Amendment

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Protocol Title: A Phase II Study of Neoadjuvant rFowlpox-PSA (L155)-TRICOM (Prostvac-F/TRICOM) in Combination with rVaccinia-PSA (L155)-TRICOM (Prostvac-V/TRICOM) in Men with Prostate Cancer Undergoing Treatment with Radical Prostatectomy

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* Signature signifies that investigators on this protocol have been informed that the collection and use of personally identifiable information at the NIH are maintained in a system of record governed under provisions of the Privacy Act of 1974. The information provided is mandatory for employees of the NIH to perform their assigned duties as related to the administration and reporting of intramural research protocols and used solely for those purposes. Questions may be addressed to the Protrak System Owner.

** I have reviewed this research project and considered the NIH Policy for Inclusion of Women and Minorities in Clinical Research. Taking into account the overall impact that the project could have on the research field involved, I feel the current plans adequately includes both sex/gender, minorities, children, and special populations, as appropriate. The current enrollment is in line with the planned enrollment report for inclusion of individuals on the basis of their sex/gender, race, and ethnicity and is appropriate and of scientific and technical merit.

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A. Obtain information by intervening or interacting with living individuals for research purposes
B. Obtaining identifiable private information about living individuals
C. Obtaining the voluntary informed consent of individuals to be subjects
D. Makes decisions about subject eligibility
E. Studying, interpreting, or analyzing identifiable private information or data/specimens for research purposes
F. Studying, interpreting, or analyzing de-identified data or specimens for research purposes
G. Some/all research activities performed outside NIH

Investigational Agents:

Drug Name:	Recombinant Vaccinia-PSA(L155)-TRICOM (PROSTVAC-V/TRICOM)	Recombinant Fowlpox-PSA(L155)-TRICOM (PROSTVAC-F/TRICOM)
IND Number:	15455	15455
Sponsor:	Center for Cancer Research, NCI	Center for Cancer Research, NCI
Manufacturer:	Bavarian Nordic, Inc.	Bavarian Nordic, Inc.

PRÉCIS

Background:

- Adenocarcinoma of the prostate is the most common cancer diagnosis in American males and follows lung cancer as the leading cause of cancer death**.**
- Vaccine strategies represent a novel therapeutic approach in the treatment for prostate cancer. One potential target for a prostate cancer vaccine is PSA, due to its restricted expression on prostate cancer and normal prostatic epithelial cells.
- A neoadjuvant approach may be of potential benefit providing prolonged protection via the patient's immune system against future recurrence.
- PROSTVAC is a vaccine that induces strong immune responses, has shown promising evidence of activity in a randomized phase II study (8.5 month improvement in median overall survival) and is currently in phase III clinical testing.
- This vaccine has been tested in locally recurrent prostate cancer with substantial inflammatory infiltrates within the prostate seen following subcutaneous and intraprostatic injection.

Objectives:

- The primary objective is to evaluate the post vaccine immunologic CD4 and CD8 cell infiltrate response of a neoadjuvant vaccine strategy in prostatectomy specimens in patients who plan to undergo radical prostatectomy.

Eligibility:

- Patients must have biopsy proven prostate cancer and are surgical candidates for radical prostatectomy
- Must be of sufficient good health to be surgical candidates for radical prostatectomy and have elected radical prostatectomy for management of their prostate cancer
- Granulocyte count $\geq$ 1,500/mm^3, Platelet $\geq$ 50,000/mm^3, Hgb $\geq$ 8 g/dL,
  Bilirubin < 1.5mg/dL, AST and ALT < 2.5xULN, Creatinine $\leq$ 1.5 X ULN
- Pre-intervention biopsy tissue must be available either from outside institution or repeat biopsy

Design:

- This study will utilize rV-PSA(L155)-TRICOM (PROSTVAC-V) as a priming vaccination followed by monthly boosting with rF-PSA (L155)-TRICOM (PROSTVAC-F) for 3 months.
- Patients will undergo radical prostatectomy after 4 months of treatment with PROSTVAC-V/F.
- The maximum accrual to the trial will be 27 patients.

SCHEMA

Schedule

Week 1	Week 3 or 5[1,3]	Week 5 or 9[1,3]	Week 9 or 13[1,3]	Week 10[2, 3]
Priming Vaccination	Booster Vaccination	Booster Vaccination	Booster Vaccination	Radical Prostatectomy

[1]Please note that above dates may be +/- 1 week to accommodate for scheduling

[2]Please note the date of radical prostatectomy may be within three months after week 10 to accommodate for scheduling and any reasonable delays due to clinical preoperative considerations. Every effort will be made to perform the surgery as close to week 10 as possible however. Patients with delay of greater than one month may receive additional monthly booster vaccines. If feasible, surgery should be scheduled within 7-14 days after the final booster. (See section **3.6** for more details)

[3]Patients who were enrolled prior to Amendment B will continue to follow the original week 5, 9 and 13 dosing schedule. However, surgery on these patients can be performed 7-14 days after the last vaccine is given.

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1 INTRODUCTION

1.1 STUDY OBJECTIVES

1.1.1 Primary

- The primary objective is to evaluate immunologic CD4 and CD8 cell infiltrate response of a neoadjuvant prime/boost vaccine strategy in prostatectomy specimens: priming with subcutaneous rVaccinia-PSA(L155)-TRICOM (rV-PSA-(L155)-TRICOM) with subsequent monthly boosts using subcutaneous rFowlpox-PSA(L155)-TRICOM (rF-PSA(L155)-TRICOM) in patients planning to undergo radical prostatectomy.

1.1.2 Secondary

- To determine the change in peripheral PSA-specific T cells in patients treated with these vaccines.
- To document any intraprostatic T_{reg} cell infiltration with CD4+FOX-P3 staining.
- To document any PSA changes secondary to vaccination, including rate of biochemical recurrence after prostatectomy.
- To document any MRI changes secondary to vaccination

1.2 BACKGROUND AND RATIONALE

1.2.1 Background

Adenocarcinoma of the prostate is the most common cancer diagnosis in American males and follows lung cancer as the leading cause of cancer death. One out of 6 men will be diagnosed with prostate cancer in his lifetime. An estimated 241,740 men were diagnosed with prostate cancer and 28,170 died from prostate cancer during 2012 in the United States. Even in cases of PSA-screening identified localized prostate cancer, 15-30% of patients treated with surgery will have biochemical recurrence (1).

Neoadjuvant therapy has been considered with the use of hormonal ablation or chemotherapy; however, limited benefit has been shown to be associated with the treatments. Multiple studies have been performed to assess the use of neoadjuvant androgen ablation therapy for prostate cancer prior to radical prostatectomy(1-3). Although patients generally demonstrated a significant rate of pathologic disease down staging and decreased positive margin rate, long-term follow-up demonstrated no improvement in biochemical recurrence and progression rates. Other therapies such as docetaxel, estramustine, and mitoxantrone have also been experimented with but none with significant impact on recurrence and survival(4-8).

A hallmark of prostate cancer is its prolonged disease course. The median time to biochemical recurrence after radical prostatectomy in patients initially considered to have localized disease is 2-3 years. The median time to detectable metastatic disease as visible by bone scan or CT scan imaging is 8 years, and time to death after developing of metastatic disease is another 5 years(9, 10).

Multiple schema exist for identification of patients with high risk for biochemical recurrence. These include D'Amico risk stratification which utilizes PSA, clinical stage, and biopsy Gleason score, nomograms such as the Kattan nomogram, and various other schema that have been

developed. In this study, patients will have a range of risk in their disease. As this is localized disease, in comparison to a metastatic prostate cancer cohort all patients should have overall low volume disease that is isolated to the prostate. PSA will be followed not only to understand risk stratification preop, but to follow for efficacy of prostatectomy postop. Although it is possible to identify patients who are at higher risk of ultimate biochemical recurrence pre-operatively, no effective neoadjuvant regimen has been identified. Currently multiple clinical trials are actively ongoing to study optimal neoadjuvant treatments for such patients. In view of the limited success in the prevention of prostate cancer recurrence, neoadjuvant vaccine strategies represent a novel therapeutic approach. In these patients, newer treatment strategies, including cancer vaccines, are being designed in an attempt to slow or prevent the development of disease recurrence.

Neoadjuvant use of a prostate cancer vaccine makes sense because the patient has a low volume of disease (organ confined) and has an intact immune system (surgical patients are younger, healthier, and have not been exposed to systemic therapies or have occult bone marrow metastasis as is often seen in men with metastatic castration-resistant prostate cancer who receive vaccine therapy after progressing on chemotherapy). Although no data currently exist with neoadjuvant vaccine in localized prostate cancer, outcomes with patients with lower metastatic burden have demonstrated a greater prolonged effect of delaying disease onset and progression. The patient population in this study has very low tumor burden only locally present and thus is the earliest patient population that could be treated. The potential benefits are a prolonged action of the prostate vaccine on preventing growth of any micrometastatic disease that may be present. This risk may be quite low in the low risk subgroups however is not zero in any patient. The vaccine has been demonstrated to be safe in multiple trials and thus even in the setting of uncertain benefit, this is a therapy that poses minimal risk to the patient population enrolled in the trial A topic of interest within this study will be to examine if neoadjuvant use demonstrates similar activation of T-cell immunity as compared to use at time of metastatic disease. CD4 and CD8 cell infiltrates were chosen as primary outcome measures as they represent the immune mechanism by which the vaccine ultimately has effect and have been used in recent trials with success (32). Previous studies (29) have already demonstrated a strong correlation between T-cell infiltrate and overall vaccine efficacy for overall survival.

1.2.2 PSA-TRICOM Vaccines

1.2.2.1 Rationale for PSA as a target

The first step in making a vaccine for tumor immunotherapy is to choose the target antigen. Because PSA is expressed in clinically detectable/relevant amounts in prostate epithelium." cells (normal and malignant), and the prostate gland is nonessential, it has been considered as a valid target for immunotherapy. The fact that PSA is secreted and not membrane bound limits its use as a target for humoral immunity, but not its use as a target of specific cellular immune system attack. Cells, including tumor cells, present endogenously expressed proteins on their surface in the form of peptide MHC complexes. Cytotoxic T lymphocytes (CTLs) recognize and are activated by specific peptides in the context of the appropriate MHC class I molecule on APC. This activation can in turn lead to killing of tumor targets by the peptide-specific CTLs. CTL activation as well as tumor recognition and killing are thus dependent on the MHC class I molecule.

1.2.2.2 Ability to generate anti-PSA response in vitro

The use of PSA as a target to elicit tumor-specific T-cell mediated lysis has been validated in vitro. Correale et al. demonstrated in vitro killing of a PSA–peptide-pulsed HLA-A2+ human cell line by a PSA-specific human CTL cell line, and this lysis was blocked by an antibody directed against MHC class I molecules. Subsequently, it has been shown that PSA-specific CTLs could be generated that lyse PSA-expressing prostate cancer cells(11). By stimulating normal HLA-A2 donor peripheral blood mononuclear cells (PBMCs) with HLA-A2–restricted PSA peptides in the presence of IL-2, CTL lines were generated that specifically killed PSA expressing HLA-A2+ prostate cell lines, HLA-A2+ cell lines pulsed with PSA peptide and HLA-A2 cells infected with rV-PSA(12, 13).

1.2.2.3 Peptide vs. vector encoded tumor-associated antigen

Immunization with a live recombinant vaccinia virus allows for the expression of foreign antigens encoded by a transgene directly in various cells of the host, including professional APC (APC that have been preincubated with the peptide of interest (ie PSA)). This method of immunization enables antigen processing and presentation of antigenic peptides along with host histocompatibility antigens and other necessary co-factors found on the APC.

One of the main advantages of using recombinant vaccinia viruses to develop cancer vaccines, as demonstrated by numerous investigators, is that when a gene for a protein is inserted into recombinant vaccinia and used as an immunogen, the recombinant protein is much more immunogenic than the use of that protein with adjuvant costimulatory molecules such as CD80, CD28, and CTLA4(14). A striking example of this was noted by Kass et al., where it was shown that two injections of carcinoembryonic antigen (CEA) protein in adjuvant generated little, if any, of an immune response to CEA in a CEA transgenic (CEA-Tg) mouse(15). This would be expected since the host is seeing CEA as a "self" antigen. However, when the recombinant vaccinia virus containing the CEA transgene (designated rV-CEA) is administered one or two times, a strong CEA-specific T-cell response is elicited(16). The likely reason for this is that a strong inflammatory response is generated by the host against vaccinia proteins. In turn, this inflammatory process apparently leads to an environment of cytokine production and T-cell proliferation that may further amplify the immune response to the transgene antigen. This process favors induction of a cell-mediated immune response and humoral responses to the transgene antigen. Because vaccinia actively replicates in the host, it can present high levels of transgene antigen to the immune system over a period of approximately 1 week, substantially increasing the potential for immune stimulation. The host immune response to the vaccinia vector then eliminates the virus.

1.2.2.4 TRICOM

Destruction of immunological targets such as tumors requires T-cell lymphocyte recognition, via the T cell receptor, of antigenic peptides presented in the context of MHC molecules on APCs. Costimulatory molecules are critical in the generation of potent T-cell responses. The initiation of an immune response requires at least two signals for the activation of naive T cells by APC. The first signal is antigen specific, delivered through the T-cell receptor via the peptide/MHC, and causes the T cell to enter the cell cycle. The second, "costimulatory," signal is required for cytokine production and proliferation. The most extensively studied pathway of costimulation is that involving the interaction of the costimulatory molecule B7.1 (CD80) expressed on APCs, with CD28 and CTLA4 on the T cell(17). A number of additional costimulatory molecules on APCs

have been identified; these include intercellular adhesion molecule-1 (ICAM-1) and leukocyte function associated antigen-3 (LFA-3), whose ligands are LFA-1 and CD2, respectively, on the surface of T cells(18, 19). One mechanism proposed for the ability of tumor cells to evade destruction by the immune system is their failure to express adequate levels of costimulatory molecules, resulting in a failure to induce T cell responses(20-23). A corollary of this hypothesis is that introduction of proper costimulatory molecules into tumors that express TAAs should enhance their ability to elicit specific anti-tumor immune responses. Several studies have demonstrated that transfected tumor cells expressing B7.1 induce potent responses against both modified and unmodified tumor cells(17). B7.1-transfected tumors either failed to grow, or, after initial growth, regressed. Furthermore, the immune response induced by B7.1-positive tumors protected animals from re-challenge with untransfected tumor. Both ICAM-1 and LFA-3 are also capable of conferring similar levels of costimulation of T cells against tumors cells in mouse models. In vivo assays showed that weakly immunogenic syngeneic tumors infected with rV-ICAM-1 were rejected by immunocompetent hosts(24). Similarly, murine colon adenocarcinoma tumor cells infected with rV-LFA-3 failed to grow when inoculated into immunocompetent hosts(15, 25).

The proper engagement of the T-cell receptor and costimulatory receptor requires the expression of both antigen and costimulatory molecules, respectively, in the same cell. Therefore, co-expression of costimulatory molecules using a single recombinant vector presents the potential of cooperation among these proteins to enhance T-cell activation. A number of preclinical studies have supported the validity of this approach. Immunization of mice with admixtures of two recombinant vaccinia viruses, one expressing B7.1 (rV-B7.1) and the other expressing CEA (rV-CEA), resulted in increased CEA-specific immune responses and enhanced protection against challenge with CEA-bearing tumors as compared to immunization with rV-CEA alone (27). Co-expression of CEA and B7.1 in a single recombinant vaccinia virus was even more effective than the admixture of rV-CEA and rV-B7.1 with respect to eliciting CEA-specific immunity (28). Similar enhancement of anti-tumor immunity was observed in murine studies using rV-MUC-1 and rV-B7.1 (29).

Multigene constructs using poxviral vectors (avipox and vaccinia) have been generated. These vectors contain the costimulatory molecule transgenes B7.1, ICAM-1, and LFA-3, and have been given the designation TRICOM, i.e., rV-TRICOM and avipox-TRICOM. Preclinical studies using TRICOM constructs have shown them to be superior to those constructs that contain one or two of the costimulatory molecules(26, 27). T-cell proliferation and anti-tumor immunity using recombinant vaccinia virus co-expressing murine TRICOM were much greater than the sum of responses seen using vaccinia virus expressing individual costimulatory molecules. In addition, CEA-Tg mice immunized with CEA-TRICOM vectors exhibited greater immune responses and anti-tumor responses than mice immunized with CEA or CEA-B7.1 vectors(27).

1.2.2.5 PSA-TRICOM vaccines

A phase I trial in 15 patients of monthly PSA-TRICOM was completed and demonstrated safety with no evidence of cardiac, renal, or any dose-limiting toxicity(28). Toxicities were limited to grade 1 local skin reactions at vaccine site, regional adenopathy, fatigue, and mild flu-like symptoms lasting a few days after vaccination. A follow-up phase II study demonstrated robust immunologic responses and a trend toward improved overall survival in those subjects who mounted the best immune response(29). A concurrent randomized 43-center phase II was also conducted in which 125 men with asymptomatic metastatic castration resistant prostate cancer

were enrolled(30). The men were randomized 2:1 in favor of the vaccination arm vs. placebo and Vaccinia-based vector was used for priming followed by six planned fowlpox-based vector boosts. The primary end point was progression free survival for which there was no statistically significant difference between the two groups (p=0.6). However, in what is becoming recognized as a hallmark of vaccine based cancer therapies, despite the similar time to progression, a statistically significant and clinically meaningful difference was seen in overall survival. At 3 years post study, PROSTVAC-VF patients had a better overall survival with 25 (30%) of 82 alive versus 7 (17%) of 40 controls, longer median survival by 8.5 months (25.1 v 16.6 months for controls), HR=0.56 (95% CI, 0.37 to 0.85, p=-0.006). An ongoing randomized placebo controlled, double blinded international study (Prospect) is enrolling patients and will establish whether PROSTVAC can improve overall survival in men with metastatic castration resistant prostate cancer.

The PROSTVAC vaccine has also been tested in the locally recurrent setting following radiation therapy (n=21). In this study, the priming vaccination with vaccinia-PSA-TRICOM was given s.c. and 3 monthly booster vaccines were given with fowlpox-PSA-TRICOM into the prostate. End of study biopsies (1 month after the 3rd / final boosting vaccine) demonstrated substantial increases in CD3, CD4 and CD8 cells within the prostate (manuscript in preparation). There have also been multiple other studies utilizing this same basic vaccine in the biochemical recurrence (31)(n=200) and localized disease setting (27, 32)(n=48).

1.2.2.6 Tumor Burden and vaccine efficacy

Multiple phase II and phase III clinical trials with prostate cancer vaccine based therapies for metastatic disease have demonstrated improvements in overall survival without concurrent changes in overall time to progression. The reason for this difference in contrast to traditional cytotoxic agents is the target of the therapy. Whereas cytotoxic agents directly decrease cancer burden by targeting the tumor, vaccine strategies target the immune system. The cytotoxic effect may end soon after the traditional cytotoxic agent is discontinued, however the anti-tumor effect continues after the vaccine is administered. In this way, even though the cytotoxic therapy may have an initial significant effect on overall tumor burden, the tumor can resume growth at the previous rate after cessation of the cytotoxic activity and lead to rapid progression. In a vaccinated patient, the immune system may demonstrate constant anti-tumor activity effectively slowing the growth rate of the tumor. An illustration extrapolated from patient data of this can be seen in **Figure 1**. Although onset of the slowed growth may take weeks to months to fully set in, the duration of action can persist for months to years. This also suggests that an optimal utilization of immune therapy would be to administer it at a point of low disease burden. **Figure 2** illustrates the differential effect potentially seen with administration of vaccine at moderate tumor burden (A), low tumor burden (B), and high tumor burden (C). The patient data from which these models were extrapolated was actual plotted data from patients on the phase 2 trial of PSA-TRICOM. What was noted in these patients as demonstrated in Figure 1 was a slowed rate of PSA velocity rather than significant decrease (30).

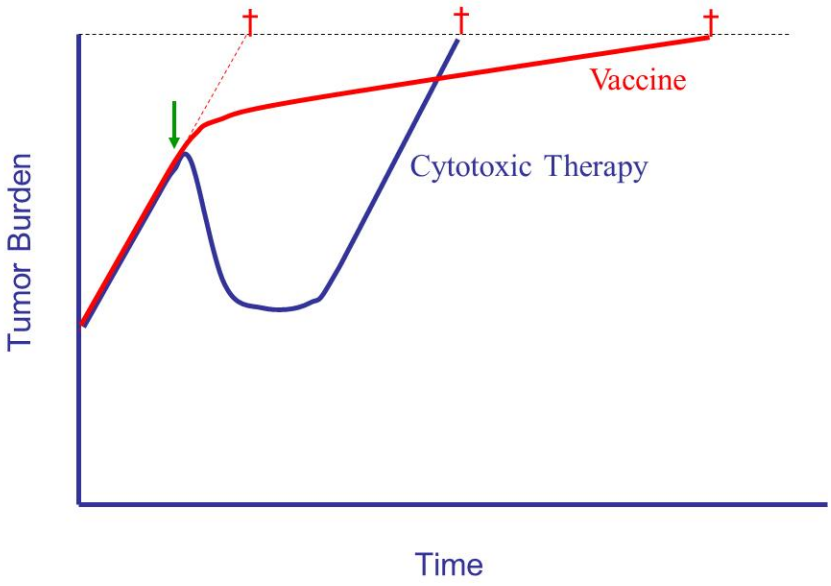

Figure 1: Different tumor burden progression patterns seen with cytotoxic versus vaccine based therapy. Curves are models based on observed cases with good vaccine efficacy.

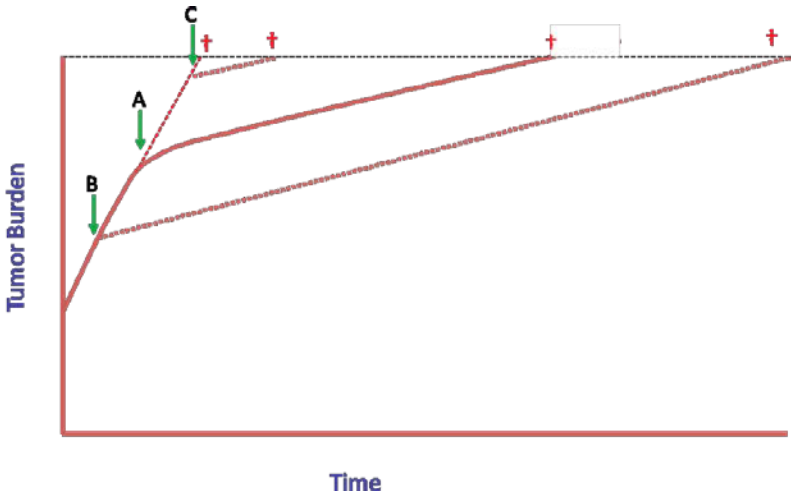

Figure 2: Immune therapy initiated at early stages of disease (B) may have a substantially improved outcome when compared to late (C) and intermediate (A) stages of disease. Curves are models based on observed cases with good vaccine efficacy.

1.2.3 Safety of Recombinant Pox-Virus–Based Vaccines

All of the components of PSA(L155)-TRICOM—the vaccinia and fowlpox vectors, the PSA antigen, and the TRICOM costimulatory molecules (B7.1, ICAM-1, and LFA-3)—have been evaluated in Phase I and II clinical safety studies(16, 33-37). Since 1991, 10 recombinant vaccinia-based vaccines and 8 recombinant fowlpox-based vaccines produced for the treatment of various cancers have been evaluated in human clinical trials at the NCI. Over 1000 cancer patients have been treated to date with these pox-virus–based vaccines. This represents a large component of the relevant safety database that supports the initiation of this proposed trial of rV-PSA(L155)-TRICOM (PROSTVAC-V/TRICOM) and rFowlpox-PSA (L155)-TRICOM (PROSTVAC-F/TRICOM). There have now been approximately 1000 patients administered recombinant vaccinia vaccines in our various trials and those of other investigators with no events of harmful shedding. A recent update of almost 1,800 vaccine cycles in about 300 patients receiving TRICOM vaccines at the NCI showed no serious vaccinia related adverse events (ASCO 2013). It should be pointed out that unlike the conventional smallpox vaccination, vaccinees in this trial (as well as in our previous trials) receive bandages and dressings to cover the vaccination site and are given careful instructions for proper maintenance (as described in detail in **Appendix D**).

One patient treated with rF-PSA(L155)-TRICOM developed grade 4 thrombotic thrombocytopenic purpura (TTP) thought to be possibly related to study drug, approximately 3.5 weeks after receiving the last dose of his vaccine. The patient had a history of hypertension, hyperlipidemia and atrial fibrillation. The patient presented with chest pain and was found to have elevated cardiac enzymes, acute renal failure and thrombocytopenia with evidence of intravascular hemolysis. He was treated for an MI and with serial plasmapheresis and hemodialysis and his myocardial infarction has resolved without sequelae and his TTP has resolved, although one month after diagnosis with TTP he continued to require hemodialysis.

1.2.4 Immune Response

Previous trials with PSA-TRICOM have evaluated immunologic parameters in clinical trials among several immunotherapy studies. A previous trial in mCRPC patients with PSA-TRICOM alone suggested that patients with greatest magnitude of T-cell-specific response against PSA had favorable clinical outcomes(29). That same trial also suggested that changes in regulatory T-cell function were also associated with improved clinical outcomes. While these findings are not surrogate markers of response, they have greatly 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.

While this response has been observed and studied in a metastatic prostate cancer model, much less is known about such a response in a localized prostate cancer scenario. Are the same associations seen or other changes in natural killer cells or cytokines of greater importance in patients with mainly localized disease? To this end, infiltrate of CD4+ and CD8+ cells will be examined in pre-vaccine biopsy specimens versus in post-vaccine prostatectomy specimens.

Immunoassays (both T-cell and antibody-based) may be useful to help define: a) if a given vaccine can elicit any immune response in the setting of localized disease, and b) the relative potency of such a response. The ELISPOT assay is relatively sensitive and quantitative. (38)

Studies have demonstrated the ELISPOT assay for IFN-gamma production to be quantitative and reproducible as a measure of human T-cell responses to vaccination. The continued use of one reproducible assay has been instrumental in our ability to evaluate and compare patients' immune responses using different vaccines and vaccine strategies in the same institution, and among different cancer centers. We have also used this ELISPOT assay to demonstrate that prostate cancer patients can mount a T-cell response to PSA post-vaccination with rV-PSA. We plan to use this assay to evaluate patients' T-cell responses to the PSA-TRICOM vaccines.

1.2.5 Summary

This study represents an initial trial of examining the use of PSA-TRICOM in the neoadjuvant setting. Several characteristics of prostate cancer make it an ideal target for immunotherapy. Its relative indolence allows sufficient time to generate immune responses, which usually take weeks or months to mount. A randomized, controlled phase II PSA-TRICOM trial utilizing a poxvirus-based vaccine platform expressing PSA and 3 costimulatory molecules (B7.1, ICAM-1, and LFA-3) demonstrated a statistically significant improvement in overall survival in vaccine-treated patients for metastatic prostate cancer. The benefits of vaccine in the NCI study however were greatest in patients with lower tumor burden. Therefore, it would be rational to investigate the benefits of vaccines in patients with earlier stage of disease who have less tumor burden. The data gathered regarding immunologic response as well as serologic response will be invaluable in determination if larger studies to examine endpoints such as biochemical recurrence are warranted.

2 ELIGIBILITY ASSESSMENT AND ENROLLMENT

2.1 ELIGIBILITY CRITERIA

2.1.1 Inclusion Criteria

2.1.1.1 Patients must have histopathological documentation of adenocarcinoma of the prostate prior to starting this study and evaluable biopsy tissue (e.g., unstained slides or blocks) available for analysis. If evaluable tissue is not available, the patient must agree to undergo a pre-vaccination prostate biopsy on study as an alternative to having available tissue available.

2.1.1.2 Patients must be a surgical candidate for radical prostatectomy based on standard workup of PSA, biopsy results, and if necessary supplemental imaging.

2.1.1.3 Patients must have chosen radical prostatectomy as their definitive treatment of choice for management of their prostate cancer.

2.1.1.4 Patients must have a performance status of 0 to 1 according to the ECOG criteria (see **Appendix A**).

2.1.1.5 No systemic steroid or steroid eye drop use within 2 weeks prior to initiation of experimental therapy. Limited doses of systemic steroids to prevent IV contrast, allergic reaction or anaphylaxis (in patients who have known contrast allergies) are allowed.

2.1.1.6 Hematological eligibility parameters (within one month of starting therapy):

- Granulocyte count $\geq$1,500/mm^3
- Platelet count $\geq$50,000/mm^3
- Hgb $\geq$ 8 g/dL

2.1.1.7 Biochemical eligibility parameters (within one month of starting therapy):

a. Hepatic function: Bilirubin < 1.5 mg/dl (OR in patients with Gilbert's syndrome, a total bilirubin ≤ 3.0 mg/dL), AST and ALT < 2.5 times upper limit of normal.

b. Creatinine ≤ 1.5 X ULN

c. Patients must be test negative for HIV, Hepatitis B and C.

2.1.1.8 Patients must not have other active invasive malignancies within the past 2 years (with the exception of non-melanoma skin cancers) or life threatening illnesses.

2.1.1.9 Patients must be willing to travel to the study site for follow-up visits.

2.1.1.10 Patients must be ≥ 18 years of age.

2.1.1.11 All patients who have received prior vaccination with vaccinia virus (for smallpox immunization) must not have a history of allergy to the vaccine.

2.1.1.12 Patients must understand and sign informed consent that explains the neoplastic nature of their disease, the procedures to be followed, the experimental nature of the treatment, alternative treatments, potential risks and toxicities, and the voluntary nature of participation.

2.1.1.13 The effects of the study agents used in this protocol on the developing human fetus are unknown. For this reason men must agree to use adequate contraception (abstinence, vasectomy, or female partner use of intrauterine device (IUD), hormonal [birth control pills, injections, or implants], tubal ligation) prior to study entry and for up to one month after the last vaccination.

2.1.2 Exclusion Criteria

2.1.2.1 Prior splenectomy.

2.1.2.2 The recombinant vaccinia vaccine should not be administered if the following apply to either recipients or, for at least 3 weeks after vaccination, their close household contacts (Close household contacts are those who share housing or have close physical contact):

- persons with active or a history of eczema or other eczematoid skin disorders
- those with other acute, chronic or exfoliative skin conditions (e.g., atopic dermatitis, burns, impetigo, varicella zoster, severe acne or other open rashes or wounds) until condition resolves
- pregnant or nursing women; children under 3 years of age
- Patients should have no evidence, as listed below, of being immunocompromised:
  - HIV positivity due to the potential for decreased tolerance and risk for severe side effects.
  - Hepatitis B or C positivity.
  - Concurrent use of topical steroids (including steroid eye drops) or systemic steroids. This is to avoid immunosuppression which may lead to potential complications with vaccinia (priming vaccination). Nasal or inhaled steroid use is permitted.

2.1.2.3 Patients with known allergy to eggs.

2.1.2.4 Other serious intercurrent illness.

2.1.2.5 Patients with a history of unstable or newly diagnosed angina pectoris, recent myocardial infarction (within 6 months of enrollment) or New York Heart Association class II–IV congestive heart failure.

2.1.2.6 Patients with significant autoimmune disease that is active or potentially life threatening if activated.

2.1.2.7 Patients with clinically significant cardiomyopathy requiring treatment.

2.2 RESEARCH ELIGIBILITY EVALUATION

- History and Physical Exam (within 1 month prior to enrollment)
- Performance Status Evaluation (within 1 month prior to enrollment)
- Pathologic Confirmation of Diagnosis. Patients that do not have previously collected biopsy material available will be asked to undergo a prostate biopsy.
- EKG
- Laboratory Evaluation (within 1 month prior to enrollment)
  - CBC with differential and platelet count
  - Acute care panel
  - Hepatic Panel
  - HIV serology
  - Hepatitis panel

2.3 REGISTRATION PROCEDURES

Authorized staff must register an eligible candidate with NCI Central Registration Office (CRO) within 24 hours of signing consent. A registration Eligibility Checklist from the web site (http://home.ccr.cancer.gov/intra/eligibility/welcome.htm) must be completed and sent via encrypted email to: NCI Central Registration Office (HOIS) ncicentralregistration-l@mail.nih.gov. After confirmation of eligibility at Central Registration Office, CRO staff will call pharmacy to advise them of the acceptance of the patient on the protocol prior to the release of any investigational agents. Verification of Registration will be forwarded electronically via e-mail to the research team. A recorder is available during non-working hours.

2.4 BASELINE STUDIES (WITHIN 4 MONTHS PRIOR TO TREATMENT)

Baseline examinations need not be repeated if they have been performed within the appropriate time frame.

- History and Physical Exam
- HLA class 1 profile
- Serum PSA (tumor marker)

- CBC
- BUN, Creatinine
- ALT, AST, Total bilirubin
- Lymphocyte Phenotyping (TBNK)
- Research Studies (See section **5** for details)
    - MRI prostate[1] (can be obtained within one year prior to treatment)
    - Immunology assays (including immune T-cell response assay, CD3, 4, 8 subsets and CD4:CD8 ratio, Class II immune responses, CTL assay, TBNK, and sera antibody analyses)
    - Prostate biopsy (performed only if previously collected tissue is not available. If biopsy was performed at screening, leftover material may be used for research study.)

[1] MRI of the prostate is strongly encouraged and will be performed prior to and after vaccination course if logistically feasible.

3 STUDY IMPLEMENTATION

3.1 STUDY DESIGN

This is a Phase II study in which a maximum of 27 patients will be enrolled. Patients with clinically localized prostate cancer will be enrolled and treated with rV-PSA(L155)-TRICOM (PROSTVAC-V/TRICOM) followed by the rFowlpox-PSA (L155)-TRICOM (PROSTVAC-F/TRICOM) boost monthly until they have had radical prostatectomy or meet off therapy criteria as listed in section **3.7.1**. At week 10 (plus up to 3 months) the patients will undergo a radical prostatectomy at the NIH Clinical Center by open or robotic-assisted laparoscopic approach as deemed appropriate by the surgeon. We will continue to follow for safety, and immunologic data.

Schedule

Week 1	**Week 3 or 5[1,3]**	**Week 5 or 9[1,3]**	**Week 9 or 13[1,3]**	**Week 10[2,3]**
Priming Vaccination	Booster Vaccination	Booster Vaccination	Booster Vaccination	Radical Prostatectomy

[1]Please note that above dates may be +/- 1 week to accommodate for scheduling

[2]Please note the date of radical prostatectomy may be within three months after week 10 to accommodate for scheduling and any reasonable delays due to clinical preoperative considerations. Patients with delay of greater than one month may receive additional monthly booster vaccines. If feasible, surgery should be scheduled within 7-14 days after the final booster.

[3]Patients who were enrolled prior to Amendment B will continue to follow the original week 5, 9 and 13 dosing schedule. However, surgery on these patients can be performed 7-14 days after the last vaccine is given.

3.2 DRUG ADMINISTRATION

3.2.1 Study drugs

Study drugs will be prepared and placed in syringes by the Clinical Center Pharmacy personnel. Both vaccines are given subcutaneously. (See sections **11.1.3** and **11.2.3** for preparation instructions.)

Week 1	rV-PSA(L155)-TRICOM	2 x 10^8 infectious units s.c.
Monthly booster	rF-PSA(L155)-TRICOM	1 x 10^9 infectious units s.c.

3.2.2 Precautions

- Prior to administration of the drugs, safe handling precautions should be thoroughly reviewed (see sections **11.1.7** and **11.2.7** in "Pharmaceutical Information").
- The proper procedure for disposing the live vaccine is a critical part of drug administration (see sections **11.1.8** and **11.2.8** in "Pharmaceutical Information").
- Patients must have a copy of the "Patient Education Sheet" (see **Appendix B**)

3.3 TREATMENT MODIFICATIONS

Patients must have recovered to ≤ grade 1 attributable toxicity 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 2 attributable toxicity persists for > 42 days, the patient will not receive further vaccine inoculations and will be removed from protocol and proceed directly to definitive therapy. Assessments will be performed during each vaccination and blood draw interval for immunologic testing as described in section **3.4.1.3**.

3.4 ON STUDY EVALUATION

3.4.1 Guidelines for Clinical and Laboratory Evaluation

3.4.1.1 A complete history and physical examination including signs and symptoms shall be done within 1 month of enrolling on trial.

3.4.1.2 Laboratory studies will include a complete blood count (hemoglobin and hematocrit values, white blood cell count, platelet count), BUN, Creatinine, ALT, AST, Total bilirubin, and serum PSA.

3.4.1.3 Immunologic studies will include ELISPOT assay, CD3, CD4, CD8 subsets and CD4:CD8 ratio, Class II immune responses, CTL assay, TBNK and sera antibody analyses. The ELISPOT assay is relatively sensitive and quantitative. By measuring cytokine release on a single-cell basis, the assay can detect a peptide-specific T-cell response against specific HLA class I binding peptides. (38) The level of cytotoxicity determined by the standard chromium release assay after *in vitro* expansion of specific T cells has been shown to correlate with the number of γ-IFN releasing cells measured by the ELISPOT assay in a study of both healthy donors and melanoma patients. We have

shown that the ELISPOT assay can be used without prolonged *ex vivo* manipulation of a patient's PBMC to measure immunologic responses in patients receiving cancer vaccines.

3.4.1.4 Testing Schedule (see **Appendix D** for tube requirements).

Baseline (Week 1)

See Section **2.4**

At time of monthly booster:

History and Physical Exam
Laboratory Evaluation
Research Immunologic Testing

Preop:

History and Physical Exam
PT/PTT/INR
EKG
Laboratory Evaluation
Research Immunologic Testing
Research MRI (if logistically feasible)

---- Surgery ----

NOTES:

* Patients will be monitored for non-laboratory value-based toxicity each visit.

* Inquiries regarding concurrent therapies will be made at each visit.

3.5 STUDY CALENDAR/LABORATORY EVALUATION

	Screening[1]	Baseline[1]	Week 1	Weeks[7] 3/5, 5/9, 9/13 (± 1 week)	Week 10[7] (+ 3 months)		Post-Surgery Follow-up[4]
					Pre-op	Surgery	
History and Physical	X	X	X[6]	X	X		X
Height	X						
Weight	X			X	X		
Performance Status	X						
HLA class 1 profile		X					
PT/PTT/INR					X		
EKG	X				X		
MRI prostate[2]		X			X		
Serum PSA (tumor marker)		X	X[6]	X	X		X
Pathology confirmation of diagnosis	X						
Immunology Assays [3]			X	X	X		X
Lymphocyte Phenotyping (TBNK)			X	X	X		X
Serum HIV antibody	X						
Serum Hepatitis panel	X						
CBC	X	X	X[6]	X	X		
Acute Care Panel	X						
Hepatic Panel	X						
BUN, Creatinine, ALT, AST, Total bilirubin		X	X[6]	X	X		
Priming Vaccination			X				
Booster Vaccination				X			
Prostate Biopsy[5]		X					
Radical Prostatectomy						X	

[1] Please see sections **2.2** and **2.4** for screening and baseline studies respectively.

[2] MRI of the prostate is strongly encouraged and will be performed prior to and after vaccination course if logistically feasible. Baseline MRI can be obtained within one year prior to treatment.

[3] Immunologic assays include ELISPOT assay, CD3, 4, 8 subsets and CD4:CD8 ratio, Class II immune responses, CTL assay, TBNK and sera antibody analysis. Research blood will be drawn prior to each vaccination and then prior to operation.

[4] Please see section **3.8** for further information on follow up evaluations occurring at 3- 6 months post op and 12 – 15 months post op, and if necessary, the safety visit occurring 4 – 5 weeks after removal from protocol therapy.

[5] For patients with no evaluable prostate tissue available at time of study enrollment.

[6] Need not be repeated if they have been performed within the appropriate time frame.

[7] Patients who were enrolled prior to Amendment B will continue to follow the original week 5, 9 and 13 dosing schedule. However, surgery on these patients can be performed 7-14 days after the last vaccine is given.

3.6 SURGICAL GUIDELINES

Patients will undergo a radical prostatectomy after week 10 of the study. The radical prostatectomy may be performed any day after week 10 or up to 12 weeks after to allow for logistical constraints, but ideally as close to week 10 as possible. Patients enrolled prior to amendment B will have their surgery within 7 to 14 days after the last dose of vaccine plus 3 months to allow for logistical constraints. The surgery performed will typically be a robotic-assisted laparoscopic radical prostatectomy with bilateral pelvic lymph node dissection according to standard surgical template, however this may vary significantly depending on the clinical scenario and judgment of the surgeon. Although strongly encouraged, a lymph node dissection may be excluded if felt to be in the best interest of the patient. Alternative approaches may also be utilized to perform the radical prostatectomy as felt appropriate by the surgeon. Acquired tissue will be analyzed by pathology per their usual protocol to provide a diagnosis and appropriate grade and staging information regarding the patient's cancer. In addition, additional slides will be kept for research analysis by immunohistochemistry as well as material may also be procured and frozen for future molecular based analysis (See section **5.1** for further details).

Patients undergo preparation that is standard of care for radical prostatectomy. The surgery will be performed per routine by the surgeon.

3.7 CRITERIA FOR REMOVAL FROM PROTOCOL THERAPY AND OFF STUDY CRITERIA

Prior to removal from study, effort must be made to have all subjects complete a safety visit approximately 30 days following the last dose of study therapy.

3.7.1 Criteria for removal from protocol therapy

Patients will be removed from the protocol therapy for the following:

1. Grade 3 or greater toxicity attributed to vaccines that does not resolve to grade 1 within 42 days from time of scheduled treatment.
2. Grade 2 or greater autoimmune disease that threatens vital organs.
3. 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.
4. Greater than 3 month delay in the performance of radical prostatectomy from week 10 or date that patient first becomes eligible for surgery.

3.7.2 Off-Study Criteria

Patients will be removed from the study for the following:

1. Patient has completed all study interventions including follow up safety visit
2. Patient requests to be taken off study. Reasons for withdrawal will be documented.
3. Noncompliance with protocol guidelines (patient removed at discretion of Principal Investigator).
4. Death

3.7.3 Off Protocol Therapy and Off Study Procedure

Authorized staff must notify Central Registration Office (CRO) when a subject is taken off protocol therapy and when a subject is taken off-study. A Participant Status Updates Form from the web site (http://home.ccr.cancer.gov/intra/eligibility/welcome.htm) main page must be completed and sent via encrypted email to: NCI Central Registration Office (HOIS) ncicentralregistration-l@mail.nih.gov.

3.8 FOLLOW-UP EVALUATION

Subjects will be seen 3 – 6 months post operatively as well as 12 – 15 months postoperatively. The following assessments will be performed at these visits:

- History and Physical Exam
- PSA
- ELISPOT assay, CD3, CD4, CD8 subsets and CD4:CD8 ratio, Class II immune responses, CTL assay.

If subjects have stopped taking the study vaccination for any of the reasons listed in section **3.7.1** prior to prostatectomy, they will be seen at NIH, if feasible, for a safety visit within 4-5 weeks of last vaccination. The safety assessments may be performed by a local physician and laboratory if patients unable to return to the NIH Clinical Center at this time.

The following assessments will be performed at the safety follow up.

- History and Physical Exam
- Laboratory Evaluation (CBC, BUN, Creatinine, ALT, AST, Total bilirubin, and serum PSA).

After the safety visit, if there are no unresolved grade 3 or higher AEs, the patient will not require further safety follow-up. If there are unresolved grade 3 – 4 AEs, patients will be followed either at the NIH clinical center or by their local physician until symptoms stabilize. In the latter case, we will obtain the physician's record of AEs.

4 CONCOMITANT MEDICATIONS/MEASURES

4.1 CONCURRENT THERAPIES

Concurrent anti-tumor therapies will not be allowed.

4.2 SUPPORTIVE CARE

1. For the vaccine administration, antiemetics and anti-diarrheal 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. Patients on this protocol should avoid being given dexamethasone or other corticosteroids (with the exception of topical and inhaled steroids) particularly in the two-week period post vaccinia treatment. If their medical condition requires this therapy, the principal investigator must be notified.

2. 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 for 3 days before receiving any dose of vaccine.
3. Symptomatic anemia should be treated with appropriate red blood cell or erythropoietin support.
4. 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/mm^3. 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/mm^3.
5. Nutritional support and psychosocial support. During cancer treatments it is sometimes difficult for patients to maintain good nutrition. If it is deemed necessary, or it could benefit the patient, the patient will be referred for a nutritional consult. Patients who are having emotional difficulty coping with their disease and/or their treatment will be referred to a social worker for evaluation and support.
6. 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 autoinoculation). The effectiveness of VIG therapy appears to be time-dependent. VIG is of no benefit in the treatment of postvaccinial encephalitis, and is contraindicated for treatment of vaccinial keratitis. At present there is no other available anti-viral treatment of proven benefit for vaccinia-related complications. VIG is an isotonic sterile solution of the immunoglobulin fraction (antibodies) of plasma from persons vaccinated with vaccinia vaccine. If symptoms develop suggestive of the acquisition of one of the previously described vaccinia complications, or a close contact occurs between a recently vaccinia-vaccinated patient and a susceptible person with one of the pre-existing medical conditions described above, the patient should report the findings immediately to the protocol investigator or other established contact, for consideration for VIG therapy.
7. 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.

5 BIOSPECIMEN COLLECTION

5.1 CORRELATIVE–IMMUNOLOGIC STUDIES

(See Appendix D for tubes and volumes)

5.1.1 Immune cell infiltrate

Slides made from prostate biopsy specimens (collected at baseline) and prostatectomy specimens (collected at surgery performed after last dose of vaccine) will be stained for CD4 and CD8 cells and digitized for analysis of immune cell infiltrate. Other markers such as CD4/FoxP3 may also be stained for further correlative studies. These studies will be performed by the Laboratory of

Tumor Immunology and Biology. At a minimum, 5 slides from one biopsy core confirmed to contain cancer will be needed for baseline quantification. Attempts will be made based on anatomic location notation or when available, MRI based location, to correlate biopsy location with pathologic location on prostatectomy specimens. When feasible, quantitation will be performed in triplicate. Quantitation will be reported as number of stained cells per micron squared of surface area. Wilcoxion matched pair signed rank test, Friedman test with Dunn's multiple comparisons, or alternative statistical models will be used as appropriate. Aperio ScanScope digital scanner systems with Aperio ImageScope software algorithms will be used. Manual quantitation will be performed in case of software malfunction. Isotype antibodies negative controls will be included in all runs. Tonsil or lymph node tissue will be positive control for CD4 and CD8. Slides will be developed with primary and secondary antibodies per manufacturer specifications for visualization of cells.

5.1.2 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 by the Laboratory of Tumor Immunology and Biology.

5.1.3 T-cell assays

We plan to examine the immune response in selected patients. Lymphocytes will be separated from heparinized blood using density gradient centrifugation. The lymphocytes will then be placed in human AB serum with 10% DMSO and stored in liquid nitrogen. When samples are available from pre- and post-treatment, the ELISPOT or other T-cell assay will be performed. The ELISPOT assay, measuring IFN-gamma production, is used to determine CTL precursor frequency to peptides from tumor associated antigens in both pre- and post-vaccination peripheral blood mononuclear cells, as previously described(32). These assays will be performed in the Laboratory of Tumor Immunology and Biology, NCI, NIH.

5.1.4 MRI study

If logistically feasible, an MRI will be performed at baseline before the first vaccine administration and after the last vaccine administration as part of the pre-operative workup. This will be done to assess for changes in the imaging characteristics of the prostate cancer pre and post vaccination.

Prior to entering the scanner the patient will answer the standard MRI safety checklist administered to all patients undergoing MRI in the Clinical Center to insure that it is safe to perform an MRI. Images will be reviewed for lesions suspicious for prostate cancer. Pre and post-vaccine images will be compared to assess for changes seen on MRI. These studies will be performed by the NCI Molecular Imaging Program.

5.1.5 Additional Assays

Blood and tissue samples may be used for additional research studies, which may include phenotypic and functional analysis of tumor and immune-cell subsets (such as the CD3, CD4, and CD8 subsets and CD4:CD8 ratio), Class II immune responses, CTL assay, TBNK and analyses for cytokines (IFN-γ, IL-10, IL-12, IL-2, IL-4, etc.), chemokines, antibodies, tumor associated antigens, tumor specific gene expression profiling using RT-PCR of the RNA extracted from the tumor and/or other markers. Many of these studies will aid in not only assessing response, but predictors of response.

All samples will be labeled with the following identifier system.

- Patient's enrollment #
- Trial number
- Patient's initials

Example: 01-ABC

These labels are used only to send the samples from the NIH Clinical Center to the NCI Frederick Central Repository. The NCI Repository will process all samples, appropriately discard the label on the blood tube, and then store the samples with unique identifiers, to which only NCI study personnel will have the code to link to patient specific clinical information. Samples will be tracked according to Section **5.2** (Sample Storage, Tracking and Disposition).

5.1.6 Analysis of pre and post treatment samples

5.1.6.1 Analysis of tissues using multiplexed immunofluorescence or multispectral imaging to identify specific markers, including markers of immune cell subsets, may be performed in collaboration with:

Clifford Hoyt
PerkinElmer
68 Elm St.
Hopkinton, MA 01748
Phone: 774-278-2249
Clifford.hoyt@perkinelmer.com

And

General Electric (GE) Global Research
Fiona Ginty, Ph.D.
Lifesciences and Molecular Diagnostics Org
Building K1-5B27
One Research Circle
Niskayuna, NY 12309
Phone: 518-387-7985
ginty@research.ge.com

5.1.6.2 RNA-based analysis may be performed in collaboration with:

- HTG Molecular Diagnostics, Inc.
  3430 E. Global Loop
  Tucson, AZ 85706
  Phone: (877) 289-2615
  Fax: (520) 547-2837

  Attention:
  John Wineman, VP Corporate Development
  Phone: (503) 545-2081
  jwineman@htgmolecular.com

  HTG Molecular Diagnostics, Inc. has a platform to obtain RNA from formalin-fixed and paraffin-embedded (FFPE) tissues and from PBMC cells. NCI would

send HTG Molecular paired coded samples of pre treatment and post treatment PBMC and FFPE slides (H&E and serial unstained slides) for analysis.

5.2 STORAGE AND TRACKING OF COLLECTED BLOOD SAMPLES

All data associated with the patient samples is protected by using a secure database. Non-clinical samples drawn at the NIH Clinical Center or collected from tissue specimens for immunological evaluation will be transported to the NCI Frederick Central Repository by the Leidos, Biomedical, Inc. couriers. The contact person for arranging transport is Jenn Marte (301.496.7214).

Samples will be tracked and managed by Central Repository database. All samples will be stored in either a -20 or -80˚C freezer. 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, 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's 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 de-identified 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.

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

Samples, and associated data, will be stored permanently unless the patient withdraws consent. The PI will report destroyed samples to the IRB if samples become unsalvageable or destroyed by environmental conditions (ex. Broken freezer or lack of dry ice in shipping container) or if a patient chooses to withdraw his/her consent. Samples will also be reported as lost if they are lost in transit or misplaced by a researcher.

6 DATA COLLECTION AND EVALUATION

6.1 DATA COLLECTION

Data from this study will be stored in the C3D and Labmatrix databases.

1. Eligible patients must be confirmed and checklist completed. Consent form must be signed prior to registration with the NCI Central Registration Office.
2. For adverse event reporting see section **7.**
3. Treatment is given according to protocol (dated notes about doses given, complications, and clinical outcomes).
4. Toxicity is assessed according to protocol (laboratory report slips, etc.)
5. Response is assessed according to protocol (X-ray, scan, lab reports, date noted on clinical assessment, as appropriate).
6. Drug Accountability Records are kept for each patient.
7. Exception will be made for the collection of Grade I toxicities.
8. Post-operatively, we will not report Grades 1 or 2 AEs that are clearly attributable to surgery. This includes, but is not limited to, anemia, nausea and pain, but will be at the PIs discretion. Post-operatively, we will not capture erectile dysfunction or incontinence as AEs.

As general anesthetics, as well as medications used for perioperative symptom management, tend to be both expected and routine, we will not capture medications administered perioperataively; however, should a patient experience a Serious Adverse Event during surgery, any medications indicated during that event would be entered into the database.

The PI will be responsible for overseeing entry of data into an in-house password protected electronic system 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. All data obtained during the conduct of the protocol will be kept in secure network drives or in approved alternative sites that comply with NIH security standards. Primary and final analyzed data will have identifiers so that research data can be attributed to an individual human subject participant.

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 breech in our plan to protect subject confidentiality and trial data has occurred, the IRB will be notified.

6.2 RESPONSE CRITERIA

6.2.1 Determination of Immunologic Response

6.2.1.1 Local Immunologic Response

The primary endpoint of this study will be to examine the ability of the vaccine to induce a local T-cell response in the prostate. CD4 and CD8 cell infiltrates will be examined in the peri-tumoral region of the prostate biopsy specimens as well as in the prostate specimen itself. Utilizing computer automated staining analysis, density of cell infiltrate will be calculated and the pre and post-vaccine values will be compared to determine response to vaccine.

6.2.1.2 Systemic Immunologic Response

In order to evaluate the potency of clinical vaccines to induce a systemic response, assays that determine an antigen-specific T lymphocyte response to vaccines have been employed. Immune response T-cell assays (such as the ELISPOT assay) have been used successfully in numerous vaccine trials. The ability to detect single cells that produce interferon gamma has led to specificities of 80-95%. ELISPOT has very good reproducibility both when the same sample is tested at two time points and when an individual is tested at different time points. We have also seen minimal variation in the flu-specific ELISPOT in patients when tested at time points about 84 days apart. Utilizing these immune response assays, the change in PSA-specific T-cell response from baseline to after the last vaccination will be considered for evidence of an immunologic response to the vaccine.

6.3 TOXICITY CRITERIA

6.3.1 CTCAE

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).

6.3.2 Toxicity Grading for Vaccinia Toxicity

- Grade 1: Generalized vaccinia extending no more than 10 cm from the vaccination site (i.e., limited to the upper arm).
- Grade 2: Any autoinoculation syndrome that resolves without sequelae; generalized vaccinia extending more than 10 cm from the vaccination site.
- Grade 3: Any toxicity that is between grades 2 and 4.
- Grade 4: Autoinoculation syndrome with sequelae (e.g., blindness); post-vaccinia encephalitis; vaccinia gangrenosum; eczema gangrenosum; Stevens-Johnson syndrome.
- Grade 5: Death

7 SAFETY REPORTING REQUIREMENTS/DATA AND SAFETY MONITORING PLAN

7.1 DEFINITIONS

7.1.1 Adverse Event

An adverse event is defined as any reaction, side effect, or untoward event that occurs during the course of the clinical trial associated with the use of a drug in humans, whether or not the event is considered related to the treatment or clinically significant. For this study, AEs will include events reported by the patient, as well as clinically significant abnormal findings on physical examination or laboratory evaluation. A new illness, symptom, sign or clinically significant laboratory abnormality or worsening of a pre-existing condition or abnormality is considered an AE. All AEs must be recorded on the AE case report form unless otherwise noted above in Section **6.1**.

All AEs, including clinically significant abnormal findings on laboratory evaluations, regardless of severity, will be followed until return to baseline or stabilization of event. Serious adverse events that occur more than 30 days after the last administration of investigational agent/intervention and have an attribution of at least possibly related to the agent/intervention should be recorded and reported as per sections **7.2**, **7.3, 7.4.**

An abnormal laboratory value will be considered an AE if the laboratory abnormality is characterized by any of the following:

- Results in discontinuation from the study
- Is associated with clinical signs or symptoms
- Requires treatment or any other therapeutic intervention
- Is associated with death or another serious adverse event, including hospitalization.
- Is judged by the Investigator to be of significant clinical impact
- If any abnormal laboratory result is considered clinically significant, the investigator will provide details about the action taken with respect to the test drug and about the patient's outcome.

7.1.2 Suspected adverse reaction

Suspected adverse reaction means any adverse event for which there is a reasonable possibility that the drug caused the adverse event. For the purposes of IND safety reporting, 'reasonable possibility' means there is evidence to suggest a causal relationship between the drug and the adverse event. A suspected adverse reaction implies a lesser degree of certainty about causality than adverse reaction, which means any adverse event caused by a drug.

7.1.3 Unexpected adverse reaction

An adverse event or suspected adverse reaction is considered "unexpected" if it is not listed in the investigator brochure or is not listed at the specificity or severity that has been observed; or, if an investigator brochure is not required or available, is not consistent with the risk information described in the general investigational plan or elsewhere in the current application. "Unexpected", also refers to adverse events or suspected adverse reactions that are mentioned in the investigator brochure as occurring with a class of drugs or as anticipated from the pharmacological properties of the drug, but are not specifically mentioned as occurring with the particular drug under investigation.

7.1.4 Serious

An Unanticipated Problem or Protocol Deviation is serious if it meets the definition of a Serious Adverse Event or if it compromises the safety, welfare or rights of subjects or others.

7.1.5 Serious Adverse Event

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 drug experience
- Inpatient hospitalization or prolongation of existing hospitalization
- 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.

7.1.6 Disability

A substantial disruption of a person's ability to conduct normal life functions.

7.1.7 Life-threatening adverse drug experience

Any adverse event or suspected adverse reaction that places the patient or subject, in the view of the investigator or sponsor, at immediate risk of death from the reaction as it occurred, i.e., it does not include a reaction that had it occurred in a more severe form, might have caused death.

7.1.8 Protocol Deviation (NIH Definition)

Any change, divergence, or departure from the IRB-approved research protocol.

7.1.9 Non-compliance (NIH Definition)

The failure to comply with applicable NIH Human Research Protections Program (HRPP) policies, IRB requirements, or regulatory requirements for the protection of human research subjects.

7.1.10 Unanticipated Problem

Any incident, experience, or outcome that:

- Is unexpected in terms of nature, severity, or frequency in relation to

  (a) the research risks that are described in the IRB-approved research protocol and informed consent document; Investigator's Brochure or other study documents, and

  (b) the characteristics of the subject population being studied; **AND**

- Is related or possibly related to participation in the research; **AND**

- Suggests that the research places subjects or others at a g*reater risk of harm* (including physical, psychological, economic, or social harm) than was previously known or recognized.

7.2 NCI-IRB AND CLINICAL DIRECTOR (CD) REPORTING

7.2.1 NCI-IRB and NCI CD Expedited Reporting of Unanticipated Problems and Deaths

The Protocol PI will report in the NIH Problem Form to the NCI-IRB and NCI CD:

- All deaths, except deaths due to progressive disease
- All Protocol Deviations
- All Unanticipated Problems
- All non-compliance

Reports must be received within 7 days of PI awareness via iRIS.

7.2.2 NCI-IRB Requirements for PI Reporting at Continuing Review

The protocol PI will report to the NCI-IRB:

1. A summary of all protocol deviations in a tabular format to include the date the deviation occurred, a brief description of the deviation and any corrective action.
2. A summary of any instances of non-compliance
3. A tabular summary of the following adverse events:
   - All Grade 2 **unexpected** events that are possibly, probably or definitely related to the research;
   - All Grade 3 and 4 events that are possibly, probably or definitely related to the research;
   - All Grade 5 events regardless of attribution;
   - All Serious Events regardless of attribution.

   NOTE: Grade 1 events are not required to be reported.

7.2.3 NCI-IRB Reporting of IND Safety Reports

Only IND Safety Reports that meet the definition of an unanticipated problem will need to be reported to the NCI IRB.

7.3 IND SPONSOR REPORTING CRITERIA

An investigator must immediately report to the sponsor, using the mandatory MedWatch form 3500a, any serious adverse event, whether or not considered drug related, including those listed in the protocol or investigator brochure and must include an assessment of whether there is a reasonable possibility that the drug caused the event.

- All Grade 5 (fatal) events (except death due to progressive disease) must be reported via email within 24 hours. A complete report must be submitted within one business day.
- All other serious adverse events including deaths due to progressive disease must be reported within one business day

Study endpoints that are serious adverse events (e.g. all-cause mortality) must be reported in accordance with the protocol unless there is evidence suggesting a causal relationship between the drug and the event (e.g. death from anaphylaxis). In that case, the investigator must immediately report the death to the sponsor.

Events will be submitted to the Center for Cancer Research (CCR) at: CCRsafety@mail.nih.gov and to the CCR PI and study coordinator.

7.3.1 Reporting Pregnancy

7.3.1.1 Paternal exposure

Male patients should refrain from fathering a child or donating sperm during the study and for one month after the last dose of PROSTVAC. Pregnancy of the patient'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 one month after the last dose should, if possible, be followed up and documented.

7.4 SAFETY REPORTING CRITERIA TO THE PHARMACEUTICAL COLLABORATORS

All events listed below must be reported in the defined timelines to CCRsafety@mail.nih.gov.

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

The Investigator should complete and submit an SAE Medwatch 3500 Form, containing all information that is required by the Regulatory Authorities, to BNIT by e-mail within 24 to 48 hours of awareness for SAEs deemed to be possibly, probably or definitely related to the study vaccines.

The SAE documentation, including the Medwatch 3500 Form and available source records, should be emailed to:

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

The following minimum information is required:

- Study number/IIT regulatory identifier
- Subject number, sex and age
- The date of report
- A description of the SAE (event, seriousness of the event)
- Causal relationship to the study drug

Follow-up information for the event should be sent within 7 days as necessary.

SAEs that are deemed unrelated or unlikely to be due to the study vaccines are submitted quarterly to BNIT at the following address:

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

7.5 INSTITUTIONAL BIOSAFETY COMMITTEE (IBC) REPORTING CRITERIA

7.5.1 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 rV-PSA(L155)-TRICOM and rF-PSA(L155)-TRICOM 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 rV-PSA(L155)-TRICOM and rF-PSA(L155)-TRICOM, 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 FDA Form 3500a.

7.5.2 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.

7.5.2.1 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.

7.5.2.2 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.

7.6 DATA AND SAFETY MONITORING PLAN

7.6.1 Principal Investigator/Research Team

The clinical research team will meet on a weekly basis 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. Adverse events will be reported as required above. Any safety concerns, new information that might affect either the ethical and or scientific conduct of the trial, or protocol deviations will be immediately reported to the IRB using iRIS and to the Sponsor*)*.

The principal investigator will review adverse event and response data on each patient 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.

7.6.2 Sponsor Monitoring Plan

As a sponsor for clinical trials, FDA regulations require the CCR to maintain a monitoring program. The CCR's program allows for confirmation of: study data, specifically data that could affect the interpretation of primary study endpoints; adherence to the protocol, regulations, and SOPs; and human subjects protection. This is done through independent verification of study data with source documentation focusing on:

- Informed consent process
- Eligibility confirmation
- Drug administration and accountability
- Adverse events monitoring
- Response assessment.

The monitoring program also extends to multi-site research when the CCR is the coordinating center.

This trial will be monitored by personnel employed by an NCI contractor. Monitors are qualified by training and experience to monitor the progress of clinical trials. Personnel monitoring this study will not be affiliated in any way with the trial conduct.

7.6.3 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 NCI-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 STATISTICAL SECTION

The primary objective of this trial is to determine the effect of prostate cancer vaccine on CD4 and CD8 cell infiltrate staining.

The primary endpoints will be the changes from baseline to after surgery of the CD4 and CD8 cell infiltrates. The fold change from baseline to post surgery biopsy will be the primary endpoint for each measure. With two primary parameters to be measured with respect to a change at one time point, the sample size will be selected to allow each test to be performed using a 0.025 two-tailed significance level, in order to allow the overall set of two tests to be very conservatively performed as if at an overall 0.05 level using a Bonferroni adjustment. With 24 total patients with full measurements on both parameters at baseline and post-surgery, there would be 80% power to detect a change from baseline equal to 0.67 standard deviations of the change (0.67 effect size) using a two-tailed 0.025 level paired t-test. In practice, instead of requiring that each test achieve a 0.025 level in order to be declared significant, if appropriate to do so, a less overly stringent Hochberg adjustment may be performed. In addition, if the differences from baseline to post-surgery are not normally distributed (that is, if $p<0.05$ by a Shapiro Wilks test) then a Wilcoxon signed rank test will be performed instead.

It is anticipated that 1-2 patients per month may enroll on this trial. Thus, it is expected that up to 2 years may be required to enroll 24 evaluable patients. In order to allow for a small number of inevaluable patients, the accrual ceiling for the trial will be set at 27 patients.

9 COLLABORATIVE AGREEMENTS

9.1 AGREEMENT TYPE

The clinical grade vaccines used in this protocol were prepared by Bavarian Nordic, Inc. as part of an ongoing CRADA with the LTIB, CCR, NCI.

Sharing of samples/data will be facilitated through approved Material Transfer Agreements (MTA).

10 HUMAN SUBJECTS PROTECTIONS

10.1 RATIONALE FOR SUBJECT SELECTION

10.1.1 Selection Based on Gender, Ethnicity, and Race

Subjects from all racial/ethnic groups are eligible for this study if they meet the eligibility criteria. To date, there is no information that suggests that differences in drug metabolism or disease response would be expected in one group compared with another. Efforts will be made to extend accrual to a representative population, but in this preliminary study, a balance must be struck between patient safety considerations and limitations on the number of individuals exposed to potentially toxic and/or ineffective treatments on one hand and the need to explore gender and ethnic aspects of clinical research on the other hand. If differences in outcome that correlate with ethnic identity are noted, accrual may be expanded or a follow-up study may be written to investigate those differences more fully.

10.1.2 Strategies/Procedures for Recruitment

Our patient accrual for this protocol would be facilitated by the Clinical Center Support Center (CCSC), developed to increase the accrual to clinical studies via community outreach as well as recruitment letters to referring physicians.

This protocol will be available through the physicians' data query (PDQ) database.

10.1.3 Justification for Exclusions

Due to impaired cellular immunity, HIV patients are at an increased risk of serious side effects from vaccinations with infectious agents and are excluded.

10.2 PARTICIPATION OF CHILDREN

Individuals 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 unknown toxicities in pediatric patients.

10.3 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 **10.5**), all subjects ≥ age 18 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 for evaluation. For those subjects that become incapacitated and do not have pre-determined substitute decision maker, the procedures described in MEC Policy 87-4 for appointing a surrogate decision maker for adult subjects who are (a) decisionally impaired, and (b) who do not have a legal guardian or durable power of attorney, will be followed.

10.4 EVALUATION OF BENEFITS AND RISKS/DISCOMFORTS

For patients with localized prostate cancer, the gold standard of care can include active surveillance, radical prostatectomy, or radiation therapy. No current optimal neoadjuvant

treatment protocol is as yet established even in high risk patients with a significant risk of biochemical recurrence. PSA vaccines using pox vectors have been studied as neoadjuvant therapy in Phase II trials in patients undergoing radiation treatment with good immune response. Potential risks include the possible occurrence of any of a range of side effects listed in section **11**.

10.4.1 Alternative Approaches or Treatments

Patients will be consented verbally and in writing regarding the risks and benefits of this trial, the treatment requirements, and alternative approaches to entering on this trial.

10.4.2 Procedure for Protecting Against or Minimizing any Potential Risks

All care will be taken to minimize side effects, but they can be unpredictable in nature and severity. This study may involve risks to patients, which are currently unforeseeable. All patients will have blood tests, examinations and scans as described in the monitoring schedule (section **3.5**). Patients will also be required to have a local physician to provide long-term care and to monitor for complications. If patients suffer any physical injury as a result of the participation in this study, immediate medical treatment is available at the Clinical Center, National Cancer Institute, Bethesda, Maryland. Although no compensation is available, any injury will be evaluated and treated in keeping with the benefits or care to which patients are entitled under applicable regulations.

10.4.3 Provisions for Monitoring Data Collection to Ensure Safety of Subjects

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.

10.4.4 Risk-Benefit Ratio in Target Population

Among normal patients who have received prior vaccination, there is an overall **0.0108%** complication rate (see table below).

The overall risk in vaccinia naïve patients is slightly higher at 0.1253%

Complication Rates—Vaccinia (cases per million vaccines)*

Complication	**Rate**
Inadvertent inn.	42
E. multiforme	10
Generalized vacc	9
E. vaccinatum	3
V. necrosum	3

Encephalitis	2
Other**	39
Total	108

*Adapted from Lane et al., J. Infect. Dis. 1970;122:303-309.

**Ind. bacterial superinfections and lesions uncomfortable enough to result in physician contact. Unusual complications ind. fetal vaccinia, melanoma at vaccine scar, and monoarticular arthritis.

However, this risk is reduced by a factor of 10 if the patient has previously received vaccinia virus, either through the smallpox vaccination program or through other recombinant virus clinical trials (MMWR, June 22, 2001/50(RR10);1-25). This reduces the overall risk of vaccinia immunization to 0.0108%. It should be noted that the complication rates predominantly include inadvertent autoinoculation, i.e., autologous transmitting of virus from inoculation site to face and hands. When the complication rates are stratified and only severe cases examined, the adjusted risk is 0.00005%.

There is an expected accrual of 27 patients for this protocol. The actual expected risk of vaccine complications (including dissemination) on a per patient basis remains 0.0108%.

In the rare cases of vaccinia immunization complications of any type (with the exception of vaccinial keratitis), the Center for Disease Control maintains a supply of VIG (see below). Contact Information: Emergency: (404) 639-2888; Routine: (404) 639-3670; Fax: (404) 639-3717.

Vaccinia immunoglobulin:

- Must be available to give vaccinia safely
- Is available from CDC Drug Service and U.S. Army
- Dose: 0.6 ml/kg IM (can be given at multiple sites/Divided doses over 24–36 hours)
- Humanized monoclonal antibodies
  - promising; not yet available
  - against neutralizing epitopes conserved between variola and vaccinia

As to the risk-benefit ratio of vaccinia virus dissemination in the target population, the overall risk has been assessed at 0.0108%. The benefit cannot be estimated, as this is an experimental therapy. Noting the patient population (non-immunocompromised, metastatic prostate cancer (failed primary therapy)), however, it can be inferred that the ratio of risk of complications from metastatic prostate cancer to the risk of vaccinia virus dissemination in the target population would be large.

10.5 RISKS/BENEFITS ANALYSIS

The risks to patients are reasonable in relation to the anticipated benefits in relation to the importance of the knowledge that may reasonably be expected to result.

10.6 CONSENT AND ASSENT PROCESS AND DOCUMENTATION

The investigational nature and objectives of this trial, the procedures involved, and their attendant risks and discomforts, potential benefits, and potential alternative therapies will be explained to the patient and a signed informed consent document obtained.

10.6.1 Telephone Re-Consent

If re-consent is required, we are requesting that consent via telephone be allowed. In this case, the informed consent document will be sent to the subject. An explanation of the study will be provided over the telephone after the subject has had the opportunity to read the consent form. The subject will sign and date the informed consent. A witness to the subject's signature will sign and date the consent. The original informed consent document will be sent back to the consenting investigator who will sign and date the consent form with the date the consent was obtained via telephone. A fully executed copy will be returned via mail for the subject's records. The informed consent process will be documented on a progress note by the consenting investigator and a copy of the informed consent document and note will be kept in the subject's research record.

11 PHARMACEUTICAL INFORMATION

11.1 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.

11.1.1 Source

Recombinant Fowlpox-PSA (L155)-TRICOM (PROSTVAC-F/TRICOM is supplied to the NIH CC Pharmacy by Bavarian Nordic, Inc.

11.1.2 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.

11.1.3 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.

11.1.4 Storage

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

11.1.5 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 2-8ºC. 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.

11.1.6 Route of Administration

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

11.1.7 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.

11.1.8 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 eyewear, 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:
    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 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:

a. *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*

b. *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

11.1.9 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.

11.2 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.

11.2.1 Source

Recombinant Vaccinia-PSA(L155)-TRICOM (PROSTVAC-V/TRICOM) is supplied to the NIH CC Pharmacy by Bavarian Nordic, Inc.

11.2.2 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.

11.2.3 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.

11.2.4 Storage

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

11.2.5 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 2-8ºC. 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 2-8ºC for up to 4 hours following preparation.

11.2.6 Route of Administration

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

11.2.7 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.

11.2.8 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*

11.2.9 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 $\geq$ 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.

11.2.10 Recombinant Vaccinia Vaccine Patient Care Implications, Contraindications and Potential Complications

11.2.10.1 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).

11.2.11Potential 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 ($\geq$ 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.

11.2.12Treatment of Vaccinia Vaccination Complications

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.

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13 APPENDICES

13.1 APPENDIX A: 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.

13.2 APPENDIX B: rVACCINIA-PSA (L155)-TRICOM (PROSTVAC-V/TRICOM) rFOWLPOX-PSA(L155)/TRICOM (PROSTVAC-F/TRICOM) 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 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 blisters on day 5 to 6 and then 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 unbandaged site because live virus could be washed onto other areas on 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 7-14 days 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 < 3 years 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 your main doctor or with a clinic nurse, call the Urology Clinic between 8 AM and 4:30 PM Monday to Friday. To speak with the research nurses, call the research nurse office during the day; during nights, weekends, and sometimes during the day, when the

research office is empty, you may leave a message for the research nurse on the answering machine. You can call Dr. Pinto any time during weekday hours. In an emergency on weekends, evenings, or holidays, you can always get in touch with the UROLOGY DOCTOR ON CALL (listed below) The on call doctor will call you back. If you have to go to an emergency room near your home, go to the hospital first, and then have the doctors there call for more information.

PHONE NUMBERS

OP3 Clinic	(301) 496-5484*
Peter Pinto, MD	(240) 760-6249*
*after clinic hours the NCI Medical Oncology physician on call through NIH page operator	(301) 496-1211

13.3 APPENDIX C: STUDY SUBJECT INSTRUCTIONS FOR PROTOCOL BNIT-PRV-301.

Study Subject Instructions for Protocol BNIT-PRV-301

Introduction

The purpose of this document is to tell you about general precautions for caring for PROSTVAC injection sites that are important and necessary for ensuring your safety and the safety of those around you.

In case you have any unanswered questions after becoming familiar with these instructions, make sure to let the study doctor or nurse know. In the course of this study, you will be receiving injections of two investigational products; one injection of PROSTVAC-V or placebo and six injections of PROSTVAC-F or placebo.

PROSTVAC-V uses vaccinia virus (the virus used in current smallpox vaccines) and PROSTVAC-F uses a fowlpox virus (a virus that can be found in birds). Both PROSTVAC-V and PROSTVAC-F are viruses that have been artificially weakened. Both viruses have been changed to include human prostate-specific antigen (PSA) and three other human molecules that are designed to help your immune system to recognize prostate cancer cells that contain PSA.

Fowlpox virus cannot multiply in human cells and does not present a safety concern.

Vaccinia virus can multiply in human cells and, very rarely, can cause complications and infect people who are in contact with you. Some people are at a higher danger of complications from vaccinia virus, this is why you **cannot** be in this study if you:

- Have an immunodeficiency or are taking immunosuppressive drugs
- Have active or chronic eczema or a skin condition that cause skin damage
- Have a member of your household who is pregnant/breastfeeding
- Are not able to avoid household contact with pregnant/breastfeeding women, immune-compromised individuals, or children under 3 years of age for approximately 3 weeks after the **first** (vaccinia) vaccination

All of the conditions listed above are known to put an individual at increased risk of developing rare, but serious complications of vaccinia virus infection. In case you have any of the listed conditions, you should notify your doctor or a study nurse and not participate in the study.

Vaccination with PROSTVAC-V or placebo (Week 1):

General precautions: These conditions apply to the first vaccination given during Week 1.

Even though the vaccinia virus in PROSTVAC is weakened, it still can be transferred to other parts of your body or to people you are in close contact with.

To prevent this from happening, you should:

- Always keep the injection site covered with a bandage (it will be marked for you and bandages will be provided for you).
- Wear a shirt with sleeves (or pants if vaccinated in the thigh) that is long enough to cover the injection site.
- Never touch or scratch the injection site.
- If you accidentally touched the uncovered injection site that has not healed, do not touch any other part of your body or face and do not rub your eyes. WASH your hands immediately with soap and water.
- Wash any items of clothing, bedding or towels that came in contact with vaccinations site separately with hot water and detergent.
- Do not allow anyone who may be at higher risk of complications from vaccinia virus (an immunosupressed person, a child under 3 years old, a pregnant woman) to come into close contact with you, with old bandages, or with potentially infected items of clothing until your injections site has healed and the scab has fallen off.

The injection site may leak a small amount of fluid and itch or be painful for seven to ten days before forming a scab. You should keep the injection site covered until the scab falls off.

You have been given a kit with everything you need to change the bandage over the injection site if it soaks through or becomes dirty. The study nurse will have explained to you how to use all the items in the kit. If you have more questions, the phone number for the study nurse is at the end of these instructions.

When changing the bandage:

- Cover the surface you are going to use with a pad from your kit and place an open biohazard bag on it
- Always wear the disposable gloves before you start changing you dressing
- After you remove the old bandage, carefully put it into the open biohazard bag. Try not to let the old bandage touch the outside of the bag. Place the pad in the biohazard bag.
- Remove the gloves as the study staff showed you (turning inside out) and place them in the open biohazard bag.
- Seal the bag and wash your hands with soap and water.
- Put a new bandage on the injections site. Try to do it without letting your fingers touch the injection site.
- Wash your hands again with soap and water.
- **Never touch your eyes, face or open skin when you are changing bandage.**

Frequent and careful hand washing by you and anyone in physical contact with you is the best way to prevent the spread of the vaccinia virus.

You can take a shower if needed while the injection site has not healed yet, but you would have to cover it with a waterproof bandage from your kit. In case the injection site accidentally gets wet, pat it dry with a gauze pad and dispose the gauze pad in a biohazard bag, then put a new bandage on and wash your hands with soap and water.

You cannot go swimming until the injection site has healed and the scab has fallen off.

Be extra careful if you:

- wear contact lenses,
- removable dentures,
- have a colostomy or any other open area on your body that needs daily care (including brushing your teeth),
- **always** wash your hands very well before handling your contact lenses, dentures, toothbrush, bandages, etc.
- **always** take care of all of these procedures ***before*** changing your dressing.

The biohazard bags with used dressings in them should be kept in a secure location and returned to the hospital or clinic for destruction the next time you have a scheduled visit. If you need more bags (or anything else), ask the study nurse or doctor.

Vaccinations with PROSTVAC-F or placebo

General Precautions: These conditions apply to the 2nd - 7th vaccinations given Weeks 3 - 21

Since the virus in PROSTVAC-F does not multiply in human cells, it does not cause any of the complications that are possible with vaccinia virus. There is no danger of infecting other parts or your body or other people, so there are no restrictions on contact with immune-compromised individuals, pregnant or breastfeeding women, or children under 3 years of age.

To care for the injection site after these vaccinations you will have to keep it covered with a simple adhesive bandage until the site heals. Adhesive bandages are included in your kit.

There are no restrictions on bathing or swimming.

For questions or concerns, contact the study personnel at:

Name: _____

Phone number: _____

13.4 APPENDIX D: INSTRUCTIONS FOR PRE-STUDY AND FOLLOW-UP BLOOD TESTS

Blood Studies	Blood Tube/Comments	Destination
CBC with differential	1 light lavender tube	CC Department of Laboratory Medicine (DLM)
Hepatic Panel, Acute Care Panel	1 4 mL SST	CC DLM
Anti-HIV-1/2	1 8 mL SST	CC TTV lab
PT/PTT/INR	4.5 mL citrate blue top	CC DLM
HBs Antigen Screening Anti-HCV Antibody	1 8 mL SST	CC Department of Transfusion Medicine (DTM)
Prostate Specific Antigen	4 mL SST	CC DLM
Lymphocyte Phenotyping, TBNK	1 light lavender tube	CC DLM
Immunology Assays	6 10 mL Na Heparin tubes 2 7 ml SST tubes	NCI-Frederick 1-301-846-5893

13.5 APPENDIX E: ABBREVIATIONS

ANA	anti-nuclear antibody
APC	antigen-presenting cells
B7-1	(CD80)
BSA	bovine serum albumin
CCR	Center for Cancer Research
CEA	carcinoembryonic antigen
CEA-Tg	CEA transgenic
CED	chicken embryo dermal
CMC	cell-mediated cytotoxicity
CR	complete response
CRADA	Cooperative Research and Development Agreement
CTA	Clinical Trials Agreement
CTC	common toxicity criteria
CTEP	Cancer Therapy Evaluation Program
CTL	cytotoxic T lymphocyte
DAR	Drug Accountability Record
DC	dendritic cells
DLT	dose limiting toxicity
DMC	Data Monitoring Committee
EBV	Epstein-Barr virus
FACS	fluorescent antibody cell surface
FP-WT	wild-type fowlpox
GM-CSF	granulocyte-macrophage colony-stimulating factor
HBSS	Hank's buffered saline
ICAM-1	intercellular adhesion molecule-1
IDB	Investigational Drug Branch
IFN-γ	interferon-gamma
IRB	Institutional Review Board
LD	longest diameter
LFA-3	leukocyte function associated antigen-3
LTIB	Laboratory of Tumor Immunology and Biology

mCRPC	**metastatic castrate resistant prostate cancer**
MOI	multiplicity of infection
MTD	maximal tolerated dose
PBM	Pharmaceutical Management Branch
PBMC	peripheral blood mononuclear cells
PD	progressive disease
PDQ	physician's data query
PR	partial response
PSA	prostate-specific antigen
r-GM-CSF	recombinant GM-CSF
SD	stable disease
TAA	tumor-associated antigen
TBNK	**T-cells, B-cells and NK cells**
Tc	Technetium
TRICOM	TRiad of COstimulatory Molecules
V-WT	wild-type vaccinia
VIG	vaccinia immunoglobulin

INSTITUTE: National Cancer Institute

STUDY NUMBER: 14-C-0112 PRINCIPAL INVESTIGATOR: Peter Pinto, M.D.

STUDY TITLE: A Phase II Study of Neoadjuvant rFowlpox-PSA (L155)-TRICOM (Prostvac-F/TRICOM) in Combination with rVaccinia-PSA (L155)-TRICOM (Prostvac-V/TRICOM) in Men with Prostate Cancer Undergoing Treatment with Radical Prostatectomy

Continuing Review Approved by the IRB on 01/23/17
Amendment Approved by the IRB on 05/05/17 (G) Date Posted to Web: 05/17/17

Standard

INTRODUCTION

We invite you to take part in a research study at the National Institutes of Health (NIH).

First, we want you to know that:

Taking part in NIH research is entirely voluntary**.**

You may choose not to take part, or you may withdraw from the study at any time. In either case, you will not lose any benefits to which you are otherwise entitled. However, to receive care at the NIH, you must be taking part in a study or be under evaluation for study participation.

You may receive no benefit from taking part. The research may give us knowledge that may help people in the future.

Second, some people have personal, religious or ethical beliefs that may limit the kinds of medical or research treatments they would want to receive (such as blood transfusions). If you have such beliefs, please discuss them with your NIH doctors or research team before you agree to the study.

Now we will describe this research study. Before you decide to take part, please take as much time as you need to ask any questions and discuss this study with anyone at NIH, or with family, friends or your personal physician or other health professional.

Why is this study being done?

Prostate cancer recurs in 15 - 30% of patients who have been treated with radical prostatectomy (removal of the prostate gland). Many clinical studies have been performed to identify a treatment given before surgery that would reduce the rate of recurrence. To date there is no proven chemotherapy or vaccine therapy in the setting of treatment before surgery that has accomplished the outcome of reduced rate of recurrence. We are testing to determine whether a vaccine, PROSTVAC-Vaccinia (V)/TRICOM, followed by a series of monthly booster shots, PROSTVAC-Fowlpox (F)/TRICOM given to patients with localized prostate cancer (cancer that has not spread) before the surgery, will lead to any changes in the number and type of immune

cells in the prostate gland. This vaccine was developed originally at the National Cancer Institute and is manufactured by the company Bavarian Nordic. This will let us know if the vaccines have had any effect on how your body responds to the cancer. Studies previously done on these types of vaccines in patients with metastatic prostate cancer (cancer that has spread) have suggested that patients with the highest number of immune cells had the best clinical outcomes. The vaccines have not been approved by the U.S. Food and Drug Administration; however, they have been used in clinical trials of over 1,000 cancer patients and have been well tolerated without major side effects. The PROSTVAC/TRICOM vaccine exposes the patient to the protein "Prostate Specific Antigen" (PSA) along with three immune system activating proteins in an attempt to activate the immune system against the PSA protein. PSA is a protein found in high amounts in prostate cancer but not in most other normal tissue of the body. In this way, the vaccine hopefully activates the immune system against prostate cancer cells without inducing immune activity against other cells in the body. Studies in men with metastatic prostate cancer have demonstrated some evidence of improved survival with the vaccine. Specific side effects are listed in the risk section of the consent.

Why are you being asked to take part in this study?

You are being asked to participate in this study because you have been diagnosed with prostate cancer that has not spread and you have decided that you would like to have your prostate gland removed as a treatment.

How many people will take part in this study?

About 27 patients will take part in this study.

Description of Research Study

Before you begin the study

You will need to have tests or procedures to find out if you can be in the study. These exams, tests or procedures are part of regular cancer care and may be done even if you do not join the study. We will need to obtain tissue from a prior prostate biopsy. If sufficient tissue is not available from any of your previous prostate biopsies, then it will be necessary to undergo a biopsy of the prostate prior to receiving the vaccine for this study. You will sign a separate consent to have these tests done and they will be described to you at that time.

During the study

If you meet the "entry criteria" of the study, after a few additional tests that are described below, you will be injected with a dose of the PROSTVAC-V/TRICOM vaccine most likely on your leg unless there is a reason to inject at an alternative site. Four weeks after that at the start of week 3 (± 1 week) after your enrollment, you will be injected with the PROSTVAC-F/TRICOM vaccine booster. You will receive a second booster vaccine at the start of week 5 (± 1 week) and a third vaccine at the start of week 9 (± 1 week). At week 10, you will have surgery to remove your

prostate. If you were enrolled on the trial prior to the amendment dated 09/23/14, you will receive your booster vaccines at the start of weeks 5, 9, and 13 (± 1 week) and you will be eligible to have surgery beginning one week after the last dose of vaccine. However, the surgery may be performed up to 3 months after week 10 or after you first become eligible for surgery if necessary. If the surgery is delayed for more than 1 month, you may receive additional monthly booster vaccines. Because you will receive this series of four vaccines, there will necessarily be a four-month delay from when you start taking the first vaccine and when you can have your surgery while on this study. This interval of delay is within the range of standard practice for this medical condition. Theoretically, any delay in standard treatment may pose a risk.

In order to monitor your progress during the study, we will perform the following tests and procedures that are part of regular cancer care. Tests performed at baseline (which is after you have enrolled but before you have taken any study medication) need not be repeated if they were done recently at screening. Your study doctor will tell you if any baseline procedures must be repeated.

These tests will be performed at baseline:

- Prostate specific antigen test (PSA)
- Tests to determine what kind of proteins are on the surface of your white blood cells

These will be performed at baseline and repeated at the time of your monthly boosters, just before you have the surgery to remove your prostate:

- Medical history and physical examination
- Tests to check your blood counts and blood chemistries
- Tests to determine how well your liver functions

You will also need the following tests for research to see how the vaccine is affecting your body and other research studies:

- MRI of the prostate (if feasible). Performed at baseline and just before you have the surgery to remove your prostate. This intervention will not expose you to any additional radiation as MRI uses magnets to image the body.
- We will collect a little more than a quarter cup of your blood at baseline, at the time of your monthly boosters, and just prior to surgery so that we may test how your immune system is responding the study therapy
- A biopsy (a small piece of tissue) from your prostate will be collected before you have received any vaccine only if there is no evaluable tissue available from a previous biopsy.
- Some of the leftover samples that are collected may be used in the future for additional studies. These may include analyses of your tumor cells and immune cells as well as measuring how much of a particular molecule the genes in your tumor produce (called gene expression profiling).

When you are finished taking the vaccine (treatment)

Post-surgery follow up

After your surgery, you will need to return to the NIH Clinical Center for follow up visits to be performed 3 to 6 months after your surgery and 6 to 12 months after your surgery. At these visits, we will perform the following tests:

- Medical history and physical examination
- Tests to check your blood PSA level
- A little more than a quarter of a cup of blood for research tests to test how your immune system has responded to study therapy

Safety visit

If you stop receiving the monthly boosters for any of the reasons listed in the stopping therapy section and you are unable to have surgery performed as part of this protocol, we would like to see you again for a safety visit within 4 to 5 weeks after you have finished taking the study drug in order to perform the following tests:

- Medical history physical examination
- Tests to check your blood counts and blood chemistries
- Tests to determine how well your liver functions

If you are unable to return to NIH for the safety visit, these may be performed by a local physician and laboratory

Surgery

Treatments covered under this study will include surgery to treat your cancer. This treatment will not be experimental. Your doctors will describe your treatment plan to you in detail before asking you to sign this consent form. Prior to your operation, your surgeon will discuss the specific details of the surgical procedure with you. We will keep some of the tumor tissue that is removed for research studies that help us understand if the vaccine therapy is useful. Once all of your questions have been answered, you will be asked to sign a separate consent for the surgery.

Birth Control

If you are the partner of a woman who can become pregnant, you will need to practice an effective form of birth control before starting study treatment, during study treatment, and for one month after you have received your last vaccine. If you think that your partner is pregnant, you should tell your study doctor or nurse at once.

Effective forms of birth control include:

- abstinence
- intrauterine device (IUD)
- hormonal [birth control pills, injections, or implants]
- tubal ligation
- vasectomy

Risks or Discomforts of Participation

Risks to be considered with PROSTVAC-TRICOM vaccines

Vaccinia Virus

The first vaccine injection you will receive will be PSA-TRICOM vaccinia (PROSTVAC-V). It is derived from the vaccinia virus. Vaccinia virus has been given to hundreds of millions of people worldwide to prevent the disease smallpox. Vaccinia immunization has resulted in the worldwide elimination of smallpox. It is a live replicating virus that infects large mammals and rodents, and usually causes only a self-limited skin infection in humans. The virus stimulates a strong immune response, which results in the body eliminating the virus. However, caution is required in its use, in that subjects or their contacts may experience inadvertent spread of vaccinia, or worse may experience more severe rare infections. The potential for these risks, and the precautions necessary to minimize these risks, are discussed further below.

In clinical studies of PSA-TRICOM, the vaccine is given by injection under the skin. Most subjects experience some redness and diffuse swelling in the surrounding area, approximately 1-4 inches (2-10 centimeters) in diameter. This lasts for 7-14 days and may be accompanied by itching and soreness. There is typically full healing and no residual scarring from subcutaneous administration. On average, vaccinia stays active in your body for approximately 10-14 days. Prior to receiving your next vaccine, you will be evaluated for evidence of bacterial infection, blisters, vesicles, (lesions seen on your skin at or around your vaccine site) or evidence of persistent vaccinia infection.

When vaccinia is given to protect against smallpox, it is usually scratched into the outer layers of the skin with a two-pronged needle. A normal reaction after this administration in a person who has been previously vaccinated with vaccinia includes appearance of a small bump (papule) in 3 days, a small blister or cluster of blisters in 5-7 days, and healing with little scarring within 2 to 3 weeks. Swollen lymph nodes ("swollen glands") and/or fever are infrequent.

You would receive your investigational vaccine by a shot under the skin rather than by scratching it onto the skin the way a traditional smallpox vaccination is given. Therefore, you may have less of a skin reaction; however, vaccination with PSA-TRICOM may produce reactions similar to those seen with the smallpox vaccine.

A potential problem associated with vaccinia vaccination is accidental spread of the virus to another area of your body. This occurs rarely (incidence 1 in 4000 in some reports), however, it is very important to protect against. You can transfer the virus to your eye and mucous membranes (inner lining) of the nose, mouth or genitals by scratching the vaccination site and then rubbing the eye or an open skin area. If you participate in this study you will have to take special care of your vaccination site and wash your hands often to prevent spreading of the virus. You will be provided with written instructions with details about the vaccination site and how to care for it, as well as how to contact the study staff if you have questions or concerns.

Because you may "shed" live virus from the vaccination site after vaccination until the vaccination site heals completely, and could spread the virus to others, you must avoid close contact with the following people for approximately 3 weeks after the first vaccination only:

- persons with weak or suppressed immune systems such as individuals with leukemia or lymphoma, individuals with AIDS, or those receiving treatment to suppress their immune system (for example, after organ transplantation).
- individuals with eczema or other significant skin rashes, itching infections, burns, chicken pox, or skin injury
- pregnant or breast-feeding women
- or, children under 3 years of age

"Close contact" means that these people share your house with you, are in physical contact with you, come in contact with your bed linens or clothes, and/or you take care of them and touch them.

For every 1 million people vaccinated with vaccinia scratched into the outer layer of the skin, the vaccinia virus was transmitted to roughly 75 of their contacts (In this study, the investigational vaccine will be injected under the skin which minimizes this risk). A dressing will be placed over the vaccination site to reduce the risk of accidental spreading. It is very important that you keep the vaccination site covered. Hand washing is also necessary.

During the reintroduction of smallpox vaccination in the past decade, several individuals thought to be at risk for heart disease experienced inflammation around the heart (myopericarditis). With careful monitoring, it was noted that approximately 1/2000 subjects, who had not been previously vaccinated, developed this condition. The incidence was lower in subjects that had been revaccinated. The symptoms of myopericarditis were usually mild and short lived. If you have poor heart function requiring treatment, you will not be able to participate in this study.

Possible adverse reactions can also be related to allergic responses the vaccine itself. An allergic reaction to the study vaccine may be development of a rash or hives within 7 to 10 days of vaccination, which usually gets better within 2 to 4 days. Rarely, a serious allergic reaction requiring hospitalization may occur.

Generalized vaccinia may be characterized by several small blisters around the vaccination site or by widely distributed lesions developing 7-12 days after immunization. This is also known as a

disseminated vaccinia infection. These tend to follow a course of healing similar to that of the inoculation site.

Serious side effects from the vaccinia vaccine are most common in young children, subjects with disorders of the immune system, and individuals with skin disorders. That is why precautions are taken to exclude such individuals from exposure.

Serious reactions such as post-vaccinia encephalomyelitis ("brain inflammation"), which can lead to coma and death, or progressive vaccinia which leads to a large unhealing sore and death are the most severe complications after vaccination. They occur almost exclusively in very young children who are exposed to vaccinia for the first time, or in subjects with impaired immunity; such individuals are not eligible for this study and must be avoided after vaccination. The death rate for people receiving revaccination with vaccinia for smallpox is about 1 in 10 million.

These serious reactions have not been seen in any subjects treated with PROSTVAC-V to date.

Vaccinia Immune Globulin (VIG) has been successful as a therapy for some but not all of these complications. VIG is an injectable antibody preparation made from the plasma of people vaccinated with the vaccinia vaccine. If symptoms develop suggestive of one of the previously described vaccinia complications, or a close contact occurs between a recently vaccinia-vaccinated subject and a susceptible person with one of the pre-existing medical conditions described above, the subject should report the findings immediately to the protocol investigator or other established contact, for consideration for VIG therapy, since VIG may work better if given early.

There are other anti-viral treatments with activity against vaccinia virus. Cidofovir, FDA approved for use in treating cytomegalovirus infections, has antiviral activity against poxviruses. However, the drug is only given intravenously under careful monitoring as it has some side effects, in particular risk for kidney toxicity.

Fowlpox Virus (empty fowlpox vector)

PROSTVAC-F (the booster doses of PSA-TRICOM) is based on fowlpox virus. Fowlpox virus naturally infects birds, not mammals, and has been researched and used in other vaccines for at least twenty years. The virus does not grow (replicate) in human cells and is not known to cause human disease. The vaccines including fowlpox virus have been given in research studies to both animals and humans for HIV, malaria and cancer. Side effects from fowlpox are mild and could include injection site reactions, fever, fatigue, anemia (low red blood cell count) and leucopenia (low white blood cell count). With any experimental compound, there is the risk of unexpected and serious or deadly complications even if they have not been seen previously.

Additional risks and side effects related to the vaccine therapy with PROSTVAC-V/TRICOM and PROSVTAC-F/TRICOM

Likely:

- Injection site reaction (pain, swelling, itching, induration, and redness)
- Tiredness, weakness

- Fever
- Shaking chills
- Nausea
- Glands, (lymph node) enlarge and become tender

Less likely:

- Headache
- Allergic reaction
- Sweating
- Wound complication
- Vomiting
- Confusion and disorientation
- Loss of appetite
- Yeast infection
- Constipation
- Cough
- Diarrhea
- Indigestion
- Fatigue
- Facial tingling
- Muscle ache
- Nausea
- Facial numbness
- Itching

Rare but serious:

- An uncommon blood condition called thrombotic thrombocytopenic purpura (TTP). One patient treated with this vaccine out of approximately 1,000 treated developed TTP. It is not known if this was related to the vaccine or from something else. This is a serious disease that is associated with low blood counts (both red blood cells that carry oxygen and platelets that help your blood clot), bleeding, fever, neurologic symptoms (such as changes in level of alertness including coma, headache, difficulty speaking confusion or paralysis) and kidney dysfunction. The symptoms are due to the formation of clots that form or spread to many organs. This can usually be treated with exchange plasmapheresis, a therapy that

removes and replaces plasma the protein containing fluid from a patient's blood. Should you go on this trial, we will follow you closely for any signs or symptoms of this disease.

- Leg weakness

Other Potential Side Effects

Additional adverse effects could be related to the immune response to the PSA and/or TRICOM proteins that are part of the vaccines. Some normal human cells (such as normal prostate cells) have these proteins on their surface. If the vaccine causes an immune reaction against these normal cells, you could develop swelling or inflammation of these tissues. While unlikely, it is also possible that if you develop a very active antibody (immune) reaction after the vaccination, you could develop an immune complex disease (or serum sickness) which can cause fevers, rashes, joint pains, and less commonly, kidney failure and severe allergic reaction inside blood vessels (vasculitis) or any part of your body. None of these symptoms have been observed to date in the approximately 1,000 subjects receiving the Bavarian Nordic vaccines, but the possibility of their occurrence exists.

Biopsy risks

A needle biopsy, which is procedure in which we will obtain a small piece of tumor tissue from your prostate, is performed on this study only if there is no previously available biopsy material available for us to use. A key part of the study is to determine if the vaccine increased the immune system in the prostate biopsy. If we do not have material from a biopsy, we will not be able to understand that. The biopsy procedure usually causes only brief discomfort at the site from which the biopsy is taken. Rarely, infection or bleeding may occur at the needle site.

Blood collection risks

Taking blood may cause some discomfort, bleeding or bruising where the needle enters the body, and in rare cases, it may result in fainting. There is a small risk of infection. Some people have not felt well when having their blood taken. Some people have felt dizzy while having their blood drawn or after. Let the nurse know if you would prefer to lie down while you have your blood drawn.

MRI risks

Because MRI uses low-energy, non-ionizing radio waves, there are no known risks or side effects.

Potential Benefits of Participation

Are there benefits to taking part in this study?

The aim of this study is to see if this experimental vaccine will cause your immune system to react against the tumor. We do not know if you will receive personal, medical benefit from taking part in this study. These potential benefits could include shrinking of your tumor or lessening of your symptoms, such as pain, that are caused by the cancer. Because there is not much information

about the drug's effect on your cancer, we do not know if you will benefit from taking part in this study, although the knowledge gained from this study may help others in the future who have cancer.

Alternative Approaches or Treatments

What other choices do I have if I do not take part in this study?

Instead of being in this study, you have these options**:**

- Getting treatment or care for your cancer without being in a study
- Taking part in another study

Please talk to your doctor about these and other options.

Research Subject's Rights

What are the costs of taking part in this study?

If you choose to take part in the study, the following will apply, in keeping with the NIH policy:

- You will receive study treatment at no charge to you. This may include surgery, medicines, laboratory testing, x-rays or scans done at the Clinical Center, National Institutes of Health (NIH), or arranged for you by the research team to be done outside the Clinical Center, NIH if the study related treatment is not available at the NIH.
- There are limited funds available to cover the cost of some tests and procedures performed outside the Clinical Center, NIH. You may have to pay for these costs if they are not covered by your insurance company.
- Medicines that are not part of the study treatment will not be provided or paid for by the Clinical Center, NIH.
- Once you have completed taking part in the study, medical care will no longer be provided by the Clinical Center, NIH.

Will your medical information be kept private?

We will do our best to make sure that the personal information in your medical record will be kept private. However, we cannot guarantee total privacy. Organizations that may look at and/or copy your medical records for research, quality assurance, and data analysis include:

- The National Cancer Institute (NCI) and other government agencies, like the Food and Drug Administration (FDA), which are involved in keeping research safe for people.

- National Cancer Institute Institutional Review Board
- Qualified representatives from Bavarian Nordic, Inc., the pharmaceutical company who produces the PROSTVAC-V/TRICOM and PROSTVAC-F/TRICOM vaccines.

A description of this clinical trial will be available on http://www.Clinicaltrials.gov, as required by U.S. Law. This Web site will not include information that can identify you. At most the Web site will include a summary of the results. You can search this Web site at any time.

Some of the analysis on your blood and tissue samples may be done by an outside facility. Your samples will be labeled with a study code number and will not include personal information. Only the investigators on this study know who the samples came from.

Stopping Therapy

Your doctor may decide to stop your therapy for the following reasons:

- if he/she believes that it is in your best interest
- if you have side effects from the treatment that your doctor thinks are too severe
- if new information shows that another treatment would be better for you

In this case, you will be informed of the reason therapy is being stopped. If therapy is stopped before you have had your surgery, you may choose to have your prostatectomy done here at the NIH if there is a protocol appropriate. If there is not a protocol for you or you prefer to have your surgery done elsewhere, we can refer you to another treatment center or to your primary physician.

You can stop taking part in the study at any time. However, if you decide to stop taking part in the study, we would like you to talk to the study doctor and your regular doctor first.

If you decide at any time to withdraw your consent to participate in the trial, we will not collect any additional medical information about you. However, according to FDA guidelines, information collected on you up to that point may still be provided to Bavarian Nordic, Inc. or designated representatives. If you withdraw your consent and leave the trial, any samples of yours that have been obtained for the study and stored at the NCI can be destroyed upon request. However, any samples and data generated from the samples that have already been distributed to other researchers or placed in the research databases can**not** be recalled and destroyed.

Conflict of Interest

The National Institutes of Health (NIH) reviews NIH staff researchers at least yearly for conflicts of interest. This process is detailed in a Protocol Review Guide. You may ask your research team for a copy of the Protocol Review Guide or for more information. Members of the research team who do not work for NIH are expected to follow these guidelines but they do not need to report their personal finances to the NIH.

Members of the research team working on this study may have up to $15,000 of stock in the companies that make products used in this study. This is allowed under federal rules and is not a conflict of interest.

The National Institutes of Health and the research team for this study are using vaccines developed by Bavarian Nordic, Inc. through a joint study with your researchers and the company. The company also provides financial support for this study.

Use of Specimens and Data for Future Research

To advance science, it is helpful for researchers to share information they get from studying human samples. They do this by putting it into one or more scientific databases, where it is stored along with information from other studies. A researcher who wants to study the information must apply to the database and be approved. Researchers use specimens and data stored in scientific databases to advance science and learn about health and disease.

We plan to keep some of your specimens and data that we collect and use them for future research and share them with other researchers. We will not contact you to ask about each of these future uses. These specimens and data will be stripped of identifiers such as name, address or account number, so that they may be used for future research on any topic and shared broadly for research purposes. Your specimens and data will be used for research purposes only and will not benefit you. It is also possible that the stored specimens and data may never be used. Results of research done on your specimens and data will not be available to you or your doctor. It might help people who have cancer and other diseases in the future.

If you do not want your stored specimens and data used for future research, please contact us in writing and let us know that you do not want us to use your specimens and/or data. Then any specimens that have not already been used or shared will be destroyed and your data will not be used for future research. However, it may not be possible to withdraw or delete materials or data once they have been shared with other researchers.

OTHER PERTINENT INFORMATION

1. Confidentiality. When results of an NIH research study are reported in medical journals or at scientific meetings, the people who take part are not named and identified. In most cases, the NIH will not release any information about your research involvement without your written permission. However, if you sign a release of information form, for example, for an insurance company, the NIH will give the insurance company information from your medical record. This information might affect (either favorably or unfavorably) the willingness of the insurance company to sell you insurance.

The Federal Privacy Act protects the confidentiality of your NIH medical records. However, you should know that the Act allows release of some information from your medical record without your permission, for example, if it is required by the Food and Drug Administration (FDA), members of Congress, law enforcement officials, or authorized hospital accreditation organizations.

2. Policy Regarding Research-Related Injuries. The Clinical Center will provide short-term medical care for any injury resulting from your participation in research here. In general, no long-term medical care or financial compensation for research-related injuries will be provided by the National Institutes of Health, the Clinical Center, or the Federal Government. However, you have the right to pursue legal remedy if you believe that your injury justifies such action.

3. Payments. The amount of payment to research volunteers is guided by the National Institutes of Health policies. In general, patients are not paid for taking part in research studies at the National Institutes of Health. Reimbursement of travel and subsistence will be offered consistent with NIH guidelines.

4. Problems or Questions. If you have any problems or questions about this study, or about your rights as a research participant, or about any research-related injury, contact the Principal Investigator, Peter Pinto, M.D., Building 10, Room 2W-5940, Telephone: 240-760-6249. You may also call the Clinical Center Patient Representative at 301-496-2626. If you have any questions about the use of your specimens or data for future research studies, you may also contact the Office of the Clinical Director, Telephone: 240-760-6070.

5. Consent Document. Please keep a copy of this document in case you want to read it again.

COMPLETE APPROPRIATE ITEM(S) BELOW:

A. Adult Patient's Consent

I have read the explanation about this study and have been given the opportunity to discuss it and to ask questions. I hereby consent to take part in this study.

Signature of Adult Patient/ Legal Representative _____ Date _____

Print Name _____

B. Parent's Permission for Minor Patient.

I have read the explanation about this study and have been given the opportunity to discuss it and to ask questions. I hereby give permission for my child to take part in this study.
(Attach NIH 2514-2, Minor's Assent, if applicable.)

Signature of Parent(s)/ Guardian _____ Date _____

Print Name _____

C. Child's Verbal Assent (If Applicable)

The information in the above consent was described to my child and my child agrees to participate in the study.

Signature of Parent(s)/Guardian _____ Date _____ Print Name _____

THIS CONSENT DOCUMENT HAS BEEN APPROVED FOR USE FROM JANUARY 23, 2017 THROUGH JANUARY 22, 2018.

Signature of Investigator _____ Date _____

Print Name _____

Signature of Witness _____ Date _____

Print Name _____