigens found in prostate cancer. Patients will undergo multiple vaccinations. Side effects of the vaccine are outlined elsewhere. Whether the vaccine will have any clinical effect is unknown; therefore, benefit for all subjects cannot be promised from vaccine in addition to standard of care (ADT+docetaxel), nor the chance of benefit accurately

predicted for all patients on study. Participation in this study is voluntary, and refusal will not result in penalty or loss of benefit to which the patient is otherwise entitled.

Participation may be discontinued at any time without penalty, and the patient will be encouraged to discuss any concerns or questions.

12.7 CONSENT PROCESS AND DOCUMENTATION

The informed consent document will be provided as a physical or electronic document to the participant or consent designee(s) as applicable for review prior to consenting. A designated study investigator will carefully explain the procedures and tests involved in this study, and the associated risks, discomforts and benefits. In order to minimize potential coercion, as much time as is needed to review the document will be given, including an opportunity to discuss it with friends, family members and/or other advisors, and to ask questions of any designated study investigator. A signed informed consent document will be obtained prior to entry onto the study.

The initial consent process as well as re-consent, when required, may take place in person or remotely (e.g., via telephone or other NIH approved remote platforms used in compliance with policy, including HRPP Policy 303) per discretion of the designated study investigator and with the agreement of the participant/consent designee(s). Whether in person or remote, the privacy of the participant will be maintained. Consenting investigators (and participant/consent designee, when in person) will be located in a private area (e.g., clinic consult room). When consent is conducted remotely, the participant/consent designee will be informed of the private nature of the discussion and will be encouraged to relocate to a more private setting if needed.

Consent will be documented with required signatures on the physical document (which includes the printout of an electronic document sent to participant) or as described below, with a manual (non-electronic) signature on the electronic document. When required, witness signature will be obtained similarly as described for the investigator and participant.

Manual (non-electronic) signature on electronic document:

When a manual signature on an electronic document is used for the documentation of consent at the NIH Clinical Center, this study will use the following to obtain the required signatures:

- Adobe platform (which is not 21 CFR Part 11 compliant); or,
- iMedConsent platform (which is 21 CFR Part 11 compliant)

During the consent process, participants and investigators will view individual copies of the approved consent document on screens at their respective locations (if remote consent); the same screen may be used when in the same location but is not required.

Both the investigator and the participant will sign the document using a finger, stylus or mouse.

Note: Refer to the CCR SOP PM-2, Obtaining and Documenting the Informed Consent Process for additional information (e.g., verification of participant identity when obtaining consent remotely) found here.

For the optional biopsy and imaging for research in the protocol, the patient will consent at the time of the procedure. If the patient refuses the optional biopsy at that time, the refusal will be documented in the medical record and in the research record.

12.7.1 Consent Process for Adults Who Lack Capacity to Consent to Research Participation

For participants addressed in section **12.4**, an LAR will be identified consistent with Policy 403 and informed consent obtained from the LAR, as described in Section **12.7**.

13 REGULATORY AND OPERATIONAL CONSIDERATIONS

13.1 STUDY DISCONTINUATION AND CLOSURE

This study may be temporarily suspended or prematurely terminated if there is sufficient reasonable cause. Written notification, documenting the reason for study suspension or termination, will be provided by the suspending or terminating party to study participants, investigator, the Investigational New Drug (IND) sponsor and regulatory authorities. If the study is prematurely terminated or suspended, the Principal Investigator (PI) will promptly inform study participants, the Institutional Review Board (IRB), and sponsor and will provide the reason(s) for the termination or suspension. Study participants will be contacted, as applicable, and be informed of changes to study visit schedule.

Circumstances that may warrant termination or suspension include, but are not limited to:

- Determination of unexpected, significant, or unacceptable risk to participants
- Demonstration of efficacy that would warrant stopping
- Insufficient compliance to protocol requirements
- Data that are not sufficiently complete and/or evaluable
- Determination that the primary endpoint has been met
- Determination of futility

Study may resume once concerns about safety, protocol compliance, and data quality are addressed, and satisfy the sponsor, IRB and as applicable, Food and Drug Administration (FDA).

13.2 QUALITY ASSURANCE AND QUALITY CONTROL

The clinical site will perform internal quality management of study conduct, data and biological specimen collection, documentation and completion. An individualized quality management plan will be developed to describe a site's quality management.

Quality control (QC) procedures will be implemented beginning with the data entry system and data QC checks that will be run on the database will be generated. Any missing data or data anomalies will be communicated to the site(s) for clarification/resolution.

Following written Standard Operating Procedures (SOPs), the monitors will verify that the clinical trial is conducted, and data are generated, and biological specimens are collected, documented (recorded), and reported in compliance with the protocol, International Council for Harmonisation Good Clinical Practice (ICH GCP), and applicable regulatory requirements (e.g., Good Laboratory Practices (GLP), Good Manufacturing Practices (GMP)).

The investigational site will provide direct access to all trial related sites, source data/documents, and reports for the purpose of monitoring and auditing by the sponsor, and inspection by local and regulatory authorities.

13.3 CONFLICT OF INTEREST POLICY

The independence of this study from any actual or perceived influence, such as by the pharmaceutical industry, is critical. Therefore, any actual conflict of interest of persons who have a role in the design, conduct, analysis, publication, or any aspect of this trial will be disclosed and managed. Furthermore, persons who have a perceived conflict of interest will be required to have such conflicts managed in a way that is appropriate to their participation in the design and conduct of this trial. The study leadership in conjunction with the NIH has established policies and procedures for all study group members to disclose all conflicts of interest and will establish a mechanism for the management of all reported dualities of interest.

13.4 CONFIDENTIALITY AND PRIVACY

Participant confidentiality and privacy is strictly held in trust by the participating investigators, their staff, and the sponsor(s). This confidentiality is extended to cover testing of biological samples and genetic tests in addition to the clinical information relating to participants. Therefore, the study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorized third party without prior written approval of the sponsor.

All research activities will be conducted in as private a setting as possible.

The study monitor, other authorized representatives of the sponsor, representatives of the Institutional Review Board (IRB), and/or regulatory agencies may inspect all documents and records required to be maintained by the investigator, including but not limited to, medical records (office, clinic, or hospital) and pharmacy records for the participants in this study. The clinical study site will permit access to such records.

The study participant's contact information will be securely stored at the clinical site for internal use during the study. At the end of the study, all records will continue to be kept in a secure location for as long a period as dictated by the reviewing IRB, Institutional policies, or sponsor requirements.

Study participant research data, which is for purposes of statistical analysis and scientific reporting, will be transmitted to and stored at the NCI CCR. This will not include the participant's contact or identifying information. Rather, individual participants and their research data will be identified by a unique study identification number. The study data entry and study management systems used by the clinical site and by NCI CCR research staff will be secured and password protected. At the end of the study, all study databases will be archived at the NIH.

To further protect the privacy of study participants, a Certificate of Confidentiality has been issued by the National Institutes of Health (NIH). This certificate protects identifiable research information from forced disclosure. It allows the investigator and others who have access to research records to refuse to disclose identifying information on research participation in any civil, criminal, administrative, legislative, or other proceeding, whether at the federal, state, or local level. By protecting researchers and institutions from being compelled to disclose

information that would identify research participants, Certificates of Confidentiality help achieve the research objectives and promote participation in studies by helping assure confidentiality and privacy to participants.

14 PHARMACEUTICAL INFORMATION

14.1 RECOMBINANT FOWLPOX-PSA(L155)TRICOM™

Other Names: PROSTVAC-F/TRICOM™; PROSTVAC-F

Classification: Recombinant fowlpox virus vector vaccine of the genus *Avipoxvirus.*

Product Description: Recombinant Fowlpox-PSA(L155)/TRICOM™ is a recombinant fowlpox virus vector vaccine containing the genes for human PSA and three co-stimulatory molecules (designated TRICOM™): B7.1, ICAM-1 (intercellular adhesion molecule-1), and LFA-3 (leukocyte function-associated antigen-3). The PSA gene coding sequence is modified to code for a single amino acid substitution [isoleucine to leucine at amino acid position 155 of the PSA antigen (designated L155)], which is designed to enhance immunogenicity. This modification occurs in a 10-mer, HLA-A2-restricted, immunodominant epitope of the antigen [designated PSA-3 (amino acids 154-163)]. An attenuated, live, plaque-purified isolate from the POXVAC-TC strain of fowlpox virus was used as the parental virus for this recombinant vaccine. A plasmid vector containing the modified PSA gene and the genes for the three co-stimulatory molecules is used for *in vivo* recombination between the plasmid vector and parental fowlpox virus genome. The recombinant vaccine is manufactured by infection of primary chicken embryo dermal (CED) cells with the recombinant fowlpox virus. Fowlpox virus can infect mammalian cells and express the inserted transgenes to stimulate both humoral and cellular immunity, but cannot replicate in non-avian species, making systemic infections unlikely.

14.1.1 How Supplied

Recombinant Fowlpox-PSA(L155)/TRICOM™ is supplied in vials containing 0.75 mL of the vaccine at a final viral concentration titer of 2 x 10^9 infectious units/mL formulated in phosphate-buffered saline containing 10% glycerol.

14.1.2 Preparation

Thaw vials completely at room temperature. Ensure the thawed contents are at the bottom of the upright vial and vortex vigorously at high power for at least ten seconds prior to dose preparation. Withdraw 0.5 mL (1 x 10^9 infectious units) into a 1 mL syringe for administration by subcutaneous injection.

14.1.3 Storage

Store intact vials of Recombinant Fowlpox-PSA(L155)/TRICOM™ at –70°C or colder.

14.1.4 Stability

Shelf-life stability studies of the intact vials are ongoing. Once the intact vials are thawed, the vaccines maintain their potency for up to 4 days when stored at 2-8°C. Do not re-freeze thawed vials. Vials of Recombinant Fowlpox-PSA(L155)/TRICOM™ are for single-use only and do not contain a preservative. Administer prepared doses as soon as possible following preparation (*i.e.*,

within one hour). If necessary, store prepared doses at 2-8ºC for up to 4 hours following preparation.

14.1.5 Route of Administration

Recombinant Fowlpox-PSA(L155)/TRICOM™ is administered by subcutaneous injection.

14.1.6 Special Handling

Fowlpox virus is classified as a Biosafety Level 1 agent. These agents are not known to cause disease in healthy human adults and are of minimal potential hazard to personnel and the environment under ordinary conditions of use. Clinicians can use techniques generally acceptable for nonpathogenic material. The recombinant vaccine is a preparation of a live virus (infectious for birds) containing DNA sequences derived from the human genome. Handle the recombinant vaccine as a hazardous biological substance and dispose of waste materials as hazardous biological waste, with incineration according to local institutional policy and according to local, state, and federal regulations. Healthcare workers handling the recombinant fowlpox vaccine should avoid direct contact with pet birds for at least 72 hours after working with the agent.

Preparation, Handling and Disposal Recommendations

1. Strictly adhere to standard microbiological practices and techniques.
2. Limit/restrict access to preparation areas while dose preparation is in progress.
3. Use appropriate infection control measures (e.g., thorough hand washing) after handling any materials.
4. Institute and follow policies for safe handling of sharps.
5. Perform all dose preparations in a certified Class II biological safety cabinet, generally using procedures, guidelines and personal protective apparel used during preparation of antineoplastic agents [*e.g.*, minimizing creation of aerosols; no eating, drinking, handling contact lenses or applying cosmetics in the work area; using appropriate personal protective apparel - gowns, sterile latex gloves (double-gloving is recommended), respirator masks, protective eye wear, hair cover].
6. Decontaminate the biological safety cabinet prior to dose preparation with sterile gauze soaked in 10% bleach solution (0.52% sodium hypochlorite solution), or other appropriate disinfectant suitable for decontamination, rinsing, then wiping down with sterile gauze soaked in 70% alcohol. Consult specific manufacturer's recommendations with respect to disinfectant concentration, contact time and method of application.
7. Have all necessary supplies on-hand before beginning the preparation procedure. Develop a detailed worksheet outlining all supplies, dose calculations, and preparation procedures, and keep it available.
8. Place an empty biohazard sharps container lined with a leak-proof biohazard bag in or near the biosafety cabinet to dispose of all waste generated.
9. Transport the agent from the freezer to the work area in leak proof bag.
10. Wipe or spray items used for dose preparation with 70% alcohol before placing in the biological safety cabinet. Disinfectants should remain in contact with the surfaces for at least five minutes prior to dose preparation. Avoid exposing the virus to disinfectants.

11. Wipe the syringe containing the prepared dose with 70% alcohol before removing it from the biological safety cabinet; transport it in a leak proof bag or container labeled with a biohazard symbol.
12. Place all waste into the sharps container lined with the leak proof biohazard bag and decontaminate the biological safety cabinet again by wiping down all surfaces with sterile gauze soaked in 10% bleach solution, or other appropriate disinfectant, rinsing, then wiping down with sterile gauze soaked in 70% alcohol. Following decontamination, dispose of personal protective apparel in the biohazard safety bag.
13. Incinerate waste according to institutional policy and according to local, state, and federal regulations.
14. Handle accidental spills similarly to antineoplastic spills and/or according to institutional policy:
    - Prevent others from entering the area and allow aerosols time to settle (approximately 10 minutes).
    - Use protective apparel, eyewear, mask, and gloves.
    - Cover spills with disposable absorbent towels.
    - Decontaminate the area with 10% bleach solution, or other appropriate disinfectant suitable for decontamination, allowing approximately a 20-minute contact time.
    - Dispose of all waste as biohazardous waste and incinerate according to institutional policy and according to local, state, and federal regulations.
15. Immediately report spills and accidents resulting in overt exposure to recombinant DNA molecules to the Institutional Biosafety Committee and NIH/OSP (Office of Science Policy). Send reports to the Office of Science Policy, National Institutes of Health, 6705 Rockledge Drive, Suite 750, MSC 7985, Bethesda, MD 20892-7985. Phone (301) 496-9838; HGTprotocols@mail.nih.gov. Provide medical evaluation, surveillance, and treatment as appropriate and maintain written records of the event.

    For more information about biohazard risk group classification and biohazard safety levels see:

    - *NIH Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines). See current version at: http://osp.od.nih.gov/sites/default/files/NIH_Guidelines.html*
    - *Biosafety in Microbiological and Biomedical Laboratories; U. S. Department of Health and Human Services Centers for Disease Control and Prevention and National Institutes of Health. See current edition at: http://www.cdc.gov/biosafety/publications/index.htm*

Patient Care Implications and Contraindications

Cover vaccination sites with a sterile dry dressing (e.g., Telfa pad). Once the injection site is healed, no further barrier is necessary. As a precaution, protect injection sites that are exhibiting evidence of weeping, oozing or ulceration with a sterile dry dressing. In these circumstances, instruct patients to avoid direct contact of the injection site with susceptible individuals (e.g.; those who may be immunocompromised by disease or therapy). Instruct patients to avoid fathering a child for at least 4 months following therapy completion with the recombinant vaccine. Instruct patients receiving fowlpox vaccines to avoid direct contact with pet birds for at

least 72 hours after vaccination or while there are any visible lesions at the injection site.

Due to the method of manufacturing (virus grown in primary chicken embryo dermal cells), patients with a history of allergy to eggs or egg products should not receive the vaccine.

14.2 RECOMBINANT VACCINIA-PSA(L155)/TRICOM™

Other Names: PROSTVAC-V/TRICOM™; PROSTVAC-V

Classification: Recombinant vaccinia virus vector vaccine of the genus *Orthopoxvirus.*

Product Description: Recombinant Vaccinia-PSA(L155)/TRICOM™ is a recombinant vaccinia virus vector vaccine containing the genes for human PSA and three co-stimulatory molecules (designated TRICOM™): B7.1, ICAM-1 (intercellular adhesion molecule-1), and LFA-3 (leukocyte function-associated antigen-3). The PSA gene coding sequence is modified to code for a single amino acid substitution [isoleucine to leucine at amino acid position 155 of the PSA antigen (designated L155)], which is designed to enhance immunogenicity. This modification occurs in a 10-mer, HLA-A2-restricted, immunodominant epitope of the antigen [designated PSA-3 (amino acids 154-163)]. An attenuated, live, derivative of the Wyeth (New York City Board of Health) strain of vaccinia virus is used as the parental virus for the recombinant vaccine. A plasmid vector containing the modified PSA gene and the genes for the three co-stimulatory molecules is used for *in vivo* recombination between the plasmid vector and parental vaccinia virus genome. The recombinant vaccine is manufactured by infection of primary chicken embryo dermal (CED) cells with the recombinant vaccinia virus. Vaccinia virus can infect mammalian cells and express the inserted transgenes, and is a potent immune stimulator, eliciting both a strong humoral and cellular immune response. Vaccinia virus is replication competent in mammalian cells, making systemic infections possible.

14.2.1 How Supplied

Recombinant Vaccinia-PSA(L155)/TRICOM™ is supplied in vials containing 0.75 mL of the vaccine at a final viral concentration titer of 4 x 10^8 infectious units/mL formulated in phosphate-buffered saline containing 10% glycerol.

14.2.2 Preparation

Thaw vials completely at room temperature. Ensure the thawed contents are at the bottom of the upright vial and vortex vigorously at high power for at least ten seconds prior to dose preparation. Withdraw 0.5 mL (2 x 10^8 infectious units) into a 1 mL syringe for administration by subcutaneous injection.

14.2.3 Storage

Store intact vials of Recombinant Vaccinia-PSA(L155)/TRICOM™ at –70°C or colder.

14.2.4 Stability

Shelf-life studies of the intact vials are ongoing. Once the intact vials are thawed, the vaccines maintain their potency for up to 4 days when stored at 2-8^{o}C. Do not re-freeze thawed vials. Vials of Recombinant Vaccinia-PSA(L155)/TRICOM™ are for single-use only and do not contain a preservative. Administer prepared doses as soon as possible following preparation (*i.e.*, within one hour). If necessary, store prepared doses at 2-8^{o}C for up to 4 hours following preparation.

14.2.5 Route of Administration

Recombinant Vaccinia-PSA(L155)/TRICOM™ is administered by subcutaneous injection.

14.2.6 Special Handling and Precautions

Vaccinia virus is classified as a Biosafety Level 2 agent. These agents are associated with human disease and are of moderate potential hazard to personnel and the environment. The recombinant vaccine is a preparation of a live virus affecting humans and contains DNA sequences derived from the human genome. Handle the recombinant vaccine as an infectious hazardous biological substance and dispose of waste materials as infectious hazardous biological waste, with incineration according to local institutional policies and according to local, state, and federal regulations.

Preparation, Handling and Disposal Recommendations

1. Prepare a biosafety manual which advises personnel of special hazards and specific instructions on practices and procedures.
2. Post warning hazard signs on access doors, identifying the agents, the biosafety level, the name and phone number of the Principal Investigator or other responsible person, and any special requirements for entry.
3. Establish policies and procedures allowing only personnel who are knowledgeable of the potential hazards and meet specific entry requirements (*e.g.*, immunization) into agent preparation or storage areas.
4. Strictly adhere to standard microbiological practices and techniques.
5. Limit/restrict access to preparation areas while dose preparation is in progress.
6. Use appropriate infection control measures (*e.g.*, thorough hand washing) after handling any materials.
7. Institute and follow policies for safe handling of sharps. Use only needle-lock syringes and needles for dose preparation. Use extreme caution to prevent autoinoculation. Do not bend, shear, or replace the needle guard from the syringe following use. Promptly place used needles and syringes in puncture-resistant containers for disposal.
8. Perform all dose preparations in a certified Class II biological safety cabinet, generally using procedures, guidelines and personal protective apparel used during preparation of antineoplastic agents [*e.g.*, minimizing creation of aerosols; no eating, drinking, handling contact lenses or applying cosmetics in the work area; using appropriate personal protective apparel - gowns, sterile latex gloves (double-gloving is recommended), respirator masks, protective eyewear, hair cover].
9. Perform all procedures carefully to minimize aerosol creation.

10. Decontaminate the biological safety cabinet prior to dose preparation with sterile gauze soaked in 10% bleach solution (0.52% sodium hypochlorite solution), or other appropriate disinfectant suitable for decontamination, rinsing, then wiping down with sterile gauze soaked in 70% alcohol. Consult specific manufacturer's recommendations with respect to disinfectant concentration, contact time and method of application.
11. Have all necessary supplies on-hand before beginning the preparation procedure. Develop a detailed worksheet outlining all supplies, dose calculations, and preparation procedures, and keep it available.
12. Place an empty biohazard sharps container in the biosafety cabinet to dispose of all waste generated.
13. Transport the agent from the freezer to the work area in leak proof bag.
14. Wipe or spray items used for dose preparation with 70% alcohol before placing in the biological safety cabinet. Disinfectants should remain in contact with the surfaces for at least five minutes prior to dose preparation. Avoid exposing the virus to disinfectants.
15. Wipe the syringe containing the prepared dose with 70% alcohol before removing it from the biological safety cabinet; transport it in a leak proof bag or container labeled with a biohazard symbol.
16. Place all waste into a sharps container lined with the leak proof biohazard bag and decontaminate the biological safety cabinet again by wiping down all surfaces with sterile gauze soaked in 10% bleach solution, or other appropriate disinfectant, rinsing, then wiping down with sterile gauze soaked in 70% alcohol. Following decontamination, dispose of personal protective apparel in the biohazard safety bag.
17. Place all waste and protective apparel in a leak proof biohazard bag, and place the bag inside a biohazard sharps container for incineration according to institutional policy and according to local, state, and federal regulations.
18. Handle accidental spills similarly to antineoplastic spills and/or according to institutional policy:
    a. Prevent others from entering the area and allow aerosols time to settle (approximately 10 minutes).
    b. Use protective apparel, eyewear, mask, and gloves.
    c. Cover spills with disposable absorbent towels.
    d. Decontaminate the area with 10% bleach solution, or other appropriate disinfectant suitable for decontamination, allowing approximately a 20-minute contact time.
    e. Dispose of all waste and protective apparel as infectious biohazardous waste and incinerate according to institutional policy and according to local, state, and federal regulations.
19. Immediately report spills and accidents resulting in overt exposure to recombinant DNA molecules to the Institutional Biosafety Committee and NIH/OSP (Office of Science Policy). Send reports to the Office of Science Policy, National Institutes of Health, 6705 Rockledge Drive, Suite 750, MSC 7985, Bethesda, MD 20892-7985. Phone (301) 496-9838; HGTprotocols@mail.nih.gov. Provide medical evaluation, surveillance, and treatment as appropriate and maintain written records of the event.

For more information about biohazard risk group classification and biohazard safety levels:

- *NIH Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines). See current version at: http://osp.od.nih.gov/sites/default/files/NIH_Guidelines.html*
- *Biosafety in Microbiological and Biomedical Laboratories; U. S. Department of Health and Human Services Centers for Disease Control and Prevention and National Institutes of Health. See current edition at: http://www.cdc.gov/biosafety/publications/index.htm*

Precautions for Healthcare Workers

Recombinant vaccinia virus transmission risk to exposed healthcare workers is unknown. To date, no reports of transmission to healthcare personnel from vaccine recipients have been published. If appropriate infection control precautions are observed (such as covering the vaccination site and washing hands after contact with the vaccination site or bandages), healthcare workers are probably at less risk of infection than laboratory workers because of the smaller volume and lower titers of virus in clinical specimens as compared with laboratory material. However, because of the potential risk for transmission, healthcare personnel who prepare or administer doses of recombinant vaccinia vaccine or have direct contact with contaminated dressings or other infectious material from participants in clinical studies must adhere to appropriate infection control measures and should be offered vaccination with the licensed vaccinia vaccine. Do not administer routine, non-emergency vaccination with the licensed vaccinia vaccine to healthcare workers, if they, or for at least three weeks after vaccination, their close household contacts (close household contacts are those who share housing or have close physical contact):

- have active eczema or a history of eczema or atopic dermatitis, or Darier's disease.
- have other acute, chronic, or exfoliative skin conditions (e.g., burns, impetigo, varicella zoster, severe acne, or other open rashes or wounds), until the condition resolves.
- are pregnant or intend to become pregnant within 4 weeks of vaccination or are breast-feeding.
- are immunodeficient or immunocompromised (by disease or therapy), including HIV infection.

Additionally, do not administer routine, non-emergency vaccination with the licensed vaccinia vaccine to healthcare workers if the vaccinee:

- has a moderate or severe acute illness, until the illness resolves.
- is less than 18 years of age, unless specifically indicated.
- is undergoing topical steroid therapy for inflammatory eye diseases or undergoing therapy with systemic steroids; potential immune suppression increases risk for vaccinia-related complications.
- has a history of allergy or serious reaction to prior vaccinia vaccination or any of the vaccine's components.
- As a precaution, the CDC recommends that individuals with known cardiac disease (e.g., previous MI, angina, CHF, cardiomyopathy, stroke or TIA) or who have ≥ 3 known risk factors for cardiac disease (e.g., hypertension, hypercholesterolemia, diabetes, first degree relative with onset of cardiac complications prior to age 50, smoker), not receive routine,

non-emergency, prophylactic vaccination with the licensed vaccinia vaccine while a possible causal relationship between vaccination and cardiac events is being evaluated.

Avoid exposure to the recombinant vaccinia vaccine, any contaminated dressings, or other infectious materials from patients, or the patient's inoculation site if you are pregnant or breast-feeding; have a history or presence of active eczema or atopic dermatitis; have acute, chronic or exfoliative skin conditions; or, are immunocompromised. More information on vaccinia precautions for healthcare workers can be obtained from http://www.cdc.gov/mmwr/preview/mmwrhtml/rr5010a1.htm#tab2 and http://www.cdc.gov/mmwr/PDF/rr/rr5207.pdf.

The CDC is the only source of the licensed vaccinia vaccine. The CDC will provide vaccinia vaccine to protect laboratory and other healthcare personnel, whose occupations place them at risk of exposure to vaccinia and other closely related orthopoxviruses, including vaccinia recombinants. The vaccine should be administered under the supervision of a physician selected by the study institution. Revaccination is recommended every 10 years. For instructions on obtaining the licensed vaccinia vaccine, contact Drug Services, National Center for Infectious Diseases, CDC at (404) 639-3670.

Recombinant Vaccinia Vaccine Patient Care Implications, Contraindications and Potential Complications

Patient Care Implications and Contraindications

Cover vaccination sites with a sterile dry dressing (*e.g.*, Telfa pad). Instruct patients on proper hand-hygiene, sterile dressing care, bathing, laundering of clothing, *etc*. Treat patient bandages or dressings removed from the vaccination site as infectious waste and dispose in appropriate biohazard containers. Do not administer the recombinant vaccinia vaccine if the recipient, or for at least three weeks after vaccination, their close household contacts (close household contacts are those who share housing or have close physical contact):

- have active eczema or a history of eczema or atopic dermatitis, or Darier's disease.
- have other acute, chronic, or exfoliative skin conditions (*e.g.*, burns, impetigo, varicella zoster, severe acne, contact dermatitis, psoriasis, herpes or other open rashes or wounds), until the condition resolves.
- are pregnant or intend to become pregnant (due to the potential risk of fetal vaccinia); or are breast-feeding (due to the potential risk of contact transmission and inadvertent inoculation). It is currently unknown if vaccinia virus or antibodies are excreted in breast milk. Patients (*i.e.*, vaccinees) should avoid fathering a child for at least 4 months following completion of therapy with the recombinant vaccine.
- are in close contact with children less than 3 years of age (due to the potential risk of contact transmission and inadvertent inoculation).
- are immunodeficient or immunocompromised (by disease or therapy), including individuals with HIV infection.

Additionally, do not administer the recombinant vaccinia vaccine if the vaccinee:

- has a moderate or severe acute illness, until the illness resolves.

- is undergoing topical steroid therapy for inflammatory eye diseases, or undergoing therapy with systemic steroids; potential immune suppression increases risk for vaccinia-related complications.
- At this time, until a more definitive causal relationship is determined, it is recommended that patients with known CHF or clinically significant cardiomyopathy, not be vaccinated with recombinant vaccinia-based vaccines, due to the potential for development of myocarditis and/or pericarditis.

  Although the CDC believes that there is no evidence to conclude that the licensed vaccinia vaccine used in the Smallpox Vaccination Program causes angina or heart attacks, it acknowledges a possible causal relationship between the vaccination and heart inflammation. The CDC continues to study the relationship, but in the meantime, recommends excluding individuals with underlying heart disease from participation in the current Smallpox Vaccination Program. Patients are being immunized with recombinant vaccinia vaccines with therapeutic intent and will be evaluated for cardiovascular risk factors and recent or symptomatic cardiac events per protocol eligibility criteria. Patients will be encouraged to minimize cardiovascular disease risks and encouraged to follow risk reduction according to standard medical practice.

Due to the method of manufacturing (virus grown in primary chicken embryo dermal cells), do not administer the recombinant vaccinia vaccine to patients with a history of allergy to eggs or egg products. Do not administer the recombinant vaccinia vaccine to patients with a history of allergy or serious reaction to prior vaccinia vaccination (*e.g.*, smallpox vaccination).

Potential Complications Associated With Recombinant Vaccinia Vaccination

When vaccinia vaccine is administered by scarification for vaccination against smallpox, expected local reactions in individuals that have not previously been vaccinated with vaccinia include the appearance of a red papule in 3-4 days, followed by vesiculation in 5-6 days, and then the formation of a pustule on days 8-9. A large area of erythema may surround the vesicle and pustule. A crusted scab usually forms by the second week and sloughs by the third week, leaving a well-formed scar. Maximal viral shedding occurs from days 4-14, but can continue until the scab is shed from the skin. Other normal local reactions can include development of local satellite lesions, regional lymphadenopathy that can persist for weeks following healing of the skin lesion, considerable local edema, and intense inflammation from the vaccination (*i.e.*, viral cellulites), which may be confused with bacterial cellulites. Systemic symptoms typically occur between 8-10 days post-vaccination and include fever, malaise, headache, chills, nausea, myalgia, local lymphadenopathy, soreness and intense erythema surrounding the vaccination site.

When administered by scarification in individuals that have previously been vaccinated with vaccinia, expected local reactions include the appearance of a clear cut pustule 6-8 days following vaccination or the development of an area of definite induration around a central lesion that may be an ulcer or scab 6-8 days following vaccination. The response to re-vaccination depends on the degree of residual immunity following previous vaccination. Similar systemic symptoms may occur, but typically at a lower frequency.

When recombinant vaccinia vaccines are administered by intradermal, intralesional, subcutaneous or intramuscular routes of injection, milder local reactions are expected, but similar systemic symptoms may occur.

There have been a number of complications reported in the literature associated with vaccinia vaccination for smallpox. Reported complications from vaccinia vaccine when given by scarification include: a) auto-inoculation of other sites with vaccinia, b) generalized vaccinia, c) eczema vaccinatum, d) progressive vaccinia (vaccinia necrosum), or e) post-vaccinial encephalitis. In a 1968 ten-state survey, cases of these complications per million vaccinations in adult recipients (≥ 20 years of age) of vaccinia primary vaccination and revaccination were:

	Primary Vaccination	Revaccination
auto-inoculation	606.1	25
generalized vaccinia	212.1	9.1
eczema vaccinatum	30.3	4.5
progressive vaccinia	none reported	6.8
postvaccinial encephalitis	none reported	4.5

Based on a 1968 national survey, the number of deaths in primary vaccinees was approximately 1 per million and the number of deaths in recipients of revaccination was approximately 0.25 per million. Deaths were most often the result of postvaccinial encephalitis or progressive vaccinia.

Information has been reported by the US Department of Defense (DoD) during the post-vaccination surveillance assessment of adverse events in military personnel following implementation of a Smallpox Vaccination Program from the period of December 13, 2002 through May 28, 2003. Although not directly comparable to historical numbers, due to differences in multiple population variables, estimated cases (number of cases per million vaccinations based on vaccination of 450,293 personnel, with a median age of 26 years and 70.5% as primary vaccinees) of these same complications per million vaccinations were:

auto-inoculation	107
generalized vaccinia	80
eczema vaccinatum	none reported
progressive vaccinia	none reported
postvaccinial encephalitis	2.2

Generally, self-limited adverse reactions that can be serious, but not life-threatening include autoinoculation, erythematous and urticarial rashes, and generalized vaccinia. More serious life-threatening complications include progressive vaccinia, eczema vaccinatum, and post-vaccinial encephalitis/encephalomyelitis. The complications of vaccinia vaccination may involve a number of different reactions:

1. **Non-specific erythematous or urticarial rashes:** These rashes can appear approximately 10 days after vaccination and may sometimes be confused with generalized vaccinia, but are generally self-limiting. Patients are usually afebrile and these benign rashes usually resolve spontaneously within 2-4 days. Erythema multiforme can present as different types of lesions, including macules, papules, urticaria, and bull's eye lesions (dark papule or vesicle surrounded by a pale zone and an area of erythema). These lesions may be extremely pruritic, lasting up to four weeks. Rarely, more serious bullous erythema multiforme (Stevens-Johnson syndrome) may occur, requiring hospitalization. Vaccinia Immune Globulin (VIG) therapy is not indicated to treat these rashes. Supportive care measures are warranted since these rashes are likely manifestations of an immune response or hypersensitivity reaction to the vaccine and are not likely to contain vaccinia virus.
2. **Bacterial Infection:** Vaccination site infection, most likely due to staphylococcus and streptococcus normal skin flora, is rare. Onset is approximately 5 days post-vaccination and is more common in children. Appropriate antibiotic therapy is required.
3. **Inadvertent Inoculation**: This can occur in the vaccinee (autoinoculation) as well as in close contacts (contact transmission). Accidental infection is the most common complication of vaccinia vaccination, accounting for approximately 50% of all complications associated with vaccination and revaccination. This usually results from autoinoculation of vaccinia virus transferred from the site of the vaccination. Sites typically involved include the face, eyelids, nose, mouth, genitalia, or rectum, but can also involve the arms, legs, and trunk. Contact transmission of vaccinia, with accompanying toxicities, may occur when a recently vaccinated individual has contact with a susceptible individual. In a 1968 ten-state survey, contact transmissions were reported to occur at a rate of 27 infections per million vaccinations. The age group in which contact transmission occurred most commonly was in children ≤ 5 years. Eczema vaccinatum as a result of contact transmission may result in a more severe syndrome than that seen in vaccinees, perhaps because of multiple simultaneous inoculations. About 30% of eczema vaccinatum cases reported in the 1968 ten-state survey were a result of contact transmission. It is possible that the number of cases of contact transmission would be greater in today's population, due to a largely unvaccinated patient population against smallpox. Contact transmission rarely results in postvaccinial encephalitis or progressive vaccinia. Most cases of inadvertent inoculation usually resolve without specific therapy and resolution of lesions follow the same course as the vaccination site in immunocompetent individuals. VIG can be used for severe cases involving extensive lesions or if comorbid conditions exist. VIG is contraindicated in the presence of isolated keratitis due to the risk of increased corneal scarring. VIG use can be considered in cases of ocular implantation, with keratitis, if vision-threatening or if other life-threatening vaccinia-related complications exist that require VIG therapy.

4. **Generalized vaccinia:** Generalized vaccinia (GV) is characterized by a disseminated maculopapular or vesicular rash of varying extent on any part of the body and typically develops 6-9 days after vaccination. The lesions follow the same course as the vaccination site lesion. The lesions are hematogenously spread and may contain vaccinia virus. In immunocompetent individuals, the rash is generally self-limiting and requires supportive care therapy. VIG treatment can be used in severe cases for patients who are systemically ill and whose condition might be toxic or who have serious underlying immunosuppressive illnesses.
   The differential diagnosis of GV includes eczema vaccinatum, erythema multiforme, inadvertent inoculation at multiple sites, and uncommonly, early stages of progressive vaccinia or other vesicular diseases (*e.g.*, severe chickenpox or disseminated herpes). Several publications have investigated the reporting of GV among those individuals who received smallpox vaccinations during 2003. Out of 38,440 vaccine recipients, 29 reports of possible GV during January 2003–December 2003 were identified but only 2 reports met the case definition. More than 75% of the reports received a final diagnosis of hypersensitivity reaction or nonspecific rash after review by dermatologists or because laboratory results were negative for vaccinia and other orthopoxviruses. Of 74 cases investigated in 753,226 smallpox vaccinations administered in December 2002 to December 2004, 50 (67.6%) met the case definition of possible GV. Cases occurred more frequently in primary vaccinees (rate, 81 per 1 million vaccinees) than in those revaccinated (rate, 32 per 1 million vaccinees). However, none met the case definition of probable or confirmed GV, including 15 with virologically negative laboratory evaluations (*e.g.*, culture, polymerase chain reaction, or skin biopsy). Twenty-one reports of postscab lesions were made between January and August 2003 among 37,542 smallpox vaccinees. The lesions (scab and/or fluid) of seven patients were tested for vaccinia virus by use of polymerase chain reaction and/or immunohistochemistry; all were found to be negative. In addition, the postscab lesions of four of the patients were biopsied. The results from two of the biopsies suggested an allergic contact dermatitis, and results of one each demonstrated chronic dermatitis and squamous cell carcinoma. None of the four biopsied lesions had histologic evidence of viropathic changes and no evidence supported smallpox vaccination as a cause for any of the lesions.
5. **Eczema vaccinatum:** Eczema vaccinatum is a serious complication in persons with eczema and other types of chronic or exfoliative skin conditions. It can also occur among eczematous contacts of recently vaccinated persons. Vaccinial lesions (generalized papular, vesicular or pustular lesions) develop on areas of the skin that are, or had at one time been, eczematous. These areas become highly inflamed and lesions may spread to healthy skin. The rash is often accompanied by fever and individuals are systemically ill. The fatality rate for untreated cases (prior to availability of VIG) has been reported from 30-40%. Following availability of VIG, mortality was reduced to approximately 7%. Early diagnosis and prompt treatment with VIG is necessary to reduce mortality.
6. **Progressive vaccinia:** Progressive vaccinia is the most serious cutaneous complication, occurring when the local vaccination lesion fails to heal and develops progressive necrosis, with destruction of large areas of skin, subcutaneous tissue, and underlying structures. Progressive lesions may spread to other skin surfaces and to bone and viscera. Progressive vaccinia is associated with a high mortality rate. This complication has been

seen in patients with a compromised immune system due to a congenital deficiency, lymphoproliferative disease, immunosuppressive treatment, or HIV infection. Management should include aggressive VIG therapy.

7. **Post-Vaccinial Encephalitis/Encephalomyelitis**: Vaccinial complications affecting the CNS are unpredictable. Post-vaccinial encephalitis typically affects children < 2 years of age and is characterized by an onset of symptoms 6-10 days following vaccination, which include seizures, hemiplegia, aphasia, and transient amnesia. Histopathological changes include generalized cerebral edema, mild lymphocytic meningeal infiltration, ganglion degenerative changes and perivascular hemorrhages. Older children and adults can develop encephalitis or encephalomyelitis characterized by an onset of symptoms 11-15 days following vaccination, which include fever, vomiting, headache, malaise, and anorexia, progressing to loss of consciousness, amnesia, confusion, disorientation, restlessness, delirium, drowsiness, seizures and coma. Histopathological changes include demyelination with lymphocytic infiltration, but limited cerebral edema. Mortality rates have ranged from 15-25%, with 25% of patients who recover being left with varying degrees and types of neurological deficits. VIG has not been shown to be effective in treating CNS disease and is not recommended. Post-vaccinial encephalitis/encephalomyelitis are diagnoses of exclusion and are not believed to be a result of replicating vaccinia virus. Although no specific therapy exists, supportive care, anticonvulsants, and intensive care might be required. A review of vaccinia-related deaths (68) during a 9-year period (1959–1966 and 1968) revealed that among first-time vaccines, 36 (52%) patients died as a result of post-vaccinial encephalitis.
8. **Fetal Vaccinia**: Fetal vaccinia is a rare, but serious complication following vaccinia vaccination during pregnancy or shortly before conception (e.g., within four weeks). To date, fewer than 50 cases have been reported and often result in fetal or neonatal death. Efficacy of VIG therapy in a viable infant or used prophylactically in women during pregnancy is unknown. The CDC has established a National Smallpox Vaccine in Pregnancy Registry. This registry will follow women during their pregnancies and their babies, after they are born, to determine the outcome of such pregnancies. The CDC can be contacted at (404) 639-8253.
9. **Myocarditis/Pericarditis:** The CDC has recommended a temporary medical deferral to the voluntary Smallpox Vaccination Program for persons with heart disease or cardiovascular risk factors (March 25, 2003) and issued "interim supplementary information" regarding evidence that smallpox vaccination may cause myocarditis and/or pericarditis (March 31, 2003) in people recently vaccinated with the smallpox vaccine. The cardiac events reported include myocardial infarction, angina, myocarditis, pericarditis, and myopericarditis. Although the CDC believes that there is no evidence to conclude that the licensed vaccinia vaccine causes angina or heart attacks, it acknowledges a possible causal relationship between the vaccination and heart inflammation. The CDC continues to study the relationship, but in the meantime, recommends that individuals with underlying heart disease be excluded from participation in the current Smallpox Vaccination Program. While it is currently not possible to fully evaluate the risk of cardiac events or the risk of myocarditis, pericarditis, or myopericarditis associated with vaccinia vaccination, it is reasonable to inform patients participating in studies using recombinant vaccinia virus of these reports and

provide relevant guidance for evaluating these events. Further investigation from the ongoing vaccine program may provide additional data regarding an association or lack of association with cardiovascular disease. Patients are being immunized with recombinant vaccinia vaccines with therapeutic intent and will be evaluated for cardiovascular risk factors and recent or symptomatic cardiac events per protocol eligibility criteria. Patients will be encouraged to minimize cardiovascular disease risks and encouraged to follow risk reduction according to standard medical practice. At this time the evidence for an association with myocarditis, pericarditis, or myopericarditis seems plausible, but a rare event. If not otherwise excluded, patients with known CHF or clinically significant cardiomyopathy requiring treatment should be excluded from protocol eligibility.

Out of a total of 540,824 military personnel vaccinated with a New York City Board of Health strain of vaccinia from December 2002 through December 2003, 67 developed symptomatic myopericarditis. In the 61 ECGs that were reviewed, an identifiable abnormality was evident in 46 (75.4%). The most common abnormalities included ST-segment changes observed evident in 40 patients (65.6%); 5 patients (8.2%) had normal variant early repolarization, and T-wave abnormalities were noted in 11 patients (18.0%). In addition, cardiac enzymes were elevated in 60 of 61 (98.4%) patients evaluated with this assay. On follow-up of 64 patients, all patients had objective normalization of electrocardiography, echocardiography, graded exercise testing, laboratory testing, and functional status; 8 (13%) reported atypical, non-limiting persistent chest discomfort. Among 37,901 health care workers vaccinated with the identical strain, 21 myo/pericarditis cases were identified; 18 (86%) were revacinees. Twelve met criteria for either myocarditis or myopericarditis, and 9 met criteria for pericarditis only (6 suspected and 3 probable). The severity of myo/pericarditis was mild, with no fatalities, although 9 patients (43%) were hospitalized.

14.3 DOCETAXEL (TAXOTERE®)

(Please see package insert for complete drug information)

14.3.1 Source

Docetaxel will be obtained from commercial sources by the local site pharmacy.

14.3.2 Formulation

TAXOTERE® (docetaxel) Injection Concentrate, Intravenous infusion (IV) is a sterile, non-pyrogenic, pale yellow to brownish-yellow, non-aqueous solution in single-use vials containing 20 mg docetaxel (anhydrous), 0.54 grams polysorbate 80 and 0.395 grams dehydrated alcohol solution per milliliter in two presentations:

1. TAXOTERE® (docetaxel) Injection Concentrate 20 mg docetaxel in 1 mL in 50/50 (v/v) ratio polysorbate 80/dehydrated alcohol in a blister pack in one carton (NDC 0075-8003-01)

2. TAXOTERE® (docetaxel) Injection Concentrate 80 mg docetaxel in 4 mL in 50/50 (v/v) ratio polysorbate 80/dehydrated alcohol in a blister pack in one carton (NDC 0075-8004-04)

14.3.3 Preparation

Dilute the concentrated drug product:

1. After removing Taxotere® from refrigeration, allow them to stand at room temperature approximately 5 minutes before proceeding
2. Use only a 21-Gauge needle to withdraw the drug product from a vial
   - Larger bore needles (e.g., 18 and 19 gauge) may result in stopper coring and introduce particulate matter into the drug product
3. Aseptically withdraw the amount of concentrated docetaxel solution required for a patient's dose with a calibrated syringe and transfer it into an appropriate parenteral product bag or bottle containing a volume of either 0.9% NS or D5W sufficient to produce a final docetaxel concentration within the range 0.3 – 0.74 mg/mL
   - Product concentrations must not exceed 0.74 mg docetaxel per milliliter
   - To minimize patient exposure to phthalate plasticizers (e.g., DEHP), which may be leached from PVC containers, prepare and store docetaxel injection for administration to patients in bottles (glass, polypropylene) or plastic bags (polypropylene, polyolefin)
4. Thoroughly mix the admixture by manual rotation and by gently inverting the product container
   - When prepared as described and stored between 2° – 25°C, docetaxel solutions are stable for 4 hours

14.3.4 Storage and Stability

Store between 2°C and 25°C. Retain in the original package to protect from bright light. Freezing does not adversely affect the product.

14.3.5 Administration Procedures

To minimize patient exposure to phthalate plasticizers (e.g., DEHP), which may be leached from PVC containers and administration sets, administer docetaxel only through polyethylene-lined administration sets

14.3.6 Incompatibilities

- Hypersensitivity to docetaxal or polysorbate 80; neutrophil counts of <1500/µL.
- Cytochrome P450 3A4 inducers, inhibitors, or substrates: May alter docetaxel metabolism.

14.3.7 Toxicity

(See package insert for complete list of side effects)

The most common adverse reactions across all docetaxel indications are infections, neutropenia, anemia, febrile neutropenia, hypersensitivity, thrombocytopenia, neuropathy, dysgeusia, dyspnea, constipation, anorexia, nail disorders, fluid retention, asthenia, pain, nausea, diarrhea, vomiting, mucositis, alopecia, skin reactions, myalgia.

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16 APPENDIX A: STUDY CALENDAR

ARM A: Sequential Docetaxel followed by PROSTVAC

	Screening/ Baseline[7]	Wk -2	Wk 1	Wk 4	Wk 7	Wk 10	Wk 13	Wk 16	Wk 19	Wk 21	Wk 24	Wk 27	Wk 30	Wk 33	Wk 36	Safety Visit	Follow-up q 12 weeks until disease progression
Treatment																	
Vaccinia-PROSTVAC									X								
Fowlpox-PROSTVAC										X	X	X	X	X	X		
Docetaxel (75 mg/m^2)[1]			X	X	X	X	X	X									
GnRH Agonist/ Antagonist		Continued at standard schedule based on patients previous dosing															X
Assessments																	
History and PE, Ht and Wt	X[3,4]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
ECOG Performance Score and ECG	X[3,4]																
Pathologic confirmation of dx[2]	X[2]																
Labs (see section 2.2 and 2.3)	X[3,4]		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Research Bloods	X		X			X	X		X		X					X	X[9]
Biopsy (optional)	X										X						
PK[5]			X														
Tc 99 scintigraphy	X[3]								X								X[8]

	Screening/ Baseline[7]	Wk -2	Wk 1	Wk 4	Wk 7	Wk 10	Wk 13	Wk 16	Wk 19	Wk 21	Wk 24	Wk 27	Wk 30	Wk 33	Wk 36	Safety Visit	Follow-up q 12 weeks until disease progression
CT-C/A/P, or MRI	X[3]								X								X[8]
Experimental Imaging (optional)[6]	X																X
Adverse Events		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Concomitant Medications		X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X

[1]Docetaxel will be given with dexamethasone premedication: Dexamethasone 8 mg PO for 3 doses at 12 h, 3 h, & 1 h before starting docetaxel OR Dexamethasone 12 mg IV 30 – 60 min before docetaxel for patients who miss ≥1 oral dexamethasone doses

[2]Pathologic confirmation will be obtained any time prior to initiation of study therapy

[3]Screening assessments obtained within 8 weeks prior to initiation of study therapy

[4]Baseline assessments obtained within 16 days prior to initiation of study therapy (these tests will not need to be repeated if they were done at screening within the appropriate timeframe)

[5]Required unless logistically not feasible:

Blood samples for the determination of docetaxel (DTX) plasma levels will be obtained from participating patients via 6mL sodium heparin tube (BD, Franklin Lakes, NJ) collected just prior to drug administration (predose), 5-min prior to end of 1-hr infusion (or just prior to infusion end), then 15min, 30min, 3hr, 6hr, 12hr, and 24hr post end of infusion (EOI). Blood draws will be permitted to have a window of +/- 5min to accommodate logistical challenges. Samples will be obtained from patients only during first dose of DTX on both study arms. Bioanalytical measurements will be conducted on an ultra HPLC-MSMS system by the Clinical Pharmacology Program (CPP).

This data will be used to monitor DTX plasma accumulation for pharmacokinetic analysis and in order to assess any drug-vaccine interactions or correlations, as well as correlations with pharmacogenomics, adverse events, and clinical response.

[6]When logistically feasible and with the patient's consent, experimental imaging including NaF PET, will be done at baseline, annually (within 1 month) or earlier if clinically indicated (at time of PSA, clinical progression, and or radiographic progression).

[7]See sections **2.2** and **2.3** for screening and baseline procedures respectively

[8]CT and Bone scan yearly (+/- 6 weeks) from end of chemo and at serological progression as defined in Section **6.3**

[9]Every 6 months after completion of therapy.

ARM B: Combined Docetaxel with PROSTVAC

	Screening/ Baseline[7]	Week -2	Week 1	Week 4	Week 7	Week 10	Week 13	Week 16	Safety Visit	Follow-up q 12 weeks until disease progression
Treatment										
Vaccinia-PROSTVAC		X								
Fowlpox-PROSTVAC			X, D1	X, D1	X, D1	X, D1	X, D1	X, D1		
Docetaxel (75 mg/m^2)[1]			X D2 or D3	X D2 or D3	X D2 or D3	X D2 or D3	X D2 or D3	X D2 or D3		
GnRH Agonist/Antagonist		Continued at standard schedule based on patients previous dosing								X
Assessments										
History and PE, Height and Weight	X[3,4]		X	X	X	X	X	X	X	X
ECOG Performance Score and ECG	X[3,4]									
Pathologic confirmation of dx[2]	X[2]									
Labs (see section **2.2** and **2.3**)	X[3,4]		X	X	X	X	X	X	X	X
Research Bloods	X		X	X			X		X	X[9]
Biopsy (optional)	X			X						
PK studies[5]			X							
Tc 99 scintigraphy	X[3]								X	X[8]
CT-C/A/P, or MRI	X[3]								X	X[8]
Experimental Imaging (optional)[6]	X									X
Adverse Events		X	X	X	X	X	X	X	X	X
Concomitant Medications		X	X	X	X	X	X	X	X	X

[1]Docetaxel will be given 20-48 hours after vaccine dose. Docetaxel will be given with dexamethasone premedication: Dexamethasone 8 mg PO for 3 doses at 12 h, 3 h, & 1 h before starting docetaxel OR Dexamethasone 12 mg IV 30 – 60 min before docetaxel for patients who miss ≥1 oral dexamethasone doses

[2]Pathologic confirmation will be obtained any time prior to enrollment

[3]Screening assessments obtained within 8 weeks prior to enrollment

[4]Baseline assessments obtained within 16 days prior to enrollment (these tests will not need to be repeated if they were done at screening within the appropriate timeframe)

[5]Required unless logistically not feasible:

Blood samples for the determination of docetaxel (DTX) plasma levels will be obtained from participating patients via 6mL sodium heparin tube (BD, Franklin Lakes, NJ) collected just prior to drug administration (predose), 5-min prior to end of 1-hr infusion (or just prior to infusion end), then 15min, 30min, 3hr, 6hr, 12hr, and 24hr post end of infusion (EOI). Blood draws will be permitted to have a window of +/- 5min to accommodate logistical challenges. Samples will be obtained from patients only during first dose of DTX on both study arms. Bioanalytical measurements will be conducted on an ultra HPLC-MSMS system by the Clinical Pharmacology Program (CPP).

This data will be used to monitor DTX plasma accumulation for pharmacokinetic analysis and in order to assess any drug-vaccine interactions or correlations, as well as correlations with pharmacogenomics, adverse events, and clinical response.

[6]When logistically feasible and with the patient's consent, experimental imaging including NaF PET, will be done at baseline, annually (within 1 month) or earlier if clinically indicated (at time of PSA, clinical progression, and or radiographic progression).

[7]See sections **2.2** and **2.3** for screening and baseline procedures respectively

[8]CT and Bone scan yearly (+/- 6 weeks) from end of chemo and at serological progression as defined in Section **6.3**

[9]Every 6 months after completion of therapy.

ARM C: PROSTVAC prior to Docetaxel

	Screening/ Baseline[6]	Vaccine Wk 1	Vaccine Wk 3	Vaccine Wk 6	Vaccine Wk 9	Vaccine Wk 12 PI discretion	Vaccine Wk 15 PI discretion	Chemo Wk 1	Chemo Wk 4	Chemo Wk 7	Chemo Wk 10	Chemo Wk 13	Chemo Wk 16	Safety Visit	Follow-up q 12 weeks until disease progression
Treatment															
Vaccinia-PROSTVAC		X													
Fowlpox-PROSTVAC			X	X	X	X	X								
Docetaxel (75 mg/m^2)								X	X	X	X	X	X		
GnRH Agonist/Antagonist	Continued at standard schedule based on modality chosen and when ADT was initiated														
Assessments															
History and PE, Height and Weight	X[2,3]		X	X	X	X	X	X	X	X	X	X	X	X	X
ECOG and ECG	X[2,3]														
Pathologic confirmation of dx[1]	X[1]														
Labs (see section **2.2** and **2.3**)	X[2,3]		X	X	X	X	X	X	X	X	X	X	X	X	X
Research Bloods	X			X	X			X			X			X	X[9]
Biopsy (optional)	X						X								
PK studies[4]								X							
Tc 99 scintigraphy	X[2]							X[8]						X	X[7]
CT-C/A/P, or MRI	X[2]							X[8]						X	X[7]
Experimental Imaging (optional)[5]	X														X
Concomitant		X	X	X	X	X		X	X	X	X	X	X	X	X

	Screening/ Baseline[6]	Vaccine Wk 1	Vaccine Wk 3	Vaccine Wk 6	Vaccine Wk 9	Vaccine Wk 12 PI discretion	Vaccine Wk 15 PI discretion	Chemo Wk 1	Chemo Wk 4	Chemo Wk 7	Chemo Wk 10	Chemo Wk 13	Chemo Wk 16	Safety Visit	Follow-up q 12 weeks until disease progression
Medications															
Adverse Events		X	X	X	X	X	X	X	X	X	X	X	X	X	X

[1]Pathologic confirmation will be obtained any time prior to enrollment

[2]Screening assessments obtained within 8 weeks prior to enrollment

[3]Baseline assessments obtained within 16 days prior to enrollment (these tests will not need to be repeated if they were done at screening within the appropriate timeframe)

[4]Required unless logistically not feasible:

Blood samples for the determination of docetaxel (DTX) plasma levels will be obtained from participating patients via 6mL sodium heparin tube (BD, Franklin Lakes, NJ) collected just prior to drug administration (predose), 5-min prior to end of 1-hr infusion (or just prior to infusion end), then 15min, 30min, 3hr, 6hr, 12hr, and 24hr post end of infusion (EOI). Blood draws will be permitted to have a window of +/- 5 minutes to accommodate logistical challenges. Samples will be obtained from patients only during first dose of DTX on allstudy arms. Bioanalytical measurements will be conducted on an ultra HPLC-MSMS system by the Clinical Pharmacology Program (CPP).

This data will be used to monitor DTX plasma accumulation for pharmacokinetic analysis and in order to assess any drug-vaccine interactions or correlations, as well as correlations with pharmacogenomics, adverse events, and clinical response.

[5]When logistically feasible and with the patient's consent, experimental imaging including NaF PET, will be done at baseline, annually (within 1 month) or earlier if clinically indicated (at time of PSA, clinical progression, and or radiographic progression).

[6]See sections **2.2** and **2.3** for screening and baseline procedures respectively

[7]CT and Bone scan yearly (+/- 6 weeks) from end of chemo and at serological progression as defined in Section **6.3**

[8]May be performed any time within 3 weeks of first doxectaxel dose, regardless of last vaccine.

[9]Every 6 months after completion of therapy.

17 APPENDIX B: PERFORMANCE STATUS CRITERIA

ECOG Performance Status Scale		**Karnofsky Performance Scale**	
Grade	Descriptions	Percent	Description
0	Normal activity. Fully active, able to carry on all pre-disease performance without restriction.	100	Normal, no complaints, no evidence of disease.
		90	Able to carry on normal activity; minor signs or symptoms of disease.
1	Symptoms, but ambulatory. Restricted in physically strenuous activity, but ambulatory and able to carry out work of a light or sedentary nature (e.g., light housework, office work).	80	Normal activity with effort; some signs or symptoms of disease.
		70	Cares for self, unable to carry on normal activity or to do active work.
2	In bed <50% of the time. Ambulatory and capable of all self-care, but unable to carry out any work activities. Up and about more than 50% of waking hours.	60	Requires occasional assistance, but is able to care for most of his/her needs.
		50	Requires considerable assistance and frequent medical care.
3	In bed >50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.	40	Disabled, requires special care and assistance.
		30	Severely disabled, hospitalization indicated. Death not imminent.
4	100% bedridden. Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.	20	Very sick, hospitalization indicated. Death not imminent.
		10	Moribund, fatal processes progressing rapidly.
5	Dead.	0	Dead.

18 APPENDIX C: VACCINIA-PROSTVAC PATIENT INSTRUCTION SHEET

1. What vaccination site reactions can you expect?
2. How should you care for the vaccination site?
3. Are there any activities I should avoid?
4. What about contact with other people?
5. Who do I contact when I have a question?

1. What vaccination site reactions can you expect?

A typical reaction in a patient who has been previously vaccinated with vaccinia includes the appearance of a small swelling in 2-3 days that may enlarge to 1-2 inches across, a small blister or pustule after 5-7 days, and healing with little scarring within 2-3 weeks. Some patients may have very little skin reaction. Often the leg that received the vaccine may become swollen. Swollen or tender lymph nodes (“glands”) in the groin may be felt. A fever to 100-101°F may occur on the second or third day. You may notice that you feel tired for 3 or 4 days. The vaccination site may itch for about 2 weeks while the scab is forming. You can take acetaminophen ("Tylenol") if you have any aches or fever but you should avoid aspirin. If fever continues for more than a day or two, you should call to speak to the clinic nurse or the research nurse.

In patients who have never received vaccinia or in some who received it a very long time ago, a red swelling is followed by a blister on day 5 to 6 and then the formation of a pustule (or "boil") 1-2 inches in diameter on day 9 to 11. A large area of redness may surround this area. A crusted scab usually forms by the second week and falls off by the third week leaving a scar roughly 1/2 inch in diameter. Fever and malaise (the "blahs") may occur during the blister and pustular phases. Swollen and tender lymph nodes may persist for months. Many of the local toxicities described (e.g., pustule and scab formation) are typical of reactions seen when vaccinia is administered via scarification or intradermal administration. These reactions may be seen, but are usually not seen when administered via subcutaneous injection.

2. How should you care for the vaccination site?

Live vaccinia virus is in skin cells at the vaccination site during the 1-2 weeks until healing has occurred. Maximal viral "shedding" from the vaccination site occurs from days 4-14, but can continue until the scab falls off from the skin. After that there is no vaccinia virus in your body. You can spread the virus to other parts of your body or to other people by touching the vaccination site and then touching your eyes, mouth, a cut or some other break in the skin. You do not pass vaccinia virus by coughing or sneezing or by sharing food or cups and dishes.

In general, frequent careful hand washing by you and by any persons in physical contact with you is the best way to prevent transmission of virus. You should also use two types of barriers over your vaccination site at all times until the scab is gone. These barriers are (1) the bandage and (2) clothing (pants or elbow length sleeves depending on the site of vaccination). These barriers should remain in place until the scab has fallen off.

For dressing care you will have a bag with some no-stick "First-Aid" or "Telfa" pads, disposable gloves, and zip-lock plastic bags. If you run out of supplies between visits, you can use a dry sterile bandage (gauze or "First-Aid" or "Telfa") from the drug store.

The no-stick pad ("First-Aid" or "Telfa" pad) dressing should be worn until the site has healed. If it remains clean and dry and is not coming off, you do not need to change it. If the dressing gets wet either from drainage from the vaccination or from water when you are showering or if it starts coming off, you should remove it and put on a clean bandage. Wear the gloves when handling the old dressings. Put the old dressing and the gloves in the zip-lock bag, then wash your hands, put on the new bandage, and wash your hands again. You do not need to wear gloves for the new bandage. You do not need to wash the vaccination site, but while the dressing is off, you may wash it lightly with a cloth, soap, and water. If you do wash, blot the site dry with a towel (don't rub), then put the wash cloth and the towel in the laundry. Do not let the shower run on the non-bandaged site because live virus could be washed onto other areas of your body. Do not put any steroid cream, medicated creams, or other ointments on the vaccination site.

Before you throw away the zip-lock bag with the old dressing and gloves in it, pour a little bleach (about a quarter cup) in the bag to help kill any virus.

Wash your hands after each step.

3. Are there any activities I should avoid or take special care?

You should not go swimming until the vaccination site has healed and you no longer need to wear a bandage on it.

If you wear contact lenses, have removable dentures, have a colostomy or any other "open" area on your body that needs daily care, always wash your hands very well before handling your contact lenses, dentures, dressings, etc. Take care of all of these procedures before changing your vaccination dressing.

4. What about contact with other people?

Remember, frequent careful hand washing by you and by any persons with whom you have physical contact is the best way to prevent transmission of virus. During the time you need to wear a bandage (for a minimum of 3 weeks after vaccination) there are several kinds of people with whom you should avoid close contact. "Close contact" means that you sleep in the same bed with the person, give the person baths, and/or touch their bare skin to change their clothes (or diapers), apply ointments, or change their bandages.

The individuals you should avoid include children ≤ 3years of age; pregnant women or nursing women; individuals with eczema, history of eczema or other skin conditions such as active cases of extensive psoriasis, severe rashes, generalized itching, infections, burns, chicken pox, or skin trauma; and/or immune suppressed individuals such as individuals with leukemia or lymphoma, with AIDS, or those receiving immunosuppressive treatment (for example, after organ transplant).

5. Who do I contact when I have a question?

If you have any questions at any time, please call. A nurse or a physician is available 24 hours a day by telephone. To speak with the research nurse, call the research nurse's office during regular business hours; if your question is not urgent, you may leave a message for the research nurse on the answering machine. If your question is urgent or if you have a question outside of the regular business hour, please contact the 3NW inpatient unit and speak with the charge nurse.

In an emergency, call 911 or report to the local emergency room/urgent care center. If you have to go to an emergency room, please ask the doctors to call NIH for more information.

PHONE NUMBERS	
3NW Inpatient unit	(301) 451-0789
12th floor Oncology Clinic	(301) 496-4026
Ravi Madan, MD	(301) 480-7168 *
James Gulley, MD, PhD	(301) 480-7164 *

*after regular business hours, please contact 3NW inpatient unit.

19 APPENDIX D: GUIDELINES FOR PROPHYLAXIS AND TREATMENT FOR HYPERSENSITIVITY REACTIONS (HSR) ASSOCIATED WITH DOCETAXEL

<table>
<tr><th>Initial Regimen</th><th>HSR Interventions
Step 1</th><th>HSR Interventions
Step 2</th><th>HSR Interventions
Step 3</th></tr>
<tr><td>For ALL patients who have not experienced an HSR associated with Docetaxel</td><td>For pts with a H/O a single HSR episode</td><td>For pts who experience a HSR after implementing Step 1 interventions</td><td>For pts with severe or repeated HSR episodes after implementing Steps 1 & 2 interventions</td></tr>
<tr><td>PREMEDICATION WITH:
Dexamethasone 8 mg PO for 3 doses at 12 h, 3 h, & 1 h before starting docetaxel
OR
Dexamethasone 12 mg IV 30 – 60 min before docetaxel for patients who miss ≥1 oral dexamethasone doses
• Reference: study protocol, Section 3.2.3</td><td colspan="2">PREMEDICATION WITH:
Dexamethasone 8 mg PO for 2 doses at 12 h & 3 h before starting docetaxel
+
Dexamethasone 12 mg IV 30 – 60 min before starting docetaxel
+
Diphenhydramine 50 mg IV push 15 – 30 min before starting docetaxel
+
Ranitidine 50 mg IV infusion 30 min before starting docetaxel</td><td>PREMEDICATION WITH:
Dexamethasone 8 – 20 mg PO for 2 doses between 24 – 36 h and at 12 hours before starting docetaxel
+
Dexamethasone 20 mg IV 30 – 60 min before starting docetaxel
+
Diphenhydramine 50 – 150 mg IV push 15 – 30 min before starting docetaxel
+
Ranitidine 50 mg IV infusion 15 – 30 min before starting docetaxel</td></tr>
<tr><td>Docetaxel 75 mg/m^2* IV over 60 minutes†</td><td>Docetaxel
75 mg/m^2* IV over 1 – 2 hours†</td><td>Docetaxel 75 mg/m^2* IV over 4 hours
• Given in two portions‡, as:
Docetaxel 37.5 mg/m^2 IV over 2 hours, q.2 h for 2 doses</td><td>Docetaxel 75 mg/m^2* IV
• Given in three portions‡, as follows:
1. Docetaxel 5 mg in 10 mL IV via syringe pump over 60 min, followed immediately afterward by:
2. Docetaxel 25 mg in 50 mL IV over 60 min, followed immediately afterward by:
3. Docetaxel 75 mg/m^2 (minus 30 mg) IV over 120 min</td></tr>
<tr><td colspan="4">PRN Orders:
Hydrocortisone 100 mg IV push q.15 min for 2 doses, PRN docetaxel reaction
+</td></tr>
</table>

Initial Regimen	HSR Interventions Step 1	HSR Interventions Step 2	HSR Interventions Step 3
For ALL patients who have not experienced an HSR associated with Docetaxel	**For pts with a H/O a single HSR episode**	**For pts who experience a HSR after implementing Step 1 interventions**	**For pts with severe or repeated HSR episodes after implementing Steps 1 & 2 interventions**
Diphenhydramine 50 mg IV push q.15 min for 2 doses, PRN docetaxel reaction + **Ranitidine** 50 mg IV, PRN docetaxel reaction + **Hydromorphone** 1 mg IV push q.15 min for 4 doses, PRN pain with docetaxel reaction + **Alum**inum **Hydrox**ide 200 mg + **Mag**nesium **Hydrox**ide 200 mg + **Simethicone** 20 mg chewable tablet 1 – 2 tablets q.3 hours, PRN epigastric discomfort (drug name in CRIS is cross-referenced with "Mylanta")			

* Initial (unmodified) docetaxel dosage is identified. Consult the study protocol or a medically responsible investigator to determine whether docetaxel dose/dosage modification is indicated.

† For each docetaxel product, drug delivery will be attempted over the administration period indicated, but rate titration is permitted. If rate titration is needed to accommodate patient tolerance, the following escalation scheme is recommended:

Initial Rate:	25 mL/h for 5 minutes
Rate Escalation Steps:	
1st	50 mL/h for 5 minutes
2nd	100 mL/h for 5 minutes
3rd	200 mL/h for 5 minutes
4th	250 mL/h until completed

‡ Docetaxel stability is concentration dependent. All docetaxel products diluted within the range 0.3 – 0.74 mg/mL are labeled to expire 4 hours after preparation was completed.