Abbreviated Title:	Biochemical Recurrent Prostate
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IBC #:	RD-15-VIII-18
Amendment:	H

Title: Prostvac in Patients with Biochemical Recurrent Prostate Cancer

Coordinating Center: National Cancer Institute (NCI)

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Investigational Agents:

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

PARTICIPATING SITES

All sites will engage in all protocol activities except for: data and specimen analysis which will be peformed by the Coordinating Center; sections delineated in the protocol as being for the NCI site only.

- Data and specimen analysis, which is performed by the Coordinating Center
- Please see individual protocol sections for other exceptions

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PRÉCIS

Background

- Androgen deprivation therapy (ADT) and surveillance are treatment options for prostate cancer patients with biochemical progression after localized therapy (biochemically recurrent prostate cancer). The primary goal in these patients is to prevent morbidity from their cancer and to do so with limited toxicity.
- Prostvac (Prostvac™; developed by the National Cancer Institute [NCI] and licensed to BN Immunotherapeutics, Mountain View, CA) is a novel candidate prostate cancer immunotherapy for the treatment of prostate cancer. It is a viral vector based therapeutic cancer vaccine that is administered via subcutaneous injections. In a randomized controlled Phase 2 trial, Prostvac therapy was associated with a prolongation of survival in men with metastatic castrate-resistant prostate cancer. A phase III trial recently completed accrual of patients in this same population.
- There is also rationale to use therapeutic cancer vaccines such as Prostvac in earlier stage prostate cancer patients to maximize the potential therapeutic effect of immune stimulating therapy.
- Analysis of previous trials using therapeutic cancer vaccines alone suggests that such therapies may alter tumor growth rate.

Objective

Primary Objective:

- Determine if the therapeutic cancer vaccine prostvac can decrease tumor growth rate as measured by PSA rise after 6 months compared to a group getting surveillance alone.

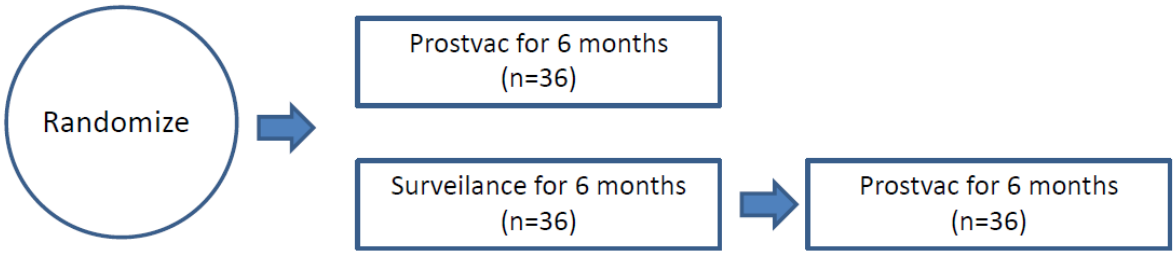
Key Eligibility Criteria

- Histologically confirmed adenocarcinoma of the prostate
- Patients with negative CT Scan and Tc-99m Bone Scan
- Patients with a PSA over 0.8 ng/ml for patients following radical prostatectomy or for patients following definitive radiation therapy: a rise in PSA of ≥ 2 ng/mL above the nadir
- Patients with a PSA doubling time of 5-15 months
- No history of active autoimmune disease or history of organ compromising autoimmune disease
- ECOG 0-1

Design

- Randomized study
- Accrual goal is 36 evaluable patients per arm; randomized 1:1 to:
  Arm A: Prostvac for 6 months with an additional optional year of maintenance for eligible patients OR
  Arm B: Surveillance for 6 months, then Prostvac for 6 months with an additional year of maintenance for eligible patients

SCHEMA



- Prostvac Schedule: q 2 weeks for 1 month, then monthly for 5 months
- Eligible patients (with a doubling time >5 months will be able to continue vaccine maintenance for up to 1 additional year of vaccine)

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1 INTRODUCTION

1.1 STUDY OBJECTIVES

1.1.1 Primary Objective

- Determine if prostvac can decrease tumor growth rate as measured by PSA rise after 6 months compared to a group on surveillance for 6 months.

1.1.2 Secondary Objectives

- Observe the effects of vaccine on PSA growth rate when prostvac is initiated after 6 months of surveillance
- Evaluate immune response including phenotypic and functional marker analysis, CD4 cells, CD 8 cells, PSA-specific T-cells measured by response to PSA peptides in CD4 and CD8 lymphotyes, natural killer cells, Regulatory T-cells, Regulatory T-cell function, cytokines, and naïve thymic emigrants.
- Associate immunologic outcomes with PSA responses/changes in growth rates.

1.1.3 Exploratory Endpoints

Beginning with amendment G, patients enrolled onto either the immediate vaccine arm or the surveillance followed by vaccine arm will be asked to complete a prostate cancer specific QOL survey with 18 questions (MAX-PC) as well as the FACT-P survey. Associations will be made between survey responses and changes in PSA kinetics.

1.2 BACKGROUND AND RATIONALE

1.2.1 Rationale for conducting the study in biochemically recurrent prostate cancer patients

Prostate cancer is the most common malignancy among men in the United States with an estimated 220,800 new diagnoses and over 27,540 deaths in 2015.[3] Although surgery or radiation can cure the majority of newly diagnosed patients, 20-40% are likely to have recurrent disease manifested as a rising PSA despite negative conventional imaging studies (CT and technetium-99m (Tc-99m) Bone Scan) used to detect metastasis. [4,5] This stage of disease is often referred to as biochemically recurrent prostate cancer.

Despite the fact that nearly 90,000 men who are diagnosed each year with prostate cancer will ultimately develop biochemically recurrent prostate cancer there is no clear standard of care for these patients.[6]. Treatments range from active surveillance to intermittent or continuous androgen deprivation therapy (ADT) and in some cases salvage radiation or surgery. Ideal effective non-hormonal treatment strategies in this population would avoid or delay the onset of metastatic castration resistant prostate cancer (mCRPC). There are two significant obstacles to developing therapies in this asymptomatic population; (1) therapies need to have minimal toxicity (2) conventional clinical endpoints (metastatic progression and overall survival) can take 5-10 years.

1.2.2 Therapeutic Cancer Vaccines in Prostate Cancer

The goal of therapeutic cancer vaccines is to generate a targeted immune response leading to immune-mediated anti-tumor activity. Sipuleucel-T is a therapeutic cancer vaccine generated from peripheral blood mononuclear cells obtained from individual patients via leukapheresis. This vaccine is generated after a patient's peripheral immune cells are collected via leukapheresis, transported to a regional processing center where they are exposed in vitro to a PAP/GM-CSF fusion protein. At the end of this process, the activated cellular product is re-infused into the patient. A full course of therapy repeats this process 3 times every 2 weeks for 1 month. [7,8] A phase III trial (n = 512) demonstrated an overall survival benefit for the vaccine (25.8 months vs. 21.7 months; P = 0.032).[7] Based on these overall survival findings, the FDA approved sipuleucel-T for the treatment of asymptomatic or minimally symptomatic mCRPC, making it the first FDA-approved therapeutic cancer vaccine for the treatment of any malignancy.

1.2.3 Prostvac

Prostvac (Prostvac™; developed by the National Cancer Institute [NCI] and licensed to BN Immunotherapeutics, Mountain View, CA), offers an alternative strategy to sipuleucel-T.[2,9] (The LTIB and BN Immunotherapeutics have an ongoing CRADA for the preclinical and clinical development of Prostvac.) To target prostate-specific antigen (PSA), Prostvac vaccine employs genetically altered poxviruses to deliver targeting information to immune cells and generate an immune response. Administered subcutaneously, the poxviruses deliver the transgenes for the tumor associated antigens (PSA) to antigen presenting cells through cellular infection. Once these pox viruses are within the cellular cytoplasm, the transgenes are processed. The end result is an antigen presenting cell expressing a PSA peptide within the major histocompatibility complex, resulting in PSA-specific cytolytic T lymphocytes activation.[10,11] (**Figure 2**) This approach does not require expensive, labor-intensive *ex vivo* preparation of patients' peripheral blood. Prostvac is thus potentially more logistically and financially feasible over the long-term than sipuleucel-T[12].

Prostvac has been investigated in 2 phase II trials in mCRPC, both of which administered the vaccine at monthly intervals until disease progression. An industry-sponsored, placebo-controlled, multicenter trial in 125 mCRPC patients randomized them 2:1 in favor of Prostvac; the placebo was an empty poxviral vector containing no transgenes. As was seen in the sipuleucel-T studies, patients receiving vaccine showed no change in short term disease progression, yet had an overall survival benefit (25.1 months with Prostvac vs. 16.6 months with placebo; $P = 0.0061$).[1] (**Figure 1**) A second phase II study of Prostvac in 32 mCRPC patients at the NCI demonstrated that the vaccine was able to generate a T-cell specific immune response[13]. Based on the findings in these trials, a phase III trial of Prostvac in mCRPC is currently underway ([11]).

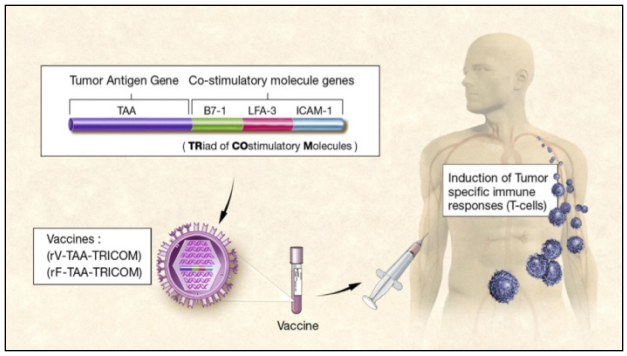

Figure 2: Poxviral vaccine strategy: Modified poxvirus contains transgenes for the tumor-associated antigen PSA and 3 T-cell costimulatory molecules.[2]

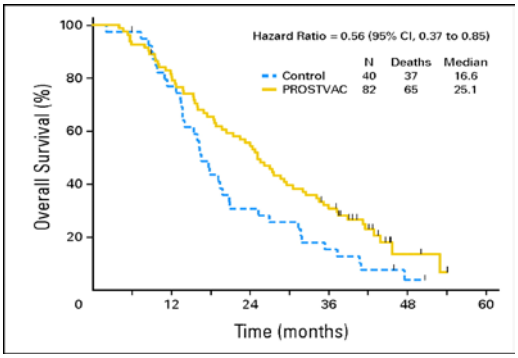

Figure 1: Prostvac improved survival in mCRPC patients in a randomized multi center phase II. [1]

Prostvac is very well-tolerated, with common side effects of grade 1 injection-site reactions or flu-like symptoms.[1,13] A favorable side effect profile and the potential ability to induce a sustained antitumor immune response, clinical trials are ongoing evaluating strategies to use Prostvac in earlier disease patients.

A phase III trial (NCT01322490) of Prostvac monotherapy, however, was reported to have not met its primary endpoint of overall survival in September, 2017. Nonetheless, the rationale for use of Prostvac in earlier stage prostate cancer remains scientifically rational and this trial will continue. Furthermore, at this point neither this trial nor the phase III trial have suggested a safety concern for Prostvac.

1.2.4 The Importance of Evaluating the Immunologic Response

Our group in the LTIB has previously evaluated immunologic parameters in clinical trials with Prostvac among several immunotherapy studies.[13,14] While the these findings are not surrogate markers of response, they have improved our knowledge of a vaccine-generated immune response and provided a better understanding of what factors are potentially important in mounting a sufficient anti-tumor immune response that could be associated with improved clinical outcomes. These and other data may allow optimization of vaccines in subsequent clinical trials. Few groups are as well positioned and have the experience as the LTIB to conduct this rigorous immune testing from clinical samples of patients treated with Prostvac.

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

Unlike previous studies involving Prostvac, which evaluated immune responses to a single 9 amino acid section of the 244 amino acid PSA protein, the immune analysis in this trial will

evaluate overlapping 15 amino acid sequences of the entire PSA protein. (**Figure 3**). This more extensive analysis will provide a more thorough understanding of the immune response initiated by Prostvac and a greater opportunity for clinical correlations. This type of analysis has not been previously prospectively performed in biochemically recurrent prostate cancer patients. Advantages of this approach include the ability to assess both CD4 and CD8 T cells, identification of multifunctional T cells (those producing ≥2 cytokines), and identification of T lymphocytes with lytic potential (CD107a expression). (**Table 1**).

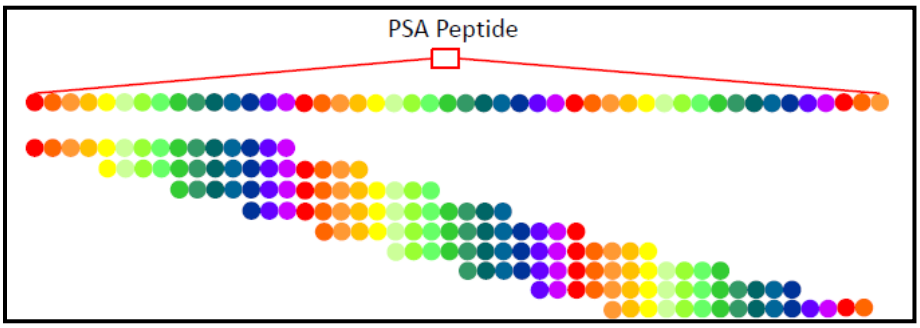

Figure 3: Overlapping PSA Peptide Assessment. This innovative approach to evaluating immune response allows for assessment of immune response to the entire PSA protein using overlapping 15 amino acid sequences. (Previous techniques have focused on assessing just one of these peptides.)

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

Table 1: Preliminary Data from a trial of Prostvac in mCRPC. This table shows how this multi peptide approach can be used in this proposal. In this previous (unpublished) trial, 2 cohorts of patients were evaluated using the proposed methods. (Responses are listed as absolute # of CD4 or CD8 T Lymphocytes Producing Cytokine or Positive for CD107a per $1x10^6$ cells plated.) This readout shows the breadth of this analysis as both CD4 and CD8 tell cells are evaluated using multiple parameters (cytokine production signifying activation – IFN-γ, IL-2 and TNF-α) and lytic potential as measured by CD107a expression. When only one peptide was used to analyze the immune responses, no immune responders were identified (unpublished).

Briefly, the methodology takes cryopreserved peripheral blood mononuclear cells (PBMCs) from patients before therapy and at the time of restaging. They are thawed and rested overnight at 37ºC, 5% CO_2 in complete media (IMDM supplemented with 10% Human AB, 2mM glutamine, 100 units/ml penicillin, and 100 µg/ml streptomycin). The next day (Day 0), PBMCs are seeded in 12 well plates ($2.5 x10^6$ in 1 mL), and stimulated with peptide mixes (0.1ug/mL per peptide); cultures were supplemented on days 3 and 5 with cytokines (IL7 and IL15, 10 ng/mL, PeproTech, Rocky Hill, NJ) and fresh media, and on day 7 are rested (with removal of cytokine and peptide). On day 11, $1x10^6$ cells were restimulated for 24 hours in 96 well plates with peptide mixes in the presence of anti-CD107a-APC (clone H4A3, BD Biosciences); brefeldin A (1ul/mL) and monensin (0.7ul/mL) (BD Biosciences) are added to cultures 2 hours after the start of the restimulation and incubated for the final 22 hours. PBMCs are then stained with anti-CD4-PerCP-Cy5.5 (clone OKT4, Biolegend), anti-CD8-AF700 (clone OKT8, Ebioscience), and anti-TNF-PE (clone MAb11), anti-IFNγ-PE-Cy7 (clone 4SB3), and anti-IL-2-BV521 (clone 5344.111) (BD Biosciences). At least $3x10^5$ events in the live gate are acquired with a BD LSR-II flow cytometer equipped with a UV, violet, blue, and red laser. FCS files are analyzed with FlowJo V.9.7 for Macintosh (TreeStar, Ashland, OR). Fluorescence minus one (FMO) controls are used for gaiting, and non-viable cells were excluded. The absolute number of $CD4^+$ or $CD8^+$ lymphocytes producing cytokine or positive for CD107a is calculated per $1x10^6$ cells plated at the start of the IVS. The background signal (obtained with the HLA peptide pool), and values obtained prior to therapy were subtracted from those obtained post-therapy. Values ≥250 are scored as positive for TAA-specific immune response following therapy. (**Figure 4**)

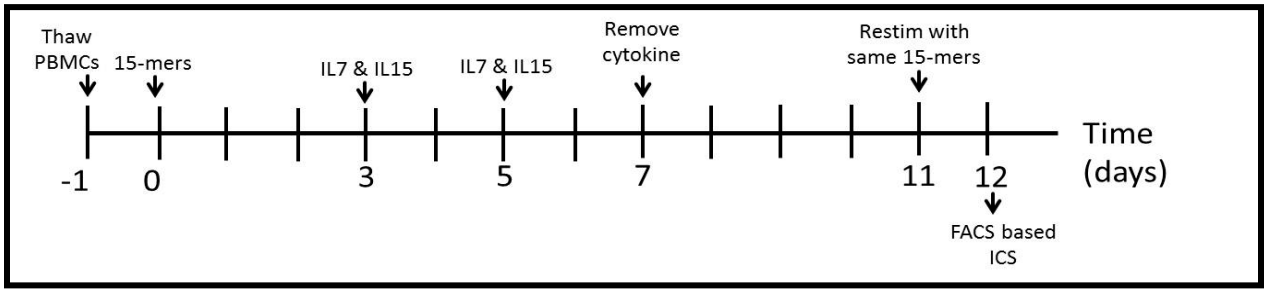

Figure 4: Methodology of Assessing PSA-specific Immune Responses

Flow Cytometry Analysis of Immune subsets (Biomarker Development)
As part of the extensive immune interrogation, peripheral immune cells will be evaluated by 30 markers to assess 127 immune subsets. (**Table 2**) This methodology has been previously described.[15] This added analysis will provide preliminary data to develop a Peripheral Immunoscore based on the frequency of specific pre-determined immune cell subsets in the

blood of patients prior to therapy. The score is calculated from the frequency of certain immune cell subsets prior to vaccine treatment. The panel of markers will also be used to measure immune response to the vaccine. The ultimate goal is to use this Peripheral Immunoscore as a biomarker that could serve as an intermediate marker of immune response in future studies. Data from this study would be used to establish the relevant markers and can be confirmed in future studies. The first step has been performed in a previous study with another pox viral vaccine in breast cancer (unpublished data; **Figure 5**)

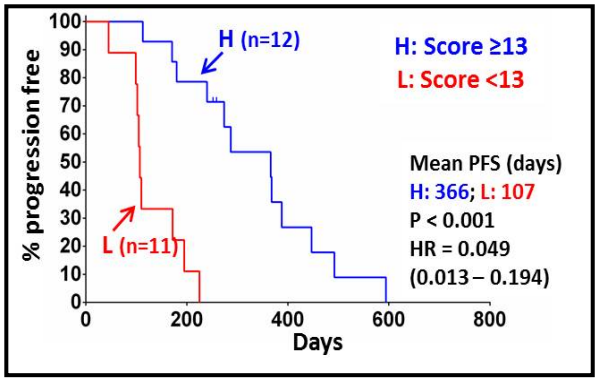

Figure 5: Preliminary Data of Immunoscore Associations with Clinical Outcomes. This data (unpublished) from a recently completed breast cancer study involving a therapeutic cancer vaccine highlights the potential of Immunoscore. In this study, patients with peripheral immunoscore (H, more than 13 parameters positive, measured prior to therapy) had a significant increase in progression free survival. This difference was only observed in the vaccine arm and not in the standard of care alone arm. A separate assessment is required in prostate cancer and will be done in this study. If similar findings are seen, the Immunoscore could be prospectively evaluated in future trials using the data from this study to establish which immune subsets are most relevant.

Table 2: Analysis of Peripheral Immune Cells

1. **CD4**: Helper T lymphocytes (32 subsets)
2. **CD8**: Cytotoxic T lymphocytes (29 subsets)
   - **Markers of PD-1 pathway and T cell activation (in CD4 and CD8):**
     - **EOMES:** activation
     - **TCR-αβ:** activation
     - **Tbet:** activation
     - **BATF:** activation/exhaustion
   - **Maturation status of T lymphocytes (in CD4 and CD8)**:
     - **Naïve**: $CD45RA^+$ $CCR7^+$
     - **Effector Memory**: $CD45RA^-$ $CCR7^-$
     - **Terminal (EMRA)**: $CD45RA^+$ $CCR7^-$
   - **T lymphocyte markers (in CD4 and CD8)**:
     - **CTLA-4:** inhibition
     - **PD-1:** activation/inhibition
     - **PD-L1:** activation/cross-inhibition
     - **TIM-3:** inhibition
     - **ICOS**: activation (only on CD4)
3. **Tregs**: Regulatory T lymphocytes ($CD4^+$ $CD25^+$ $FoxP3^+$ $CD127^-$) (7 subsets)
   - **CD45RA**: Tregs highly expandable *in vitro*
   - **CTLA-4**: Treg suppression
   - **CD49d:** "contaminating" effector lymphocytes (non-Tregs)
   - **ICOS**: Treg suppression
   - **PD-1:** activation/inhibition
   - **PD-L1:** cross-inhibition
4. **B lymphocytes**: $CD19^+$ (5 subsets)
   - **CTLA-4:** inhibition
   - **TIM-3:** inhibition
   - **PD-1:** activation/inhibition
   - **PD-L1:** cross-inhibition
5. **NK**: Natural killer cells ($CD56^+$ $CD3^-$) (20 subsets)
   - **$CD16^+$ $CD56^{br}$**: Functional intermediate, lytic and cytokine production
   - **$CD16^+$ $CD56^{dim}$**: Mature NK, cytokine production
   - **$CD16^-$ $CD56^{br}$:** Immature, abundant in human placenta

- **CD16^{-} CD56^{dim}**: non-lytic, non-cytokine production
- **TIM-3:** activation
- **PD-1:** activation/inhibition
- **PD-L1:** cross-inhibition

6. **NK-T**: CD56^{+} CD3^{+} (4 subsets)
    - **TIM-3:** activation
    - **PD-1:** activation/inhibition
    - **PD-L1:** cross-inhibition
7. **cDCs (Conventional DCs):** CD3^{-}CD56^{-}CD1c^{+}CD303^{-} (5 subsets)
8. **pDCs (plasmacytoid DCs):** CD3^{-}CD56^{-}CD1c^{-}CD303^{+} (5 subsets)
    - **Markers of DC activation**
        - **CD83:** activation
        - **TIM-3:** inhibition
        - **PD-1:** activation/inhibition
        - **PD-L1:** cross-inhibition
9. **MDSCs**: Myeloid-derived suppressor cells (CD11b^{+} HLA-DR$^{low/-}$ CD33^{+}) (20 subsets)
    - **CD14**: Common Myeloid Marker (high in monocytes, dim in granulocytes)
    - **CD15**: Granulocyte marker
    - **CD16**: most immature monocytic MDSCs
    - **PD-1:** activation/inhibition
    - **PD-L1:** cross-inhibition

1.2.5 Rationale for Changes in Growth Rate as Primary Endpoint

Monotherapy with a therapeutic cancer vaccine or immune check point inhibitor has previously demonstrated improved survival without short-term changes in progression. [3,9,14,15]. Therefore, short term clinical end points are unlikely to determine the clinical potential of vaccines in patients with prostate cancer, especially given the anticipated heterogeneity of the population and likelihood for prolonged survival after enrollment. A previous analysis of prostate cancer clinical trials at the NCI suggested that a therapeutic cancer vaccine may alter the tumor growth rate after treatment, thereby having no effects on delaying short-term progression yet improving long term outcome. This data suggested that the decreased growth rate of the tumor continued even after the vaccine was discontinued; perhaps suggesting a sustained vaccine-initiated immune response impacted the tumor growth rate. This is contrasted with cytotoxic chemotherapy which led to initial reductions in tumor size, but then growth rate resumed at the same pre-treatment rate once cytotoxic therapy was discontinued.[16] (**Figure 6**) Furthermore, unpublished data from an ECOG trial in non-metastatic prostate cancer demonstrates that a reduced growth rate was seen after treatment with a vaccine and was demonstrable within 100 days of vaccine initiation. (**Figure 7**). Together, these data suggest that vaccines may be able to impact tumor growth rates thereby potentially improving survival, and such changes can be seen as early as 3 months after initiating therapy.

The prospective use of tumor growth rate changes after treatment with a vaccine may introduce a new perspective and method in the assessment of therapeutic cancer vaccines and check point inhibitors. Given the indolent nature of biochemically recurrent prostate cancer, a 50% reduction in the growth rate could have substantial implications on survival.[16,17]

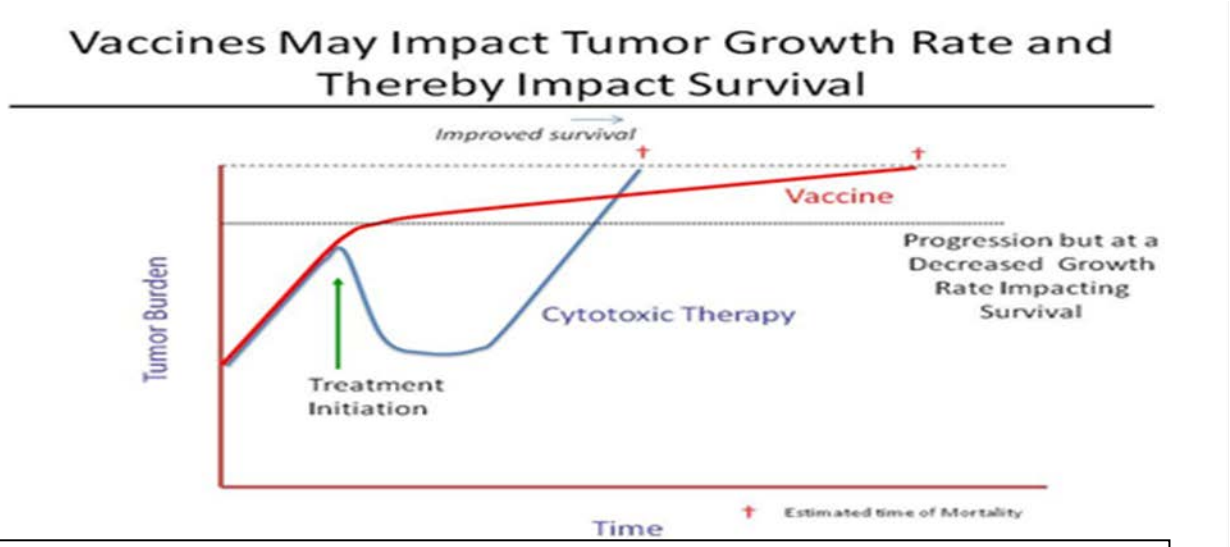

Figure 6: Retrospective data suggests that therapeutic cancer vaccines can alter the growth rate of tumors over time leading to an impact on overall survival.

1.2.6 Rationale for Using a 6-Month Endpoint to Evaluate Change in the Growth Rate

An analysis of the ECOG trial 9802, which treated castration-sensitive non-metastatic prostate cancer patients (n=29) suggests a vaccine targeting PSA alters tumor growth rate, as measured by PSA (log g (growth rate) +/- SE (standard error)), within 80 to100 days (**Figure 7**). [18] This retrospective analysis used the tumor growth rate equation determined that by day 80 after Prostvac therapy was initiated a statistically significant change in tumor growth rate was detectable compared to baseline.[16] This altered growth rate was sustained at day 100 as well. These data support the hypothesis that vaccines exert their therapeutic effect not by reducing tumor burden, but by altering tumor growth rate. (The current study differs E9802 in that it is randomized to immediate or delayed vaccine with a primary focus on the immune impact of the vaccine on tumor growth rates.)

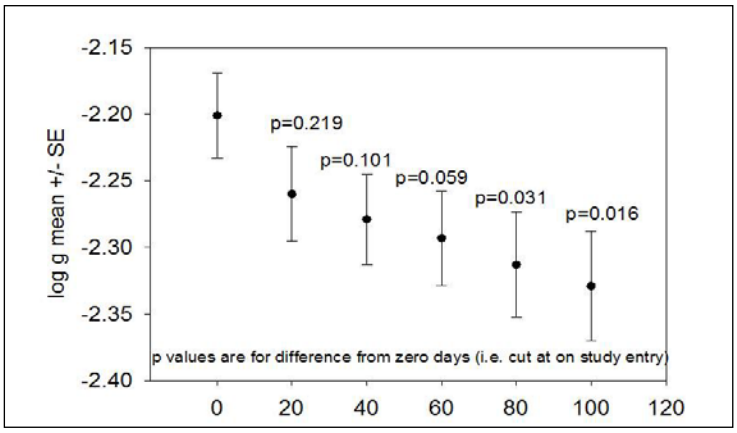

Figure 7: Prostvac can affect growth rate in 80 days. An analysis of the ECOG trial 9802, which treated castration-sensitive non-metastatic prostate cancer patients (n=29) suggests Prostvac alters tumor growth rate, as measured by PSA *(log g (growth rate) +/- SE (standard error)),* within 80 to100 days. This retrospective analysis used the tumor growth rate equation determined that by day 80 after Prostvac therapy was initiated a statistically significant change in tumor growth rate was detectable compared to baseline. This altered growth rate was sustained at day 100 as well. These data support the hypothesis that vaccines exert their therapeutic effect not by reducing tumor burden, but by altering tumor growth rate.

This trial will provide an opportunity to prospectively define the mechanism by which vaccines can impact tumor growth rate.

1.2.7 Tumor Growth Kinetics

The regression-growth equation

Drs. Stein and Fojo at the NCI have developed an equation based on the assumption that the change of a tumor's quantity during therapy results from 2 independent component processes: an exponential (first-order kinetics) decrease/regression and an exponential regrowth of the tumor[19]. The equation is $f(t) = exp(-d * t)+exp(g * t) - 1$ (A) where exp is the base of the natural logarithm, $e = 2.7182$, and $f(t)$ is the tumor (or in MTC calcitonin) measurement at time t in days, normalized to (divided by) the tumor measurement at day 0, the time at which treatment is commenced. Rate constant d (**d**ecay, in days^{-1}) represents the exponential decrease/regression of

the serum tumor marker (i.e. PSA) signal during therapy. Rate constant *g* (**g**rowth, also in days^{-1}) represents the exponential growth/regrowth of the tumor during treatment. These rate constants may be expressed in terms of half-lives and doubling times. Thus, *d* equals $\ln_2$ (0.693) divided by the time it takes for the regressing part to shrink by half, whereas *g* equals $\ln_2$ divided by the time for the growing component to double.[16]
Two earlier papers depict theoretical curves depicting the separate components of Equation (A) and how these combine together to give the time dependence of the tumor size, *f* (18,19). When the data showed a continuous decrease from the time of treatment start, so that only the regression parameter *d* was found to differ significantly from zero with $P < 0.05$, Equation (A) was replaced by the following reduced form, with the growth rate constant eliminated: $f(t) = exp(-d * t)$ (B) When tumor measurements showed a continuous increase, so that only the growth parameter *g* was found to differ significantly from zero with $P < 0.05$, Equation (A) was replaced by the following reduced form, with the decay constant eliminated: $f(t) = exp(g * t)$.[19,20]

1.2.7.1 Data Analysis

For each patient an attempt to fit Equation (A) to each data set for which more than one data point is available. Curve fitting will be performed using Sigmaplot (Systat Software), or by using the Solver routine in an Excel spreadsheet. We will extract parameters g and d with their associated Student's t and P values[19]. Data will be analyzed in Excel (Microsoft) and in Sigmaplot 9.0. Linear regressions to evaluate the relationship between the growth rate constant, *g*, or other parameters will be implemented using the polynomial linear routine of Sigmaplot 9.0. Sample comparisons were performed by Student's *t*-test, using SigmaStat 3.5 (Systat Software), with *P* set at 0.05 for significance.[16]

2 ELIGIBILITY ASSESSMENT AND ENROLLMENT

2.1 ELIGIBILITY CRITERIA

2.1.1 Inclusion Criteria

2.1.1.1 Histopathological documentation of prostate cancer confirmed in either the Laboratory of Pathology at the National Institutes of Health (NIH) Clinical Center, Walter Reed National Military Medical Center, MSKCC, DFCI or BIDMC prior to enrollment. If no pathologic specimen is available, patients may enroll with a pathologist's report showing a histologic diagnosis of prostate cancer and a clinical course consistent with the disease.

2.1.1.2 Biochemical progression after definitive radiation or surgery defined as follows:

- For patients following definitive radiation therapy: a rise in PSA of ≥ 2 ng/mL above the nadir (per RTOG-ASTRO consensus criteria).
- For patients following radical prostatectomy: rising PSA after surgical procedure. (Patients must have a PSA ≥ 0.8 ng/ml)

2.1.1.3 ECOG performance status of 0–1 (Karnofsky ≥80%, see **APPENDIX A**).

2.1.1.4 Patients must have a PSA doubling time of 5-15 months.

2.1.1.5 Patients must have a rising PSA as confirmed by 3 values done at least 1 week apart and over no less than 1 month.

2.1.1.6 Recovery from acute toxicity related to prior therapy, including surgery and radiation, or no toxicity ≥ grade 2.

2.1.1.7 Negative CT scan/MRI and bone scan for metastatic prostate cancer.

2.1.1.8 Hematological eligibility parameters (within 16 days before starting therapy; see **APPENDIX D**):

- Granulocyte count ≥ 1000/mm^3
- Platelet count ≥ 100 000/mm^3
- Hgb ≥ 10 g/dL

2.1.1.9 Biochemical eligibility parameters (within 16 days before starting therapy):

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

2.1.1.10 No other active malignancies within the past 36 months (with the exception of nonmelanoma skin cancers or carcinoma in situ of the bladder) or life-threatening illnesses, in the opinion of the investigator.

2.1.1.11 Willing to travel to the NIH, MSKCC, DFCI, BIDMC for follow-up visits.

2.1.1.12 18 years of age or older.

2.1.1.13 Able to understand and sign informed consent.

2.1.1.14 Baseline testosterone ≥ 100 ng/dl

2.1.1.15 PSA ≤ 30 ng/mL.

2.1.1.16 The effects PROSTVAC on the developing human fetus are unknown. For this reason, men must agree to use adequate contraception (hormonal or barrier method of birth control; abstinence) prior to study entry, for the duration of study therapy and at least one month post therapy. Should a woman become pregnant or suspect she is pregnant while her partner is participating in this study, she should inform her treating physician immediately.

2.1.2 Exclusion Criteria

2.1.2.1 Immunocompromised status due to:

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

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

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

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

2.1.2.5 History of prior chemotherapy

2.1.2.6 History of prior immunotherapy within the last 3 years

2.1.2.7 Major surgery within 4 weeks prior to enrollment (Day 1 visit).

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

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

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

2.1.2.11 Previous serious adverse reactions to smallpox vaccination

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

2.1.2.13 Receipt of an investigational agent within 28 days (or 56 days for an antibody-based therapy) before the first planned dose of study drugs.

2.1.2.14 Patients who test positive for HBV or HCV

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

2.1.3 Recruitment Strategies

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

2.2 SCREENING EVALUATION

A. Pathological confirmation of diagnosis by either the Laboratory of Pathology at the NIH, Walter Reed National Military Medical Center, MSKCC, DFCI, or BIDMC may

be obtained anytime prior to enrollment (if specimen is available). If no pathologic specimen is available, patients may enroll with a pathologist's report showing a histologic diagnosis of prostate cancer and a clinical course consistent with the disease.

B. The following parameters will be obtained within 8 weeks prior to enrollment:
   1. HIV test
   2. Hepatitis B and C
   3. Tc-99 whole-body scintigraphy
      CT (or MRI may be substituted at investigator's discretion) of chest, abdomen and pelvis

C. The following parameters will be obtained within 16 days prior to enrollment (cycle 1 day 1 for immediate treatment arm [Arm A], or M-6 visit for surveillance first arm [Arm B]):
   4. History and physical examination with ECOG
   5. Serum PSA
   6. Complete blood count plus differential and platelet count
   7. Hepatic and Acute Care
   8. Testosterone level

2.3 REGISTRATION PROCEDURES

Authorized staff must register an eligible candidate with NCI Central Registration Office (CRO) within 24 hours of receiving a signed 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 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. When relevant, treatment assignment is conveyed to the Pharmacy at the same time. Verification of Registration will be forwarded electronically via e-mail to the research team. A recorder is available during non-working hours.

2.3.1 For Participating Site Registration

Registration will be a two-part process as patients are screened on this protocol. A protocol registration form will be supplied by the NCI study coordinator and updates will be provided as needed. Subject eligibility and demographic information is required for registration. To initially register a subject, after the participant has signed consent, complete the top portion of the form and send to NCI study coordinator. Once eligibility is confirmed, after completion of screening studies, complete the remainder of the form which is the eligibility checklist, indicating that the patient is being registered for treatment and send to NCI study coordinator. In addition, source documents supporting the eligibility criteria must be sent to the NCI study coordinator. The NCI study coordinator will notify you either by e-mail or fax that the protocol registration form has been received which will include the unique patient/subject ID number. Questions about eligibility should be directed to the NCI study coordinator or NCI PI. Questions related to registration should be directed to the NCI study coordinator.

Subjects that do not meet screening criteria should be removed from the study following the procedure in section **3.7.3**.

2.3.2 Treatment Assignment and Randomization Procedures

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Cohorts

Number	Name	Description
1	Prostate Cancer	Male patients diagnosed with prostate cancer (non-metastatic), with demonstrated biochemical progression, a PSA doubling time of 5-15 months, and with a rising PSA.

Arms

Letterr	Name	Description
A	PROSTVAC treatment	PROSTVAC treatment for 6 months with an additional optional year of maintenance for eligible patients
B	Delayed PROSTVAC treatment	Surveillance for 6 month followed by PROSTVAC treatment for 6 months with an additional year of maintenance for eligible patients

Randomization and Arm Assignment

Subjects in Cohort 1 will be randomized on a 1:1 basis to **Arm A** or to **Arm B**. The computerized randomization will be peformed by the NCI Central Registration Office. CRO staff will send a secured e-mail with the Verification of Registration of the patient and treatment arm patient is randomized to. This process will be completed within 15–30 minutes of sending the eligibility checklist. CRO will keep track of all randomization data in Clinical Data Registry (CDR) of CRO.

2.4 BASELINE EVALUATION

A. The following parameters will be obtained **within 8 weeks** prior to treatment intiation:
   1. Baseline electrocardiogram (EKG) on all patients, and appropriate cardiologic evaluation, as clinically indicated, to provide baseline function and identify any patients who should be monitored closely for cardiac risks associated with vaccinia vaccination.

B. The following parameters will be obtained **within 16 days** prior to start of treatment (these tests will not need to be repeated if they were done at screening within the appropriate timeframe):
   1. Clinical evaluation
      - History and physical examination
      - ECOG performance status (see **APPENDIX A**)
      - Height, weight

2. Laboratory studies
   - Serum PSA
     CBC/differential, with platelet count
   - Serum testosterone level
   - Serum chemistries (Na^+, K^+, Cl^-, CO_2, glucose, BUN, creatinine, albumin, calcium, magnesium, phosphorus, alkaline phosphatase, ALT, AST, total bilirubin, LDH, CK, uric acid, total protein)
   - Lymphocyte phenotyping CD3/CD4/CD8
   - Urinalysis in patients unless no feasible (i.e. patient has incontinence)
   - PAP (prostatic acid phosphatase), at the discretion of the PI

3 STUDY IMPLEMENTATION

3.1 STUDY DESIGN

- This is a randomized trial of PROSTVAC for 6 months vs. surveillance for 6 months followed by PROSTVAC for 6 months in patients with nmCSPC prostate cancer. 72 evaluable patients will be enrolled with an accrual ceiling of 110 patients
- PROSTVAC will be administered identical to the Phase III dosing with vaccine given week 1 (vaccinia-PROSTVAC, $2x10^8$ infectious units subcutaneously) and then fowlpox-vaccine ($1x10^9$ infectious units subcutaneously) on weeks 3 and 5 followed by monthly fowlpox-vaccine ($1x10^9$ infectious units subcutaneously) for a total of 5 months (including vaccinia, vaccine is administered over 6 months).
- The primary endpoint of this trial will be to determine if PROSTVAC can decrease tumor growth rate as measured by PSA rise after 6 months compared to a group getting surveillance alone. This time frame should be sufficient to evaluate immune effect on the re-growth rate given that the ECOG 9802 analysis demonstrated a significant change in tumor growth rate after starting PROSTVAC.
- Secondary endpoints include observing the effects of vaccine on PSA growth rate when prostvac is initiated after 6 months of surveillance, immune response evaluation will include CD4 cells, CD 8 cells, PSA-specific T-cells, natural killer cells, Regulatory T-cells, Regulatory T-cell function, cytokines, naïve thymic emigrants, associate immunologic outcomes with PSA responses/changes in growth rates.
- <u>For patients who have a PSA doubling time greater than 5 months (measured over the previous 6 months of treatment), they may receive maintenance vaccine every 3 months for an additional year. For patients who are on-study at the time of this amendment, they will be given an option to receive 1 year of vaccine maintenance (4 doses, administered every 3 months).</u>
- When possible, patients will be followed for TTP beyond the treatment period.

3.1.1 Protocol Stopping Rules

This study will utilize the Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 for grading systemic toxicity (see section **6.3**).

- One occurrence of grade 5 toxicity by the NCI-CTCAE version 4.0 attributable to the treatment regimen.
- Two occurrences of grade 4 toxicity by the NCI-CTCAE version 4.0 attributable to the treatment regimen.

If either of the above occurs, the Principal Investigator will halt accrual to the trial and will discuss with the NCI Institutional Review Board (IRB) whether any changes need to be made to the protocol.

3.2 DRUG ADMINISTRATION

Patients will receive PROSTVAC identical to Phase III dosing (NCT01322490) which consists of a single immunization of PROSTVAC-V (vaccinia-PROSTVAC, $2x10^8$ infectious units subcutaneously) in Week 1, followed by immunizations of PROSTVAC-F (fowlpox-PROSTVAC $1x10^9$ infectious units subcutaneously) administered on weeks 3 and 5 followed by monthly fowlpox-vaccine ($1x10^9$ infectious units subcutaneously) for a total of 5 months (including vaccinia, vaccine is administered over 6 months).

The preferred PROSTVAC-V/F subcutaneous injection site is the upper thigh. If given on the arm, which is not preferred, the vaccine should be administered subcutaneously in the back of the upper arm.

3.3 TREATMENT MODIFICATIONS

3.3.1 Vaccine

3.3.1.1 Dosing delay

- Patients must have recovered to ≤ grade 1 toxicity related to vaccine (or baseline) 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 nonautoimmune toxicity attributable to the vaccine persists for > 42 days, the patient will not receive further vaccine inoculations and will be removed from the protocol treatment and followed for resolution of toxicity and immune endpoints.
- Patients who develop grade 3 injection site reactions will have their vaccine held until injection site reaction resolves to grade 2 or less.
- Patients who develop ≥ a grade 2 allergic or autoimmune disease that threatens vital organ function or any ≥ grade 3 autoimmunity, not related to a therapeutic response, will be removed from the protocol treatment and followed for resolution of toxicity and immune endpoints.
- Patients who develop any grade 4 toxicity attributable to the study drug(s) will be removed from the protocol treatment and followed for resolution of toxicity and immune endpoints.
- If a scheduled vaccine dose is missed due to scheduling or logistical issues, the vaccine may be given within 14 days of the appointed time

3.3.1.2 Dose modifications

No dose modifications are allowed with this vaccine.

3.4 QUESTIONNAIRES

3.4.1 Evaluating Quality of Life in this Population

Little has been formally evaluated about the impact of PSA kinetics on patients with BCR. Beginning with amendment G, patients enrolled onto either the immediate vaccine arm or the surveillance followed by vaccine arm will be asked to complete a prostate cancer specific QOL survey with 18 questions (MAX-PC) as well as the FACT-P survey at the following time points:

Immediate vaccine Arm
Baseline; 3 months; 6 months; 9 months; approximately q3-4 months in follow-up

Surveillance Arm
-6 months; -3 months; Baseline/start of vaccine; 3 months; 6 months; 9 months; approximately q3-4 months in follow-up

Each survey will take approximately 5 minutes to complete and they will be administered in english. The surveys being used have been published and vetted as appropriate QOL tools[21-23] . This analysis will be done in an exploratory fashion as part of a collaboration with Dr. Alicia Morgans who is an expert in prostate cancer QOL studies.

3.5 PROTOCOL EVALUATION (SEE APPENDIX C)

3.5.1 All patients who are deemed eligible and who sign an informed consent will be enrolled in this trial.

3.5.2 A complete history and physical examination, including ECOG performance status, will be done within 16 days before enrollment.

3.5.3 Laboratory Studies

(See also **APPENDIX C**)

- Serum PSA
- CBC/differential with platelet count
- Serum testosterone level
- Serum chemistries (Na^+, K^+, Cl^-, CO_2, glucose, BUN, creatinine, albumin, calcium, magnesium, phosphorus, alkaline phosphatase, ALT, AST, total bilirubin, LDH, CK, uric acid, total protein)
- HIV/Hepatitis B/Hepatitis C tests (within 8 weeks prior to start of enrollment)
- Lymphocyte phenotyping CD3/CD4/CD8

3.5.4 Radiographic assessment

Patients will undergo scheduled radiographic assessment for metastatic disease at 6 month intervals. Radiologic studies consisting of bone scan, CT scan of chest, and CT scan of abdomen/pelvis will be performed at baseline. MRI may be substituted for CT scan at the discretion of the investigator.

Patients in this trial may have the option to take part in a PET imaging study at the NIH in Bethesda, MD, which may utilize relevant data from this study.

3.5.5 Assessment for PROSTVAC administration

Dosing and administration of PROSTVAC will be performed in the NIH Day Hospital, MSKCC, DFCI, or BIDMC. Patients will be monitored with vital signs (blood pressure, heart rate, respiratory rate, temperature) prior to and within 1 hour after the initial vaccine treatment. On subsequent visits (where rF-PROSTVAC is administered) patients will have vital signs checked prior to vaccine and then be monitored for 30 minutes after vaccine treatments. (Post-vaccine vital signs will not be required except for the first vaccination.) Documentation of any patient reported symptoms occurring between dosing will be included in the assessment. Laboratory assessments will be conducted as per **APPENDIX C**.

3.6 CONCURRENT THERAPIES

Concurrent hormonal therapy will not be allowed. Concurrent anticancer treatment with chemotherapy, systemic glucocorticoids (topical and inhaled steroids allowed), radiation therapy, major surgical procedures for prostate cancer, and nonprotocol-related immunotherapy will not be permitted.

Concurrent systemic corticosteroid use (daily or every other day for continued use > 14 days) will also not be allowed.

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 28 days following the last dose of study therapy.

3.7.1 Criteria for removal from protocol therapy

Patients will be removed from treatment for the following:

- Clinical or radiographic progression of disease.
- Grade 3 or greater toxicity attributed to treatment that does not resolve to grade 1 within 42 days from time of scheduled treatment.
- Grade 2 or greater autoimmune disease that threatens vitals organs.
- Any Grade 4 toxicity that is possibly, probably or definitely related to the protocol treatment will require a patient to be off-treatment
- Intercurrent illness or medical circumstances. If at any time the constraints of this protocol are detrimental to the patient's health, the patient may be removed from protocol therapy and reasons for withdrawal will be documented.
- Any grade of seizure will require a patient to be off-treatment.
- Requirement for androgen deprivation therapy
- Completion of protocol therapy including an approximately 28 day safety visit as outlined in section **3.8** (when logistically feasible)

3.7.2 Off-Study Criteria

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

3.7.3 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 ncicentralregistration-l@mail.nih.gov.

3.7.3.1 For Participating Sites

The Participant Status Updates Form will be supplied by the NCI study coordinator. Send the completed form to the NCI study coordinator.

3.8 FOLLOW-UP EVALUATIONS

After subjects have stopped taking the study medication for any of the reasons listed in Section **3.7**, they will be seen at the study site, if logistically feasible, for a safety visit within 4-5 weeks of drug discontinuation. The safety assessments may be performed by a local physician and laboratory if patients unable to return to the study site at this time.

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

- History and Physical Examination
- Serum chemistries (Na+, K+, Cl-, CO2, glucose, BUN, creatinine, albumin, calcium, magnesium, phosphorus, alkaline phosphatase, ALT, AST, total bilirubin, LDH, CK, uric acid, total protein)
- CBC/differential with platelet count
- Serum PSA level
- Serum PAP
- Adverse event reporting

After the safety visit, if there are no unresolved grade 3 or higher AEs, we may, when feasible contact the patient annually to find out how they are doing and to determine survival status. If there are unresolved grade 3 – 4 AEs, patients will be followed either at the study site or by their local physician. In the latter case, we will obtain the physician's record of AEs.

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

4 BIOSPECIMEN COLLECTION

4.1 CORRELATIVE STUDIES FOR RESEARCH

4.1.1 Immunologic Parameters

- Antibodies to PSA, vaccinia, fowlpox may be tested
- Leukocyte CD3, CD4, CD8 subsets; CD4:CD8 ratio will be drawn at baseline and monthly prior to vaccination while the patient remains on trial.
- The results of the HIV antibody need to be available before treatment to determine eligibility
- Additional studies will include but are no limited to quantitative and qualitative assessments of regulatory T-cells, Natural Killers cells, Myeloid Derived Suppressor Cells, anti-glycan antibodies and Naïve T-cell/new thymic emigrants.
- Assessment of levels of cytokines.
- Immunologic studies will be repeated more frequently if clinically indicated, and any abnormalities potentially related to treatment will be followed until they have resolved, or have been determined not to be treatment-related.

4.1.2 Immunologic Assays

4.1.2.1 CD4 T Cell Proliferation Assay

It is planned that all patients will undergo exploratory analysis of the ability to detect CD4-positive responses using a whole-protein PSA assay, as well as a peptide mix with 63 different 15-mer peptides by ELISPOT and/or ELISA.

4.1.2.2 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.

4.1.2.3 Flow cytometry analysis of thymic emigrant

To determine recent thymic emigrants, flow cytometry analysis will be performed on peripheral blood mononuclear cells. Cells will be resuspended in staining buffer (phosphate-buffered saline containing 3% fetal bovine serum) and stained for 30 minutes at 4°C with the combination of following antibodies: APC-H7-conjugated anti-CD4, PE-CY7-conjugated anti-CD3; FITC-conjugated CD45RA, PE-conjugated CD31, PerCP-CY5.5-conjugated Ki-67, AF-700-conjugated-CD197, V450-conjugated CD8, APC-conjugated CD103, V500-conjugated CD27 all purchased from BD Pharmingen, San Diego, CA). After that, FoxP3 intra-cellular staining will be performed on the cells stained with anti-CD4 and anti-CD25. They will be fixed and permeabilyzed using a fix/perm kit (eBioscience, San Diego, CA) according to the manufacturer's manual, and will be labeled with FITC-conjugated anti-Foxp3 antibody (236A/E7 clone) or its isotype control antibody (eBioscience). Flow cytometry will be performed on a Becton Dickinson LSRII (BD Biosciences) device.

4.1.2.4 Natural Killer (NK) CELLS

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

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

4.1.2.6 Regulatory T Cells

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

4.1.3 Additional Assays

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

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 **4.2**.

If there are inadequate samples for analysis because of loss or destruction of those samples, the NCI PI will report this to the IRB.

4.1.4 For Participating Sites – Collection and Shipping of Research Blood Samples

For patients at participating sites, research samples consisting of 6 green top tubes and 2 red top tubes will be drawn at baseline, Cycle 1 Day 1, Cycle 2 Day 1, Cycle 6 Day 1, and Cycle 9 Day 1. The green top tubes will be sent via overnight Fed-Ex to the NCI Frederick Repository at the address below:

Leidos Biomedical Research
Attn: Theresa Burks

1050 Boyles Street
Bldg. 469/Room 121
Frederick, MD 21702
Phone 301-846-5125 or 301-846-1707

The red top tubes will be spun down and stored in a -80℃ freezer at partipating sites. These serum samples will be batched and then shipped to the NCI Frederick Repository (address above) at intervals.

Once a patient's treatment schedule has been determined, Caroline Jochems at the Laboratory of Tumor Immunology and Biology/NIH should be notified at jochemscm@mail.nih.gov for planning purposes. Please see **APPENDIX F** for further details.

End of study research blood draw is optional for patients based on logistics and investigator discretion.

4.2 SAMPLE STORAGE, TRACKING AND DISPOSITION

The Clinical Support Laboratory, Leidos Biomedical Research, Inc.-Frederick, processes and cryopreserves samples in support of IRB-approved, NCI clinical trials. All data associated with the patient samples is protected by using a secure database. All samples drawn at the NIH Clinical Center will be transported to the NCI Frederick Central Repository by couriers.

Samples will be tracked and managed by Central Repository database. All samples will be stored in either a -20˚C or -80˚C freezer. These freezers are located at NCI Frederick Central Repository in Frederick, Maryland.

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, backup, and emergency support capability and monitoring to ensure long-term integrity of the specimens for research.

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 to 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, 3 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.

Samples will be used for research analysis, including immunologic monitoring as outlined in section **4.1.2**. All specimens for analysis will be requested from Leidos Biomedical, Inc. and will be delivered by Leidos Biomedical, Inc. couriers to the Laboratory of Tumor Immunology and Biology.

4.2.1 Protocol Completion/Sample Destruction

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

Samples and associated data will be stored permanently unless the patient withdraws consent. If the patient withdraws consent the participant's data will be excluded from future distributions, but data that have already been distributed for approved research use will not be able to be retrieved. The NCI 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. Freezer problems, lost samples or other problems associated with samples will also be reported to the IRB, the NCI Clinical Director, and the office of the CCR, NCI.

5 SUPPORTIVE CARE

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

Other supportive care with blood components, antibiotics, analgesics, general medical therapy, etc., will be delivered as required. Any patients taking antibiotics for any reason must complete that course of therapy and be free of evidence of further infection before receiving any dose of vaccine.
Symptomatic anemia should be treated with appropriate red blood cell or erythropoietin support. Thrombocytopenia should be treated conservatively. In the absence of bleeding or a planned invasive procedure, platelet transfusions should be given for a platelet count below 10,000/mm3. If invasive procedures are planned or the patient develops bleeding, platelet transfusions should be administered in accordance with the standard of practice, usually maintaining a platelet count of > 50,000/mm3.

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

5.1 EXCLUDED MEDICATIONS

While patients on protocol treatment, all medications required for the health of the patient are allowed with the following exceptions**:**

- Concurrent chemotherapy
- Concurrent radiation therapy
- Concurrent immunotherapy
- Concurrent anti-cancer radionuclides
- Concurrent systemic corticosteroid use (daily or every other day for continued use > **14 days**; See section **3.6**)
- Concomitant use of secondary hormonal treatments

5.2 TREATMENT OF VACCINIA VACCINATION COMPLICATION

5.2.1 Vaccinia Immune Globulin:

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 an investigational agent available through the CDC's Strategic National Pharmaceutical Stockpile under an IND protocol by contacting the CDC's Smallpox Vaccine Adverse Events Clinician Information Line at 1-877-554-4625. 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 intravenous formulation of VIG is available through the CDC, which has a lower level of aggregated protein, allowing it to be used by either the IM or IV route. This formulation will

most likely be preferred for administration and investigators will be instructed by the CDC regarding appropriate dosing and method of administration based on 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.

5.2.2 Cidofovir (Vistide®, Gilead Sciences):

Cidofovir is an FDA-approved antiviral drug for the treatment of CMV retinitis among patients with AIDS. Cell-based in vitro studies and animal model studies have demonstrated antiviral activity of this agent against certain orthopoxviruses. Currently, efficacy in the treatment of vaccinia-related complications in humans is unknown. According to the CDC, "VIG is recommended as first line of therapy. Cidofovir may be considered as a secondary treatment, and will only be released by the CDC after all inventories of VIG have been exhausted, after a patient fails to improve with VIG treatment, or as a last effort for a patient who is otherwise near death." [Medical Management of Smallpox (Vaccinia) Vaccine Adverse Reactions: Vaccinia Immune Globulin and Cidofovir. Last updated February 11, 2003. Available at: http://www.bt.cdc.gov/agent/smallpox/vaccination/mgmt-adv-reactions.asp].

6 DATA COLLECTION AND EVALUATION

6.1 DATA COLLECTION

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

Data will be secured in NCI C3D database. Data will be collected using protocol-specific case report forms, and verified for accuracy and completeness. Hard copies of data will be stored in locked secured areas and data will be entered onto a secured electronic data base. The following protocol-specific study forms will be complete and stored: eligibility checklist (developed by Central Registration Office, CRO). A copy of all serious AE forms will be kept in the research record.

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

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

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

6.1.4 Drug Accountability Records are kept for each patient.

The PI at each site will be responsible for overseeing entry of data into an in-house password protected electronic system (C3D) 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.

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

An abnormal laboratory value will be recorded in the database as an AE **only** 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.

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

Loss or destruction of data: Should we become aware that a major breach in our plan to protect subject confidentiality and trial data has occurred, the IRB will be notified.

6.2 RESPONSE CRITERIA

Patients will not undergo scheduled radiographic assessment for metastatic disease unless clinically indicated for increasing PSA or symptoms.

6.2.1 Disease Progression

6.2.1.1 PSA will not be used to measure progression of disease. Patients will be offered androgen deprivation therapy (ADT) as clinically indicated. Starting ADT will be done at the discretion of the investigator, although this will result in treatment termination and removal from the clinical trial. Patients may go on ADT at any time, although this may result in removal from the trial. PSA will be used to calculate PSA kinetics/tumor growth kinetics.

6.2.1.2 Development of a new bone lesion on bone scan.

6.2.1.3 Development of a soft tissue mass, identified on CT scan or physical exam, consistent with metastatic prostate cancer. If identified on physical exam, the lesion may be biopsied to confirm the presence of prostate cancer.

6.2.1.4 Development of urethral, ureteral, or spinal cord obstruction secondary to tumor.

6.2.1.5 Development of cytologically positive pleural effusion or lymphangitic spread in the lungs.

6.2.1.6 Symptoms which in the opinion of the investigator are consistent with clinical progression.

6.3 TOXICITY CRITERIA

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

7 SAFETY REPORTING REQUIREMENTS/DATA AND SAFETY MONITORING PLAN

7.1 DEFINITIONS

7.1.1 Adverse Event

Any untoward medical occurrence in a human subject, including any abnormal sign (for example, abnormal physical exam or laboratory finding), symptom, or disease, temporally associated with the subject's participation in research, whether or not considered related to the subject's participation in the research.

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 *greater 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 NCI 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 NCI 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 NCI GUIDANCE FOR REPORTING EXPEDITED ADVERSE EVENTS, PROTOCOL DEVIATIONS AND NON-COMPLIANCE FOR MULTI-CENTER TRIALS

7.3.1 Serious Adverse Events

DFCI PI, BIDMC PI and MSKCC PI must immediately report to the NCI PI and NCI Study Coordinator, 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 within 24 hours of PI awareness of the event.

If the event also needs to be reported using the CCR Problem Report Form **(APPENDIX E)**, the NCI Study Coordinator will contact the participating sites (DFCI and MSKCC).

As the CCR is the IND Sponsor of the study, then in addition to reporting to the NCI PI the DFCI PI and MSKCC PI must submit the report to the CCR as per section **7.4**.

7.3.2 Deviations, Unanticipated Problems and Non-Compliance

DFCI PI and MSKCC PI must report any protocol deviations, unanticipated problems, or non-compliance to the NCI PI within 7 days of PI awareness using the CCR Problem Report Form (See **APPENDIX E**). The report must be sent to their IRB in accordance with their institutional policies.

7.4 IND Sponsor Reporting Criteria

During the first 30 days after the subject receives investigational agent/intervention, the investigator must immediately report to the sponsor, using the mandatory MedWatch form 3500a, or equivalent, 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. For serious adverse events that occur more than 30 days after the last administration of investigational agent/intervention, only report those that have an attribution of at least possibly related to the agent/intervention.

Required timing for reporting per the above guideline:

Deaths (except death due to progressive disease) must be reported via email within 24 hours. A complete report must be submitted within one business day.

Other serious adverse events as well as deaths due to progressive disease must be reported within one business day

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

7.4.1 Reporting Pregnancy

7.4.1.1 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.

7.5 Safety Reporting Criteria to the Pharmaceutical Collaborators

7.5.1 Reporting to Bavarian Nordic, Inc.

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

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

7.6 INSTITUTIONAL BIOSAFETY COMMITTEE (IBC) REPORTING CRITERIA

7.6.1 Serious Adverse Event Reports to IBC

The site Principal Investigator (or delegate) will notify the site's IBC of any unexpected fatal or life-threatening experience associated with the use of PROSTVAC Vaccine as soon as possible but in no event later than 7 calendar days of initial receipt of the information. Serious adverse events that are unexpected and associated with the use of the PROSTVAC Vaccine, but are not fatal or life-threatening, much be reported to the IBC as soon as possible, but not later than 15 calendar days after the investigator's initial receipt of the information. Adverse events may be reported by using the FDA Form 3500a.

7.6.2 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 information described below shall be submitted. 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.

7.6.2.1 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.

7.6.2.2 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.

7.7 DATA AND SAFETY MONITORING PLAN

7.7.1 Principal Investigator/Research Team

The NCI Principal Investigator, NCI lead associate investigator and the NCI research nurse will meet weekly at each clinic and also conference calls will be held every 6 weeks with each participating sites to review all adverse events for each subject in this trial and to determine dose limiting toxicities and escalation rules.

Unexpected adverse events and/or serious adverse events will be reported to the NCI's Institutional Review Board (IRB) and sponsor/FDA as outlined above. If trends are noted and/or risks warrant it, accrual will be interrupted, dose levels expanded and/or the protocol and/or consent will be modified accordingly.

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.

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.

7.7.2 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 a CCR 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.

7.7.3 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 CONSIDERATIONS

The primary endpoint of this trial is to determine if prostvac can slow the growth rate of prostate cancer relative to a group of patients on surveillance. With 36 evaluable patients with a growth rate value from 0 to 6 months on the Prostvac arm and 36 evaluable patients on the surveillance arm (72 total patients) there would be adequate patients to have 80% power to test for a difference between the two arms of a change in growth rate from baseline equal to 2/3 SD of the change (0.67 SD effect size) using a 0.05 significance level two-tailed two group t-test. In practice, if the distributions of the growth rate constants are not normally distributed ($p<0.05$ by a Shapiro-Wilks test), then a Wilcoxon rank sum test may be used instead of a two-sample t-test. In order to allow for a small number of inevaluable subjects as well as to accommodate screen failures, the accrual ceiling will be set at 110. (It estimated that it will take 4 years to complete accrual to this trial.)

<u>Secondary Endpoints</u>
In addition, using the surveillance arm, two related endpoints will be explored, both the actual magnitude of the change in the growth rate parameter as well as the fraction who exhibit a substantial, 50% decline in the growth rate as a result of treatment. For the 36 patients who will be enrolled on the surveillance arm, the growth rate determined at 12 months (after 6 months of vaccine) will be subtracted from that obtained at 6 months (after surveillance) to determine the actual change in the growth rate for receiving vaccine after 6 months of surveillance. With 36 patients, there is 83% power to detect a difference in the values at the two time points equal to 1/2 of the SD of the change (.50 effect size) assuming that a two tailed 0.05 alpha level paired t-test is used.

As an additional secondary exploration, using all 36 patients on the surveillance arm, the fraction that experiences a 50% decline relative to the time of starting vaccine will be determined. Using an exact binomial test, 36 patients will have 79% power to test whether the fraction exceeds a null fraction of 30% and is consistent with 50% who have 50% declines, with a one-tailed 0.05 alpha level test. In practice, the fraction with a 50% decline will have a 95% confidence interval created about the result in order to describe what values it would be consistent with. Exploratory analyses will also be performed to compare the growth rate changes at additional time points, such as 10 and 12 months.

Immune response generated by prostvac will also be evaluated and will be compared to the fraction of patients on the two arms who have a substantial T cell response as measured by exposure to overlapping PSA peptides. The patients will be considered to have had a T cell response if there is at least a 2 fold increase in immune response compared to baseline. This response is expected to be minimal during the first 6 months on the surveillance arm and more pronounced on the arm receiving Prostvac initially. With 36 patients per arm, there is 80% power to detect a difference between 10% with immune response on the surveillance arm and 40% on the Prostvac arm, with a two-sided 0.05 significance level using Fisher's exact test. Should only 29 patients per arm have evaluable immune response data, there would be 81% power to detect a difference between 10% and 45% with immune responses.

Other secondary endpoints include performing immune response evaluation including CD4 cells, CD8 cells, PSA-specific T-cells, natural killer cells, Regulatory T-cells, Regulatory T-cell function, cytokines, and naïve thymic emigrants; and associating immunologic outcomes with PSA responses/changes in growth rates. (This could be done every 6 months). Each of these endpoints will be analyzed using appropriate nonparametric methods when applicable and the results from these analyses will be presented without formal adjustment for multiple comparisons**,** but in the context of the number of such evaluations undertaken.

Beginning with amendment G, patients enrolled onto either the immediate vaccine arm or the surveillance followed by vaccine arm will be asked to complete a prostate cancer specific QOL survey with 18 questions (MAX-PC) as well as the FACT-P survey at the following time points:

Immediate vaccine Arm
Baseline; 3 months; 6 months; 9 months; approximately q3-4 months in follow-up

Surveillance Arm
-6 months; -3 months; Baseline/start of vaccine; 3 months; 6 months; 9 months; approximately q3-4 months in follow-up.

The surveys will each be scored using the appropriate conventional scoring method to arrive at a total score for each instrument at each of the indicated time points. The changes in total score from baseline to each of the time points will be determined (for the surveillance arm, changes from -6 months and -3 months will also be determined), and each of the changes will be tested for statistical significance from zero change using a paired test (either paired t-test or Wilcoxon signed rank test depending on whether all the changes are normally distributed or not). The tests will be performed with exploratory intent and the results of the tests will be reported without

formal adjustment for multiple comparisons. In addition to evaluating the actual changes, the correlation between the two measures (MAX-PC and FACT-P) at each time point will be determined using Spearman correlation and will be reported as an exploratory result along with an interpretation of the magnitude of the correlation. Finally, the correlation of the changes in the scores with the change in growth rate at the corresponding time points will be determined and reported as an exploratory finding, along with interpretation of the magnitude of the correlation.

8.1.1 Halting Rules

The study will be halted for review of changes to the protocol and consent if either of the following is met:

- One occurrence of grade 5 toxicity attributed to the treatment regimen.
- Two occurrences of grade 4 toxicity attributed to the treatment regimen.

9 COLLABORATIVE AGREEMENTS

9.1 COLLABORATIVE RESEARCH AND DEVELOPMENT AGREEMENT (CRADA)

This study will be conducted under a Collaborative Research and Development Agreement (CRADA) with Bavarian Nordic, Inc., CRADA# 02377.

9.2 MULTI-INSTITUTIONAL GUIDELINES

9.2.1 IRB Approvals

Dana-Farber Cancer Institute (DFCI) and Beth Israel Deaconess Medical Center will obtain local IRB approval from DFCI/HCC IRB while Memorial Sloan Kettering Cancer Center (MSKCC) will use the NCI IRB as the IRB of record.

The NCI PI will provide the NCI IRB with a copy of the DFCI's approved yearly continuing review. The NCI PI will ensure that no patient is entered into the trial at participating institutions without full IRB approval of the study. Registration will be halted at any participating institution in which a current continuing approval is not on file at the NCI IRB.

9.2.2 Amendments and Consents

The NCI PI will provide the NCI IRB with copies of all amendments, consents and approvals from DFCI. The NCI PI will also provide the NCI IRB with copy MSKCC's site-specific consent document.

9.3 RESPONSIBILITIES OF THE COORDINATING CENTER (NCI)

- The Coordinating Center is responsible for assuring that each participating institution has an OHRP assurance and must maintain copies of IRB approvals from each participating site.

- The Coordinating Center is responsible for the coordination, development, submission, and approval of the protocol as well as its subsequent amendments. The protocol must not be rewritten or modified by anyone other than the Coordinating Center PI. There will be only one version of the protocol, and each participating institution will use that document. The Coordinating Center PI is responsible for assuring that all participating institutions are using the correct version of the protocol.
- The Coordinating Center PI is responsible for the overall conduct of the study at all participating institutions and for monitoring its progress.
- The Coordinating Center PI is responsible for the timely review of Adverse Events (AE) to assure safety of the patients.
- The Coordinating Center PI will be responsible for the review of and timely submission of data for study analysis.
- The Coordinating Center is responsible for central patient registration.
- The Coordinating Center is responsible for assuring that IRB approval has been obtained at each participating site prior to the first patient registration from that site.
- The Coordinating Center will maintain documentation of AE reports.
- Selected patient records may be audited on-site at participating sites.

10 HUMAN SUBJECT 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 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. Women are not eligible for this study as this diease occurs only in men.

10.1.2 Strategies/Procedures for Recruitment

Patient accrual for this protocol will be facilitated by Web-based recruitment strategies. This protocol will be listed on www.clinicaltrials.gov.

10.1.3 Justification for Exclusions

Due to impaired cellular immunity with the concomitant increased risk of serious side effects from vaccinations with infectious agents, the Centers for Disease Control and Prevention recommends that HIV infected patients be excluded, in addition, patients with chronic hepatitis infection, including B and C, because of potential immune impairment.

10.2 PARTICIPATION OF CHILDREN

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

10.3 PARTICIPATION OF NIH SUBJECTS UNABLE TO GIVE CONSENT

10.3.1 NCI

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 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 and on-going participation in research in the event that they become incapacitated or cognitively impaired during the course of the study. The PI or AI will contact the NIH Ability to Consent Assessment Team(ACAT) for evaluation as needed for the following: an independent assessment of whether an individual has the capacity to provide consent; assistance in identifying and assessing an appropriate surrogate when indicated; and/or an assessment of the capacity to appoint a surrogate. For those subjects that become incapacitated and do not have pre-determined substitute decision maker, the procedures described in MAS Policy 87-4 and NIH HRPP SOP 14E 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.

10.3.2 DFCI, BIDMC and MSKCC

If a subject becomes decisionally impaired, informed consent will be sought from legally authorized representative (LAR) to allow for on-going participation in the research and, should the subject re-gain capacity to consent for themselves, they will be consented again to allow for on-going participation in the protocol.

DFCI, BIDMC and MSKCC will follow local law and/or policies to identify the appropriate LAR for subjects who have lost capacity.

10.4 EVALUATION OF BENEFITS/RISKS/DISCOMFORTS

There is no standard therapy for patients with prostate cancer and rising PSA following local definitive therapy. Potential risks of vaccine and in this patient population include the range of side effects outlined in section **11**. PROSTVAC has been well tolerated in previous large trials.

10.4.1 Alternative Approaches or Treatments

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

10.4.2 Procedures to Eliminate or Minimize Potential Risks

This study may involve unforeseeable risks for patients, such as side effects whose exact nature and severity are unpredictable. Scrupulous care will be taken to minimize such side effects. All patients will be given blood tests, physical examinations, and scans, as described in the monitoring schedule (**APPENDIX C**), and must have a local physician to provide long-term care and monitoring for complications. No compensation is available, but any injury will be evaluated and treated in keeping with the benefits or care to which patients are entitled under applicable regulations.

10.4.3 Provisions for Monitoring Data Collection to Ensure Subject Safety

As information is gathered from this trial, clinical results will be shared with patients. Laboratory and clinical data will be frequently gathered and any new significant finding(s) found during the course of the research, which may affect a patient's willingness to participate further, will be explained.

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

10.5 Risks/Benefits Analysis

This study involves clinical research with an experimental vaccine designed to generate an immune response against antigens found in prostate cancer. Patients will undergo multiple vaccinations. Side effects of the vaccine are outlined elsewhere (see section **11**). Whether the vaccine will have any clinical effect is unknown; therefore, benefit cannot be promised, nor the chance of benefit accurately predicted. Potential benefits for all patients on study, including those patients that may become decisionally impaired while on study, could include shrinking of tumor or lessening of symptoms, such as pain, that are caused by the cancer. Participation in this study is voluntary, and refusal will not result in penalty or loss of benefit to which the patient is otherwise entitled. Participation may be discontinued at any time without penalty, and the patient will be encouraged to discuss any concerns or questions.

10.6 Consent 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 obtained. Any experimental invasive procedure will require a separate consent form. All Principal Investigators and Associate Investigators listed in this protocol who have clinical privileges are permitted to obtain informed consent.

10.6.1 Telephone Re-Consent Procedure

Reconsent on this study may be obtained via telephone according to the following procedure: 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

10.6.2 Informed Consent of Non-English Speaking Subjects

10.6.2.1 NCI

If there is an unexpected enrollment of a research participant for whom there is no translated extant IRB approved consent document, the principal investigator and/or those authorized to obtain informed consent will use the Short Form Oral Consent Process as described in MAS Policy M77-2, OHSRP SOP 12, 45 CFR 46.117 (b) (2), and 21 CFR 50.27 (b) (2). The summary that will be used is the English version of the extant IRB approved consent document. Signed copies of both the English version of the consent and the translated short form will be given to the subject or their legally authorized representative and the signed original will be filed in the medical record.

Unless the PI is fluent in the prospective subject's language, an interpreter will be present to facilitate the conversation (using either the long translated form or the short form). Preferably someone who is independent of the subject (i.e., not a family member) will assist in presenting information and obtaining consent. Whenever possible, interpreters will be provided copies of the relevant consent documents well before the consent conversation with the subject (24 to 48 hours if possible).

We request prospective IRB approval of the use of the short form process for non-English speaking subjects and will notify the IRB at the time of continuing review of the frequency of the use of the Short Form.

10.6.2.2 DFCI and BIDMC

DFCI and BIDMC will follow their own standard operating procedures regarding the consent of unexpected non-English Speaking Subjects.

10.6.2.3 MSKCC

If a patient indicates that their preferred language is one other than English, an interpreter, either in person or by phone can be provided. If a fully translated IRB approved protocol specific consent is available, standard consenting procedures are followed with the assistance of an interpreter. If a fully translated consent is not available, a patient may be consented with a short form consent written in their language. In that case, a bilingual witness is required in addition to the interpreter. The consent discussion occurs between the consenting professional and the participant/patient with the help of the interpreter. Family and/or friends may not serve as an interpreter.

The short form is signed by the participant/patient and the witness. The English Summary/ Consent is signed by the consenting professional and the witness. The participant/patient receives a copy of the signed short form and the English consent, and is then registered to the protocol and may begin protocol activities.

As this study is considered therapeutic, MSK policy requires that patients receive a fully translated study specific consent. Therefore, if a patient consents with the short form, a fully translated consent needs to be requested. Once received, the NCI IRB will review the fully translated document and, following IRB approval, the patient is reconsented with the fully translated document. Standard consenting procedures will be followed with the assistance of an interpreter.

11 PHARMACEUTICAL AND INVESTIVATIONAL DEVICE INFORMATION

PROSTVAC-F and PROSTVAC-V will be supplied to the sites by the manufacturer, Bavarian Nordic, Inc.

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 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.

11.1.2 Preparation

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

11.1.3 Storage

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

11.1.4 Stability

Shelf-life stability studies of the intact vials are ongoing. Once the intact vials are thawed, the vaccines maintain their potency for up to 4 days when stored at 2-8ºC. 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.

11.1.5 Route of Administration

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

11.1.6 Special Handling

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

Preparation, Handling and Disposal Recommendations

1. Strictly adhere to standard microbiological practices and techniques.
2. Limit/restrict access to preparation areas while dose preparation is in progress.
3. Use appropriate infection control measures (e.g., thorough hand washing) after handling any materials.
4. Institute and follow policies for safe handling of sharps.
5. Perform all dose preparations in a certified Class II biological safety cabinet, generally using procedures, guidelines and personal protective apparel used during preparation of antineoplastic agents [*e.g.*, minimizing creation of aerosols; no eating, drinking, handling contact lenses or applying cosmetics in the work area; using appropriate personal protective

apparel - gowns, sterile latex gloves (double-gloving is recommended), respirator masks, protective eye wear, hair cover].

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

    For OSP submission by the Sponsor: 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.

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

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

Patient Care Implications and Contraindications

Cover vaccination sites with a sterile dry dressing (e.g., Telfa pad). Once the injection site is healed, no further barrier is necessary. As a precaution, protect injection sites that are exhibiting evidence of weeping, oozing or ulceration with a sterile dry dressing. In these circumstances, instruct patients to avoid direct contact of the injection site with susceptible individuals (e.g.; those who may be immunocompromised by disease or therapy). Instruct patients to avoid fathering a child for at least 4 months following therapy completion with the recombinant vaccine. Instruct patients receiving fowlpox vaccines to avoid direct contact with pet birds for at least 72 hours after vaccination or while there are any visible lesions at the injection site.

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

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 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.

11.2.2 Preparation

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

11.2.3 Storage

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

11.2.4 Stability

Shelf-life studies of the intact vials are ongoing. Once the intact vials are thawed, the vaccines maintain their potency for up to 4 days when stored at 2-8ºC. 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.

11.2.5 Route of Administration

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

11.2.6 Special Handling and Precautions

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

Preparation, Handling and Disposal Recommendations

1. Prepare a biosafety manual which advises personnel of special hazards and specific instructions on practices and procedures.
2. Post warning hazard signs on access doors, identifying the agents, the biosafety level, the name and phone number of the Principal Investigator or other responsible person, and any special requirements for entry.

3. Establish policies and procedures allowing only personnel who are knowledgeable of the potential hazards and meet specific entry requirements (*e.g.*, immunization) into agent preparation or storage areas.
4. Strictly adhere to standard microbiological practices and techniques.
5. Limit/restrict access to preparation areas while dose preparation is in progress.
6. Use appropriate infection control measures (*e.g.*, thorough hand washing) after handling any materials.
7. Institute and follow policies for safe handling of sharps. Use only needle-lock syringes and needles for dose preparation. Use extreme caution to prevent autoinoculation. Do not bend, shear, or replace the needle guard from the syringe following use. Promptly place used needles and syringes in puncture-resistant containers for disposal.
8. Perform all dose preparations in a certified Class II biological safety cabinet, generally using procedures, guidelines and personal protective apparel used during preparation of antineoplastic agents [*e.g.*, minimizing creation of aerosols; no eating, drinking, handling contact lenses or applying cosmetics in the work area; using appropriate personal protective apparel - gowns, sterile latex gloves (double-gloving is recommended), respirator masks, protective eyewear, hair cover].
9. Perform all procedures carefully to minimize aerosol creation.
10. Decontaminate the biological safety cabinet prior to dose preparation with sterile gauze soaked in 10% bleach solution (0.52% sodium hypochlorite solution), or other appropriate disinfectant suitable for decontamination, rinsing, then wiping down with sterile gauze soaked in 70% alcohol. Consult specific manufacturer's recommendations with respect to disinfectant concentration, contact time and method of application.
11. Have all necessary supplies on-hand before beginning the preparation procedure. Develop a detailed worksheet outlining all supplies, dose calculations, and preparation procedures, and keep it available.
12. Place an empty biohazard sharps container in the biosafety cabinet to dispose of all waste generated.
13. Transport the agent from the freezer to the work area in leak proof bag.
14. Wipe or spray items used for dose preparation with 70% alcohol before placing in the biological safety cabinet. Disinfectants should remain in contact with the surfaces for at least five minutes prior to dose preparation. Avoid exposing the virus to disinfectants.
15. Wipe the syringe containing the prepared dose with 70% alcohol before removing it from the biological safety cabinet; transport it in a leak proof bag or container labeled with a biohazard symbol.
16. Place all waste into a sharps container lined with the leak proof biohazard bag and decontaminate the biological safety cabinet again by wiping down all surfaces with sterile gauze soaked in 10% bleach solution, or other appropriate disinfectant, rinsing, then wiping down with sterile gauze soaked in 70% alcohol. Following decontamination, dispose of personal protective apparel in the biohazard safety bag.
17. Place all waste and protective apparel in a leak proof biohazard bag, and place the bag inside a biohazard sharps container for incineration according to institutional policy and according to local, state, and federal regulations.
18. Handle accidental spills similarly to antineoplastic spills and/or according to institutional policy:

a. Prevent others from entering the area and allow aerosols time to settle (approximately 10 minutes).
b. Use protective apparel, eyewear, mask, and gloves.
c. Cover spills with disposable absorbent towels.
d. Decontaminate the area with 10% bleach solution, or other appropriate disinfectant suitable for decontamination, allowing approximately a 20-minute contact time.
e. Dispose of all waste and protective apparel as infectious biohazardous waste and incinerate according to institutional policy and according to local, state, and federal regulations.

19. Immediately report spills and accidents resulting in overt exposure to recombinant DNA molecules to the site's Institutional Biosafety Committee (IBC) by site's PI and NIH Office of Science Policy (OSP) by the Sponsor. Provide medical evaluation, surveillance, and treatment as appropriate and maintain written records of the event.

    For OSP submission by the Sponsor: 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.
    For more information about biohazard risk group classification and biohazard safety levels:
    - *NIH Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines). See current version at: http://osp.od.nih.gov/sites/default/files/NIH_Guidelines.html*
    - *Biosafety in Microbiological and Biomedical Laboratories; U. S. Department of Health and Human Services Centers for Disease Control and Prevention and National Institutes of Health. See current edition at: http://www.cdc.gov/biosafety/publications/index.htm*

Precautions for Healthcare Workers

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

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

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

- has a moderate or severe acute illness, until the illness resolves.
- is less than 18 years of age, unless specifically indicated.
- is undergoing topical steroid therapy for inflammatory eye diseases or undergoing therapy with systemic steroids; potential immune suppression increases risk for vaccinia-related complications.
- has a history of allergy or serious reaction to prior vaccinia vaccination or any of the vaccine's components.
- As a precaution, the CDC recommends that individuals with known cardiac disease (e.g., previous MI, angina, CHF, cardiomyopathy, stroke or TIA) or who have $\geq$ 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.

Recombinant Vaccinia Vaccine Patient Care Implications, Contraindications and Potential Complications

Patient Care Implications and Contraindications

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

- have active eczema or a history of eczema or atopic dermatitis, or Darier's disease.
- have other acute, chronic, or exfoliative skin conditions (*e.g.*, burns, impetigo, varicella zoster, severe acne, contact dermatitis, psoriasis, herpes or other open rashes or wounds), until the condition resolves.

- are pregnant or intend to become pregnant (due to the potential risk of fetal vaccinia); or are breast-feeding (due to the potential risk of contact transmission and inadvertent inoculation). It is currently unknown if vaccinia virus or antibodies are excreted in breast milk. Patients (*i.e.*, vaccinees) should avoid fathering a child for at least 4 months following completion of therapy with the recombinant vaccine.
- are in close contact with children less than 3 years of age (due to the potential risk of contact transmission and inadvertent inoculation).
- are immunodeficient or immunocompromised (by disease or therapy), including individuals with HIV infection.

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

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

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

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

Potential Complications Associated with Recombinant Vaccinia Vaccination

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

10 days post-vaccination and include fever, malaise, headache, chills, nausea, myalgia, local lymphadenopathy, soreness and intense erythema surrounding the vaccination site.

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

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

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

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

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

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

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

Generally, self-limited adverse reactions that can be serious, but not life-threatening include autoinoculation, erythematous and urticarial rashes, and generalized vaccinia. More serious life-

threatening complications include progressive vaccinia, eczema vaccinatum, and post-vaccinial encephalitis/encephalomyelitis. The complications of vaccinia vaccination may involve a number of different reactions:

1. **Non-specific erythematous or urticarial rashes:** These rashes can appear approximately 10 days after vaccination and may sometimes be confused with generalized vaccinia, but are generally self-limiting. Patients are usually afebrile and these benign rashes usually resolve spontaneously within 2-4 days. Erythema multiforme can present as different types of lesions, including macules, papules, urticaria, and bull's eye lesions (dark papule or vesicle surrounded by a pale zone and an area of erythema). These lesions may be extremely pruritic, lasting up to four weeks. Rarely, more serious bullous erythema multiforme (Stevens-Johnson syndrome) may occur, requiring hospitalization. Vaccinia Immune Globulin (VIG) therapy is not indicated to treat these rashes. Supportive care measures are warranted since these rashes are likely manifestations of an immune response or hypersensitivity reaction to the vaccine and are not likely to contain vaccinia virus.
2. **Bacterial Infection:** Vaccination site infection, most likely due to staphylococcus and streptococcus normal skin flora, is rare. Onset is approximately 5 days post-vaccination and is more common in children. Appropriate antibiotic therapy is required.
3. **Inadvertent Inoculation**: This can occur in the vaccinee (autoinoculation) as well as in close contacts (contact transmission). Accidental infection is the most common complication of vaccinia vaccination, accounting for approximately 50% of all complications associated with vaccination and revaccination. This usually results from autoinoculation of vaccinia virus transferred from the site of the vaccination. Sites typically involved include the face, eyelids, nose, mouth, genitalia, or rectum, but can also involve the arms, legs, and trunk. Contact transmission of vaccinia, with accompanying toxicities, may occur when a recently vaccinated individual has contact with a susceptible individual. In a 1968 ten-state survey, contact transmissions were reported to occur at a rate of 27 infections per million vaccinations. The age group in which contact transmission occurred most commonly was in children ≤ 5 years. Eczema vaccinatum as a result of contact transmission may result in a more severe syndrome than that seen in vaccinees, perhaps because of multiple simultaneous inoculations. About 30% of eczema vaccinatum cases reported in the 1968 ten-state survey were a result of contact transmission. It is possible that the number of cases of contact transmission would be greater in today's population, due to a largely unvaccinated patient population against smallpox. Contact transmission rarely results in postvaccinial encephalitis or progressive vaccinia. Most cases of inadvertent inoculation usually resolve without specific therapy and resolution of lesions follow the same course as the vaccination site in immunocompetent individuals. VIG can be used for severe cases involving extensive lesions or if comorbid conditions exist. VIG is contraindicated in the presence of isolated keratitis due to the risk of increased corneal scarring. VIG use can be considered in cases of ocular implantation, with keratitis, if vision-threatening or if other life-threatening vaccinia-related complications exist that require VIG therapy.
4. **Generalized vaccinia:** Generalized vaccinia (GV) is characterized by a disseminated maculopapular or vesicular rash of varying extent on any part of the body and typically develops 6-9 days after vaccination. The lesions follow the same course as the vaccination site lesion. The lesions are hematogenously spread and may contain vaccinia virus. In

immunocompetent individuals, the rash is generally self-limiting and requires supportive care therapy. VIG treatment can be used in severe cases for patients who are systemically ill and whose condition might be toxic or who have serious underlying immunosuppressive illnesses.

The differential diagnosis of GV includes eczema vaccinatum, erythema multiforme, inadvertent inoculation at multiple sites, and uncommonly, early stages of progressive vaccinia or other vesicular diseases (*e.g.*, severe chickenpox or disseminated herpes). Several publications have investigated the reporting of GV among those individuals who received smallpox vaccinations during 2003. Out of 38,440 vaccine recipients, 29 reports of possible GV during January 2003–December 2003 were identified but only 2 reports met the case definition. More than 75% of the reports received a final diagnosis of hypersensitivity reaction or nonspecific rash after review by dermatologists or because laboratory results were negative for vaccinia and other orthopoxviruses. Of 74 cases investigated in 753,226 smallpox vaccinations administered in December 2002 to December 2004, 50 (67.6%) met the case definition of possible GV. Cases occurred more frequently in primary vaccinees (rate, 81 per 1 million vaccinees) than in those revaccinated (rate, 32 per 1 million vaccinees). However, none met the case definition of probable or confirmed GV, including 15 with virologically negative laboratory evaluations (*e.g.*, culture, polymerase chain reaction, or skin biopsy). Twenty-one reports of postscab lesions were made between January and August 2003 among 37,542 smallpox vaccinees. The lesions (scab and/or fluid) of seven patients were tested for vaccinia virus by use of polymerase chain reaction and/or immunohistochemistry; all were found to be negative. In addition, the postscab lesions of four of the patients were biopsied. The results from two of the biopsies suggested an allergic contact dermatitis, and results of one each demonstrated chronic dermatitis and squamous cell carcinoma. None of the four biopsied lesions had histologic evidence of viropathic changes and no evidence supported smallpox vaccination as a cause for any of the lesions.

5. **Eczema vaccinatum:** Eczema vaccinatum is a serious complication in persons with eczema and other types of chronic or exfoliative skin conditions. It can also occur among eczematous contacts of recently vaccinated persons. Vaccinial lesions (generalized papular, vesicular or pustular lesions) develop on areas of the skin that are, or had at one time been, eczematous. These areas become highly inflamed and lesions may spread to healthy skin. The rash is often accompanied by fever and individuals are systemically ill. The fatality rate for untreated cases (prior to availability of VIG) has been reported from 30-40%. Following availability of VIG, mortality was reduced to approximately 7%. Early diagnosis and prompt treatment with VIG is necessary to reduce mortality.
6. **Progressive vaccinia:** Progressive vaccinia is the most serious cutaneous complication, occurring when the local vaccination lesion fails to heal and develops progressive necrosis, with destruction of large areas of skin, subcutaneous tissue, and underlying structures. Progressive lesions may spread to other skin surfaces and to bone and viscera. Progressive vaccinia is associated with a high mortality rate. This complication has been seen in patients with a compromised immune system due to a congenital deficiency, lymphoproliferative disease, immunosuppressive treatment, or HIV infection. Management should include aggressive VIG therapy.
7. **Post-Vaccinial Encephalitis/Encephalomyelitis**: Vaccinial complications affecting the CNS are unpredictable. Post-vaccinial encephalitis typically affects children < 2 years of

age and is characterized by an onset of symptoms 6-10 days following vaccination, which include seizures, hemiplegia, aphasia, and transient amnesia. Histopathological changes include generalized cerebral edema, mild lymphocytic meningeal infiltration, ganglion degenerative changes and perivascular hemorrhages. Older children and adults can develop encephalitis or encephalomyelitis characterized by an onset of symptoms 11-15 days following vaccination, which include fever, vomiting, headache, malaise, and anorexia, progressing to loss of consciousness, amnesia, confusion, disorientation, restlessness, delirium, drowsiness, seizures and coma. Histopathological changes include demyelination with lymphocytic infiltration, but limited cerebral edema. Mortality rates have ranged from 15-25%, with 25% of patients who recover being left with varying degrees and types of neurological deficits. VIG has not been shown to be effective in treating CNS disease and is not recommended. Post-vaccinial encephalitis/ encephalomyelitis are diagnoses of exclusion and are not believed to be a result of replicating vaccinia virus. Although no specific therapy exists, supportive care, anticonvulsants, and intensive care might be required. A review of vaccinia-related deaths (68) during a 9-year period (1959–1966 and 1968) revealed that among first-time vaccines, 36 (52%) patients died as a result of post-vaccinial encephalitis.

8. **Fetal Vaccinia**: Fetal vaccinia is a rare, but serious complication following vaccinia vaccination during pregnancy or shortly before conception (e.g., within four weeks). To date, fewer than 50 cases have been reported and often result in fetal or neonatal death. Efficacy of VIG therapy in a viable infant or used prophylactically in women during pregnancy is unknown. The CDC has established a National Smallpox Vaccine in Pregnancy Registry. This registry will follow women during their pregnancies and their babies, after they are born, to determine the outcome of such pregnancies. The CDC can be contacted at (404) 639-8253.
9. **Myocarditis/Pericarditis:** The CDC has recommended a temporary medical deferral to the voluntary Smallpox Vaccination Program for persons with heart disease or cardiovascular risk factors (March 25, 2003) and issued "interim supplementary information" regarding evidence that smallpox vaccination may cause myocarditis and/or pericarditis (March 31, 2003) in people recently vaccinated with the smallpox vaccine. The cardiac events reported include myocardial infarction, angina, myocarditis, pericarditis, and myopericarditis. Although the CDC believes that there is no evidence to conclude that the licensed vaccinia vaccine causes angina or heart attacks, it acknowledges a possible causal relationship between the vaccination and heart inflammation. The CDC continues to study the relationship, but in the meantime, recommends that individuals with underlying heart disease be excluded from participation in the current Smallpox Vaccination Program. While it is currently not possible to fully evaluate the risk of cardiac events or the risk of myocarditis, pericarditis, or myopericarditis associated with vaccinia vaccination, it is reasonable to inform patients participating in studies using recombinant vaccinia virus of these reports and provide relevant guidance for evaluating these events. Further investigation from the ongoing vaccine program may provide additional data regarding an association or lack of association with cardiovascular disease. Patients are being immunized with recombinant vaccinia vaccines with therapeutic intent and will be evaluated for cardiovascular risk factors and recent or symptomatic cardiac events per protocol eligibility criteria. Patients will be encouraged to minimize cardiovascular disease risks and encouraged to follow risk reduction according to standard medical practice. At

this time the evidence for an association with myocarditis, pericarditis, or myopericarditis seems plausible, but a rare event. If not otherwise excluded, patients with known CHF or clinically significant cardiomyopathy requiring treatment should be excluded from protocol eligibility.

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

Treatment 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.

12 REFERENCES

1. Kantoff PW, Schuetz TJ, Blumenstein BA, et al. Overall survival analysis of a phase II randomized controlled trial of a Poxviral-based PSA-targeted immunotherapy in metastatic castration-resistant prostate cancer. Journal of clinical oncology : official journal of the American Society of Clinical Oncology 2010;28:1099-105.

2. Madan RA, Arlen PM, Mohebtash M, Hodge JW, Gulley JL. Prostvac-VF: a vector-based vaccine targeting PSA in prostate cancer. Expert opinion on investigational drugs 2009;18:1001-11.

3. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2015. CA: a cancer journal for clinicians 2015;65:5-29.

4. Roehl KA, Han M, Ramos CG, Antenor JA, Catalona WJ. Cancer progression and survival rates following anatomical radical retropubic prostatectomy in 3,478 consecutive patients: long-term results. The Journal of urology 2004;172:910-4.

5. Kupelian PA, Mahadevan A, Reddy CA, Reuther AM, Klein EA. Use of different definitions of biochemical failure after external beam radiotherapy changes conclusions about relative treatment efficacy for localized prostate cancer. Urology 2006;68:593-8.

6. Paller CJ, Antonarakis ES. Management of biochemically recurrent prostate cancer after local therapy: evolving standards of care and new directions. Clinical advances in hematology & oncology : H&O 2013;11:14-23.

7. Kantoff PW, Higano CS, Shore ND, et al. Sipuleucel-T immunotherapy for castration-resistant prostate cancer. The New England journal of medicine 2010;363:411-22.

8. Patel PH, Kockler DR. Sipuleucel-T: a vaccine for metastatic, asymptomatic, androgen-independent prostate cancer. The Annals of pharmacotherapy 2008;42:91-8.

9. Madan RA, Bilusic M, Heery C, Schlom J, Gulley JL. Clinical evaluation of TRICOM vector therapeutic cancer vaccines. Seminars in oncology 2012;39:296-304.

10. Arlen PM, Mohebtash M, Madan RA, Gulley JL. Promising novel immunotherapies and combinations for prostate cancer. Future oncology 2009;5:187-96.

11. Essajee S, Kaufman HL. Poxvirus vaccines for cancer and HIV therapy. Expert opinion on biological therapy 2004;4:575-88.

12. Longo DL. New therapies for castration-resistant prostate cancer. The New England journal of medicine 2010;363:479-81.

13. Gulley JL, Arlen PM, Madan RA, et al. Immunologic and prognostic factors associated with overall survival employing a poxviral-based PSA vaccine in metastatic castrate-resistant prostate cancer. Cancer immunology, immunotherapy : CII 2010;59:663-74.

14. Vergati M, Cereda V, Madan RA, et al. Analysis of circulating regulatory T cells in patients with metastatic prostate cancer pre- versus post-vaccination. Cancer immunology, immunotherapy : CII 2011;60:197-206.

15. Farsaci B, Jochems C, Grenga I, et al. Identification by digital immunohistochemistry of intratumoral changes of immune infiltrates after vaccine in the absence of modifications of PBMC immune cell subsets. International journal of cancer Journal international du cancer 2014;135:862-70.

16. Stein WD, Gulley JL, Schlom J, et al. Tumor regression and growth rates determined in five intramural NCI prostate cancer trials: the growth rate constant as an indicator of therapeutic efficacy. Clinical cancer research : an official journal of the American Association for Cancer Research 2011;17:907-17.

17. Madan RA, Gulley JL, Fojo T, Dahut WL. Therapeutic cancer vaccines in prostate cancer: the paradox of improved survival without changes in time to progression. The oncologist 2010;15:969-75.

18. DiPaola RS, Chen YH, Bubley GJ, et al. A National Multicenter Phase 2 Study of Prostate-specific Antigen (PSA) Pox Virus Vaccine with Sequential Androgen Ablation Therapy in Patients with PSA Progression: ECOG 9802. European urology 2014.

19. Stein WD, Yang J, Bates SE, Fojo T. Bevacizumab reduces the growth rate constants of renal carcinomas: a novel algorithm suggests early discontinuation of bevacizumab resulted in a lack of survival advantage. The oncologist 2008;13:1055-62.

20. Stein WD, Wilkerson J, Kim ST, et al. Analyzing the pivotal trial that compared sunitinib and IFN-alpha in renal cell carcinoma, using a method that assesses tumor regression and growth. Clinical cancer research : an official journal of the American Association for Cancer Research 2012;18:2374-81.

21. Roth AJ, Rosenfeld B, Kornblith AB, et al. The memorial anxiety scale for prostate cancer: validation of a new scale to measure anxiety in men with with prostate cancer. Cancer 2003;97:2910-8.

22. Victorson DE, Beaumont JL, Rosenbloom SK, Shevrin D, Cella D. Efficient assessment of the most important symptoms in advanced prostate cancer: the NCCN/FACT-P Symptom Index. Psychooncology 2011;20:977-83.

23. Roth A, Nelson CJ, Rosenfeld B, et al. Assessing anxiety in men with prostate cancer: further data on the reliability and validity of the Memorial Anxiety Scale for Prostate Cancer (MAX-PC). Psychosomatics 2006;47:340-7.

13 APPENDIX A: PERFORMANCE STATUS CRITERIA

ECOG Performance Status Scale		**Karnofsky Performance Scale**	
Grade	Descriptions	Percent	Descriptions
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 light or sedentary work (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 > 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 > 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.

14 APPENDIX B: VACCINIA-PSA(L155)/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 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 < 3years of age; pregnant women or nursing women; individuals with eczema, history of eczema or other skin conditions such as active cases of extensive psoriasis, severe rashes, generalized itching, infections, burns, chicken pox, or skin trauma; and/or immune suppressed individuals such as individuals with leukemia or lymphoma, with AIDS, or those receiving immunosuppressive treatment (for example, after organ transplant).

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

NCI Patients:

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 Hematology/Oncology 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. Ravi Madan or Dr. James Gulley any time during weekday hours. In an emergency on weekends, evenings, or holidays, you can always get in touch with the MEDICAL ONCOLOGY 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	
3 South East	(301) 451-1152
12th floor Oncology Clinic	(301) 496-4026*
Ravi Madan, MD	(301)480-7168 *
James Gulley, MD, PhD	(301)480-8870 *
*after clinic hours the NCI Medical Oncology physician On call through NIH page operator	(301) 496-1211

Participating Sites:

If you have any questions at any time, please contact your institution's prinicipal investigator (PI).

Memorial Sloan Kettering Cancer Center (MSKCC)
Site PI: Susan Slovin, MD, PhD
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15 APPENDIX C : FOLLOW-UP EVALUATIONS

STUDY CALENDAR

Surveillance ARM – PROSTVAC: surveillance for 6 months followed by vaccinia-PROSTVAC, $2x10^8$ infectious units subcutaneously in week 1 and fowlpox-vaccine $1x10^9$ infectious units subcutaneously given in week 3, week 5 and then monthly for a total of 6 months of treatment. After this 6-month treatment period is complete, patiens will then have the option of fowlpox-vaccine $1x10^9$ q 3 months for 1 year while in follow-up).

	Screening/ Baseline[11]	Surveillance for 6 months							1 cycle = 28 days; C=Cycle; D=Day									
		M -6	M -5	M-4	M-3	M-2	M-1	C1 D1	C1 D15	C2 D1	C3 D1	C4 D1	C5 D1	C6 D1	C7 D1	C8 D1	C9+ D1[6,7]	End of study
Informed Consent / Registration	X																	
Randomization	X																	
TREATMENT																		
rV-PROSTVAC								X										
rF-PROSTVAC									X	X	X	X	X	X			X[9]	
EVALUATION																		
History and PE, Height and Weight	X		X		X		X		X	X	X	X	X	X	X	X	X[1]	X
ECOG Performance Score	X																	
Pathologic confirmation of dx	X[2]																	
Laboratory Testing																		
CBC with differential	X							X	X	X	X	X	X	X	X	X	X[1]	X
Serum chemistries (Na+, K+, Cl-, CO2, glucose, BUN, creatinine, albumin, calcium, alkaline phosphatase, ALT, AST, total bilirubin, LDH,)	X							X	X	X	X	X	X	X	X	X	X[1]	X
CK, uric acid, total protein, magnesium, phosphorus,	X																	

	Screening/ Baseline[11]	Surveillance for 6 months						1 cycle = 28 days; C=Cycle; D=Day										
		M -6	M -5	M-4	M-3	M-2	M-1	C1 D1	C1 D15	C2 D1	C3 D1	C4 D1	C5 D1	C6 D1	C7 D1	C8 D1	C9+ D1[6,7]	End of study
Urinalysis	X																	
Serum PSA and Testosterone	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X[1]	X
Hep B/HepC, HIV	X[3]																	
Lymphocyte Phenotyping (CD4/CD8)[12]	X							X	X	X	X	X	X	X	X	X	X[1]	X
PAP (prostatic acid phosphatase)	X							X	X	X	X	X	X	X	X	X	X	X
ECG	X[5]																	
Tc 99 scintigraphy	X[4]							X							X[6]			
CT-C/A/P, or MRI	X[4]							X							X[6]			
Research Bloods	X							X		X				X			X[7]	X[8]
FACT- P survey[10]		X[10]						X[10]			X[10]			X[10]			X[10]	
MSK Anxiety Survey[10]		X[10]						X[10]			X[10]			X[10]			X[10]	
Adverse Events	X		X		X		X	X →										
Concomitant Medications	X							X →										

Footnotes:

[1] HPE, CBC, Chem, PSA, and Lymphocyte Phenotyping will be obtained every cycle. For week 3 and week 5, these tests can be done +/- 3 days and for cycle 3 to cycle 6, they can be done +/- 14 days for logistical reasons. Vaccine can be given +/- 3 days for week 3 and week 5 and for cycle 3 to cycle 6, it can be given +/- 14 days for logistical reasons. Research blood will be drawn +/- 3 days for C1D1, C2D1 and +/- 14 days for C6D1 and C9D1 for logistical reasons.

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

[3] HepB/HepC/HIV status will be obtained within 8 weeks prior to enrollment

[4] Screening Tc99 sctintigraphy and CT-CAP or MRI will be obtained within 8 weeks prior to enrollment

[5] Baseline ECG will be obtained within 8 weeks prior to enrollment

[6] Restaging will be done every 6 months (+/- 14 days) or earlier if clinically indicated. If, however, in the opinion of the investigator re-staging scans are not required (in patients with a PSA DT > 6 months over the previous 6 months) then re-staging scans can be deferred, document in chart to assure compliance. However, once PSA DT is less than 6 months, scans must be performed.) Re-staging scans may exclude CT Chest.

[7] Patients will continue to be followed after Cycle 9 until progression or ADT is clinically indicated. All visits will include evaluations listed for cycle 9 except research blood. Research blood will not be drawn after Cycle 9.
[8]optional
[9]Patients will have the option to initiate 1 year of vaccine maintenance within 3 months of cycle 6 vaccine, administered every 12 weeks. This year will include follow-up every 6 weeks during this period for PSA checks on non-vaccine visits. (Patients seen every 6 weeks during 1 year maintenance period). Patients will be required to have a PSADT greater than 5 months, as measured over the prior 6 months in order to start and continue vaccine during the 1 year maintenance period.
[10]Surveys are optional at all time points and can be done every 3 months for the first 9 months and every 3-4 months therafter while patients are on-study. (If surveys are not done, reason will be documented.)
[11] Refer to sections **2.2** for screening evaluation and **2.4** for baseline evaluation
[12] Not performed at DFCI site.

-All patient visits can be delayed by 2 weeks for logistical issues except for week 3 and 5 where the window is 3 days.

Immediate Vaccine ARM– PROSTVAC (vaccinia-PROSTVAC, $2x10^8$ infectious units subcutaneously in week 1 and fowlpox-vaccine $1x10^9$ infectious units subcutaneously given in week 3, and week 5, then monthly for a total of 6 months of treatment. After this 6-month treatmentperiod is complete, patiens will then have the option of fowlpox-vaccine $1x10^9$ q 3 months for 1 year))

	Screening/ Baseline[11]	Treatment	1 cycle = 28 days; C=Cycle; D=Day									
		C1 D1	C1 D15	C2 D1	C3 D1	C4 D1	C5 D1	C6 D1	C7 D1	C8 D1	C9+ D1[6,7]	End of study
Informed Consent / Registration	X											
Randomization	X											
TREATMENT												
rV-PROSTVAC		X										
rF-PROSTVAC			X	X	X	X	X	X			X[9]	
EVALUATION												
History and PE**,** Height and Weight	X		X	X	X	X	X	X	X	X	X[1]	X
ECOG Performance Score	X											
Pathologic confirmation of dx	X[2]											
Laboratory Testing												
CBC with differential	X		X	X	X	X	X	X	X	X	X[1]	X
Serum chemistries (Na+, K+, Cl-, CO2, glucose, BUN, creatinine, albumin, calcium, magnesium, phosphorus, alkaline phosphatase, ALT, AST, total bilirubin, LDH.	X		X	X	X	X	X	X	X	X	X[1]	X
CK, uric acid, total protein, magnesium, phosphorus,	X											
Urinalysis	X											
Serum PSA and Testosterone	X	X	X	X	X	X	X	X	X	X	X[1]	X
Hep B/HepC, HIV	X[3]											

	Screening/ Baseline[11]	Treatment	1 cycle = 28 days; C=Cycle; D=Day									
		C1 D1	C1 D15	C2 D1	C3 D1	C4 D1	C5 D1	C6 D1	C7 D1	C8 D1	C9+ D1[6,7]	End of study
Lymphocyte Phenotyping (CD4/CD8)[12]	X			X	X	X	X	X	X	X	X[1]	X
PAP (prostatic acid phosphatase)	X	X	X	X	X	X	X	X	X	X	X	X
Research bloods	X			X				X			X[7]	X[8]
ECG	X[5]											
FACT- P survey[10]		X[10]			X[10]			X[10]			X[10]	
MSK Anxiety Survey[10]		X[10]			X[10]			X[10]			X[10]	
Tc 99 scintigraphy	X[4]								X[6]			
CT-C/A/P, or MRI	X[4]								X[6]			
Adverse Events	X	→	→	→	→	→	→	→				
Concomitant Medications	X	→	→	→	→	→	→	→				

Footnotes:
[1]HPE, CBC, Chem, PSA, and Lymphocyte Phenotyping will be obtained every cycle including maintenance vaccine dosing. For week 3 and week 5, these tests can be done +/- 3 days and for cycle 3 to cycle 6, they can be done +/- 14 days for logistical reasons. Vaccine can be given +/- 3 days for week 3 and week 5 and for cycle 3 to cycle 6, it can be given +/- 14 days for logistical reasons. Screening labs within 16 days can be used for C1D1 serum PSA, testosterone, and PAP. Research blood will be drawn +/- 3 days for C2D1 and +/- 14 days for C6D1 and C9D1 days for logistical reasons.
[2] Pathologic confirmation will be obtained anytime prior to enrollment
[3] HepB/HepC/HIV status will be obtained within 8 weeks prior to enrollment
[4] Screening Tc99 sctintigraphy and CT-CAP or MRI will be obtained within 8 weeks prior to enrollment
[5] Baseline ECG will be obtained within 8 weeks prior to enrollment
[6] Restaging will be done every 6 months (+/- 14 days) or earlier if clinically indicated. If, however, in the opinion of the investigator re-staging scans are not required (in patients with a PSA DT > 6 month over the previous 6 months, document in chart to assure compliance) then re-staging scans can be deferred. However, once PSA DT is less than 6 months, scans must be performed.) Re-staging scans may exclude CT Chest.
[7] Patients will continue to be followed after Cycle 9 until progression or ADT is clinically indicated. All visits will include evaluations listed for cycle 9 except research blood. Research blood will not be drawn after Cycle 9..

[8]optional
[9] Patients will have the option to initiate 1 year of vaccine maintenance within 3 months of cycle 6 vaccine, administered every 12 weeks. This year will include follow-up every 6 weeks during this period for PSA checks on non-vaccine visits. (Patients seen every 6 weeks during 1 year maintenance period). Patients will be required to have a PSADT greater than 5 months, as measured over the prior 6 months in order to start and continue vaccine during the 1 year maintenance period.
[10]Surveys are optional at all time points and can be done every 3 months for the first 9 months and every 3-4 months therafter while patients are on-study. (If surveys are not done, reason will be documented.)
[11] Refer to sections **2.2** for screening evaluation and **2.4** for baseline evaluation
[12] Not performed at DFCI site.

-All patient visits can be delayed by 2 weeks for logistical issues except for week 3 and 5 where the window is 3 days.

16 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, Mineral Panel, Acute Care Panel, LDH, CK, Uric Acid, Total Protein	1 4 mL SST	CC DLM
Anti-HIV-1/2	1 8 mL SST	CC TTV lab
Testosterone, total	1 red top tube	CC DLM
Prostate Specific Antigen	4 mL SST	CC DLM
Lymphocyte Phenotyping, TBNK (Optional based on site. Required at NCI but not MSK or DFCI.)	1 light lavender tube	CC DLM
Immunology Assays	6 10 mL Na Heparin tubes 2 8 ml SST tubes	NCI-Frederick 1-301-846-5893

17 APPENDIX E: CCR PROBLEM REPORT FORM

<table>
<tr><td>NCI Protocol #:</td><td colspan="2">Protocol Title:</td></tr>
<tr><td></td><td colspan="2">Report version: (select one)
____Initial Report
____Revised Report
____Follow-up</td></tr>
<tr><td>Site Principal Investigator:</td><td colspan="2"></td></tr>
<tr><td>Date of problem:</td><td colspan="2">Location of problem: (e.g., patient’s home, doctor’s office)</td></tr>
<tr><td colspan="3">Who identified the problem? (provide role (not name of person): nurse, investigator, monitor, etc…)</td></tr>
<tr><td>Brief Description of Subject (if applicable)
(Do NOT include personal identifiers)</td><td colspan="2">Sex: ___ Male ___ Female Age:
___ Not applicable (more than subject is involved)</td></tr>
<tr><td colspan="3">Diagnosis under study:</td></tr>
<tr><td colspan="3">Name the problem: (select all that apply)
[ ] Adverse drug reaction
[ ] Abnormal lab value
[ ] Death
[ ] Cardiac Arrest/ code
[ ] Anaphylaxis
[ ] Sepsis/Infection</td></tr>
</table>

[ ] Blood product reaction

[ ] Unanticipated surgery/procedure

[ ] Change in status (e.g. increased level of care required)

[ ] Allergy (non-medication)

[ ] Fall

[ ] Injury/Accident (not fall)

[ ] Specimen collection issue

[ ] Informed consent issue

[ ] Ineligible for enrollment

[ ] Breach of PII

[ ] Tests/procedures not performed on schedule

[ ] Other, brief 1-2 word description: _____

Detailed Description of the problem: (*Include any relevant treatment, outcomes or pertinent history*):

***Is this problem unexpected?** *(see the definition of unexpected in the protocol))*
__YES __NO **Please explain:**

***Is this problem related or possibly related to participation in the research?**
__YES __NO **Please explain:**

***Does the problem <u>suggest</u> the research places subjects or others at a greater risk of harm than was previously known or recognized?** __YES __NO
Please explain:

Is this problem? (*select all that apply*)

[ ] An Unanticipated Problem* that is: [ ] Serious [ ] Not Serious [ ] A Protocol Deviation that is: [ ] Serious [ ] Not Serious [ ] Non-compliance **Note if the 3 criteria starred above are answered, "YES", then this event is also a UP.*
Is the problem also *(select one)* [ ] AE [ ] Non-AE
Have similar problems occurred on this protocol at your site? __YES __NO **If "Yes", how many? ____ Please describe:**
Describe what steps you have already taken as a result of this problem:
In addition to the NCI IRB, this problem is also being reported to: (*select all that apply*) [ ] Local IRB [ ] Study Sponsor [ ] Manufacturer : _____ [ ] Institutional Biosafety Committee [ ] Data Safety Monitoring Board [ ] Other: _____ [ ] None of the above, not applicable

INVESTIGATOR'S SIGNATURE:	**DATE:**

18 APPENDIX F: INSTRUCTIONS FOR COLLECTION AND SHIPPING OF RESEARCH BLOOD SAMPLES AT OUTSIDE SITES

Collection:

PBMCS: 6 green top (Na heparin, 10 mL) tubes, BD Vacutainer Ref #366480

Serum: 2 red serum separator (8 mL) tubes, Greiner Bio-One Vacutainer Ref #455071

* Blood is collected at **4 time points (with an optional 5th time point in Arm B)**: baseline, Cycle 1 Day 1, Cycle 2 Day 1, Cycle 6 Day 1, and Cycle 9 Day 1. In addition, an end of study research blood draw can be done at the discretion of the investigator and based on logistics for willing patients.

Shipping:

PBMCs are to be sent on the day of draw; serum is to be frozen and batch-shipped at intervals. NCI does not provide lab kits/shipping supplies. Please send samples using generic ambient (PBMCs) and dry ice (serum) Category B sample shippers, and include a completed transmittal form (blank form provided) with each shipment. Please ship FedEx Overnight using FedEx account number 251310645.

****Please note, PBMCs cannot be shipped within 72 hours after a bone scan or MUGA scan injection due to the Technetium-99 radioisotope or within 24 hours after a Sodium Fluoride PET scan injection due to the Fluorine-18 radioisotope. If a bone scan, MUGA scan, or PET scan (or other nuclear medicine study) is scheduled, PBMCs must be drawn prior to injection of radioisotopes.**

1) Pack blood vials in Category B shipping materials (EXAKT-PAK as described below or equivalent):

For ambient PBMCs:

EXAKT-PAK for Vials Category B D-Pak MD8702V06 (Accommodates 6 vials)

Includes Inner pack (Ambient) and Insulated Cooler
With 2 cool packs per cooler, part #CP1003, room temperature or slightly cooled (not frozen) to keep samples between 18 and 30ºC.

For frozen serum:

EXAKT-PAK Frozen Category B D-Pak MD8703V06

Dry ice required (not included)

2) Send email notification of upcoming shipment, and include completed transmittal or manifest with shipment.

- Emails should be sent to the following individuals:

  Theresa Burks, burkst@mail.nih.gov

  Myrna Rauckhorst, mrauckhorst@mail.nih.gov

  Caroline Jochems, jochemscm@mail.nih.gov

  Ravi Madan, madanr@mail.nih.gov

3) Ship **FedEx Overnight** using FedEx account number **251310645**.

4) **NO** specimens should be shipped on Fridays or the day before a Federal holiday.

Shipping Address:

Leidos Biomedical Research, Inc.

Attn: Theresa Burks

1050 Boyles Street

Bldg. 469/Room 121

Frederick, MD 21702

Phone 301-846-5125, or 301-846-1707

Paperwork/Labeling:

- Please label research tubes with a coding mechanism. The following information should be contained on the label:
  - site name
  - patient enrollment number (provided by the Central Registration Office upon registration/randomization) and initials
  - arm – list whether patient is randomized to Arm A or Arm B
  - NCI protocol #16-C-0035
  - Date Drawn
  - Time point – list sample as either baseline, C1D1, C2D1, D6D1, or C9D1

Example:

Site name – 01-ABC–Arm A

Protocol # NCI 16-C-0035

Date Drawn: 08/01/2015

Time point: baseline

- Please complete a transmittal form for each PBMC timepoint and include with sample shipment. Blank transmittal form will be provided. For batch-shipped serum, please provide an Excel spreadsheet manifest listing all included samples. Information that should be listed on the manifest includes Subject ID, Sample ID, Sample Type (i.e. serum), Collection Date/Time, Study Day, Number of Vials, and any Comments.

Shipping Supplies:

NCI does not provide lab kits/shipping supplies. Please send samples using generic ambient (PBMCs) and dry ice (serum) Category B sample shippers (such as EXAKT-PAK or equivalent).

EXAKT-PAK supplies available at:
https://www.exaktpak.com/store/
EXAKT Technologies, Inc.
Home office: 7002 N. Broadway Extension
Oklahoma City, OK 73116-9006
405-848-5800

19 APPENDIX G: SAMPLE FORM

ATTN: Ms. Theresa Burks/ Leidos-Frederick, Attn: CML 469/121, Bldg 1050 Boyles Street, Frederick MD 21702, Phone 301-846-5125

PROJECT ID: 001.016.0039.0003 **CSAS: 21682** **SOURCE CODE: SD007**

PROTOCOL: 16-C-0035 Prostvac in Patients with Biochemical Recurrent Prostate Cancer. Sample from external center (*insert Site Name here*)

INVESTIGATOR: Ravi Madan, M.D.
CONTACT: Jennifer Marte, 301-480-0276, martej@mail.nih.gov
RESEARCH NURSE: Myrna Rauckhorst RN, 240-760-7971, page 102-14681, mrauckhorst@mail.nih.gov
BACK-UP CONTACT: Caroline Jochems, 301-402-6274 or 496-4343; jochemscm@mail.nih.gov

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RESEARCH INFORMATION: 16-C-0035

Patient enrollment # + initials + arm: _____

ADDITIONAL SAMPLE INFORMATION (Time point, Cycle, etc.) : _____

DATE SAMPLE DRAWN: _____

DATE SAMPLE PROCESSED: _____

[ ] Whole Blood _____ Draw time: _____ Tubes Sent_____

[ ] Serum _____ Draw time: _____ Tubes Sent ____

LAB USE ONLY

Lab ID# _____

Lab ID# _____

Kopp Lab SAMPLE DESCRIPTION: **16C0035 + Pt. Enrollment # +3 pts initials+Arm #**

Fisher Biosciences Repository Input Sample Description:

CCMM number + 16-C-0035

PROCESSING OR TESTING REQUESTED ON FRESH SAMPLE: Check all that apply:

Cryopreservation: __X___

Updated 9-27-17

20 APPENDIX H: SURVEYS

MAX-PC survey:

TABLE 4
The Modified 18-Item Memorial Anxiety Scale for Prostate Cancer

YOUR FEELINGS ABOUT PROSTATE CANCER AND PROSTATE SPECIFIC ANTIGEN TESTS
We would like to better understand how patients cope with aspects of their treatment for prostate cancer and the medical tests frequently involved in their care.

I. Below is a list of comments made by men about prostate cancer. Please indicate by circling the number next to each item how frequently these comments were true for you *during the past week;* not at all, rarely, sometimes, often.

	Not at all	Rarely	Sometimes	Often
1. Any reference to prostate cancer brought up strong feelings in me.	0	1	2	3
2. Even though it's a good idea, I found that getting a PSA test scared me.	0	1	2	3
3. Whenever I heard about a friend or public figure with prostate cancer, I got more anxious about my having prostate cancer.	0	1	2	3
4. When I thought about having a PSA test, I got more anxious about my having prostate cancer.	0	1	2	3
5. Other things kept making me think about prostate cancer.	0	1	2	3
6. I felt kind of numb when I thought about prostate cancer.	0	1	2	3
7. I thought about prostate cancer even though I didn't mean to.	0	1	2	3
8. I had a lot of feelings about prostate cancer, but I didn't want to deal with them.	0	1	2	3
9. I had more trouble falling asleep because I couldn't get thoughts of prostate cancer out of my mind.	0	1	2	3
10. I was afraid that the results from my PSA test would show that my disease was getting worse.	0	1	2	3
11. Just hearing the words "prostate cancer" scared me.	0	1	2	3

II. For the next three questions, please indicate how frequently these situations have *EVER* been true for you.

	Not at all	Rarely	Sometimes	Often
12. I have been so anxious about my PSA test that I have thought about delaying it.	0	1	2	3
13. I have been so worried about my PSA test result that I have thought about asking my doctor to repeat it.	0	1	2	3
14. I have been so concerned about my PSA test result that I have thought about having the test repeated at another lab to make sure they were accurate.	0	1	2	3

III. Listed below are a number of statements concerning a person's beliefs about their own health. In thinking about the *past week*, please indicate how much you agree or disagree with each statement: strongly agree, agree, disagree, or strongly disagree. Please circle the number of your answer.

	Strongly agree	Agree	Disagree	Strongly disagree
15. Because cancer is unpredictable, I feel I cannot plan for the future.	0	1	2	3
16. My fear of having my cancer getting worse gets in the way of my enjoying life.	0	1	2	3
17. I am afraid of my cancer getting worse.	0	1	2	3
18. I am more nervous since I was diagnosed with prostate cancer	0	1	2	3

PSA: prostate specific antigen.

FACT-P Survey:

Below is a list of statements that other people with your illness have said are important. **Please circle or mark one number per line to indicate your response as it applies to the <u>past 7 days</u>.**

	PHYSICAL WELL-BEING	**Not at all**	**A little bit**	**Some-what**	**Quite a bit**	**Very much**
GP1	I have a lack of energy	0	1	2	3	4
GP2	I have nausea	0	1	2	3	4
GP3	Because of my physical condition, I have trouble meeting the needs of my family	0	1	2	3	4
GP4	I have pain	0	1	2	3	4
GP5	I am bothered by side effects of treatment	0	1	2	3	4
GP6	I feel ill	0	1	2	3	4
GP7	I am forced to spend time in bed	0	1	2	3	4

	SOCIAL/FAMILY WELL-BEING	**Not at all**	**A little bit**	**Some-what**	**Quite a bit**	**Very much**
GS1	I feel close to my friends	0	1	2	3	4
GS2	I get emotional support from my family	0	1	2	3	4
GS3	I get support from my friends	0	1	2	3	4
GS4	My family has accepted my illness	0	1	2	3	4
GS5	I am satisfied with family communication about my illness	0	1	2	3	4
GS6	I feel close to my partner (or the person who is my main support)	0	1	2	3	4
Q1	*Regardless of your current level of sexual activity, please answer the following question. If you prefer not to answer it, please mark this box* ☐ *and go to the next section.*					
GS7	I am satisfied with my sex life	0	1	2	3	4

	EMOTIONAL WELL-BEING	**Not at all**	**A little bit**	**Some-what**	**Quite a bit**	**Very much**
GE1	I feel sad	0	1	2	3	4
GE2	I am satisfied with how I am coping with my illness	0	1	2	3	4
GE3	I am losing hope in the fight against my illness	0	1	2	3	4
GE4	I feel nervous	0	1	2	3	4
GE5	I worry about dying	0	1	2	3	4
GE6	I worry that my condition will get worse	0	1	2	3	4

	FUNCTIONAL WELL-BEING	**Not at all**	**A little bit**	**Some-what**	**Quite a bit**	**Very much**
GF1	I am able to work (include work at home)	0	1	2	3	4
GF2	My work (include work at home) is fulfilling	0	1	2	3	4
GF3	I am able to enjoy life	0	1	2	3	4
GF4	I have accepted my illness	0	1	2	3	4
GF5	I am sleeping well	0	1	2	3	4
GF6	I am enjoying the things I usually do for fun	0	1	2	3	4
GF7	I am content with the quality of my life right now	0	1	2	3	4

	ADDITIONAL CONCERNS	**Not at all**	**A little bit**	**Some-what**	**Quite a bit**	**Very much**
C2	I am losing weight	0	1	2	3	4
C6	I have a good appetite	0	1	2	3	4
P1	I have aches and pains that bother me	0	1	2	3	4
P2	I have certain parts of my body where I experience pain	0	1	2	3	4
P3	My pain keeps me from doing things I want to do	0	1	2	3	4
P4	I am satisfied with my present comfort level	0	1	2	3	4
P5	I am able to feel like a man	0	1	2	3	4
P6	I have trouble moving my bowels	0	1	2	3	4
P7	I have difficulty urinating	0	1	2	3	4
BL2	I urinate more frequently than usual	0	1	2	3	4
P8	My problems with urinating limit my activities	0	1	2	3	4
BL5	I am able to have and maintain an erection	0	1	2	3	4