Amendment

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Protocol Title: A Randomized Phase II Trial Combining Vaccine Therapy with PROSTVAC /TRICOM and Flutamide, vs. Flutamide Alone in Men with Androgen Insensitive, Non Metastatic (D0.5) Prostate Cancer

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

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

CTEP#: **7354**
NCI Protocol#: **07-C-0107**
OSP#: **0603-764**
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Amendment: **P**

PROTOCOL TITLE

A Randomized Phase II Trial Combining Vaccine Therapy with PROSTVAC /TRICOM and Flutamide, vs. Flutamide Alone in Men with Androgen Insensitive, Non Metastatic (D0.5) Prostate Cancer

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Drug Name:	PROSTVAC®-V/TRICOM™ (vaccinia) [a recombinant vaccinia virus containing the genes for PSA (L155) and three human T-cell costimulatory molecules (TRICOM)]
NSC Number:	717170
IND Number:	10,915
Sponsor:	Cancer Therapy Evaluation Program (CTEP)/ National Cancer Institute

Drug Name:	PROSTVAC®-F/TRICOM™ (fowlpox) [a recombinant fowlpox virus containing the genes for PSA (L155) and three human T-cell costimulatory molecules (TRICOM)]
NSC Number:	717171
IND Number:	10,915
Sponsor:	Cancer Therapy Evaluation Program (CTEP)/ National Cancer Institute

Drug Name:	Flutamide (Eulexin®)
Commercially Available:	Manufactured by Schering- Plough, Corp., Kenilworth, NJ

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PRÉCIS

Background:

- There is no standard of care for prostate cancer patients progressing on hormone therapy with a rising serum PSA level without evidence of metastatic disease.
- We have completed a phase II trial in which men with this stage of disease were randomized to receive a pox vector PSA vaccine vs. the antiandrogen nilutamide.
- The median time to treatment failure on nilutamide was 7.6 months
- 12 patients on the vaccine arm had nilutamide added at the time of PSA progression.
- The median time for treatment failure after the addition of nilutamide was 13.9 months, for a total of 25.9 months from initiation of vaccine therapy.
- This suggests that the combination of hormone therapy with vaccine therapy may lead to an improved clinical benefit compared to hormone therapy alone
- Due to the increased toxicity of nilutamide compared to other antiandrogens and the patients prior exposure to bicalutamide therapy, we plan to use flutamide as a second line hormonal manipulation in the below study.

Objectives (Primary):

- To determine if use of a combination of vaccine plus flutamide may be associated with a trend toward improvement in time to treatment failure compared to flutamide alone.

Eligibility:

- Must have non metastatic androgen insensitive prostate cancer with a rising PSA with castrate levels of testosterone and no evidence of metastatic disease on CT scan or bone scan.
- Hgb $\geq$ 9 Gm/dL
- Lymphocyte count $\geq 500/mm^3$.
- Hepatic function: Bilirubin $\leq$ 1.5 mg/dl OR patients with Gilbert's syndrome, a total bilirubin $\leq$ 3.0 mg/dL, AST and ALT< 2.5 times upper limit of normal

Design:

- Flutamide will be administered at a dose of 250 mg PO tid every day in both arms A and B.
  rV-PSATRICOM will be administered s.c. on day 1 in Arm B.
  rF-PSATRICOM will be administered s.c. on day 29 & every 4 weeks in Arm B.
- For patients with declining PSA no restaging will be done unless they develop symptoms consistent with metastatic disease.
- For patients with rising PSA, once 2 consecutive PSA rises are seen from the last imaging studies, CT and bone scans will be done at their next scheduled visit. They will then be re-staged (CT and bone scans) at 3 month intervals as long as PSA continues to rise.
- After 3 months of therapy, patients receiving the flutamide alone (arm A) may cross over to receive vaccine if they develop a rising PSA and scans are without metastatic disease. The vaccine may commence 4 weeks after flutamide is stopped if the PSA continues to rise. If there is an antiandrogen withdrawal response (a decline in PSA 28 days after the discontinuation of flutamide), PSA serum levels will be checked every 28 days and vaccine may commence when the serum PSA levels begin to rise (if scans are negative for metastatsis). Patients on arm B will have flutamide discontinued and may continue vaccine therapy. At this point patients may continue to receive treatment on study until the

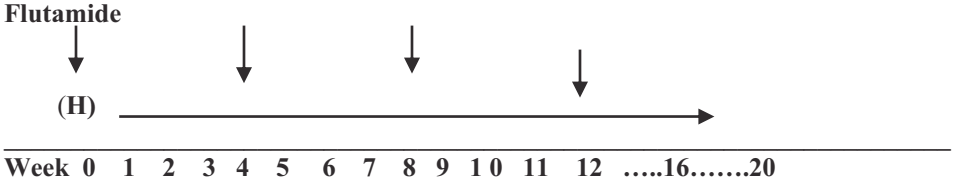
development of disease on scans or a second occurrence of rising PSA levels in the absence of clinical progression.

- Patients who have been on study for 2 years or more with stable disease and who are not getting vaccine, clinic visits may be scheduled every 8 weeks. (Patients receiving monthly vaccine will continue to have monthly visits.)
- For patients who have stable disease and attend clinic every 8 weeks, once 2 consecutive PSA rises are seen, a CT and bone scan will be done at their next visit in 4 weeks. They will then be restaged (CT and bone scans) at 3 month intervals as long as PSA continues to rise.

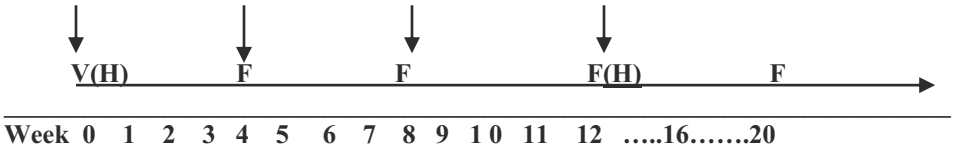
SCHEMA

This randomized Phase II study proposes to evaluate the role of second line antiandrogen therapy and a pox vector based PSA vaccine in a population of patients with prostate cancer who have a rising serum PSA despite castrate levels of testosterone and prior antiandrogen therapy but no metastatic disease on bone or CT scans. We will look for a potential clinical benefit using an initial vaccination with 2 x 10^8 pfu PROSTVAC-TRICOM -V on day 1 followed by monthly boosting vaccinations with 1 x 10^9 pfu PROSTVAC-TRICOM –F. The duration of each treatment cycle is approximately 28 days. In addition to the vaccine therapy, 2^{nd} line antiandrogen therapy with flutamide will be given orally on a daily basis beginning with the first vaccine on day 1. This will be compared to patients receiving flutamide alone. Patients may continue on therapy until the development of measurable disease on scans or if they develop toxicity requiring stopping of their treatment. For patients with declining PSA no restaging will be done unless they develop symptoms consistent with metastatic disease. For patients with rising PSA, once 2 consecutive PSA rises are seen from the last imaging studies, CT and bone scans will be done at their next scheduled visit. They will then be re-staged (CT and bone scans) at 3 month intervals as long as PSA continues to rise. After the initial 3 month evaluation, if a patient has a rising serum PSA level (based on Bubley criteria JCO, 1999) but no evidence of metastatic disease, patients in Arm A will have flutamide discontinued and may begin to receive vaccine alone 4 weeks after cessation of flutamide (if there is no evidence of an antiandrogen withdrwal response) as described above, since the standard care would be discontinuation of flutamide for rising serum PSA. If there is an antiandrogen withdrawal response (a decline in PSA 28 days after the discontinuation of flutamide), PSA serum levels will be checked every 28 days and vaccine may commence when the serum PSA levels begin to rise (if scans are negative for metastatsis). Those patients who have rising serum PSA levels on Arm B without evidence of metastatic disease will have flutamide discontinued, but may continue to receive vaccine treatment. Since there is no standard of care aside from antiandrogen discontinuation for these patients and they would have already received 2^{nd} line therapy, patients can continue to receive treatment on study until there is evidence of disease on bone scan or CT scan or until there is a second occurrence of rising PSA levels in the absence of clinical progression. For patients who have been on study for 2 years or more with stable disease and who are not getting vaccine, clinic visits may be scheduled at 8 week intervals. For these patients who have rising PSA, once 2 consecutive PSA rises are seen, a CT and bone scan will be done at their next visit in 4 weeks. They will then be restaged (CT and bone scans) at 3 month intervals as long as PSA continues to rise. Patients will be stratified based on a "projected" PSA doubling time of >10 months vs. ≤ 10 months using last 2 serum PSA levels 1 month apart prior to enrolling on study.

Arm A (n=33)
Flutamide



Arm B (n=33)
Vaccine (monthly) + Flutamide (daily)



Stratification of patients- projected PSA doubling time of >10 months vs ≤ 10 months (based upon last 2 serum PSA levels prior to enrollment 1 month apart)

At week 12, restaging CT Chest/Abdomen/Pelvis and Bone scans will be performed in all patients, and when clinically indicated (by consecutive rises in PSA or new symptoms) thereafter. Patients may continue on therapy if scans do not demonstrate metastastic prostate cancer.

Legend
H = **Flutamide** 250 mg po t.i.d, commencing on day 1 in both arms
V= PROSTVAC-V /TRICOM 2 x 10^8 pfu s.c
F= PROSTVAC-F /TRICOM 1 X 10^9 pfu s.c

↓ Blood draw for Immunologic studies- a. Elispot assay (see section **3.4.6** and **APPENDIX C**).

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1 INTRODUCTION

1.1 Study Objectives

1.1.1 Primary

The primary objective of this randomized pilot trial is to determine if use of a combination of vaccine plus flutamide may be associated with a trend toward improvement in time to treatment failure (defined as a rising PSA (Bubley criteria, JCO 1999), development of metastatic disease, or removal from treatment due to excessive toxicity) compared to patients receiving flutamide alone.

1.1.2 Secondary

Secondary objectives are 1) determining preliminary evidence of any patterns of immunologic effects which differ by treatment including the immunologic effects of flutmaide withdrawal on patients continuing on vaccine following a rising PSA on flutamide, 2) estimating toxicity noted on the arms and comparing the results, 3) evaluating, in a preliminary fashion, the effect of vaccine on development of metastatic disease after PSA progression, and 4) evaluating PSA responses and immune responses in patients who have had flutamide stopped at the time of PSA progression and either continue vaccine (Arm B) or have vaccine initiated (arm A) at time of flutamide discontinuation, 5) assessing response rate in patients randomized to both arms, as well as responses in those patients after cross over from the flutamide arm to vaccine only (time of disease progression will be defined as from the first date that vaccine is initiated in those patients who have had flutamide discontinued until the first notation of clinical progression).

1.2 Background and Rationale for the Current Trial

1.2.1 Prostate Cancer Background

Adenocarcinoma of the prostate is the most common cancer diagnosis in American males, and the second leading cause of cancer death. One out of 11 men will develop clinically significant prostate cancer in his lifetime. During 2005, an estimated 232,900 men will be diagnosed with prostate cancer and 30,350 will die from prostate cancer in the United States (**(1)**). While the majority of patients are now diagnosed with clinically localized disease, 30-40% of patients will progress within 10 years after local therapy (radiation or surgery) as evidenced by a rise in PSA.(**(1)**-**(5)**)

Widespread monitoring of PSA following definitive therapy has resulted in the diagnosis of large number of patients with only biochemical recurrence, defined as a rise in PSA without evidence of metastatsic disease on exam or X-rays. Many of these patients will undergo androgen deprivation therapy (ADT) (**(6)**-**(10)**). Unfortunately, most of these patients will eventually develop a second rise in PSA. The majority of patients will eventually develop metastatic disease on imaging studies. Many patients will first have a rise in PSA on hormonal therapy

without radiographic evidence of disease (stage D0.5) Treatment options for stage D0.5 patients include additional hormonal manipulations, observation, cytotoxic chemotherapy and enrollment onto clinical trials with investigational agents; however, there is currently no standard of care. Three randomized studies have evaluated the natural history of Stage D0.5 prostate cancer. Bianco et al. evaluated 63 biochemical recurrent castrate prostate cancer patients. The median time to development of metastatic disease was 9.0 months (**(62)**). Another study by Smith et al. evaluated 201 patients with this disease stage. In this study the median time to metastasis was 30 months (**(63)**). Finally, in our previous published study at the NCI, 42 patients were evaluated. 30% of patients developed metastatic disease at 9 months (**(20)**).

Development of vaccines strategies designed to break tolerance and generate a sustained potent immune response against prostate cancer thus represents a novel therapeutic approach. Pre-clinical and clinical studies with a range of vaccines have demonstrated that the induction of T-cell responses directed against a self-antigen can lead to anti-tumor activity in the absence of toxicity (**(11)**, **(17)**, **(19)**, **(44)**). (see pages 10,11 for detailed discussion of these references) PSA is a potential target for a prostate cancer vaccine due to its restricted expression on prostate cancer and normal prostatic epithelium. Since PSA is a "self" antigen, vaccines and vaccine strategies must be developed to enhance the immunogenicity of PSA.

The proposed vaccine strategy utilizes a third-generation vaccine. We plan to administer this vaccine with hormone therapy (flutamide) vs flutamide alone to see if there is a synergy between vaccine and hormones that translates into an improved clinical response in patients with nonmetastatic androgen insensitive prostate cancer. There is mounting data in the literature that provides a rationale for this strategy. Mercarder et al. demonstrated that androgen ablative therapy induces profuse T cell infiltration of benign glands and tumors in human prostates. T cell infiltration is readily apparent after 7-28 days of therapy and is comprised predominantly of a response by CD4+ T cells and comparatively fewer CD8+ T cells. Recruitment/activation of antigen-presenting cells in treated prostate tissues may contribute to local T cell activation. The induction of T cell infiltration in prostate tissues treated with androgen ablation may have implications for the immunotherapeutic treatment of prostate cancer (**(21)**). To understand the T cell response to prostate cancer, Drake et al., created transgenic mice that express a model antigen in a prostate-restricted pattern and crossed these animals to TRAMP mice that develop spontaneous prostate cancer (**(47)**). Adoptive transfer of prostate-specific CD4 T cells showed that, in the absence of prostate cancer, the prostate gland is mostly ignored. Tumorigenesis allows T cell recognition of the prostate gland--but this recognition is tolerogenic, resulting in abortive proliferation and ultimately in hyporesponsiveness at the systemic level. Androgen ablation was able to mitigate this tolerance--allowing prostate-specific T cells to expand and develop effector function after vaccination. These results suggest that immunotherapy for prostate cancer may be most efficacious when administered after androgen ablation (**(47)**). Finally, in the NCI 00C-0137 study we examined this concept of combining androgen ablative therapy with immunotherapy (**(20)**). Men with hormone refractory prostate cancer with no measurable disease on scans were randomized to receive a pox vector PSA vaccine vs. the androgen receptor antagonist nilutamide. Forty-two patients were randomized to receive vaccine vs. antiandrogen therapy with nilutamide (21 patients per arm). The median time to treatment failure in the vaccine arm was 9.9 months with 13/21 decreases in PSA velocity vs. 7.6 months with 16/21 decreases in PSA velocity in the nilutamide arm (p=0.28). Eight patients from the

nilutamide arm had vaccine added at the time of PSA progression. The median time to treatment failure with combined therapy was 5.2 months, with a median duration from entry on study of 15.9 months. Twelve patients who progressed on the vaccine arm had nilutamide added at the time of PSA progression. The median time for treatment failure with combined therapy was 13.9 months and a median of 25.9 months from initiation of therapy. The study, however, was not designed to measure the statistical significance between the treatment with hormone only vs hormone added following initial vaccine therapy. Eight HLA-A2 positive patients on the vaccine arm and 3 HLA-A2 positive patients on the nilutamide arm were evaluated for induction of PSA-specific T-cell responses prior to and following 3 monthly cycles of therapy (**(20)**). (see page 11-12 for additional details)

1.2.2 Vaccines

The first step in making a vaccine for tumor immunotherapy is to choose the target antigen. PSA is expressed essentially only in prostatic epithelial cells (normal and malignant), and the prostate gland is nonessential. Therefore, PSA is a valid target for immunotherapy. The fact that PSA is secreted and not membrane bound limits the use of PSA as a target for humoral immunity, but not the use of PSA as a target of specific cellular immune system attack. Cells, including tumor cells, present endogenously expressed proteins on their surface in the form of peptide MHC complexes. Cytotoxic T lymphocytes (CTLs) recognize and are activated by specific peptides in the context of the appropriate MHC class I molecule on antigen-presenting cells (APCs). This activation can in turn lead to killing of tumor targets by the peptide-specific CTLs. CTL activation as well as tumor recognition and killing are thus dependent on the MHC class I molecule. One allele of MHC class I molecule, HLA-A2, is present in about 50% of the population in the U.S. and several HLA-A2 restricted peptides from PSA have previously been identified.(**(11)**;**(12)**)

Ability to Generate PSA-Specific T cells In-Vitro

The use of PSA as a target to elicit tumor-specific T-cell mediated lysis has been validated *in vitro*. Correale et al. demonstrated *in vitro* killing of a PSA–peptide-pulsed HLA-A2+ human cell line by a PSA-specific human CTL cell line, and lysis was blocked by an antibody directed against MHC class I molecules.(**(13)**)

It has also been shown that PSA-specific CTLs could be generated that lyse PSA-expressing prostate cancer cells.(**(11)-(13)**) By stimulating normal HLA-A2 donor peripheral blood mononuclear cells (PBMCs) with HLA-A2–restricted PSA peptides in the presence of IL-2, CTL lines were generated that specifically killed PSA expressing HLA-A2+ prostate cell lines, HLA-A2+ cell lines pulsed with PSA peptide and HLA-A2 cells infected with rV-PSA.(**(12)**)

Peptide vs. Vector Encoded Tumor-Associated Antigen

An advantage of recombinant poxvirus in developing cancer vaccines is the demonstration that recombinant proteins derived from genes inserted and transcribed in viral genomes are more immunogenic than protein in adjuvant.(**(14)-(16)**) A striking example of this was noted by Kass et al., where it was shown that two injections of CEA protein in adjuvant generated little, if any, of an immune response to CEA in a CEA transgenic (CEA-Tg) mouse.(**(14)**) This would be

expected since the host is seeing CEA as a "self" antigen. However, when the recombinant vaccinia virus containing the CEA transgene (designated rV-CEA) is administered one or two times, a strong CEA-specific T-cell response is elicited. This is likely due to a strong inflammatory response generated by the host against vaccinia proteins. In turn, this inflammatory process apparently leads to an environment of cytokine production and T-cell proliferation that may further amplify the immune response to the transgene antigen. This process favors induction of a cell-mediated immune response to the transgene antigen.

Pox-Viral PSA Vaccines

The use of pox viral vectors to stimulate an immune response to PSA has been evaluated in several clinical trials. Three Phase I clinical trials have evaluated the safety and biological effects of using a vaccinia vector containing the gene for PSA in patients with prostate cancer. In the first study, rV-PSA (Prostvac) was administered to six patients with recurrent disease who previously had undergone radical prostatectomy. Toxicity was minimal, and dose limiting toxicity was not observed. One patient had anti-PSA IgG antibody activity induced following vaccination [**(44)**]. In a larger Phase I trial conducted at the Dana-Farber Cancer Center [**(17)**], 33 patients were vaccinated with Prostvac. The final 10 patients received the cytokine GM-CSF along with the Prostvac vaccinations. Immunologic assays were performed on seven of the 10 patients; five of the seven were noted to have at least a 2-fold increase in T-cell precursors specific for the PSA-3 peptide. In four of these five patients with an increased PSA-specific immune response, there was stabilization of serum PSA levels for 6–11+ months [**(17)**]. Gulley et al. published the results of the third Phase I clinical trial of rV-PSA in 42 patients with metastatic androgen independent prostate cancer [**(11)**]. There was no significant treatment-related toxicity apart from injection site reactions, and the authors demonstrated immunological responses in selected patients as evidenced by an increase in the proportion of PSA-specific T cells following vaccination. Furthermore, they showed that these patients' T cells could lyse PSA-expressing tumor cells in vitro. There was also a suggestion of improved time to progression when GM-CSF was added to the vaccine regimen.

The Eastern Cooperative Oncology Group reported a randomized Phase II study in which 64 patients with rising PSA following definitive local therapy with no evidence of disease on scans were randomized to receive four vaccines with rV-PSA (designated V) and/or rF-PSA (designated A for avipox).(**(18)**) The arms were thus AAAA (arm A), AAAV (arm B), and VAAA (arm C). This study has recently been updated with a median follow-up time of 50 months. The median time to PSA progression is 9.2 and 9.1 months for arms A and B respectively, compared to 18.2 months for arm C (rV-PSA prime and rF-PSA boost; p=0.15 by Log Rank Test). The median time to clinical progression has still not been reached for any treatment group with 80% of men in arms A and B free of disease progression compared to 90% of men in arm C free of clinical progression (p=0.73 by log rank test). These results suggest that men with hormone-dependent prostate cancer and a rising PSA may derive long-term clinical benefit from vaccinations with poxviruses expressing PSA (**(18)**).

NCI 00-C-0154 and NCI 00-C-0137 are two Phase II clinical trials at the NCI using rV-PSA mixed with a recombinant vaccinia containing the T-cell costimulatory molecule B7.1 along with

booster vaccinations of avipox-PSA. Gulley et al. showed that the combination of vaccine (rV-PSA plus rV-B7.1 prime followed by rF-PSA booster vaccines) with external beam radiation therapy was safe and was able to generate an immune response to PSA in the majority of patients [**(19)**]. Thirteen of 17 evaluable patients had at least a 3-fold increase in the number of circulating PSA-specific T cells following vaccine, whereas none of eight evaluable patients in the radiation alone arm had any measurable increase in their number of PSA-specific T cells. In the NCI 00C-0137 study we examined this concept of combining androgen ablative therapy with immunotherapy (**(20)**). Men with hormone refractory prostate cancer with no measurable disease on scans were randomized to receive a pox vector PSA vaccine vs. the androgen receptor antagonist nilutamide. Forty-two patients were randomized to receive vaccine vs. antiandrogen therapy with nilutamide (21 patients per arm). The vaccine consisted of recombinant vaccinia viruses containing the PSA and B7.1 costimulatory genes as prime vaccinations and avipox-PSA as boosters. After 6 months, patients with a rising PSA and no metastasis may receive a combination of both treatments. Three patients on nilutamide were removed from study secondary to grade 3 toxicities; no grade 3 toxicities were attributed to vaccine. The median time to treatment failure in the vaccine arm was 9.9 months with 13/21 decreases in PSA velocity vs. 7.6 months with 16/21 decreases in PSA velocity in the nilutamide arm (p=0.28). Eight patients from the nilutamide arm had vaccine added at the time of PSA progression. The median time to treatment failure with combined therapy was 5.2 months, with a median duration from entry on study of 15.9 months. Twelve patients on the vaccine arm who progressed had nilutamide added at the time of PSA progression. The median time for treatment failure with combined therapy was 13.9 months and a median of 25.9 months from initiation of therapy. The study, however, was not designed to measure the statistical significance between the treatment with hormone only vs hormone added following initial vaccine therapy. Eight HLA-A2 positive patients on the vaccine arm and 3 HLA-A2 positive patients on the nilutamide arm were evaluated for induction of PSA-specific T-cell responses prior to and following 3 monthly cycles of therapy. No PSA-specific T-cell responses were noted to the nilutamide treatment. However, 4 of the 8 vaccine patients were observed to have a minimum of a 2-fold increase in PSA-specific T-cell frequency following 3 monthly vaccinations. One patient had a greater than 9-fold response. Two patients on the vaccine arm were evaluated for immunologic responses to the vaccine following multiple monthly fowlpox-PSA boosts. Patient LS2149 had a nearly 17-fold increase in PSA-specific T-cell precursors following 14 months of treatment and patient DS7075 mounted a nearly 15-fold increase following 11 months of treatment (**(20)**).

Diversified Prime and Boost Regimens

Preclinical studies have demonstrated the advantage of a prime vaccination with recombinant vaccinia and boost with a recombinant avipox as compared to the continued use of either vector alone.(**(21)**;**(22)**) A clinical trial in patients with metastatic CEA-expressing carcinomas has subsequently indicated an immunologic benefit of priming with rV-CEA and giving multiple boosts with avipox-CEA vaccine.(**(23)**) This pilot trial also suggested clinical benefit with this strategy.(**(24)**) Patients were randomized to receive vaccinations priming with vaccinia-CEA (V) and boosting with 3 monthly avipox-CEA (A) vaccinations (designated VAAA regimen), or they received the 3 monthly avipox-CEA vaccinations followed by a fourth rV-CEA vaccine (designated AAAV regimen). In each group, patients were evaluated for immunologic responses using the overnight ELISPOT assay. Patients with clinical responses to their regimen continued to receive multiple boosts with avipox-CEA. After 2+ years of follow-up, 6 out of 9 patients

randomized to the VAAA regimen exhibited stable disease with some patients receiving up to 24 monthly vaccinations. All 9 of the patients randomized to the AAAV arm had progressed at the 2-year follow-up. The results of the comparison in survival of these two groups were statistically significant (p=0.05). Furthermore, there was a statistically significant correlation between CEA-specific immunologic responses and overall survival.((24)) Although this was a hypothesis-generating trial that was done in different tumor types, we believe the above clinical data, the data obtained in the ECOG trial comparing PSA vaccines (VAAA vs. AAAA vs. AAAV)((25);(26)), and the substantial preclinical data support the hypothesis that priming with vaccinia followed by boosting with a fowlpox vector is likely to be more potent than the use of either vaccine alone. We thus plan to use rV-PSA(3A)/TRICOM as a prime vaccination and rF-PSA(3A)/TRICOM as booster vaccinations.

Recombinant Human GM-CSF as a Biologic Adjuvant

To further enhance the response of the immune system to a vaccine, therapy has often involved the co-administration of an adjuvant with the vaccine. Adjuvants change the character and number of APCs in the area of the vaccination, act as a depot for the vaccine thus prolonging the time the antigen is presented to APCs, or alter the pathway by which the protein being presented is processed. Cytokines are of particular interest because they may affect specific arms of the immune system.

GM-CSF has a variety of effects on the immune response. GM-CSF upregulates class II MHC expression on macrophages, enhances the maturation of dendritic cells (DC), stimulates DC migration, and produces a localized inflammatory response at the site of injection and a systemic response in the bone marrow. GM-CSF has been shown in numerous preclinical and clinical trials to enhance primary immune responses due to enhanced APC efficiency.((27)-(30))

Several clinical trials sponsored by the NCI have been completed employing poxvirus-based vaccines with GM-CSF, and others are ongoing to study the safety of rF-GM-CSF in humans. The study of ALVAC-CEA/B7.1 with GM-CSF revealed that the addition of 250 micrograms of GM-CSF as an immunologic adjuvant was well tolerated.((31)) This trial has enrolled 55 patients in the GM-CSF arm and noted 6 patients with grade 2 toxicities that included transient elevations of hepatic transaminases and leukopenia. There was no renal toxicity. In an ongoing trial using rV-PSA, rV-B7.1, rF-PSA with GM-CSF and low-dose IL-2 in patients with localized prostate cancer undergoing radiation therapy (RT), the data from 29 patients reveal only 1 transient creatinine elevation and proteinuria in two patients related to radiation therapy.((32)) In another study using rV-PSA, rV-B7.1, rF-PSA with GM-CSF and low-dose IL-2 vs. hormones in metastatic prostate cancer patients, only one transient (1 week) elevation of serum creatinine, 2 proteinurias and 1 significant hematuria—secondary to the patient's pre-existing bladder outlet obstruction were noted in a total of 31 patients.((33))

In 2010, Gulley et al. published the results the phase II trial of PSA-TRICOM in patients with metastatic castration resistant prostate cancer (Gulley JL. Cancer Immunol Immunother. 2010). This trial was designed to evaluate the role of GM-CSF with PSA-TRICOM. While this was a small study (n=32), surprisingly, it appeared that the proportion of patients responding immunologically and the magnitude of their response was similar regardless of whether they

received GM-CSF or not. In this trial, overall survival was also analyzed. There was no suggestion that patients who did not receive GM-CSF had worse survival.

Given uncertain benefit of GM-CSF, GM-CSF is being evaluated in a randomized phase 3 clinical trial (NCT01322490). The absence of high level evidence limits the enthusiasm for its use. Therefore, sargramostim (GM-CSF) will be given to patients enrolled at the NCI site only.

As of amendment P, sargramostim (GM-CSF) will no longer be given to patients enrolled at the NCI site because there has not been found to be definitive benefit of the Sargramostim injections to this point. Other studies using the RF-PSA TRICOM are not currently using Sargramostim injections; the subjects in the multicenter sites are also not required to take/give Sargramostim.

TRICOM

Costimulatory molecules are critical in the generation of T-cell responses, especially when weak immunogens such as tumor-associated antigens are being employed. The initiation of an immune response requires at least two signals for activation of naïve T cells by APCs. The first signal is antigen specific, delivered through the T-cell receptor via the peptide/MHC, and causes the T cell to enter the cell cycle. The second, "costimulatory," signal is required for cytokine production and proliferation. Without this second signal, the antigen-specific T cell may undergo anergy or apoptosis. Tumor cells lack costimulatory molecules and thus in the natural states are poor APCs for T cells. At least three distinct molecules normally found on the surface of professional APCs have been reported to be capable of providing the second signal critical for T-cell activation: B7-1, ICAM-1, and LFA-3. Both antigen and costimulatory molecules must be expressed in the same cell to properly engage the TCR and costimulatory receptor. In order to achieve this, multigene constructs using pox viral vectors (avipox and vaccinia) have been generated. These vectors contain the costimulatory molecule transgenes B7-1, ICAM-1, and LFA-3, and have been given the designation TRICOM (TRIad of COstimulatory Molecules).

Preclinical studies using TRICOM constructs have shown them to be superior to those constructs that contain one or two of the costimulatory molecules.(**(34)-(36)**) T-cell proliferation and anti-tumor immunity using recombinant vaccinia virus co-expressing murine TRICOM were much greater than the sum of responses seen using vaccinia virus expressing individual costimulatory molecules. In addition, CEA-Tg mice immunized with CEA-TRICOM vectors exhibited greater immune responses and anti-tumor responses than mice immunized with CEA or CEA-B7.1 vectors.(**(35)**)

Agonist Epitopes

Cancer immunity in humans may depend on the development of an effective immune response directed to "self" molecules. This, of course, has the inherent problem of breaking "tolerance" in order to generate and propagate specific T cells directed against "self" tumor-associated antigens. In an attempt to circumvent this situation, novel peptides have been constructed to increase the immune response directed against "self" antigens. Our previous studies have demonstrated that the PSA molecule contains an epitope capable of eliciting PSA-specific cytolytic T cell responses in vitro.(**(37)**;**(38)**) This 9-mer peptide has been designated PSA-3. In clinical trials using the rV-PSA vaccine, it was demonstrated that patients can mount an immune response post-vaccination to the PSA-3 epitope.(**(39)**;**(40)**)

PSA(L155) incorporates a single amino acid substitution of a leucine for an isoleucine at position

number 155 in the native PSA-3 epitope. When compared with the native PSA-3 epitope, PSA(L155) demonstrated enhanced binding to the MHC-class I A2 allele. In addition, T cells activated with the PSA(L155) peptide showed higher levels of lysis of human prostate cancer cells than those generated with the native peptide.((41)) Levels of interferon-gamma are traditionally used to assess T-cell stimulation status. T-cells stimulated with DC pulsed with the PSA(L155) peptide also produced almost 4-fold higher levels of IFN-gamma than DC pulsed with the native PSA-3 peptide. IL-2 production was also increased by a similar amount in the PSA(L155) generated T cells vs. the PSA-3 generated T cells. Recombinant vaccinia viruses were also constructed containing the entire PSA transgene with and without the single amino acid substitution that constitutes the PSA(L155) epitope. DC infected with the recombinant vector containing the agonist amino acid change within the entire PSA gene (designated rV-PSA(L155)) were more effective than DC infected with the rV-PSA vector in enhancing IFN-gamma production by T cells and in the induction of T cells capable of killing cells expressing native PSA.((42))

The advantage of using agonist epitopes has now been demonstrated in clinical trials. In patients with metastatic melanoma, objective clinical responses were demonstrated in patients vaccinated using an agonist epitope to the gp-100 melanoma associated antigen given in combination with IL-2.((43);(44)) In addition, a higher proportion of patients generated an immune response against the native gp-100 when given the vaccine with the agonist epitope than one with the native epitope.((44)) An agonist peptide epitope to CEA has been shown to have clinical activity in patients with advanced CEA-expressing tumors.((45)) Patients received 2 monthly vaccinations with DC loaded with the CEA agonist peptide. Two of 12 patients experienced complete responses (CR), one patient had a mixed response, and two had stable disease (SD). Clinical response in this trial correlated with CEA-specific T-cell responses.((45)) Still, while there have been increases seen in tumor-associated antigen specific T-cells seen in the majority of patients on these trials, it is only a minority of patients with advanced disease that have sustained clinical benefit. It is possible that by further manipulation the immune response could be strengthened leading to a more robust clinical response seen in a higher proportion of patients.

1.2.3 Safety Considerations

Preclinical Safety of Recombinant Poxvirus-Based Vaccines

Preclinical immunotoxicology studies with pox-TRICOM vectors have previously been reported.((46)) CEA-Tg mice were injected with tumor cells that formed experimental metastasis. Fourteen days after tumor transplant, mice were vaccinated with rV-CEA-TRICOM s.c., followed by 3 weekly boosts of s.c. rF-CEA-TRICOM. Mice also received GM-CSF with the vaccinations and subsequent IL-2. Control mice with tumors were not vaccinated and died within 10 weeks. Immunotoxicology was performed at 1-year post-tumor transplant in mice cured of tumors, as well as in healthy age- and sex-matched controls. Parameters examined included: (a) In-Life Body weight, (b) Histopathology-50 Tissues per mouse, (c) Urinalysis-11 parameters, (d) serum chemistry-9 parameters, and (e) blood chemistry-7 panels. Neo-antibodies were studies specific for: B7.1, ICAM-1, LFA-3, GM-CSF, CEA, Vaccinia, and Fowlpox.Scl-70, ssDNA, dsDNA, Sm/nRNP, Histone, and CIC were studied for autoantibodies. No differences were found in any category between the treated group and the control group.((47)) Tissues

examined after treatment of CEA-Tg mice as outlined above showed no pathological effect coincident to the positive therapeutic effect of being cured of a CEA-positive tumor.

The Toxicology Branch of the NCI conducted a study of rV-PSA, replicating poxvirus, in rhesus macaques.(**(48)**) The prostate gland of the rhesus is structurally and functionally similar to the human prostate; furthermore, there is 94% homology between the amino acid sequences of rhesus and human PSA. Three groups of four male rhesus monkeys were vaccinated by skin scarification once every 4 weeks for 3 vaccinations with nonrecombinant vaccinia virus (TBC-Wy) at 10^8 pfu/vaccination or with rV-PSA at 1 x 10^7 or 1 x 10^8 pfu/vaccination. Vaccination-associated clinical observations during the first 3 months of the study were limited to erythema at the vaccination site, regional lymphadenopathy, and low grade fever, which are typical symptoms following vaccinia inoculation. There was no evidence of any serious adverse effects. A complete necropsy with histopathology was performed on each animal. No treatment-related effects were noted in any of the parameters measured in this study.

Clinical Safety of Recombinant Poxvirus-Based Vaccines

Since 1991, 10 recombinant vaccinia-based vaccines and 8 recombinant fowlpox-based vaccines produced by Therion Biologics Corp. for the treatment of various cancers have been evaluated in human clinical trials sponsored by CTEP, DCTD, NCI. Over 700 cancer patients, most with metastatic disease, have been treated to date with these poxvirus–based vaccines in 29 CTEP sponsored or Therion Biologics Corporation sponsored clinical trials.(49) There have been no serious adverse events related to the vaccine when administered subcutaneously.(49) These trials represent a large component of the relevant safety database that supports the initiation of this proposed trial of rV- PSA(L155)/TRICOM and rF-PSA(L155)-TRICOM. Significant safety experience in humans includes: (a) vaccinia- and/or fowlpox-based vaccines safely administered by a variety of routes including intradermal (by injection or scarification), s.c., intramuscular, intravenous, and intratumoral at doses up to 2 x 10^9 pfu (vaccinia) or 6 x 10^9 pfu (fowlpox); (b) vaccinia-based and fowlpox-based vaccines containing costimulatory molecules, alone or in combination with CEA or PSA antigens, have been administered without serious adverse effects as outlined below.

Pox viral vectors containing PSA, either alone or with B-7.1 or TRICOM, have been evaluated in 9 different Phase I and II clinical studies comprising over 200 vaccinated patients with advanced prostate cancer. There have been no serious toxicities attributed to the vaccine.(**(17)**,**(19)**,**(20)**,**(23)**,**(44)**) (clinical and immunologic data to these referenced trials are presented on pages 8-12)

A completed Phase I clinical trial at Georgetown University evaluated the safety of rV- and rF-CEA(6D)/TRICOM vectors.(**(50)**) Fifty-nine patients were accrued, completing all 6 dose escalation cohorts as well as a seventh and eighth cohort with GM-CSF. Only mild treatment-related toxicity has been observed to date. Another trial at Fox Chase Cancer Center completed enrollment with 62 patients in a CEA(6D)/TRICOM based trial. A third trial at Duke University incorporates DC infected with rF-CEA(6D)/TRICOM, then given intravenously. This trial has enrolled 15 patients (as of 8-20-04). To date, we are unaware of any significant toxicity associated with these vectors, further supporting the safety profile seen in patients tested in the earlier pox viral studies.

There were two recently completed Phase I clinical trials utilizing rV- and rF-vectors containing PSA(L155)/TRICOM. In the first trial, 10 patients with androgen insensitive prostate cancer were enrolled. They received rV-PSA(L155)/TRICOM 2 x10^8 pfu s.c. on day 1 and rF-PSA(L155)/TRICOM 1 x 10^9 pfu s.c. on day 29. Patients were followed 1 month following the second vaccination. No serious toxicities were observed in any of these patients. The second trial recently completed at the NCI Clinical Center enrolled 15 patients with no DLT noted (**(23)**). A follow-up phase II clinical trial is ongoing with 29 patients enrolled and no serious toxicities attributed to vaccine seen to date (**(23)**). However, in a Therion Biologics sponsored Phase II study, one patient treated with rF-PSA(L155)-TRICOM developed grade 4 thrombotic thrombocytopenic purpura (TTP) thought to be possibly related to study drug, approximately 3.5 weeks after receiving the last dose of his vaccine. The patient had a history of hypertension, hyperlipidemia and atrial fibrillation. The patient presented with chest pain and was found to have elevated cardiac enzymes, acute renal failure and thrombocytopenia with evidence of intravascular hemolysis. He was treated for an MI and with serial plasmapheresis and hemodialysis and his myocardial infarction has resolved without sequelae and his TTP has resolved, although one month after diagnosis with TTP he continued to require hemodialysis.

1.2.4 Androgen Suppression and Immune Response

Since the seminal work of Huggins and Hodges in 1941 establishing androgen dependence of prostate cancer proliferation, androgen deprivation has served as the cornerstone in the management of metastatic prostate cancer (**(51)**). Androgen deprivation can be achieved surgically by orchiectomy, medically by a LHRH agonist such as leuprolide, or a combination of LHRH agonist plus an androgen receptor antagonist (ARA), e.g., bicalutamide for a CAB. Despite initial response rates of 80 to 90%, virtually all men progress to androgen insensitive disease. When antiandrogen withdrawal fails, one therapeutic option is the use of alternative ARA as second-line hormonal therapy (**(52)**,**(53)**). The nonsteroidal antiandrogens approved by the FDA and commonly used in the United States are flutamide (Eulexin), bicalutamide (Casodex) and nilutamide (Nilandron). They bind to the AR and inhibit the stimulatory action of testosterone and dihydrotestosterone (DHT). DHT is the primary androgen that stimulates the growth of prostate tissue including prostate cancer. It is unclear why patients may respond to a second ARA agent after initial progression, but it is presumed that specific antiandrogens interact differently with the AR on prostate cancer cells (**(54)**).

Mercarder et al. demonstrated that androgen ablative therapy induces profuse T cell infiltration of benign glands and tumors in human prostates (**(21)**). This data is discussed in detail in section **1.2.1**, page 10. The induction of T cell infiltration in prostate tissues treated with androgen ablation may have implications for the immunotherapeutic treatment of prostate cancer (**(21)**). To understand the T cell response to prostate cancer, Drake et al., demonstrated that tumorigenesis allows T cell recognition of the prostate gland--but this recognition is tolerogenic, resulting in abortive proliferation and ultimately in hyporesponsiveness at the systemic level (see section **1.2.1**, p.10 for more detail discussion). Androgen ablation was able to mitigate this tolerance--allowing prostate-specific T cells to expand and develop effector function after vaccination. These results suggest that immunotherapy for prostate cancer may be most efficacious when administered after androgen ablation (**(47)**). In the NCI 00C-0137 study we examined this concept of combining androgen ablative therapy with immunotherapy (**(20)**). Men

with hormone refractory prostate cancer with no measurable disease on scans were randomized to receive a pox vector PSA vaccine vs. the androgen receptor antagonist nilutamide. The median time to treatment failure on nilutamide was 7.6 months, with 3 of these patients coming off study secondary to drug toxicity. However, 12 patients on the vaccine arm had nilutamide added at the time of PSA progression. The median time for treatment failure after the addition of nilutamide was 13.9 months, for a total of 25.9 months from initiation of vaccine therapy. This suggests that the addition of hormone therapy following initial vaccine therapy may lead to an improved clinical benefit compared with hormone therapy alone. (**(20)**)

1.2.5 PSA Doubling Time (PSADT)

D'amico et al. have demonstrated that the change in the PSA level during the year before diagnosis has been suggested to be significantly associated with Prostate cancer specific mortality. Following radiation therapy, Prostate cancer specific death and all cause death following PSA failure were noted for patients with a short PSA DT (ie 12 months or less) (**(60)**). Lee et al. reviewed the medical records of 621 men with nonmetastatic prostate cancer treated with radiation therapy and hormone therapy between 1989 and 2003. Only hormone therapy duration ($p = 0.008$) and PSADT </=8 months (<0.001) were significantly associated with time to true clinical failure. The estimated 5-year rate of any clinical failure was 9.4% for men with a PSADT >8 months and 60.4% for men with a PSA doubling time </=8 months ($p < 0.001$) (**(61)**). Therefore, based on various reports using a PSADT of greater than 12 months or 8 months as a good prognostic indicator, we plan to stratify using greater than 10 months vs. 10 months or less to differentiate patients who are at a greater risk of disease progression in our proposed study.

1.2.6 Rationale for treating castrate-resistant, non-metastatic prostate cancer patients.

This study is based on a previous study employing an earlier version of a vector-based vaccine targeting PSA and the anti-androgen nilutamide. The results of that study suggested that the combination of anti-androgen and vaccine could improve time to progression and overall survival. (**(64)**;**(65)**). This previous trial did not require patients to have been treated with a previous anti-androgen prior to enrollment. In addition, a similar proportion of the patients who had no previous anti-androgen had a sustained response as those who had progressed on anti-androgen (**(64)**). The premise that hormonal therapy will enhance immune response as previously outlined (Section **1.2.4**) remains valid, regardless of whether patients have progressed on a previous anti-androgen or not.

Based on the original trial design in the previous trial, the estimated time to progression was 7.6 months (**(64)**). This includes both PSA progression and new metastatic lesions. Additionally, this included a proportion of patients who had not progressed on previous anti-androgens. Overall there is great variability reported in the literature for time to progression with D0.5 prostate cancer and there is no clear data on time to PSA-progression in this population (D0.5 Prostate Cancer). A large study followed over 3000 prostate cancer patients in the PSA era from Johns Hopkins. The data from 91 patients who eventually developed metastatic disease suggested that time from D0.5 disease to metastasis was only 8 months in patients treated with primarily with radical prostatectomy. (**(66)**). This is consistent with the previous study here that

demonstrated a median time to progression of 7.6 months, which included PSA progression. This time to progression (7.6 months) also served as the statistical basis for this study.

1.2.7 Rationale for treating patients previously treated with flutamide in the (neo)adjuvant setting.

Many patients have been previously treated with flutamide, but still should be considered for re-treatment with flutamide and thus should be considered for this protocol. Some patients may have been treated with this agent previously as part of a neoadjuvant or adjuvant hormonal treatment for patients undergoing primary radiation therapy. For some patients, flutamide is administered for up to 6-8 weeks in an effort to prevent a "flare" after initial GnRH agonist. Others may have discontinued flutamide as part of an intermittent treatment approach. From a clinical stand point, if the patient has not had a rising PSA on previous flutamide, they could be re-treated with this agent with reasonable expectation of response. (**(67)**) Therefore, eligible patients may have been treated with previous flutamide as long as the patient did not have a rising PSA while on flutamide. Patients will be required to be at least 1 year removed from previous flutamide exposure.

1.2.8 Summary

In this Phase II clinical trial we will seek to translate the following observations from the laboratory and prior clinical trials into the clinic in patients with advanced incurable tumors.

- Pox viral vectors can induce a PSA-specific T-cell response in patients with advanced prostate cancer.
- Heterologous prime and boost regimens are superior in terms of generalizing immune responses; and this may translate into improved clinical responses.
- The use of agonist epitopes within the TAA can induce a better immune response than native peptides and have been associated with clinical responses.
- The use of GM-CSF does not add significant toxicity and in pre-clinical models is essential for induction for optimal immune responses*.
- Hundreds of patients have been treated on clinical trials with recombinant pox viral vectors, and there have been no dose limiting toxicities attributed to these vaccines. However, on a Phase II study sponsored by Therion Biologics, one patient treated with rF-PSA(L155)-TRICOM developed grade 4 thrombotic thrombocytopenic purpura (TTP) thought to be possibly related to study drug, approximately 3.5 weeks after receiving the last dose of his vaccine.
- Androgen ablative therapy induces profuse T cell infiltration of benign glands and tumors in human prostates. T cell infiltration is readily apparent after 7-28 days of therapy
- Patients appear to have prolonged responses when a second line antiandrogen agent is added to a PSA vaccine after an initial priming with the vaccine compared to those patients receiving the antiandrogen as monotherapy.

* As of amendment P, sargramostim (GM-CSF) will no longer be given to patients enrolled at the NCI site because there has not been found to be definitive benefit of the Sargramostim

injections to this point. Other studies using the RF-PSA TRICOM are not currently using Sargramostim injections; the subjects in the multicenter sites are also not required to take/give Sargramostim.

2 ELIGIBILITY ASSESSMENT AND ENROLLMENT

2.1 Eligibility Criteria

2.1.1 Inclusion Criteria

A. Histopathological documentation of prostate cancer confirmed in the Laboratory of Pathology at the NIH Clinical Center or participating institute's Department of Pathology prior to starting this study. 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. Must have non-metastatic androgen insensitive prostate cancer with a rising PSA with castrate levels of testosterone and no evidence of metastatic disease on CT scan or bone scan. A rising PSA is defined as two consecutively rising PSA levels, separated by at least 1 month apart, with the last measurement that is > 1ng/ml. Patients on nilutamide therapy must undergo nilutamide withdrawal for at least 4 weeks and still show evidence of a rising PSA. Following treatment with bicalutamide, patients must undergo withdrawal for at least 6 weeks and still show evidence of a rising PSA.
C. Life expectancy greater than or equal to 6 months.
D. ECOG performance status of 0-1.
E. No systemic steroid or steroid eye drop use within 2 weeks prior to initiation of experimental therapy.
F. Hematological eligibility parameters (See **APPENDIX C**.)
   - Granulocyte count ≥1,500/mm^3
   - Platelet count ≥100,000/mm^3
   - Hgb ≥ 9 Gm/dL
   - Lymphocyte count ≥ 500/mm^3.
G. Biochemical eligibility parameters (within 16 days of starting therapy)
   - Hepatic function: Bilirubin ≤ 1.5 mg/dl, OR patients with Gilbert's syndrome, a total bilirubin ≤ 3.0 mg/dL, AST and ALT< 2.5 times upper limit of normal
H. No other active malignancies within the past 3 years (with the exception of non-melanoma skin cancers or carcinoma in situ of the bladder) or life threatening illnesses.
I. Willing to travel to the NIH for follow-up visits.
J. 18 years of age or greater.
K. Able to understand and sign informed consent.
L. Must agree to use effective birth control (such as a condom) or abstinence during and for a period of 4 months after the last vaccination therapy. Patients must be willing to remain on chemical castration therapy, unless they have had surgical castration.
M. Patients must have recovered from acute toxicities related to prior therapy or surgery.
N. Parameters for assessment of baseline renal function:
   - Serum creatinine ≤ 1.5 x upper limit of normal OR creatinine clearance on a 24-h

urine collection of ≥ 60 mL/min.

2.1.2 Exclusion Criteria

A. Patients should have no evidence of being immunocompromised as listed below.
   - Human immunodeficiency virus positivity due to the potential for decreased tolerance and may be at risk for severe side effects
   - Concurrent use of topical steroids (including steroid eye drops) or systemic steroids. Nasal or inhaled steroid use is permitted
   - Patients who have undergone allogenic peripheral stem cell transplantion or solid organ transplantation requiring immunosuppression

B. Patients who test positive for active Hepatitis B or Hepatitis C infection

C. Patients should have no autoimmune diseases that have required treatment such as, Addison's disease, Hashimoto's thyroiditis, or systemic lupus erythematous, Sjogren syndrome, scleroderma, myasthenia gravis, Goodpasture syndrome, active Grave's disease.

D. History of allergy or untoward reaction to prior vaccination with vaccinia virus or to any component of the vaccinia vaccine regimen.

E. 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) are: persons with active or a history of eczema or other eczematoid skin disorders; those with other acute, chronic or exfoliative skin conditions (e.g., atopic dermatitis, burns, impetigo, varicella zoster, severe acne, or other open rashes or wounds) until condition resolves; pregnant or nursing women; children 3 years of age and under; and immunodeficient or immunosuppressed persons (by disease or therapy), including HIV infection. See **APPENDIX B**.

F. Serious intercurrent medical illness (e.g., one that requires treatment) which would interfere with the ability of the patient to carry out the treatment program, including, but not limited to, inflammatory bowel disease, Crohn's disease, ulcerative colitis, or active diverticulitis.

G. Patients with cardiac disease that have fatigue, palpitation, dyspnea or angina with ordinary physical activity (New York Heart Association class 2 or greater) are not eligible.

H. Patients with a history of congestive heart failure or who have objective evidence of congestive heart failure by physical exam or imaging are not eligible.

I. Patients with pulmonary disease that have fatigue or dyspnea with ordinary physical activity are not eligible.

J. Concurrent chemotherapy.

K. No known brain metastasis, or with a history of seizures, encephalitis, or multiple sclerosis.

L. Patients with a serious hypersensitivity reaction to egg products are not eligible.

M. Prior splenectomy.

N. Patients who have received prior flutamide therapy in the last year. (Patients treated with flutamide in the neoadjuvant or adjuvant setting or those previously treated with flutamide who did not have a rising PSA on treatment would be allowed to enroll on the protocol.)

2.2 Research Eligibility Evaluation

A. The following tests may be obtained anytime prior to enrollment:
   1. Pathological confirmation of diagnosis and PSA expression in the Laboratory of Pathology at NIH Clinical Center or participating institute's Department of Pathology prior to starting this study will be required. However, 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 start of enrollment:
   1. HIV test
   2. Hepatitis B and C

C. The following parameters will be obtained within 28 days prior to start of enrollment:
   1. Tc-99 whole body scintigraphy
   2. CT of chest/abdomen /pelvis
   3. 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

D. The following parameters will be obtained within 16 days prior to start of treatment:
   1. Clinical Evaluation
      - History and physical examination
      - ECOG performance status (see **APPENDIX A**)
      - Height, Weight

   2. Laboratory studies
      - Complete blood count plus differential and platelet count
      - Acute care panel (electrolytes, BUN, creatinine)
      - Liver panel (AST/ALT/total bilirubin)
      - Alkaline phosphatase, LDH
      - Urinalysis, 24 hour urine collection to evaluate creatinine clearance, proteinuria, and urine electrolytes
      - ANA
      - Serum PSA and PAP
      - Lymphocyte phenotyping CD4/CD8 (This will be performed for patients enrolled at the NCI site only)

2.3 Patient Registration and Treatment Randomization

All patients must have completed an eligibility checklist. Projected PSA doubling time is a stratification factor in the randomization (see formula in Section **5.4**). Patients must be registered with Central Registration Information Services within 24 hours of signing the consent. Authorized staff must register patients by faxing the eligibility checklist from the web site (http://home.ccr.cancer.gov/intra/eligibility/welcome.htm) to the Central Registration Office at (301) 480-0757.

For randomization, authorized staff should call 301-402-1732 between the hours of 8:30 a.m. and 5:00 p.m., Monday through Friday. A recorder is available during non-working hours.

For Participating site Registration:

All patients must be registered through the NCI Central Registration Office (CRO). The CRO is open from 8:30am to 5:30pm EST Monday through Friday, excluding federal holidays. A protocol registration form and cover memo will be supplied by the Coordinating Center, NCI CCR and updates will be provided as needed. Subject eligibility and demographic information is required for registration. To register a subject, fax the completed registration checklist and cover memo to the CRO at 301-480-0757. Please indicate on the protocol registration form whether the patient is screening or is eligible to start treatment. The CRO will notify you either by e-mail or fax that the protocol registration form has been received. The CRO will assign a unique patient/subject ID number for each subject that will be used to enter data into the C3D data base. Questions about eligibility should be directed to the Coordinating Center's Research Nurse, Sheri McMahon, 301-496-9812, sheri.mcmahon@nih.gov. Technical questions about the form should be directed to the Central Registration Office (301-402-1732).

A copy of the registration checklist form should be faxed to Coordinating Center Research Team at 301-480-1779.

3 STUDY IMPLEMENTATION

3.1 Study Design (see Schema)

This randomized Phase II study proposes to evaluate the role of second line antiandrogen therapy and a pox vector based PSA vaccine in a population of patients with prostate cancer who have a rising serum PSA despite castrate levels of testosterone and prior antiandrogen therapy but no metastatic disease on bone or CT scans. Patients will be randomized on a 1:1 basis to Arm A (flutamide alone) or Arm B (flutamide + vaccine).

Arm A: Flutamide 250 mg orally TID beginning on day 1.

Arm B: Flutamide 250 mg orally TID beginning on day 1.
PROSTVAC-V/TRICOM (vaccinia) 2 x 10^8 pfu subcutaneously on day 1 of the first cycle only, followed by monthly (every 4 week) boosting vaccinations with PROSTVAC-F/TRICOM (fowlpox) 1 x 10^9 pfu subcutaneously on day 1 of each cycle. The duration of each treatment cycle is approximately 28 days.

Patients may continue on the therapy they were randomized to until the development of measurable disease on scans, rising serum PSA, or if they develop toxicity requiring stopping of their treatment. For patients with declining PSA no restaging will be done unless they develop symptoms consistent with metastatic disease. For patients with rising PSA, once 2 consecutive

PSA rises are seen from the last imaging studies, CT and bone scans will be done at their next scheduled visit. They will then be re-staged (CT and bone scans) at 3 month intervals as long as PSA continues to rise.

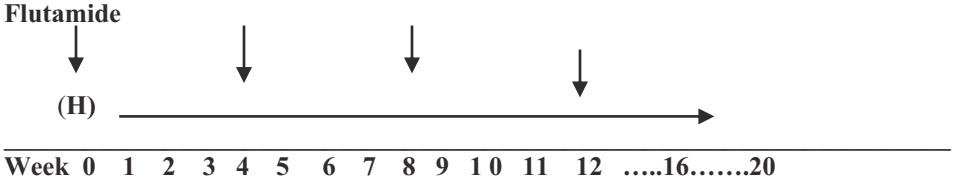
1. Patients on either arm who develop clinical progression (i.e. evidence of metastatic disease on scans) will be taken off study.

2. Patients who do not develop clinical progression (as defined above), but develop biochemical recurrence (i.e rising serum PSA levels) will undergo the following:

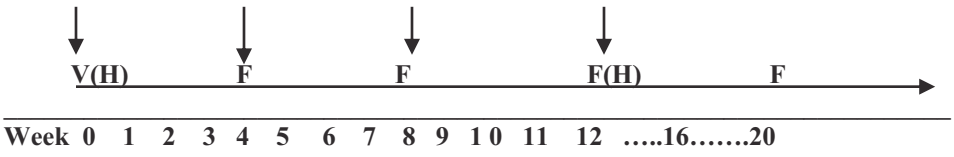
- After 3 months of therapy, patients randomized to the flutamide alone (arm A) may cross over to receive vaccine if they develop a rising PSA and scans are without metastatic disease. The vaccine may commence 4 weeks after flutamide is stopped (if the PSA continues to rise. If there is an antiandrogen withdrawal response (a decline in PSA 28 days after the discontinuation of flutamide), PSA serum levels will be checked every 28 days and vaccine may commence when the serum PSA levels begin to rise (if scans are negative for metastatsis and if patients continue to meet the eligibility criteria as outlined in section **2.1**.
- Patients randomized to Arm B will have flutamide discontinued, but may continue to receive vaccine treatment until the development of disease on scans or a second occurrence of rising PSA levels in the absence of clinical progression.

3. If patients continue to develop biochemical progression following the maneuvers outlined in #2 above, they will come off-treatment, but can remain on study until the development of metastatic disease on scans.

Arm A (n=33)
Flutamide



Arm B (n=33)
Vaccine (monthly) + Flutamide (daily)



Stratification of patients- projected PSA doubling time of >10 months vs ≤ 10 months (based upon last 2 serum PSA levels prior to enrollment 1 month apart)

At week 12, restaging CT Chest/Abdomen/Pelvis and Bone scans will be performed in all patients. For patients with declining PSA no restaging will be done unless they develop

symptoms consistent with metastatic disease. For patients with rising PSA, once 2 consecutive PSA rises are seen from the last imaging studies, CT and bone scans will be done at their next scheduled visit. They will then be re-staged (CT and bone scans) at 3 month intervals as long as PSA continues to rise. Patients may continue on therapy if scans do not demonstrate metastastic prostate cancer. For patients who have been on study for 2 years or more with stable disease and who are not getting vaccine, clinic visits may be scheduled at 8 week intervals. For these patients who have rising PSA, once 2 consecutive PSA rises are seen, a CT and bone scan will be done at their next visit in 4 weeks. They will then be restaged (CT and bone scans) at 3 month intervals as long as PSA continues to rise.

Legend
H = **Flutamide** 250 mg po t.i.d, commencing on day 1 in both arms
V= PROSTVAC-V /TRICOM 2 x 10^8 pfu s.c
F= PROSTVAC-F /TRICOM 1 X 10^9 pfu s.c

↓ Blood draw for Immunologic studies- a. Elispot assay (see section **3.4.6** and **APPENDIX C**).

3.2 Drug Administration

Patients will receive vaccines at the NIH Clinical Center or other participating institution (see special handling procedures, section **8**). Study drugs will be prepared and placed in syringes by the Clinical Center Pharmacy personnel at the NIH or Institution's Pharmacy Designates at the participating institution. Please section **3.1** for dose, schedule and route of administration.

3.2.1 Precautions

- Prior to administration of the drugs, safe handling precautions should be thoroughly reviewed (see precautions and special handling subsections of section **8**, "Pharmaceutical Information").
- The proper procedure for disposing the live vaccine is a critical part of drug administration (see the disposal sections of section **8**, "Pharmaceutical Information"
- Patients must be instructed and receive a copy of the "Patient Instruction Sheet" (see **APPENDIX B**).

3.3 Treatment Modifications

Vaccine Dose Modification: None

Flutamide Dose Modification: None

Criteria for individual patient retreatment
Patients with grade 3 non-autoimmune toxicity due to the treatment regimen may resume treatment provided that the toxicity has decreased to baseline or grade 1 toxicity within 42 days of scheduled retreatment. Patients with grade 4 toxicity will not be treated.

Patients with ≥ grade 3 autoimmune toxicity will not be treated and will be removed from study.

3.4 Protocol Evaluation: (See APPENDIX C)

3.4.1 All patients who are deemed eligible and who sign the informed consent form will be enrolled onto this trial.

3.4.2 A complete history and physical examination including ECOG performance status shall be done within 16 days of enrollment.

3.4.3 Laboratory studies (within 16 days prior to on-study date)

- Serum PSA and PAP
- CBC/differential, with platelet count
- Serum chemistries (Na+, K+, Cl-, CO_2, glucose, BUN, creatinine, alkaline phosphatase, ALT, AST, total bilirubin, LDH)
- Hepatitis B, C / HIV panel (within 8 weeks prior to day 1)
- Urinalysis
- EKG

3.4.4 Immunologic Parameters

- IFN-gamma ELISPOT assay for PSA-specific T lymphocytes (HLA-A2 patients only)
- Antibodies to PSA, vaccinia, fowlpox, and ANA titer
- 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
- HLA class I expression (HLA typing) with A2 subtyping (obtain anytime prior to enrollment)
- 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.

3.4.5 Radiographic examinations

Radiologic studies consisting of bone scan, CT scan of chest/abdomen/pelvis will be performed at baseline. For patients with declining PSA no restaging will be done unless they develop symptoms consistent with metastatic disease. For patients with rising PSA, once 2 consecutive PSA rises are seen from the last imaging studies, CT and bone scans will be done at their next scheduled visit. (Therefore, restaging scans would be done at an interval no less than 3 months.) They will then be re-staged (CT and bone scans) at 3 month intervals as long as PSA continues to rise.

3.4.6 Collection of Resarch (Immunologic) Blood Samples – Only for patients enrolled at the NIH*

Research blood will be drawn at baseline, prior to enrollment and at every monthly visit for every patient on the study. In addition, patients who are HLA-A2 will have apheresis requested for immunologic testing at baseline and around weeks 12, 24 and 36 while on vaccine as described in section **5.2.5**. Furthermore, up to 15 patients on Arm A (flutamide alone) who go on to receive vaccine will have apharesis performed prior to and around weeks 12, 24 and 36 while on vaccine. Apharesis will allow us to obtain sufficient PBMC samples from patients to be able to peform not only the standard Elispot using the PSA-3 peptide, but to also look at other epitopes that may be important in evaluating an antigen cascade effect from the therapy administered. This will allow us to gain important immunologic information to determine if patients are not only mounting immune responses to the antigens in the vaccine, but to also immune responses to other antigens present on the tumor cells. At the time points when apheresis in not obtained, we will obtain 6 green top (10ml) tubes. These will be used for ELISPOT assays (as described in **APPENDIX E**). In addition, 2 SST (tiger) top tubes will be drawn for antibody testing (as described in **APPENDIX D**).

*As of amendment P, all immunologic studies will be optional and at PI discretion.

Blood samples may be used for other research studies which may include phenotypic and functional analysis of immune cell subsets, and analysis for cytokines, chemokines, antibodies, tumor-associated antigens and / or other markers.

Immunologic blood samples will be processed at:

Clinical Services Program
NCI Frederick Cancer Research and Development Center
PO Box B
Frederick MD 21702
301-846-1707

On days samples are drawn, Jennifer Bangh at CSP should be notified (phone: [301] 846-5893; fax [301] 846-6222). She will arrange courier delivery of the specimens to the processing lab. The weekly patient lists of samples drawn will be emailed to Sandra Doren at dorens@mail.ih.gov and Jen Bangh at jb478s@nih.gov and Theresa Burks (burkst@mailnih.gov).

3.4.7 Storage and Tracking of Collected Blood Samples

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 the Leidos Biomedical Research, Inc. 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.

American Type Culture Collection (ATCC) manages the NCI Frederick Central Repositories under subcontract to Leidos Biomedical Research, Inc., Frederick, Inc. NCI Frederick Central Repositories store, among other things, biological specimens in support of NIH clinical studies.

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

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

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

Sample data is stored in the BioSpecimen Inventory System (BSI) II. This inventory tracking system is used to manage the storage and retrieval of specimens as well as maintain specimen data. BSI is designed for controlled, concurrent access. It provides a real-time, multi-user environment for tracking millions of specimens. The system controls how and in what order database updates and searches are performed. This control prevents deadlocks and race conditions. For security, BSI has user password access, 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 **APPENDIX E**. All specimens for analysis will be requested from Leidos Biomedical Research, Inc. and will be delivered by Leidos Biomedical Research, Inc. couriers to the Laboratory of Tumor Immunology and Biology.

3.4.8 Protocol Completion/Sample Destruction

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

Samples and associated data will be stored permanently unless the patient withdraws consent. The PI will report destroyed samples to the IRB if samples become unsalvageable or destroyed

by environmental conditions (i.e., 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.

3.5 Concurrent Therapies

Patients who have not undergone bilateral surgical orchiectomy will be required to stay on a gonadotropin releasing hormone agonist or antagonist. This also applies to patients who have PSA progression and are taken off of flutamide. However, concurrent anticancer treatment with chemotherapy, other hormonal therapies, alternative medicines, systemic glucocorticoids (topical and inhaled steroids allowed), radiation therapy, major surgical procedures or non-protocol related immunotherapy will not be permitted.

3.6 Off Study Criteria

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

Off Study Criteria: Progressive Disease, Patient's Decision, Toxicity, Medically Indicated, and PI Discretion

1. Clinical progression of disease as described in section **5.2**.
2. 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. In this event, the reasons for withdrawal will be documented.
3. Patient's request to be taken off study. In this event, the reasons for withdrawal will be documented.
4. If patients are non-compliant with the protocol guidelines, they may be removed from the study at the discretion of the principal investigator.
5. Any Grade 4 toxicity that is possibly, probably or definitely related to drug will require a patient to be off-treatment.
6. Any Grade 3 treatment non autoimmune related toxicity that does not resolve to Grade 1 or baseline within 42 days and is possibly, probably or definitely related to drug will require a patient to be off-treatment.

3.7 Off-Study Procedures

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

For Participating Sites:

All subjects must be registered through the NCI Central Registration Office (CRO). The CRO is open from 8:30am to 5:30pm EST Monday through Friday, excluding federal holidays. An off-

study form will be supplied by the CCR study coordinator. Send the completed off-study form to the CCR study coordinator.

3.8 Post-Treatment Evaluation

The Biologic Response Modifiers Advisory Committee has recommended that long-term follow-up extend over a period of 15 years. Relevent information will be reported to the FDA. Patients will be enrolled onto the "Follow-Up Study of Subjects Previously Enrolled in Poxviral Vector Gene Transfer Studies" once off treatment. For patients enrolled at participating Institutions other than the NIH, attempts will be made to enroll patients on this follow up protocol using telephone enrollment as described in the Long Term Follow Up protocol (04-C-0274).

4 SUPPORTIVE CARE

For both the administration of vaccine and flutamide, antiemetics, stool softeners and anti-diarrheal agents may be administered as required, but are not anticipated to be needed and should not be used prophylactically on the first cycle. The selection of the specific antiemetic regimen is at the discretion of the treating physician. Antiemetic regimens should 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/mm^3. If invasive procedures are planned or the patient develops bleeding, platelet transfusions should be administered in accordance with the standard of practice, usually maintaining a platelet count of > 50,000/mm^3.

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

4.1 Treatment of Vaccinia Vaccination Complications

4.1.1 Vaccinia Immune Globulin (VIG):

First-line treatment of some of the complications of vaccinia caused by dissemination of vaccinia virus (severe cases of inadvertent inoculation involving extensive lesions or if comorbid conditions exist, severe cases of generalized vaccinia in patients that are systemically ill and

whose condition might be toxic or who have serious underlying immunosuppressive illnesses, eczema vaccinatum, and progressive vaccinia) is with VIG.

VIG is contraindicated, however, for the treatment of isolated vaccinial keratitis. VIG is a sterile solution of the immunoglobulin fraction of pooled plasma from individuals inoculated with vaccinia vaccine. VIG is 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.

4.1.2 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].

The CDC has informed the NCI/CTEP that cidofovir will not be supplied through Strategic National Pharmaceutical Stockpile to investigators involved in CTEP-sponsored protocols utilizing recombinant vaccinia-based vaccines. This agent will only be provided by the CDC in

the occurrence of an emergency public health event. Thus, investigators should obtain cidofovir for second-line therapy through commercial sources if necessary. NCI/CTEP investigators may use the CDC cidofovir IND protocol as a "guideline" when providing cidofovir for treatment under an off-label use. The CDC will provide their IND protocol for the use of cidofovir related to adverse reactions post vaccinia vaccination to NCI/CTEP for distribution to investigators of NCI-sponsored protocols upon request. The CDC Clinician Information Line at 1-877-554-4625 should still be consulted regarding appropriateness of therapy and guidance.

5 DATA COLLECTION AND EVALUATION

5.1 Data Collection

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

5.1.2 Data will be secured in the Cancer Central Clinical Database (C3D) based on software produced by Oracle Corporation. Data will be collected using protocol-specific case report forms, verified for accuracy and completeness, and submitted to Clinical Data Update System (CDUS) through the file transfer protocol (FTP) from C3D every 2 weeks. 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 Harris Orkand Information Services). A copy of all serious AE forms will be kept in the regulatory binder.

Multi-center participating site will enter data remotely into web-based C3D system. The site's investigator is responsible for maintaining all source documentation related to the study including films/CDs, ECG, and other records.

The coordinating center (NCI) is responsible for compiling and submitting CDUS data to CTEP for all participants and for providing data to the Principal Investigator for review.

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

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

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

5.1.6 Drug Accountability Records are kept for each patient.

The PI will be responsible for overseeing entry of data into an in-house password protected electronic system and ensuring data accuracy, consistency and timeliness. The principal investigator, associate investigators/research nurses and/or a contracted data manager will assist

with the data management efforts. All data obtained during the conduct of the protocol will be kept in secure network drives or in approved alternative sites that comply with NIH security standards. Primary and final analyzed data will have identifiers so that research data can be attributed to an individual human subject participant.

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

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

5.2 Response Crite ria

5.2.1 Methods of Measuring Disease Progression (note: patient's must have non-metastatic disease as an eligibility criteria-see **2.1.1** B Inclusion Criteria. New lesions by RECIST Criteria will be for definition of disease progression. Rising serum PSA levels will also be used for determining disease progression-see **5.2.1.3** and **5.2.2**)

All patients in this study must be assessed for response to treatment, even if there are major treatment deviations. Each patient will be assigned one of the following categories: 1) complete response; 2) partial response; 3) stable disease; 4) progressive disease; and 5) not evaluable (early death from malignant disease, early death from toxicity, early death due to other causes, or unknown-not assessable, insufficient data). This protocol will use CTEP's Response Evaluation Criteria on Solid Tumors (RECIST) for assessing response. A quick reference to the RECIST guidelines can be downloaded at the following URL: http://ctep.cancer.gov/guide. A copy of this quick reference is found in D.

For patients with declining PSA no restaging will be done unless they develop symptoms consistent with metastatic disease. For patients with rising PSA, once 2 consecutive PSA rises are seen from the last imaging studies, CT and bone scans will be done at their next scheduled visit. (Therefore, restaging scans would be done at an interval no less than 3 months.) They will then be re-staged (CT and bone scans) at 3 month intervals as long as PSA continues to rise.

1. Patients on either arm who develop clinical progression (i.e. evidence of metastatic disease on scans) will be taken off study.

2. Patients who do not develop clinical progression (as defined above), but develop biochemical recurrence (i.e rising serum PSA levels) will undergo the following:

- Patients randomized to Arm A will have flutamide discontinued and may begin to receive the vaccine as described above following a 4 week delay to determine if there is evidence of an antiandrogen withdrawal response (if they continue to meet the eligibility criteria as outlined in section **2.1**)
- Patients randomized to Arm B will have flutamide discontinued, but may continue to receive vaccine treatment.

3. If patients continue to develop biochemical progression following the maneuvers outlined in #2 above, they will come off-treatment, but can remain on study until the development of metastatic disease on scans.

5.2.1.1 CT and MRI are the best currently available and reproducible methods to measure target lesions selected for response assessment. Conventional CT or MRI should be performed with cuts of 10 mm or less in slice thickness contiguously. Spiral CT should be performed using a 5 mm contiguous reconstruction algorithm.

5.2.1.2 Due to the high reported false positive rates of bone scans (30%), patients with a falling PSA (see section 5.2.2.2) with new lesions on bone scan not clinically determined to be related to metastasis (i.e. trauma), and no other evidence of disease progression may remain on trial until the progressive metastatic nature of these lesions has been confirmed on subsequent bone scans.

5.2.1.3 PSA serum levels will be used as a method of measuring disease response or progression. After the initial 3 month evaluation, if a patient has a rising serum PSA level (see section **5.2.2.2**) but no evidence of metastatic disease, patients in Arm A will have flutamide discontinued and may begin to receive vaccine alone as described above, since the standard care would be discontinuation of flutamide for rising serum PSA. Those patients who have rising serum PSA levels on Arm B without evidence of metastatic disease will have flutamide discontinued, but may continue to receive vaccine treatment until the development of disease on scans or a second occurrence of rising PSA levels in the absence of clinical progression. If the serum PSA begins or continues to rise off flutamide (Bubley criteria, JCO 1999), vaccine therapy will be discontinued. Since there is no standard of care aside from antiandrogen discontinuation for these patients and they would have already received 2nd line therapy, patients can stay on study until there is evidence of disease on bone scan or CT scan.

5.2.2 Biochemical Responses

5.2.2.1 **PSA responses will be determined as below:**

5.2.2.1.1 Complete response PSA < 0.2 confirmed by a second reading at least 4 weeks later.

5.2.2.1.2 PSA decline of > 50%.
All patients with a decline of PSA of at least 50% (confirmed by a second value at least 4 weeks after the first) and who have no other evidence of disease progression will be reported in this category. Patients will be reported with both PSA decline only and PSA decline and stable disease on scans.

5.2.2.2 **Time to PSA Progression**
In the absence of evidence of clinical progression on scans, we believe that time to PSA progression may also be an important variable to report. In this trial, PSA progression will be evaluated based on the PSA level at the time treatment is initiated compared to the PSA level at week 12 and monthly thereafter while the patient continues on trial. If

at least a 50% decline in PSA has been achieved, the end date is the time that the PSA has increased 50% above the nadir and a minimum of 5ng/ml. For patients without a PSA fall of this magnitude (or no fall in PSA), the endpoint for progression will be calculated at a time a 25% increase in PSA over nadir has been achieved with a minimum increase of 5 ng/ml. (see section **5.2.1.3**)

5.2.3 Duration of Overall Response (Biochemical Response)

5.2.3.1 The duration of overall response is measured from the time measurement criteria are met for CR or PR (whichever status is recorded first) until the first date that recurrence or PD is objectively documented.

5.2.4 Reporting of Results

5.2.4.1 All patients included in the study must be assessed for response to treatment, even if there are major protocol treatment deviations or if they are ineligible. Each patient will be assigned one of the following categories: (1) complete response, (2) partial response, (3) stable disease, (4) progressive disease, (5) early death from malignant disease, (6) early death from toxicity, (7) early death because of other cause, or (8) unknown (not assessable, insufficient data).

5.2.4.2 All of the patients who met the eligibility criteria will be included in the main analysis of the response rate. Patients in response categories 4–9 will be considered as failing to respond to treatment (disease progression). Thus an incorrect treatment schedule or drug administration does not result in exclusion from the analysis of the response rate. Precise definitions for categories 4–9 will be protocol specific.

5.2.4.3 All conclusions should be based on all eligible patients.

5.2.4.4 Sub analyses may then be performed on the basis of a subset of patients, excluding those for whom major protocol deviations have been identified (e.g., early death due to other reasons, early discontinuation of treatment, major protocol deviations, etc.). However, these sub analyses may not serve as the basis for drawing conclusions concerning treatment efficacy, and the reasons for excluding patients from the analysis should be clearly reported.

5.2.4.5 The 95% confidence intervals should be provided.

5.2.5 Assay for Immunologic Response

In order to evaluate the potency of clinical vaccines, assays that determine an antigen-specific T lymphocyte response to vaccines have been employed. The ELISPOT assay for IFN production (see **APPENDIX E**) has shown correlation with the results of the CTL assay.(55, 56) The CTL assay has been used successfully in numerous vaccine trials. The ability to detect single cells that produce interferon gamma has led to specificities of 80-95%. Schmittel et al. have shown that the ELISPOT has very good reproducibility both when the same sample is tested at two time points and when an individual is tested at different time points.(**(57)**) We have also seen minimal variation in

the flu-specific ELISPOT in patients when tested at time points about 84 days apart.(58)

The primary immunologic endpoint for evaluating the immune response will be the frequency of interferon gamma-releasing T cells specific to PSA-3A, a HLA-A2 restricted epitope of PSA, as measured by the ELISPOT assay. Only the patients who are HLA-A2 positive will undergo ELISPOT analysis. It is planned that all patients will undergo exploratory analysis of the ability to detect CD4 positive responses using a whole protein PSA assay.

In all patients undergoing apheresis, 5 x 10^8 to 2 x 10^9 mononuclear cells will be obtained by a single-access (single venipuncture) "four-pass" mononuclear cell procedure on the Haemonetics V-50 instrument, during which 2.0 liters of whole blood would be process at a flow rate of about 70-80 ml/min. The total duration of the procedure is 90 min to 2 hours. Patients will be required to have a minimum HCT of 28% and a platelet count of at least 75,000 to undergo a Haemonetics procedure. Apheresis will be requested for immunologic testing in patients receiving vaccine at baseline and around weeks 12, 24, and 36(see section **3.4.6**).

Anti-PSA antibodies will be measured prior to and following vaccinations using an ELISA assay as previously described (**(17)**). In addition, tests for anti-vaccinia antibody, anti-fowlpox antibody, anti-GM-CSF antibody, anti-B7.1 antibody, anti-ICAM-1 antibody and anti-LFA-3 antibody will be run in selected patients as previously described. (**(35)**)

As of amendment P, sargramostim (GM-CSF) will no longer be given to patients enrolled at the NCI site because there has not been found to be definitive benefit of the Sargramostim injections to this point. Other studies using the RF-PSA TRICOM are not currently using Sargramostim injections; the subjects in the multicenter sites are also not required to take/give Sargramostim. Thus, with amendment P, tests for anti-GM-CSF antibody will no longer be performed.

5.3 Toxicity Criteria

Common Terminology Criteria for Adverse Events, Version 4.0(CTCAE)
This study will utilize the CTCAE version 4.0 for toxicity and adverse drug experience reporting beginning January 1, 2011. A copy of the CTCAE version 4.0 will be available from the CTEP webpage,-http://ctep.cancer.gov/reporting/ctc.html. All appropriate treatment areas will have access to a copy of the CTCAE version 4.0.

Toxicity Grading for Vaccinia Toxicity(**(11)**) (see Section **8.2**)
Grade 1: Cutaneous reaction extending no more than 10 cm from the vaccination site (i.e., limited to the upper arm).
Grade 2: Any autoinoculation syndrome that resolves without sequelae; Generalized vaccinia extending more than 10 cm from the vaccination site.
Grade 3: Any toxicity that is between grade 2 and 4.

Grade 4: Autoinoculation syndrome (e.g. blindness); post vaccinia encephalitis; vaccinia gangrenosum; eczema gangrenosum; Stevens-Johnson syndrome.

Dose Limiting Toxicities for Flutamide include Grade 4 diarrhea, Grade 4 hepatic toxicity, both which would require discontinuation of the drug. Patients with grade 3 non-autoimmune toxicity due to Flutamide, may resume treatment provided that the toxicity has decreased to baseline or grade 1 toxicity within 42 days of scheduled re-treatment. However, patients with recurrent Grade 3 toxicities will be taken off drug. Grade 4 toxicity would require coming off study.

5.4 Statistical Considerations

The primary objective of this randomized trial is to determine if use of a combination of vaccine plus flutamide may be associated with a trend toward improvement in time to treatment failure in patients with stage D0.5 prostate cancer compared to that which would occur with flutamide alone.

Secondary objectives are 1) determining preliminary evidence of any patterns of immunologic effects which differ by treatment including the immunologic effects of flutmaide withdrawal on patients continuing on vaccine following a rising PSA on flutamide, 2) estimating toxicity noted on the arms and comparing the results, 3) evaluating, in a preliminary fashion, the effect of vaccine on development of metastatic disease after PSA progression, and 4) evaluating PSA responses and immune responses in patients who have had flutamide stopped at the time of PSA progression and either continue vaccine (Arm B) or have vaccine initiated (arm A) at time of flutamide discontinuation

The trial will be conducted as a randomized pilot trial with two arms as follows:

Arm A: flutamide
Arm B: flutamide + vaccine

Patients will be stratified based on a projected PSA doubling time (PSADT) of > 10 months vs. ≤ 10 months using the last 2 PSA values 1 month apart prior to enrollment on study.

$$\text{PSADT} = \frac{\log(2) \times t}{\log(\text{final PSA}) - \log(\text{initial PSA})}$$

t=time from initial to final PSA determination
log (2) = 0.301

Time to treatment failure (TTF) will be the primary endpoint of this trial, where a treatment failure would be defined as a rising PSA (Bubley Criteria, JCO, 1999), development of metastatic disease, or removal from treatment due to excessive toxicity. Because PSA will be measured monthly and metastatic disease will be evaluated every 3 months, this will be sufficiently frequent for the primary evaluation to be made using standard actuarial methods (Kaplan-Meier plots with a log-rank p-value determinations).

The previously published trial of Nilutamide in a similarly eligible population of patients (stage

D0.5 prostate cancer) conducted at the NCI (protocol 00-C-0137) identified a 7.6 month median time to treatment failure, using the definition above. In the current trial, Flutamide will be used instead of Nilutamide because it has a potentially more favorable toxicity profile. The objective will be to determine, in the context of a randomized phase 2.5 trial, if the addition of vaccine to flutamide alone may be associated with an improvement in time to treatment failure.

The study will be randomized in a 1:1 fashion, and will enroll 33 eligible patients on each of the two arms (66 total), in order to have 80% power to detect a difference between a hypothesized median TTF of 7.6 months on flutamide alone and an increase of 6 months, to 13.6 months, with flutamide + vaccine, using a one-tailed 0.1 alpha level test. This calculation was made using nQuery Advisor version 5, assuming 36 months accrual, an additional 12 months of follow-up for the last patient enrolled, and hazards of 0.0912 per month for flutamide alone and 0.0510 for flutamide and vaccine, with a resulting hazard ratio of 1.788.

The ELISPOT assay will be performed in HLA-A2 patients. The log changes in precursor T cell frequency from pre-treatment to that after 3, 6, and 9 months of therapy will be determined and evaluated for statistical significance, both within an arm and between arms. Since this assay will only be available on a subset of patients, the power to detect differences is unknown and may be limited, and the results will be considered secondary, exploratory and possibly biased. The results will be carefully reported with necessary caveats. In addition, since there is no evidence at this time that HLA-A2 patients will have a significantly different time to treatment failure than those with other HLA types, this parameter will not be used to stratify the randomization.

Toxicity evaluations will take place during each cycle of therapy and worst grades of toxicity, by type, will be compared in a secondary analysis between the study arms.

Early stopping Rule- Should 2 patients in the first 10 patients enrolled on both arms of the trial exhibit a grade 4 toxicity of any type, regardless of the number of patients enrolled (from 2 to 10), then the trial will be temporarily closed to accrual to reevaluate the use of the agents in this combination. The exact upper 90% confidence bound about 2/10 is 45%, while that about 1/10 is 34%. Thus, while 1/10 is marginally consistent with one third of the patients having toxicity, 2/10 is consistent with more than this amount; thus 2 patients with toxicity early in the study are more than would be tolerated in a phase II study of this type.

Those patients who experience a PSA progression while on their initial, randomized therapy will stop flutamide treatment when this is determined. If they were randomized to receive vaccine + flutamide, they will begin to receive vaccine (alone), and if randomized to flutamide alone, they will start vaccine alone instead. The time to development of metastatic disease from the date of discontinuation of flutamide will be evaluated as a secondary endpoint for each arm, using Kaplan-Meier curves. Descriptive analyses of the pattern of change in PSA over time will also be performed.

It is expected that approximately 20-25 patients per year may be enrolled onto this trial. Thus, in order to obtain 66 evaluable patients, accrual of approximately three years will be expected. In order to allow for a very small number of patients who are inevaluable early after enrollment, the accrual ceiling will be set at 70 patients.

Following amendment F, the study will be continue to be randomized in a 1:1 fashion, and will enroll 31 eligible patients on each of the two arms (62 total), in order to have 80% power to detect a difference between a hypothesized median TTF of 10.0 months on flutamide alone and an increase of 8 months, to 18.0 months, with flutamide + vaccine, using a one-tailed 0.1 alpha level test. This difference assumes that patients enrolled on the study will be equally divided between those who have progressed on combined androgen therapy as well as those who have just begun combined androgen therapy. This calculation was made using nQuery Advisor version 5, assuming 60 months accrual, an additional 24 months of follow-up for the last patient enrolled, and hazards of 0.0693 per month for flutamide alone and 0.0385 for flutamide and vaccine, with a resulting hazard ratio of 1.80.

Following amendment F, patients will be stratified by combined androgen therapy status (progressed; beginning) as well as projected PSA doubling time (<=10 vs. > 10 months).

As of the date of Amendment F, 13 patients have enrolled onto the study in approximately 2 years. In order to accrue a total of 62 patients in 5 years, the remaining 49 patients would need to be accrued in 3 years, or approximately 16-18 patients per year. In order to allow for a small number of inevaluable patients, the accrual ceiling will be set at 65.

Following Amendment K, only the patients at NCI will receive sargramostim (GM-CSF). Given the relatively small amount of patients which remain on the trial, descriptive and exploratory analysis will be done comparing patients who received GM-CSF and those who did not.

Following Amendment P, patients at NCI will not receive sargramostim injections. There has not been found to be definitive benefit of the Sargramostim injections to this point. Other studies using the RF-PSA TRICOM are not currently using Sargramostim injections; and the subjects in the multicenter sites are also not required to take/give Sargramostim.

5.5 Multi-Institutional Guidelines

IRB Approvals:

The NCI IRB must approve the addition of each participating site to the protocol and will require a copy of the local IRB approval from each participating site before NCI IRB approval will be granted.

Each site will need the local approval of their IRB in order to participate in this trial; in addition protocol changes will need to be communicated in accordance to with their local practice.

Participating institution will provide the NCI PI with copies of the initial local IRB approvals and semi-annual or annual continuing review approvals. The NCI PI will then furnish these to the NCI IRB. Registration will be halted at any participating institution in which a current continuing approval is not on file at the NCI IRB. Only one version of the protocol will be the correct version; amendments must be initiated through the coordinating center; amendments will be submitted to the NCI IRB and to the IRB of participating institution. The center will be responsible for its own IRB submissions.

Amendments and Consents:
The NCI PI will provide the NCI IRB with copies of all amendments, consents and approvals from each participating institution.

Patient Registration:
Participating institution must register patients with the CCR Central Registration as specified under section **2.3**. Such patients will be treated, monitored, and managed according to the guidelines of this protocol, after signing a current version of the informed consent. A copy of the registration checklist and off-study forms should be faxed to Coordinating Center Research Team at 301-480-1779.

Agent Ordering and Agent Accountability:

NCI supplied agents may be requested by the Principal Investigator (or their authorized designee) at each participating institution. Pharmaceutical Management Branch (PMB) policy requires that agent be shipped directly to the institution where the patient is to be treated. PMB does not permit the transfer of agents between institutions (unless prior approval from PMB is obtained.) The CTEP assigned protocol number must be used for ordering all CTEP supplied investigational agents. The responsible investigator at each participating institution must be registered with CTEP, DCTD through an annual submission of FDA form 1572 (Statement of Investigator), Curriculum Vitae, Supplemental Investigator Data Form (IDF), and Financial Disclosure Form (FDF). If there are several participating investigators at one institution, CTEP supplied investigational agents for the study should be ordered under the name of one lead investigator at that institution.

Active CTEP-registered investigators and investigator-designated shipping designees and ordering designees can submit agent requests through the PMB Online Agent Order Processing (OAOP) application < https://eapps-ctep.nci.nih.gov/OAOP/pages/login.jspx >. Access to OAOP requires the establishment of a CTEP Identity and Access Management (IAM) account < https://eapps-ctep.nci.nih.gov/iam/ > and the maintenance of an "active" account status and a "current" password. Alternatively, site personnel can fax completed Clinical Drug Requests (NIH-986) to the Pharmaceutical Management Branch at (301) 480-4612. For questions about drug orders, transfers, returns, or accountability, call (301) 496-5725 Monday through Friday between 8:30 am and 4:30 pm (ET) or email PMBAfterHours@mail.nih.gov anytime.

Agent Inventory Records - The investigator, or a responsible party designated by the investigator, must maintain a careful record of the inventory and disposition of all agents received from DCTD using the NCI Drug Accountability Record Form (DARF). (See the NCI Investigator's Handbook for Procedures for Drug Accountability and Storage.)

Contact information: Pharmaceutical Management Branch (http://ctep.cancer.gov) 6130 Executive Blvd, Rm 7419, Rockville, MD 20852, 301-496-5725, FAX 301-480-4612.

Data Collection and Toxicity Reporting:
Participating institution must submit research and clinical data to the Coordinating Center of theCenter for Cancer Research, NCI on a monthly basis. Data will be entered into the C3D database at participating facilities, training of designated data entry staff will be provided. Required data to be entered into C3D and/or maintained at the study site include, not exclusively:

prior disease related therapies, with dates, disease type, stage, disease sites, lab results, RECIST measurements, ECG, Scan reports and images on CD, and adverse events. Supporting eligibility source documentations should be reviewed and the eligibility checklist for registration needs to be sent within 3 business days to the Coordinating Center for eligibility review.

Concurrent medications need to be entered into the database.

All > grade 2 adverse events at least possibly attributed to research and not in the consent must be submitted as soon as possible so that they maybe reported to the NCI IRB within 7 days of receipt (see section **7.1**). Reporting to local IRB should be done as stipulated by local IRB guidelines. All data collection forms and records may be audited on-site at participating institutions and should be made available at the time of the study audit.

CTEP Multicenter Guidelines:
CTEP will perform a site visit every 3 years at participating facilities.

This protocol will adhere to the policies and requirements of the CTEP Multicenter Guidelines. The specific responsibilities of the Principal Investigator and the Coordinating Center (Study Coordinator) and the procedures for auditing are presented in **APPENDIX G**.

- The Principal Investigator/Coordinating Center is responsible for distributing all IND Action Letters or Safety Reports received from CTEP to all participating institutions for submission to their individual IRBs for action as required.

- Except in very unusual circumstances, each participating institution will order DCTD-supplied agents directly from CTEP. Agents may be ordered by a participating site only after the initial IRB approval for the site has been forwarded by the Coordinating Center to the CTEP PIO (PIO@ctep.nci.nih.gov) except for Group studies.

5.6 Data Safety Monitoring Plan

5.6.1 Principal Investigator/Research Team

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

All data will be collected in a timely manner and reviewed by the principal investigator or a lead associate investigator. Adverse events will be reported as required in section **7**. Any safety concerns, new information that might affect either the ethical and or scientific conduct of the trial, or protocol deviations will be immediately reported to the IRB using iRIS and if applicable to the Sponsor.

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

research staff.

The members of the site specific research team will review all adverse events for each subject in this trial in a timely manner. Unexpected adverse events and/or serious adverse events will be reported to the NCI's Institutional Review Board (IRB), site specific IRB and Cancer Therapy Evaluation Program (CTEP) (see section **7.1.1**). The study PI will review all safety data. If trends are noted and/or risks warrant it, accrual will be interrupted and/or the protocol and/or consent will be modified accordingly. In addition, the data will be reviewed regularly by the drug monitor at CTEP.

5.6.2 Safety Monitoring Committee (SMC)

This protocol will require oversight from the Safety Monitoring Committee (SMC). Initial review will occur as soon as possible after the annual 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.

5.6.3 Confidentiality of Trial Documents and Subject Records

Confidentiality will be maintained as much as possible, consistent with applicable regulations. Names of participants or identifying material will not be released with outpatient permission, except when such release is required by law. No patient's name or identifying information will be released in any publication or presentation. Records are maintained according to current legal requirements, and are made available for review according to the requirements of the Food and Drug Administration (FDA) or other authorized user, only under guidelines established by the Federal Privacy Act.

5.6.4 Submission of Data to NCI's Clinical Data Update System (CDUS):

This protocol will be monitored by the NCI's CDUS according to guidelines. Data will be submitted to CTEP quarterly. Data will be secured in the NCI C3D Database.

6 HUMAN SUBJECTS PROTECTIONS

6.1 Rationale for Subject Selection

6.1.1 Selection Based on 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.

6.1.2 Strategies/Procedures for Recruitment

Our patient accrual for this protocol would be facilitated by the Clinical Center Support Center (CCSC), developed to increase the accrual to clinical studies via community outreach as well as recruitment letters to referring physicians. This protocol will be available through the physicians' data query (PDQ) database.

6.1.3 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.

6.2 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.

6.3 Participation of NIH Subjects Unable to Give Consent

Adults unable to give consent are excluded from enrolling in the protocol. However re-consent may be necessary and there is a possibility, though unlikely, that subjects could become decisionally impaired. For this reason and because there is a prospect of direct benefit from research participation (section **6.4**), all subjects will be offered the opportunity to fill in their wishes for research and care, and assign a substitute decision maker on the "NIH Advance Directive for Health Care and Medical Research Participation" form so that another person can make decisions about their medical care in the event that they become incapacitated or cognitively impaired during the course of the study. Note: The PI or AI will contact the NIH Ability to Consent Assessment Team for evaluation. For those subjects that become incapacitated and do not have pre-determined substitute decision maker, the procedures described in MEC Policy 87-4 for appointing a surrogate decision maker for adult subjects who are (a) decisionally impaired, and (b) who do not have a legal guardian or durable power of attorney, will be followed.

6.4 Evaluation of Benefits/Risks/Discomforts

PSA vaccine using pox vectors are currently being used in Phase II clinical trials; preliminary results demonstrate, in some patients, stabilization or decreases in rising PSA levels without the

development of measurable metastatic disease. Potential risks include the possible occurrence of any of a range of side effects listed in section **8.2**.

6.4.1 Alternative Approaches or Treatments

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

6.4.2 Procedure for Protecting Against or Minimizing any Potential Risks

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

6.4.3 Provisions for Monitoring Data Collection to Ensure Safety of Subjects

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

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

6.5 Risks/Benefits Analysis

This study involves clinical research with an experimental vaccine designed to generate an immune response against antigens found in prostate cancer. Currently, there is no standard of care therapy for patients who have androgen independent prostate cancer but no measurable disease on scans. Second line antiandrogen therapy (i.e. flutamide) is commonly used in the community and patients will receive flutamide either alone or in combination with vaccine therapy. However alternative treatments include other hormonal therapy or other clinical trials. The side effects of the vaccines and flutamide and apheresis are outlined elsewhere (see section **8** and **5.2.2** respectively.) Whether the vaccine will have any clinical effect is unknown, therefore, benefit cannot be promised nor can the chance of benefit be accurately predicted. A patient's participation in this study is voluntary and refusal will not result in penalty or loss of benefit to

which patient is otherwise entitled.

Participation may be discontinued at any time without penalty and the patient will be encouraged to ask questions.

6.6 Consent and Assent Process and Documentation

The investigational nature and objectives of this trial, the procedures involved, and their attendant risks and discomforts, potential benefits, and potential alternative therapies will be explained to the patient and a signed informed consent document obtained. Moreover, any experimental invasive procedure will require a separate consent form. All associate investigators who have clinical privileges listed in this protocol are permitted to obtain informed consent.

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 and a copy of the informed consent document and note will be kept in the subject's research record.

7 ADVERSE EVENTS: LIST AND REPORTING REQUIREMENTS

7.1 Expedited Adverse Event Reporting to CTEP

Expedited AE reporting for this study must use CTEP-AERS (CTEP Adverse Event Reporting System), accessed via the CTEP web site (https://eapps-ctep.nci.nih.gov/ctepaers). The reporting procedures to be followed are presented in the "CTEP, NCI Guidelines: Adverse Event Reporting Requirements" which can be downloaded from the CTEP web site: (http://ctep.cancer.gov/protocolDevelopment/electronic_applications/adverse_events.htm).

In the rare occurrence when Internet connectivity is lost, a 24-hour notification is to be made to CTEP by telephone at 301-897-7497. Once Internet connectivity is restored, the 24-hour notification phoned in must be entered electronically into CTEP-AERS by the original submitter at the site.

7.1.1 All expedited adverse events reports must also be sent to the local Institutional Review Board (IRB), according to IRB policy and procedure. Multi-institutional sites will be required to send all SAE and CTEP-AERS reports in accordance with the guidelines below, and the NCI Adverse Event form (see **APPENDIX F**) to the NCI either

electronically or via fax (301-480-1779).

7.1.2 Adverse Event Reporting in human gene transfer trials must be completed and sent to the NIH Office of Science Policy (OSP). Completed reports may be sent on the OSP template at: http://osp.od.nih.gov/office-biotechnology-activities/biomedical-technology-assessment/hgt/gemcris. They may also be sent via e-mail (HGTprotocols@mail.nih.gov), fax (301-496-9839), or U.S. mail to: 6705 Rockledge Drive, Suite 750 Bethesda, Maryland 20892-7985.

7.2 NCI-IRB and NCI Clinical Director Reporting

7.2.1 NCI-IRB and NCI Clinical Director Expedited Reporting of Unanticipated Problems and Deaths

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

- 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.
These reports will be reviewed, entered into the C3D protocol database, circulated and reviewed at the next scheduled IRB meeting, and then filed in the IRB protocol file.

Reports of adverse events will be made utilizing the NCI Common Terminology Criteria for Adverse Events version 4.0 (CTCAEv4). This reference is located at http://ctep.info.nih.gov/reporting/ctc.html.

7.2.2 NCI-IRB Requirements for PI Reporting at Continuing Review

For reporting of adverse events at time of **continuing review**, the NCI-IRB requires a summary report of adverse events that have occurred on the protocol since the previous continuing review. The method of presentation should provide the NCI-IRB with the information necessary to clearly identify risks to participants and to make a risk:benefit determination. The summary report is based on the following guidance: any unexpected severity and/or unexpected frequency of expected events needs to be reported and interpreted in relation to the risk:benefit of study participants in the narrative.

The protocol PI will report to the NCI-IRB:

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

research;
- All Grade 3 and 4 events that are possibly, probably or definitely related to the research;
- All Grade 5 events regardless of attribution;
- All Serious Events regardless of attribution.

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

Based on protocol-associated risks to participants, the NCI-IRB retains the authority to establish more frequent Continuing Review periods more frequently than the customary annual review period.

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.

7.2.4 DEFINITIONS

7.2.4.1 Protocol Deviation (NIH Definition)

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

7.2.4.2 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.

7.2.4.3 Unanticipated Problem

Any incident, experience, or outcome that:

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

  (a) the research risks that are described in the IRB-approved research protocol and informed consent document; Investigator's Brochure or other study documents, and
  (b) the characteristics of the subject population being studied; AND
- Is related or possibly related to participation in the research; AND
- Suggests that the research places subjects or others at a g*reater risk of harm* (including physical, psychological, economic, or social harm) than was previously known or recognized.

7.3 Routine Adverse Event Reporti ng

All Adverse Events **must** be reported in routine (CTMS or CDUS) study data submissions. **AEs reported through** CTEP-AERS **must <u>also</u> be reported in routine study data submissions.**

7.4 Secondary Malignancy

A *secondary malignancy* is a cancer caused by treatment for a previous malignancy (*e.g.*, treatment with investigational agent/intervention, radiation or chemotherapy). A secondary malignancy is not considered a metastasis of the initial neoplasm.
CTEP requires all secondary malignancies that occur following treatment with an agent under an NCI IND/IDE be reported via CTEP-AERS. Three options are available to describe the event:

- Leukemia secondary to oncology chemotherapy (*e.g.*, acute myelocytic leukemia [AML])
- Myelodysplastic syndrome (MDS)
- Treatment-related secondary malignancy

Any malignancy possibly related to cancer treatment (including AML/MDS) should also be reported via the routine reporting mechanisms outlined in each protocol.

7.5 Second Malignancy

A second malignancy is one unrelated to the treatment of a prior malignancy (and is **NOT** a metastasis from the initial malignancy). Second malignancies require **ONLY** routine reporting via CDUS unless otherwise specified.

7.6 NCI Guidance for Reporting Expedited Adverse Events for Multi-Center Trials

The site PI must immediately report to the coordinating center PI 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 48 hours of PI awareness of the event. The Site PI must also report any protocol deviations to the coordinating center PI within 7 days of PI awareness. Participating centers must also submit the report to their IRB in accordance with their institutional policies.

7.7 Expedited Reporting Guidelines

Phase 2 and 3 Trials Utilizing an Agent under a CTEP IND: CTEP-AERS Reporting Requirements for Adverse Events That Occur Within 30 Days[1] of the Last Dose of the Investigational Agent

	Grade 1	Grade 2	Grade 2	Grade 3	Grade 3	Grades 4 & 5[2]	Grades 4 & 5[2]

	Unexpected and Expected	Unexpected	Expected	Unexpected		Expected		Unexpected	Expected
				with Hospitalization	without Hospitalization	with Hospitalization	without Hospitalization		
Unrelated Unlikely	Not Required	Not Required	Not Required	10 Calendar Days	Not Required	10 Calendar Days	Not Required	10 Calendar Days	10 Calendar Days
Possible Probable Definite	Not Required	10 Calendar Days	Not Required	10 Calendar Days	10 Calendar Days	10 Calendar Days	Not Required	24-Hour; 5 Calendar Days	10 Calendar Days

[1] **Adverse events with attribution of possible, probable, or definite that occur greater than 30 days after the last dose of treatment with an agent under a CTEP IND require reporting as follows:**

CTEP-AERS 24-hour notification followed by complete report within 5 calendar days for:

- Grade 4 and Grade 5 unexpected events

CTEP-AERS 10 calendar day report:

- Grade 3 unexpected events with hospitalization or prolongation of hospitalization
- Grade 5 expected events

[2] Although a CTEP-AERS 24-hour notification is not required for death clearly related to progressive disease, a full report is required as outlined in the table.

December 15, 2004

Note: All deaths on study require both routine and expedited reporting regardless of causality. Attribution to treatment or other cause must be provided.

- Expedited AE reporting timelines defined:
  - ➢ "24 hours; 5 calendar days" – The investigator must initially report the AE via CTEP-AERS within 24 hours of learning of the event followed by a complete CTEP-AERS report within 5 calendar days of the initial 24-hour report.
  - ➢ "10 calendar days" - A complete CTEP-AERS report on the AE must be submitted within 10 calendar days of the investigator learning of the event.
- Any medical event equivalent to CTCAE grade 3, 4, or 5 that precipitates hospitalization (or prolongation of existing hospitalization) must be reported regardless of attribution and designation as expected or unexpected with the exception of any events identified as protocol-specific expedited adverse event reporting exclusions.

- Any event that results in persistent or significant disabilities/incapacities, congenital anomalies, or birth defects must be reported via CTEP-AERS if the event occurs following treatment with an agent under a CTEP IND.

- Use the NCI protocol number and the protocol-specific patient ID provided during trial registration on all reports.

- This study will utilize the CTCAE version 4.0 for toxicity and adverse drug experience reporting. (See section **5.3)**

- A list of agent-specific expected adverse events can be found in the Pharmaceutical section **8**.

- All "Serious" or life-threatening events (Grades 4 and 5) and the first occurrence of any previously unknown reactions (regardless of grade) should be reported to Dr. Ravi

Madan immediately by telephone 301-222-7762 or 301-496-1211 (after hours).

7.8 Comprehensive Adverse Events and Potential Risks List (CAEPR)

for

PROSTVAC-V/TRICOM [Recombinant Vaccinia-PSA(L155)/TRICOM] (NSC 717170)
PROSTVAC-F/TRICOM [Recombinant Fowlpox-PSA(L155)/TRICOM] (NSC 717171)

The Comprehensive Adverse Event and Potential Risks list (CAEPR) provides a single list of reported and/or potential adverse events (AE) associated with an agent using a uniform presentation of events by body system. In addition to the comprehensive list, a subset, the Specific Protocol Exceptions to Expedited Reporting (SPEER), appears in a separate column and is identified with bold and italicized text. This subset of AEs (SPEER) is a list of events that are protocol specific exceptions to expedited reporting to NCI via CTEP-AERS (except as noted below). Refer to the 'CTEP, NCI Guidelines: Adverse Event Reporting Requirements' http://ctep.cancer.gov/protocolDevelopment/electronic_applications/docs/aeguidelines.pdf for further clarification.

The CAEPR does not provide frequency data; refer to the Investigator's Brochure for this information. Below is the CAEPR for PROSTVAC-VF/TRICOM.

> **NOTE**: Report AEs on the SPEER **ONLY IF** they exceed the grade noted in parentheses next to the AE in the SPEER. If this CAEPR is part of a combination protocol using multiple investigational agents and has an AE listed on different SPEERs, use the lower of the grades to determine if expedited reporting is required.

Version 1.3, May 1, 2012[1]

Adverse Events with Possible Relationship to PROSTVAC-VF/TRICOM (CTCAE 4.0 Term)		**Specific Protocol Exceptions to Expedited Reporting (SPEER)** (formerly known as ASAEL)
BLOOD AND LYMPHATIC SYSTEM DISORDERS		
	Blood and lymphatic system disorders - Other (lymphadenopathy)	***Blood and lymphatic system disorders - Other (lymphadenopathy) (Gr 2)***
GASTROINTESTINAL DISORDERS		
	Constipation	***Constipation (Gr 2)***
	Diarrhea	
	Nausea	***Nausea (Gr 2)***
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS		
	Chills	***Chills (Gr 2)***
	Edema limbs	***Edema limbs (Gr 2)***
	Fatigue	***Fatigue (Gr 2)***
	Fever	***Fever (Gr 2)***

	Injection site reaction	***Injection site reaction (Gr 2)***
	Pain	***Pain (Gr 2)***
MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS		
	Arthralgia	***Arthralgia (Gr 2)***
NERVOUS SYSTEM DISORDERS		
	Dizziness	***Dizziness (Gr 2)***
SKIN AND SUBCUTANEOUS TISSUE DISORDERS		
	Pruritus	***Pruritus (Gr 2)***

[1]This table will be updated as the toxicity profile of the agent is revised. Updates will be distributed to all Principal Investigators at the time of revision. The current version can be obtained by contacting PIO@CTEP.NCI.NIH.GOV. Your name, the name of the investigator, the protocol and the agent should be included in the e-mail.

[2]Infection includes all 75 sites of infection under the INFECTIONS AND INFESTATIONS SOC.

Also reported on PROSTVAC-VF/TRICOM trials but with the relationship to PROSTVAC-VF/TRICOM still undetermined:

BLOOD AND LYMPHATIC SYSTEM DISORDERS - Thrombotic thrombocytopenic purpura
CARDIAC DISORDERS - Myocardial infarction
GASTROINTESTINAL DISORDERS - Abdominal pain; Colitis; Dyspepsia; Vomiting
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS - Edema face; Gait disturbance
INFECTIONS AND INFESTATIONS – Infection[2]
INJURY, POISONING AND PROCEDURAL COMPLICATIONS - Wound complication
INVESTIGATIONS - Weight loss
METABOLISM AND NUTRITION DISORDERS - Anorexia
MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS - Back pain; Bone pain; Joint effusion; Myalgia; Pain in extremity
NERVOUS SYSTEM DISORDERS - Headache; Paresthesia
PSYCHIATRIC DISORDERS - Confusion
RENAL AND URINARY DISORDERS - Acute kidney injury
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS - Cough; Dyspnea
SKIN AND SUBCUTANEOUS TISSUE DISORDERS - Hyperhidrosis; Rash maculo-papular; Urticaria
VASCULAR DISORDERS - Hot flashes

Notes: 1- PROSTVAC-VF/TRICOM when used in combination with other agents, either commercial or investigational, could be associated with changes in the frequency or severity of known events or the emergence of new patterns of events.

2- Other potential risks or complications associated with the use of the vaccinia vaccine strain from which the attenuated recombinant vector is derived, include those observed during the smallpox vaccination programs:

- Inadvertent inoculation (autoinoculation and direct contact transmission)
- Non-specific erythematous or urticarial rashes (generally self-limiting) and rarely, more serious bullous erythema multiforme (Stevens-Johnson syndrome)
- Generalized vaccinia (disseminated maculopapular or vesicular rash of varying extent on any part of the body)

- Eczema vaccinatum (vaccinial lesion development on areas of the skin that are, or had at one time been, eczematous)
- Progressive vaccinia (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)
- Post-vaccinial encephalitis/encephalomyelitis
- Fetal vaccinia
- Myocarditis/pericarditis

3- The inclusion of co-stimulatory molecules in these agents may theoretically stimulate autoimmunity or exacerbate existing disease in susceptible individuals.

7.9 Record Ke eping

All patients must have signed an Informed Consent and an on-study confirmation of eligibility form will be filled out before entering on the study.

Complete records must be maintained on each patient, which will consist of the hospital chart with any supplementary information obtained from outside laboratories, radiology reports or physician's records. These records will serve as the primary source material that forms the basis for the research record. All relevant data will also be entered on a computer database from which formal analyses are done. The primary source documentation will assure the following: on-study information, including patient eligibility data and patient history; flow sheets, records of adverse events, specialty forms for pathology, radiation, or surgery; and off-study summary sheets, including a final assessment by the treating physician.

7.10 Regulator y Issues

The agent(s) supplied by CTEP, DCTD, NCI used in this protocol is/are provided to the NCI under a Collaborative Agreement (CRADA, CTA, CSA) between the Pharmaceutical Company(ies) (hereinafter referred to as "Collaborator(s)") and the NCI Division of Cancer Treatment and Diagnosis. Therefore, the following obligations/guidelines, in addition to the provisions in the "Intellectual Property Option to Collaborator" (http:// ctep.cancer.gov/industry) contained within the terms of award, apply to the use of the Agent(s) in this study:

1. Agent(s) may not be used for any purpose outside the scope of this protocol, nor can Agent(s) be transferred or licensed to any party not participating in the clinical study. Collaborator(s) data for Agent(s) are confidential and proprietary to Collaborator(s) and shall be maintained as such by the investigators. The protocol documents for studies utilizing investigational Agents contain confidential information and should not be shared or distributed without the permission of the NCI. If a copy of this protocol is requested by a patient or patient's family member participating on the study, the individual should sign a confidentiality agreement. A suitable model agreement can be downloaded from: http://ctep.cancer.gov.
2. For a clinical protocol where there is an investigational Agent used in combination with (an)other investigational Agent(s), each the subject of different collaborative agreements,

the access to and use of data by each Collaborator shall be as follows (data pertaining to such combination use shall hereinafter be referred to as "Multi-Party Data".):

a. NCI will provide all Collaborators with prior written notice regarding the existence and nature of any agreements governing their collaboration with NIH, the design of the proposed combination protocol, and the existence of any obligations that would tend to restrict NCI's participation in the proposed combination protocol.

b. Each Collaborator shall agree to permit use of the Multi-Party Data from the clinical trial by any other Collaborator solely to the extent necessary to allow said other Collaborator to develop, obtain regulatory approval or commercialize its own investigational Agent.

c. Any Collaborator having the right to use the Multi-Party Data from these trials must agree in writing prior to the commencement of the trials that it will use the Multi-Party Data solely for development, regulatory approval, and commercialization of its own investigational Agent.

1. Clinical Trial Data and Results and Raw Data developed under a Collaborative Agreement will be made available exclusively to Collaborator(s), the NCI, and the FDA, as appropriate and unless additional disclosure is required by law or court order. Additionally, all Clinical Data and Results and Raw Data will be collected, used and disclosed consistent with all applicable federal statutes and regulations for the protection of human subjects, including, if applicable, the Standards for Privacy of Individually Identifiable Health Information set forth in 45 C.F.R. Part 164.

4. When a Collaborator wishes to initiate a data request, the request should first be sent to the NCI, who will then notify the appropriate investigators (Group Chair for Cooperative Group studies, or PI for other studies) of Collaborator's wish to contact them.

5. Any data provided to Collaborator(s) for Phase 3 studies must be in accordance with the guidelines and policies of the responsible Data Monitoring Committee (DMC), if there is a DMC for this clinical trial.

6. Any manuscripts reporting the results of this clinical trial must be provided to CTEP by the Group office for Cooperative Group studies or by the principal investigator for non-Cooperative Group studies for immediate delivery to Collaborator(s) for advisory review and comment prior to submission for publication. Collaborator(s) will have 30 days from the date of receipt for review. Collaborator shall have the right to request that publication be delayed for up to an additional 30 days in order to ensure that Collaborator's confidential and proprietary data, in addition to Collaborator(s)'s intellectual property rights, are protected. Copies of abstracts must be provided to CTEP for forwarding to Collaborator(s) for courtesy review as soon as possible and preferably at least three (3) days prior to submission, but in any case, prior to presentation at the meeting or publication in the proceedings. Press releases and other media presentations must also be

forwarded to CTEP prior to release. Copies of any manuscript, abstract and/or press release/ media presentation should be sent to:

Email: ncicteppubs@mail.nih.gov

The Regulatory Affairs Branch will then distribute them to Collaborator(s). No publication, manuscript or other form of public disclosure shall contain any of Collaborator's confidential/ proprietary information.

7.10.1 The responsible investigator at each participating institution must be registered with CTEP, DCTD through an annual submission of FDA form 1572 and a CV.

7.10.2 The investigator, or a responsible party designated by the investigator, must maintain a careful record of the inventory and disposition of all drugs received from DCTD using the NCI Drug Accountability Record (DAR) Form. (See the NCI Investigator's Handbook for Procedures for Drug Accountability and Storage.)

Submission of Data to NCI's Clinical Data Update System (CDUS):
This protocol will be monitored by the NCI's CDUS according to guidelines. Data will be submitted to CTEP quarterly. Data will be secured in the NCI C3D Database.

The NCI/DCT Case Report Form and the NCI database will be used to report to CTEP.

A summary of the completed study will be submitted to IDB/CTEP within 2 months of study completion. A status report will be submitted and presented at upcoming NCI meetings as requested.

7.10.3 The Guide to Preventing Conflicts of Interest in Human Subjects Research at NIH has been distributed to all investigators on this study.

8 PHARMACEUTICAL INFORMATION

8.1 Recombinant Fowlpox-PSA(L155)/TRICOM™ (NSC 717171)

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.

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 pfu/mL formulated in phosphate-buffered saline containing 10% glycerol.

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 pfu) into a 1 mL syringe for administration by subcutaneous injection.

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

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.

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

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 ware, hair cover].
6. Decontaminate the biological safety cabinet prior to dose preparation with sterile gauze soaked in 10% bleach solution (0.52% sodium hypochlorite solution), or other appropriate disinfectant suitable for decontamination, rinsing, then wiping down with sterile gauze soaked in 70% alcohol. Consult specific manufacturer's recommendations with respect to disinfectant concentration, contact time and method of application.
7. Have all necessary supplies on-hand before beginning the preparation procedure. Develop a detailed worksheet outlining all supplies, dose calculations, and preparation procedures, and keep it available.
8. Place an empty biohazard sharps container lined with a leak-proof biohazard bag in or near the biosafety cabinet to dispose of all waste generated.
9. Transport the agent from the freezer to the work area in leak proof bag.
10. Wipe or spray items used for dose preparation with 70% alcohol before placing in the biological safety cabinet. Disinfectants should remain in contact with the surfaces for at least five minutes prior to dose preparation. Avoid exposing the virus to disinfectants.
11. Wipe the syringe containing the prepared dose with 70% alcohol before removing it from the biological safety cabinet; transport it in a leak proof bag or container labeled with a biohazard symbol.
12. Place all waste into the sharps container lined with the leak proof biohazard bag and decontaminate the biological safety cabinet again by wiping down all surfaces with sterile gauze soaked in 10% bleach solution, or other appropriate disinfectant, rinsing, then wiping down with sterile gauze soaked in 70% alcohol. Following decontamination, dispose of personal protective apparel in the biohazard safety bag.
13. Incinerate waste according to institutional policy and according to local, state, and federal regulations.
14. Handle accidental spills similarly to antineoplastic spills and/or according to institutional policy:
    - Prevent others from entering the area and allow aerosols time to settle (approximately 10 minutes).
    - Use protective apparel, eyewear, mask, and gloves.
    - Cover spills with disposable absorbent towels.
    - Decontaminate the area with 10% bleach solution, or other appropriate disinfectant suitable for decontamination, allowing approximately a 20-minute contact time.
    - Dispose of all waste as biohazardous waste and incinerate according to institutional policy and according to local, state, and federal regulations.
15. Immediately report spills and accidents resulting in overt exposure to recombinant DNA molecules to the Institutional Biosafety Committee and NIH/OSP (Office of Science Policy). Send reports to the Office of Science Policy, National Institutes of Health, 6705 Rockledge

Drive, Suite 750, MSC 7985, Bethesda, MD 20892-7985. Phone (301) 496-9838. Provide medical evaluation, surveillance, and treatment as appropriate and maintain written records of the event.

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

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

Patient Care Implications and Contraindications

Cover vaccination sites with a sterile dry dressing (e.g., Telfa pad). Once the injection site is healed, no further barrier is necessary. As a precaution, protect injection sites that are exhibiting evidence of weeping, oozing or ulceration with a sterile dry dressing. In these circumstances, instruct patients to avoid direct contact of the injection site with susceptible individuals (e.g.; those who may be immunocompromised by disease or therapy). Instruct patients to avoid fathering a child for at least 4 months following therapy completion with the recombinant vaccine. Instruct patients receiving fowlpox vaccines to avoid direct contact with pet birds for at least 72 hours after vaccination or while there are any visible lesions at the injection site. **Due to the method of manufacturing (virus grown in primary chicken embryo dermal cells), patients with a history of allergy to eggs or egg products should not receive the vaccine.**

8.2 Recombinant Vaccinia-PSA(L155)/TRICOM ™(NSC 717170)

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.

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 pfu/mL formulated in phosphate-buffered saline containing 10% glycerol.

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 pfu) into a 1 mL syringe for administration by subcutaneous injection.

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

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.

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

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 eye ware, hair cover].
9. Perform all procedures carefully to minimize aerosol creation.
10. Decontaminate the biological safety cabinet prior to dose preparation with sterile gauze soaked in 10% bleach solution (0.52% sodium hypochlorite solution), or other appropriate disinfectant suitable for decontamination, rinsing, then wiping down with sterile gauze soaked in 70% alcohol. Consult specific manufacturer's recommendations with respect to disinfectant concentration, contact time and method of application.
11. Have all necessary supplies on-hand before beginning the preparation procedure. Develop a detailed worksheet outlining all supplies, dose calculations, and preparation procedures, and keep it available.
12. Place an empty biohazard sharps container in the biosafety cabinet to dispose of all waste generated.
13. Transport the agent from the freezer to the work area in leak proof bag.
14. Wipe or spray items used for dose preparation with 70% alcohol before placing in the biological safety cabinet. Disinfectants should remain in contact with the surfaces for at least five minutes prior to dose preparation. Avoid exposing the virus to disinfectants.
15. Wipe the syringe containing the prepared dose with 70% alcohol before removing it from the biological safety cabinet; transport it in a leak proof bag or container labeled with a biohazard symbol.
16. Place all waste into a sharps container lined with the leak proof biohazard bag and decontaminate the biological safety cabinet again by wiping down all surfaces with sterile gauze soaked in 10% bleach solution, or other appropriate disinfectant, rinsing, then wiping down with sterile gauze soaked in 70% alcohol. Following decontamination, dispose of personal protective apparel in the biohazard safety bag.
17. Place all waste and protective apparel in a leak proof biohazard bag, and place the bag inside a biohazard sharps container for incineration according to institutional policy and according to local, state, and federal regulations.
18. Handle accidental spills similarly to antineoplastic spills and/or according to institutional policy:
    a. Prevent others from entering the area and allow aerosols time to settle (approximately 10 minutes).
    b. Use protective apparel, eyewear, mask, and gloves.
    c. Cover spills with disposable absorbent towels.

d. Decontaminate the area with 10% bleach solution, or other appropriate disinfectant suitable for decontamination, allowing approximately a 20-minute contact time.
e. Dispose of all waste and protective apparel as infectious biohazardous waste and incinerate according to institutional policy and according to local, state, and federal regulations.

19. Immediately report spills and accidents resulting in overt exposure to recombinant DNA molecules to the Institutional Biosafety Committee and NIH/OSP (Office of Science Policy). Send reports to the Office of Science Policy, National Institutes of Health, 6705 Rockledge Drive, Suite 750, MSC 7985, Bethesda, MD 20892-7985. Phone (301) 496-9838. Provide medical evaluation, surveillance, and treatment as appropriate and maintain written records of the event.

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

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

Precautions for Healthcare Workers

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

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

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

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

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

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

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

Recombinant Vaccinia Vaccine Patient Care Implications, Contraindications and Potential Complications

Patient Care Implications and Contraindications

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

- have active eczema or a history of eczema or atopic dermatitis, or Darier's disease.
- have other acute, chronic, or exfoliative skin conditions (*e.g.*, burns, impetigo, varicella zoster, severe acne, contact dermatitis, psoriasis, herpes or other open rashes or wounds), until the condition resolves.
- are pregnant or intend to become pregnant (due to the potential risk of fetal vaccinia); or are breast-feeding (due to the potential risk of contact transmission and inadvertent inoculation). It is currently unknown if vaccinia virus or antibodies are excreted in breast milk. Patients

(*i.e.*, vaccinees) should avoid fathering a child for at least 4 months following completion of therapy with the recombinant vaccine.

- are in close contact with children less than 3 years of age (due to the potential risk of contact transmission and inadvertent inoculation).
- are immunodeficient or immunocompromised (by disease or therapy), including individuals with HIV infection.

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

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

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

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

Potential Complications Associated With Recombinant Vaccinia Vaccination

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

When administered by scarification in individuals that have previously been vaccinated with

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

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

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

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

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

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

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

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

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

systemically ill and whose condition might be toxic or who have serious underlying immunosuppressive illnesses.

The differential diagnosis of GV includes eczema vaccinatum, erthyema 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 orthopox viruses. 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.

Cidofovir (Vistide®, Gilead Sciences): Cidofovir is an FDA-approved antiviral drug for the treatment of CMV retinitis among patients with AIDS. Cell-based *in vitro* studies and animal model studies have demonstrated this agent's antiviral activity against certain orthopoxviruses. Currently, its efficacy in the treatment of vaccinia-related complications in humans is unknown. According to the CDC, VIG is recommended as first line of therapy. Cidofovir may be considered as a secondary treatment, and will only be used when VIG therapy is not effective [Medical Management of Smallpox (Vaccinia) Vaccine Adverse Reactions: Vaccinia Immune Globulin and Cidofovir. Last updated September 28, 2009. Available at: http://www.bt.cdc.gov/agent/smallpox/vaccination/mgmt-adv-reactions.asp]. The CDC has informed the NCI/CTEP that cidofovir will not be supplied through Strategic National Pharmaceutical Stockpile to investigators involved in CTEP-sponsored protocols utilizing recombinant vaccinia-based vaccines. Thus, investigators should obtain cidofovir for second-line therapy through commercial sources if necessary. Investigators should consult the CDC Clinician Information Line at 1-877-554-4625 or the Director's Emergency Operations Center (DEOC) at 770-488-7100 regarding appropriateness of therapy and guidance.

8.3 Flutam ide

(Please see FDA-approved packet insert for Flutamide for complete agent information)

Description
Flutamide is an orally administered, nonsteroidal, competitive antagonist of testosterone and other androgens at androgenic receptors and may alter the nuclear translocation of the androgen/receptor complex. Flutamide has no intrinsic activity at glucocorticoid, mineralocorticoid, androgenic, estrogenic or progestin receptors.

Source
Flutamide is available generically and will be purchased by the NIH Clinical Center Pharmacy Department from commercial sources.

Formulation
The product is formulated as capsules for oral administration containing flutamide 125 mg.

Storage
Store flutamide capsules between 2°–30°C (35.6°–86°F).

Stability
The commercial product bears a manufacturer's expiration date.
Dosage and Administration
Patients will take flutamide 250 mg (2 capsules) orally, three times daily (approximately every 8 hours).

Adverse Effects:
Gastrointestinal Events

Diarrhea, nausea, and vomiting are the most common gastrointestinal adverse effects, occurring in 11%–12% of patients. The major difference in adverse effects attributed to flutamide given as a single agent compared to flutamide + an LHRH agonist, is a higher incidence of nausea and vomiting associated with the combination compared to monotherapy (4%–5% *vs.* 11%–12%, respectively).

Gastrointestinal adverse effects include nausea, vomiting, diarrhea, and abdominal discomfort and may be related to lactose that is used as an excipient in some product formulations.

Increased appetite, upset stomach, constipation, anorexia, and indigestion have been reported in 4–6% of patients who received flutamide.

Endocrinological Events
Gynecomastia with galactorrhea within 2–8 months (average 3.5 months) after starting treatment and breast tenderness are the most frequently reported adverse effects associated during flutamide plus LHRH agonist treatment. Gynecomastia is reported to occur in patients who receive flutamide as a single agent.

Hepatic Events
Hepatotoxicity, including increased liver function test results (commonly transaminases; less commonly alkaline phosphatase, bilirubin, gamma glutamyl transferase), cholestatic hepatitis with jaundice, hepatic encephalopathy, hepatitis, hepatic necrosis, and hepatic failure and rarely death are described with flutamide use.

Jaundice and hepatitis have been reported to occur in 1–5% of patients following therapeutic doses of flutamide, and slight elevations of serum aminotransferases may commonly occur.

Hepatic injury has been reversible after prompt discontinuation in some patients. Approximately half of the reported cases occurred within the initial 3 months of flutamide treatment.

Hematological Events
Anemia (6%), leukopenia (3%), neutropenia, thrombocytopenia (1%), and sulfhemoglobinemia have been associated with flutamide administration.
Flutamide is an analog of toludine, clinically significant methemoglobinemia has been rarely reported.

Thrombophlebitis and pulmonary embolism have occurred rarely and may be related to previous treatment with estrogens.

Kidney and Genitourinary Events
Hot flashes, intense amber to yellow-green urine discoloration, transiently increased BUN and creatinine are described with administration of flutamide.
Both impotence and loss of libido and no effect on libido, penile erection, and sexual performance have been reported at daily flutamide doses up to 1500 mg for up to 12 weeks.

Neurological Events
Drowsiness, confusion, nervousness, headache, dizziness, insomnia, weakness, depression, anxiety, and a manic-like syndrome have been reported rarely (< 1%) in patients during flutamide administration.

Cardiovascular Events
Myocardial infarction and myocardial ischemia have occurred rarely during flutamide administration.

Rare cases of hypertension (1% of 294 patients) have been reported during flutamide treatment among patients who received a LHRH agonist concomitantly.

Ocular Events
Blurred vision has been rarely reported with flutamide therapy.

Dermatologic Events
Photosensitivity after sun exposure, with erythematous pruritic papulovesicular rashes and eruptions, and pruritus and ecchymoses are described with administration of flutamide. Rarely, a photoallergic reaction with erythema and vesiculo-erosive necrolytic lesions. Encourage patients to apply topical sunscreens to sun-exposed skin and use protective clothing against exposure to sunlight or UV light until their individual tolerance is determined.

Drug Interactions:
Increases in prothrombin time and International Normalized Ratio have been noted after flutamide was initiated in patients who were receiving **warfarin** therapy.

Flutamide is metabolically activated by cytochrome P450 CYP1A2, and to a lesser extent by CYP3A4, CYP1A1, and CYP1B1. Flutamide has been shown experimentally to competitively inhibit CYP1A1, CYP1A2, CYP1B1.

9 REFERENCES

(1) Jemal A, Tiwari RC, Murray T, Ghafoor A, Samuels A, Ward E et al. Cancer Statistics, 2005. CA Cancer J Clin 2005; 55(1):8-29.

(2) Therasse P, Arbuck SG, Eisenhauer EA, Wanders J, Kaplan RS, Rubinstein L et al. New guidelines to evaluate the response to treatment in solid tumors. European Organization for Research and Treatment of Cancer, National Cancer Institute of the United States, National Cancer Institute of Canada. J Natl Cancer Inst 2000; 92(3):205-216.

(3) Dillioglugil O, Leibman BD, Kattan MW, SealeHawkins C, Wheeler TM, Scardino PT. Hazard rates for progression after radical prostatectomy for clinically localized prostate cancer. Urology 1997; 50(1):93-99.

(4) Stamey TA, Yemoto CM, McNeal JE, Sigal BM, Johnstone IM. Prostate cancer is highly predictable: A prognostic equation based on all morphological variables in radical prostatectomy specimens. Journal of Urology 2000; 163(4):1155-1160.

(5) Paulson DF, Moul JW, Walther PJ. Radical Prostatectomy for Clinical Stage T1-2N0M0 Prostatic Adenocarcinoma - Long-Term Results. Journal of Urology 1990; 144(5):1180-1184.

(6) Kelly, W. K. and Scher, H. I.: Prostate specific antigen decline after antiandrogen withdrawal: the flutamide withdrawal syndrome. J Urol, 149: 607, 1993

(7) Grossmann, M. E., Huang H. and Tindall, D.: Androgen receptor signaling in androgen-refractory prostate cancer. J Natl Cancer Inst; 93: 1687, 2001

(8) Taplin, M. E., Bubley, G. J., Shuster, T. D., Frantz, M. E., Spooner, A. E., Ogata G. K. et al: Mutation of the androgen-receptor gene in metastatic androgen-independent prostate cancer. NEJM 332: 1393, 1995

(9) Schellhammer, P. F., Venner, P., Haas, G. P., Small, E. J., Nieh, P. T., Seabaugh, D. R. et al: Prostate specific antigen decrease after withdrawal of antiandrogen therapy with bicalutamide of flutamide in patients receiving combined androgen blockade. J Urol 157: 1731, 1997

(10) Herrada, J., Dieringer, P. and Logothetis C. Characterization of patients with androgen-independent prostatic carcinoma whose serum prostate specific antigen decreased following flutamide withdrawal. J Urol 155:620, 1996

(11) Gulley J, Chen AP, Dahut W, Arlen PM, Bastian A, Steinberg SM et al. Phase I study of a vaccine using recombinant vaccinia virus expressing PSA (rV-PSA) in patients with metastatic androgen-independent prostate cancer. Prostate 2002; 53(2):109-117.

(12) Correale P, Walmsley K, Zaremba S, Zhu M, Schlom J, Tsang KY. Generation of human cytolytic T lymphocyte lines directed against prostate-specific antigen (PSA) employing a PSA oligoepitope peptide. J Immunol 1998; 161(6):3186-3194.

(13) Correale P, Walmsley K, Nieroda C, Zaremba S, Zhu M, Schlom J et al. In vitro generation of human cytotoxic T lymphocytes specific for peptides derived from prostate-specific antigen. J Natl Cancer Inst 1997; 89(4):293-300.

(14) Kass E, Schlom J, Thompson J, Guadagni F, Graziano P, Greiner JW. Induction of protective host immunity to carcinoembryonic antigen (CEA), a self-antigen in CEA transgenic mice, by immunizing with a recombinant vaccinia-CEA virus. Cancer Res 1999; 59(3):676-683.

(15) Bernards R, Destree A, Mckenzie S, Gordon E, Weinberg RA, Panicali D. Effective Tumor-Immunotherapy

Directed Against An Oncogene- Encoded Product Using A Vaccinia Virus Vector. Proceedings of the National Academy of Sciences of the United States of America 1987; 84(19):6854-6858.

(16) Irvine K, Kantor J, Schlom J. Comparison of a CEA-recombinant vaccinia virus, purified CEA, and an anti-idiotypic antibody bearing the image of a CEA epitope in the treatment and prevention of CEA- expressing tumors. Vaccine Research 1993; 2(2):79-94.

(17) Eder JP, Kantoff PW, Roper K, Xu GX, Bubley GJ, Boyden J et al. A phase I trial of a recombinant vaccinia virus expressing prostate-specific antigen in advanced prostate cancer. Clin Cancer Res 2000; 6(5):1632-1638.

(18) Kaufman HL, Wang W, Manola J, et al. Phase II prime/boost vaccination using poxviruses expressing PSA in hormone dependent prostate cancer: Follow-up clinical results from ECOG 7897 Program/Proceedings, American Society of Clinical Oncology 2005, 24:4501a.

(19) Gulley J, Dahut W, Arlen P, Bastian A, Rucker S, Marte J et al. A PSA-based vaccine in a randomized phase II study of patients with localized prostate cancer receiving standard radiotherapy. Clin Cancer Res 11(9):3353-62, 2005.

(20) Arlen P, Gulley J, Novik L, Rucker S, Bastian A, Marte J et al. A randomized phase II trial of either vaccine therapy (recombinant pox viruses expressing PSA and the B7.1 costimulatory molecule) versus hormone therapy (nilutamide) in patients with hormone refractory prostate cancer and no radiographic evidence of disease. J Urol 4(2):539-46, 2005.

(21) Mercader M, Bodner BK, Moser MT, Kwon PS, Park ES, Manecke RG, Ellis TM, Wojcik EM, Yang D, Flanigan RC, Waters WB, Kast WM, Kwon ED. T cell infiltration of the prostate induced by androgen withdrawal in patients with prostate cancer. Proc Natl Acad Sci U S A.;98(25):14565-70, 2001.

(22) Dahut WL, Gulley J, Arlen P, Liu Y, Katherine Fedenko, Fedenko K, Steinberg S, Wright J, Parnes H, Chen C, Jones E, Parker K, and Figg WD. A randomized Phase II trial of docetaxel plus thalidomide in androgen-independent prostate cancer. J. Clin. Onc. 22(13):2532-2539,2004

(23) James Gulley, Nushin Todd, William Dahut, Jeffrey Schlom, Philip Arlen.
A phase II study of a PSA-TRICOM vaccine, and the role of GM-CSF, in patients (pts) with metastatic androgen insensitive prostate cancer (AIPC). Proceedings Am Soc Clin Onc 23, 2504A. 2005.

(24) Grosenbach DW, Barrientos JC, Schlom J, Hodge JW. Synergy of vaccine strategies to amplify antigen-specific immune responses and antitumor effects. Cancer Res 2001; 61(11):4497-4505.

(25) Hodge JW, McLaughlin JP, Kantor JA, Schlom J. Diversified prime and boost protocols using recombinant vaccinia virus and recombinant non-replicating avian pox virus to enhance T-cell immunity and antitumor responses. Vaccine 1997; 15(6-7):759-768.

(26) Marshall JL, Hoyer RJ, Toomey MA, Faraguna K, Chang P, Richmond E et al. Phase I study in advanced cancer patients of a diversified prime-and-boost vaccination protocol using recombinant vaccinia virus and recombinant nonreplicating avipox virus to elicit anti-carcinoembryonic antigen immune responses. J Clin Oncol 2000; 18(23):3964-3973.

(27) Slack R, Ley L, Chang P, Arlen P, Tsang K, Schlom J et al. Association Between CEA-Specific T Cell Responses (TCR) Following Treatment with Vaccinia-CEA (V) and Alvac-CEA (A) and Survival in Patients (Pts) with CEA-Bearing Cancers. Proceedings Am Soc Clin Onc . 2001.
Ref Type: Abstract

(28) Kaufman H, Wang W, Manola J, DiPaola RS, Ko Y, Williams SD et al. Prime/boost vaccination using poxviruses expressing PSA in D0 prostate cancer: preliminary results of ECOG 7897, a randomized phase II clinical trial. Proceedings Am Soc Clin Onc , A12. 2002.
Ref Type: Abstract

(29) Kaufman H, Wang W, Manola J, DiPaola RS, Ko Y, Sweeney C et al. Phase II Randomized Study of Vaccine Treatment of Advanced Prostate Cancer (E7897): A Trial of the Eastern Cooperative Oncology Group. 8-11-2003.
Ref Type: Unpublished Work

(30) Morrissey PJ, Bressler L, Park LS, Alpert A, Gillis S. Granulocyte-Macrophage Colony-Stimulating Factor Augments the Primary Antibody-Response by Enhancing the Function of Antigen- Presenting Cells. Journal of Immunology 1987; 139(4):1113-1119.

(31) Kass E, Panicali DL, Mazzara G, Schlom J, Greiner JW. Granulocyte/macrophage-colony stimulating factor produced by recombinant avian poxviruses enriches the regional lymph nodes with antigen-presenting cells and acts as an immunoadjuvant. Cancer Res 2001; 61(1):206-214.

(32) Dranoff G, Jaffee E, Lazenby A, Golumbek P, Levitsky H, Brose K et al. Vaccination with Irradiated Tumor-Cells Engineered to Secrete Murine Granulocyte-Macrophage Colony-Stimulating Factor Stimulates Potent, Specific, and Long-Lasting Antitumor Immunity. Proceedings of the National Academy of Sciences of the United States of America 1993; 90(8):3539-3543.

(33) Kwak LW, Young HA, Pennington RW, Weeks SD. Vaccination with syngeneic, lymphoma-derived immunoglobulin idiotype combined with granulocyte/macrophage colony- stimulating factor primes mice for a protective T-cell response. Proceedings of the National Academy of Sciences of the United States of America 1996; 93(20):10972-10977.

(34) von Mehren M, Arlen P, Gulley J, Rogatko A, Cooper HS, Meropol NJ et al. The influence of granulocyte macrophage colony-stimulating factor and prior chemotherapy on the immunological response to a vaccine (ALVAC-CEA B7.1) in patients with metastatic carcinoma. Clin Cancer Res 2001; 7(5):1181-1191.

(35) Hodge JW, Sabzevari H, Yafal AG, Gritz L, Lorenz MGO, Schlom J. A triad of costimulatory molecules synergize to amplify T-cell activation. Cancer Research 1999; 59(22):5800-5807.

(36) Terasawa H, Tsang KY, Gulley J, Arlen P, Schlom J. Identification and characterization of a human agonist cytotoxic T-lymphocyte epitope of human prostate-specific antigen. Clin Cancer Res 2002; 8(1):41-53.

(37) Terasawa H, Tsang KY, Gulley J, Arlen P, Schlom J. Identification and characterization of a human agonist cytotoxic T-lymphocyte epitope of human prostate-specific antigen. Clinical Cancer Research 2002; 8(1):41-53.

(38) Rosenberg SA, Yang JC, Schwartzentruber DJ, Hwu P, Marincola FM, Topalian SL et al. Immunologic and therapeutic evaluation of a synthetic peptide vaccine for the treatment of patients with metastatic melanoma. Nature Medicine 1998; 4(3):321-327.

(39) Rosenberg SA, Yang JC, Schwartzentruber DJ, Hwu P, Topalian SL, Sherry RM et al. Recombinant Fowlpox viruses encoding the anchor-modified gp100 melanoma antigen can generate antitumor immune responses in patients with metastatic melanoma. Clinical Cancer Research 2003; 9(8):2973-2980.

(40) Fong L, Hou YF, Rivas A, Benike C, Yuen A, Fisher GA et al. Altered peptide ligand vaccination with Flt3 ligand expanded dendritic cells for tumor immunotherapy. Proceedings of the National Academy of Sciences of the United States of America 2001; 98(15):8809-8814.

(41) Hodge JW, Grosenbach DW, Aarts WM, Poole DJ, Schlom J. Vaccine therapy of established tumors in the absence of autoimmunity. Clin Cancer Res 2003; 9(5):1837-1849.

(42) Hodge JW, Schlom J, Donohue SJ, Tomaszewski JE, Wheeler CW, Levine BS et al. A recombinant vaccinia virus expressing human prostate-specific antigen (PSA): safety and immunogenicity in a non-human primate. Int J Cancer 1995; 63(2):231-237.

(43) Kaufman HL, DiPaola RS, von Mehren M, Marshall J, Lyerly HK, Streicher H et al. Safety profile of therapeutic pox virus-based vaccines for cancer. Proceedings Am Soc Clin Onc 23, 2513A. 2004.
Ref Type: Abstract

(44) Sanda MG, Smith DC, Charles LG, Hwang C, Pienta KJ, Schlom J et al. Recombinant vaccinia-PSA (PROSTVAC) can induce a prostate-specific immune response in androgen-modulated human prostate cancer. Urology 1999; 53(2):260-266.

(45) Marshall J, Gulley JL, Arlen P, Beetham P, Tsang K, Slack R et al. A Phase I Study of Sequential Vaccinations With Fowlpox-CEA (6D)-TRICOMTRICOM (B7.1B7-1/ICAM-1/LFA-3) Alone and Sequentially With Vaccinia-CEA (6D)-TRICOMTRICOM, With and Without GM-CSF, in Patients With CEA-expressing Carcinomas. J. Clin. Oncol 23(4):720-31, 2005.

(46) Arlen P, Gulley J, Parker C, Skarupa L, Panicali D, Beetham P et al. A pilot study of concurrent docetaxel plus PSA pox-vaccine versus vaccine alone in metastatic androgen independent prostate cancer (AIPC). Proceedings Am Soc Clin Onc 22, 1701A. 2003.
Ref Type: Abstract

(47) Drake CG, Doody AD, Mihalyo MA, Huang CT, Kelleher E, Ravi S, Hipkiss EL, Flies DB, Kennedy EP, Long M, McGary PW, Coryell L, Nelson WG, Pardoll DM, Adler AJ. Androgen ablation mitigates tolerance to a prostate/prostate cancer-restricted antigen.Cancer Cell. (3):239-49,2005.

(48) Hodge JW, Schlom J, Donohue SJ, Tomaszewski JE, Wheeler CW, Levine BS et al. A recombinant vaccinia virus expressing human prostate-specific antigen (PSA): safety and immunogenicity in a non-human primate. Int J Cancer 1995; 63(2):231-237.

(49) Kaufman HL, DiPaola RS, von Mehren M, Marshall J, Lyerly HK, Streicher H et al. Safety profile of therapeutic pox virus-based vaccines for cancer. Proceedings Am Soc Clin Onc . 2004.
Ref Type: Abstract

(50) Marshall J, Gulley JL, Arlen P, Beetham P, Tsang K, Slack R et al. A Phase I Study of Sequential Vaccinations With Fowlpox-CEA (6D)-TRICOMTRICOM (B7.1B7-1/ICAM-1/LFA-3) Alone and Sequentially With Vaccinia-CEA (6D)-TRICOMTRICOM, With and Without GM-CSF, in Patients With CEA-expressing Carcinomas. J Clin Oncol. 23(4):720-31, 2005.

(51) Huggins C, Hodges C. Studies on prostatic cancer: The effect of castration, of estrogen and of androgen injection on serum phosphatases in metastatic carcinoma of the prostate. Cancer Res 1941; 1:293-299.

(52) Kojima S, Suzuki H, Akakura K, et al. Alternative antiandrogens to treat prostate cancer relapse after initial hormone therapy. J Urol 2004; 171(2):679-683.

(53) Kassouf W, Tanguay S, Aprikian A. Nilutamide as second-line hormone therapy for prostate cancer after androgen ablation fails. J Urol 2003; 169(5):1742-1744

(54) Scher HI, Liebertz C, Kelly WK, et al. Bicalutamide for advanced prostate cancer: the natural versus treated history of disease. J Clin Oncol 1997; 15(8):2928-2938.

(55) Miyahira Y, Murata K, Rodriguez D, Rodriguez JR, Esteban M, Rodrigues MM et al. Quantification of Antigen-Specific Cd8(+) T-Cells Using An Elispot Assay. Journal of Immunological Methods 1995; 181(1):45-54.

(56) Scheibenbogen C, Lee KH, Mayer S, Stevanovic S, Moebius U, Herr W et al. A sensitive ELISPOT assay for detection of CD8+ T lymphocytes specific for HLA class I-binding peptide epitopes derived from influenza proteins in the blood of healthy donors and melanoma patients. Clinical Cancer Research 1997; 3(2):221-226.

(57) Schmittel A, Keilholz U, Scheibenbogen C. Evaluation of the interferon-gamma ELISPOT-assay for

quantification of peptide specific T lymphocytes from peripheral blood. Journal of Immunological Methods 1997; 210(2):167-174.

(58) von Mehren M, Arlen P, Tsang KY, Rogatko A, Meropol N, Cooper HS et al. Pilot study of a dual gene recombinant avipox vaccine containing both carcinoembryonic antigen (CEA) and B7.1 transgenes in patients with recurrent CEA-expressing adenocarcinomas. Clin Cancer Res 2000; 6(6):2219-2228.

(59) Arlen P, Tsang KY, Marshall JL, Chen A, Steinberg SM, Poole D, Horan Hand P, Schlom J, and Hamilton JM. The use of a rapid ELISPOT assay to analyze peptide-specific immune responses in carcinoma patients to peptide vs. recombinant poxvirus vaccines. *Cancer Immunol. Immunother*. 49: 517-529, 2000.

(60) D'Amico AV, Cote K, Loffredo M, Renshaw AA, Schultz D. Determinants of prostate cancer specific survival following radiation therapy during the prostate specific antigen era.
J Urol. 2003 Dec;170(6 Pt 2):S42-6; discussion S46-7.

(61) Lee AK, Levy LB, Cheung R, Kuban D. Prostate-specific antigen doubling time predicts clinical outcome and survival in prostate cancer patients treated with combined radiation and hormone therapy. Int J Radiat Oncol Biol Phys. 2005 May 28; [Epub ahead of print]

(62) F. J. Bianco, Z. A. Dotan, M. W. Kattan, A. J. Stephenson, H. I. Scher, J. A. Eastham, P. T. Scardino, P. T. Scardino. Natural History of Biochemically-Recurrent Castrate-Resistant Disease in Men treated with maximal androgen blockage for a Rising PSA after Radical Prostatectomy ASCO- 1st Annual Prostate Cancer Symposium, 2005 abstract #278

(63) M. Smith, F. Kabbinavar, F. Saad, A. Hussain, Y. Hei, E. Small, R. Cook, C. Higano Natural History of Rising Serum PSA in Men with Castrate Nonmetastatic Prostate Cancer ASCO- 1st Annual Prostate Cancer Symposium, 2005abstract #268

(64) PM Arlen, JL Gulley, N Todd, R Lieberman, SM Steinberg, S Morin, A Bastian, J Marte ,KY Tsang, P Beetham, DW Grosenbach, J Schlom, W Dahut. Antiandrogen, vaccine and combination therapy in patients with nonmetastatic hormone refractory prostate cancer
J Urol. 2005;174(2):539-46.

(65) RA Madan, JL Gulley, J Schlom, SM Steinberg, DJ Liewehr, WL Dahut, PM Arlen. Analysis of overall survival in patients with nonmetastatic castration-resistant prostate cancer treated with vaccine, nilutamide, and combination therapy. Clin Cancer Res. 2008;14(14):4526-31.

(66) DV Makarov, EB Humphreys, LA Mangold, MA Carducci, AW Partin, MA Eisenberger, PC Walsh PC, BJ Trock. The natural history of men treated with deferred androgen deprivation therapy in whom metastatic prostate cancer developed following radical prostatectomy. J Urol. 2008;179(1):156-61.

(67) N Sharifi, JL Gulley, WL Dahut. Androgen deprivation therapy for prostate cancer. JAMA. 2005;294(2):238-44

10 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 work of a light or sedentary nature (e.g., light housework, office work).	80	Normal activity with effort; some signs or symptoms of disease.
		70	Cares for self, unable to carry on normal activity or to do active work.
2	In bed <50% of the time. Ambulatory and capable of all self-care, but unable to carry out any work activities. Up and about more than 50% of waking hours.	60	Requires occasional assistance, but is able to care for most of his/her needs.
		50	Requires considerable assistance and frequent medical care.
3	In bed >50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.	40	Disabled, requires special care and assistance.
		30	Severely disabled, hospitalization indicated. Death not imminent.
4	100% bedridden. Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.	20	Very sick, hospitalization indicated. Death not imminent.
		10	Moribund, fatal processes progressing rapidly.
5	Dead.	0	Dead.

11 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 a blisters on day 5 to 6 and then formation of a pustule (or "boil") 1-2 inches in diameter on day 9 to 11. A large area of redness may surround this area. A crusted scab usually forms by the second week and falls off by the third week leaving a scar roughly 1/2 inch in diameter. Fever and malaise (the "blahs") may occur during the blister and pustular phases. Swollen and tender lymph nodes may persist for months. Many of the local toxicities described (e.g., pustule and scab formation) are typical of reactions seen when vaccinia is administered via scarification or intradermal administration. These reactions may be seen, but are usually not seen when administered via subcutaneous injection.

2. How should you care for the vaccination site?

Live vaccinia virus is in skin cells at the vaccination site during the 1-2 weeks until healing has occurred. Maximal viral "shedding" from the vaccination site occurs from days 4-14, but can continue until the scab falls off from the skin. After that there is no vaccinia virus in your body. You can spread the virus to other parts of your body or to other people by touching the vaccination site and then touching your eyes, mouth, a cut or some other break in the skin.You do not pass vaccinia virus by coughing or sneezing or by sharing food or cups and dishes.
In general, frequent careful hand washing by you and by any persons in physical contact with you is the best way to prevent transmission of virus. You should also use two types of barriers over your vaccination site at all times until the scab is gone. These barriers are (1) the bandage and (2) clothing (pants or elbow length sleeves depending on the site of vaccination). These barriers should remain in place until the scab has fallen off.

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

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

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

Wash your hands after each step.

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

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

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

4. What about contact with other people?

Remember, frequent careful hand washing by you and by any persons with whom you have physical contact is the best way to prevent transmission of virus. During the time you need to wear a bandage (for 7-14 days after vaccination) there are several kinds of people with whom you should avoid close contact. "Close contact" means that you sleep in the same bed with the person, give the person baths, and/or touch their bare skin to change their clothes (or diapers), apply ointments, or change their bandages.

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

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

If you have any questions at any time, please call. A nurse or a physician is available 24 hours a

day by telephone. To speak with 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
12 C Oncology Clinic	(301) 496-4026*
Ravi Madan, MD	(301) 222-7762*
James Gulley, MD, PhD	(301) 435-2956*

*after clinic hours the NCI Medical Oncology physician On call through NIH page operator (301) 496-1211

12 APPENDIX C: Protocol Evaluation

	Baseline[1]	W0	W4	W8	W12	Continued treatment and LTFU[8]
History and Physical or Medical Assessment[1]	X		X	X	X	X
Height, Weight, BSA	X		X	X	X	X
Performance Status	X		X	X	X	X
CT Scan of chest/abdomen/pelvis [6]	X				X	
Tc-99 whole body scintigraphy[6]	X				X	
Serum PSA (tumor marker)	X		X	X	X	X
Serum PAP	X		X	X	X	X
Immunology Assays[2]	X		X	X	X	X
ANA titer[2]	X					
Urinalysis[3]	X					
Serum HIV antibody[7]	X					
Serum Hepatitis panel [7]	X					
Lymphocyte Subsets [9]	X		X	X	X	X
CBC with differential	X		X	X	X	X
BUN, Creatinine, ALT, AST, Alk Phosphatase, Total bilirubin, LDH, electrolytes, and glucose	X	X	X	X	X	X
Apheresis[4]	X				X	
EKG[6]	X					
HLA-A2[5]	X					

[1]Baseline: H & P and laboratory studies should be completed within 16 days of initiating treatment.
[2] Immunologic assays include ELISPOT assay (in HLA-A2 patients), CD3, 4, 8 subsets and CD4:CD8 ratio. Research blood will be drawn at baseline, weeks 4, 8, 12, and every 4 weeks while on study (See section **3.4.4** and **APPENDIX E**). ANA titer will be drawn only at baseline and when clinically indicated. As noted in Section **3.4.6,** as of amendment P, all immunologic studies will be optional and at the discretion of the investigator.
[3] Urinalysis will be required at baseline. A 24-hour urine collection for creatinine clearance, protein, and electrolytes may be required if clinically indicated. In patients who are not able to obtain an accurate creatinine clearance, a calculated creatinine clearance and urinalysis will be done.
[4]Apheresis will be requested for immunologic testing from patients at baseline and around weeks 12, 24 and 36 (see section **3.4.6**). Apheresis will be performed only in HLA-A2 positive patients. As noted in Section **3.4.6,** as of amendment P, all immunologic studies will be optional and at the discretion of the investigator.
[5]Typing for HLA-A2 may be obtained anytime prior to receiving the first vaccine.
[6]Baseline radiographic studies should be obtained within 28 days of initiating treatment. For patients with declining PSA no restaging will be done unless they develop symptoms consistent with metastatic disease. For patients with rising PSA, once 2 consecutive PSA rises are seen from the last imaging stuides, CT and bone scans will be done at their next scheduled visit. (Therefore, restaging scans would be done at an interval no less than 3 months.) They will then be re-staged (CT and bone scans) at 3 month intervals as long as PSA continues to rise. Baseline 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.
[7]Serum HIV antibody and serum hepatitis panel within 8 weeks prior to start of enrollment.
[8]Patients who continue on trial after week 12 will continue to get follow-up evaluations. Patients receiving monthly vaccine will continue to have monthly visits. Patients who have been on study for 2 years or more with stable disease and who are not getting vaccine, clinic visits may be scheduled at 8 week intervals. Patients may undergo annual follow-up examinations at the NIH Clinical Center or our research nurse will obtain the information from their local physician for the first 5 years following

examination. These inquiries will focus on clinical information pertaining to development of de novo cancer, neurologic, autoimmune, and hematologic disorders. In addition, medical problems including information on unexpected hospitalizations and medications will be collected.

[9]Lymphocyte subsets will be performed for patients enrolled at the NCI site only. As noted in Section **3.4.6,** as of amendment P, all immunologic studies will be optional and at the discretion of the investigator.

13 APPENDIX D: Instructions for pre-study and follow-up blood tests

Blood Studies:	**Blood tube/Comments:**	**Destination**:
CBC, WBC with differential	1 purple top	Hem/Onc Lab Bldg. 10
BUN, Creatinine SGOT, Alk. Phos, Bilirubin, Albumin Chem 20	1 gold top	Hem/Onc Lab Bldg. 10
CD4:CD8	1 purple top	Hem/Onc Lab Bldg. 10
Hepatitis B and C	1 brown & black tiger top Bldg. 10	Hem/Onc Lab
Serum for HIV antibody run in house"	HIV Consent "Protocol Pt. Please	Hem/Onc Lab Bldg. 10
Serum for PSA	1 gold top	Hem/Onc Lab Bldg. 10
Lymphocyte Subsets	1 purple top	Hem/Onc Lab Bldg. 10
HLA-A2	1 yellow top ACD solution A	NIH Clinical Center Bldg. 10 - HLA Lab
Immunology Assays	6 10ml green tops 2 SST (tiger) top tubes Apheresis product	NCI-Frederick (Leidos) (1-301-846-5893)
Urinalysis	specimen container	Hem/Onc Lab Bldg. 10
ANA	1 7cc red top	Hem/Onc Lab Bldg. 10

14 APPENDIX E: ELISPOT ASSAY

We plan to examine the immune response in selected patients (HLA-A2 positive). Lymphocytes will be separated from heparinized blood using density gradient centrifugation. The lymphocytes will then be placed in human AB serum with 10% DMSO and stored in liquid nitrogen. When samples are available from pre- and post-treatment, the ELISPOT assay will be performed. The ELISPOT assay, measuring γ-IFN production, is used for determination of CTL precursor frequency to PSA-3 peptide [VISNDVCAQV] in both pre- and post-vaccination peripheral blood mononuclear cells (PBMC).(59) Briefly, 96-well milliliter HA plates (Millipore Corporation, Bedford, MA) are coated with 100μl/well of capture MAb against human γ-IFN at a concentration of 10 μg/ml for 12h at RT. Plates are blocked for 30 min with RPMI 1640 plus 10% human Ab serum. 2 x 10^5 PBMC are added to each well. PSA-3A pulsed C1R-A2 cells are added into each well as antigen presenting cells (APC) at an effector: APC ratio of 1:1. Unpulsed C1R-A2 cells are used as a negative control. HLA-A2 binding Flu matrix peptide 59-66 is used as a positive peptide control.(59) We also perform each sample with six replicates to control for variability. In addition, each sample is run with a flu peptide control (pre- and post-vaccine) as well as samples from a "normal" control HLA-A2 positive individual with previously determined levels of flu-specific T-cell precursors. Cells are incubated for 24h and lysed with phosphate buffered saline (PBS)-Tween (0.05%). Biotinylated anti γ-IFN antibody diluted to 2 μg/ml in PBS-Tween containing 1% bovine serum albumin (BSA) is added and incubated overnight in 5% CO_2 at 37°C. Plates are washed 3 times and developed with avidin alkaline phosphatase (GIBCO/BRL, Grand Island, NY) for 45 min. After washing the plates 3 times, each well is examined for positive dots. This assay will be performed in the Laboratory of Tumor Immunology and Biology, NCI, NIH. The number of dots in each well will be counted by two separate investigators in a *blinded manner*, and the frequency of responding cells is determined. It is planned that all patients will undergo exploratory analysis of the ability to detect CD4 positive responses using a whole protein PSA assay.

15 APPENDIX F: NCI-IRB SERIOUS UNEXPECTED ADVERSE EVENT REPORT FORM

Definition of a serious unexpected adverse event: For the purposes of this form, a serious adverse event is any untoward medical occurrence that results in death, is life-threatening, requires or prolongs hospitalization, causes persistent or significant disability/incapacity, results in congenital anomalies/birth defects, or in the opinion of the investigators represents other significant hazards or potentially serious harm to research subjects or others. A serious adverse event is considered unexpected if it is not described in the Package Insert or in the Investigator's Brochure (for FDA investigational agents), in the protocol, or in the informed consent document.

Please complete the information requested below and forward it to the NCI-IRB with a copy to Office of the Clinical Director, as soon as possible, but no later than 10 working days. In addition, continue to follow FDA and the NIH Office of Science Policy (OSP) reporting requirements and procedures if your research involves an IND/IDE or gene transfer.

Protocol number: _____ Protocol Title:
_____
Principal Investigator:
_____
Preferred Contact Information:
_____
Date of Event: _____
Is this a multi-center trial? Yes [ ] No [ ]. If so, is NCI the Coordinating Center? Yes [ ] No [ ]
Location of the event: NIH [ ] Elsewhere [ ] Name of Institution: _____

CTCAE Category: _____ Grade: _____ Toxicity: _____ Severity (If more explanation is required):
_____
Is the Adverse Event Attributable to the research? Yes [ ] No [ ]
Was this unexpected? Yes [ ] No [ ]
Was this in the Consent Form? Yes [ ] No [ ]
Have similar unexpected adverse events occurred on this protocol? Yes [ ] No [ ]
If "Yes", how many? ____ Describe:
_____
_____
What steps do you plan to take as a result of the adverse event reported above? Provide documentation to the IRB for review and approval of any of the steps checked below.

[ ] no action required
[ ] amend protocol
[ ] amend consent document
[ ] inform current subjects
[ ] terminate or suspend protocol
[ ] other (describe)

Is this a sponsored trial? Yes [ ] No [ ]. If yes then you may sign below and attach the required sponsor AE reporting form to the NCI-IRB Adverse Event Report Form. If no, please continue on this form.

Is NCI the Coordinating Center for a Multi-institutional trial? Yes [ ] No [ ]. If yes and this event occurred at an outside facility, you may attach a copy of the AE report. If No, continue on this form.

Signature of the Principal Investigator: _____ Date: _____
Or PI Designated Responsible MD

NCI-IRB SERIOUS UNEXPECTED ADVERSE EVENT REPORT FORM – Continuation Sheet

Brief description of subject(s) (do not include identifiers such as names or SS#s)
Sex: Age: Diagnosis:
MRN:
Brief description of the nature of the serious unexpected adverse event to include: drug, cycle, start date, last dose, narrative of event in relation to time, and a list of the other serious unexpected related events within the study (attach description separately if more space is needed):

_____
_____
_____
_____
_____
_____
_____

FDA Category for this serious unexpected adverse event:

[ ] death
[ ] disability / incapacity
[ ] life-threatening
[ ] congenital anomaly / birth defect
[ ] hospitalization-initial or prolonged
[ ] required intervention to prevent permanent impairment
[ ] other:

Relationship of Serious Unexpected Adverse Event to research:

[ ] 1 = Unrelated (clearly not related to the research)
[ ] 2 = Unlikely (doubtfully related to the research)
[ ] 3 = Possible (may be related to the research)
[ ] 4 = Probable (likely related to the research)
[ ] 5 = Definite (clearly related to the research)

Signature of the Principal Investigator: _____ Date: _____
Or PI Designated Responsible MD

16 APPENDIX G: CTEP MULTICENTER GUIDELINES

If an institution wishes to collaborate with other participating institutions in performing a CTEP sponsored research protocol, then the following guidelines must be followed.

Responsibility of the Protocol Chair

- The Protocol Chair will be the single liaison with the CTEP Protocol and Information Office (PIO). The Protocol Chair 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 Protocol Chair. There will be only one version of the protocol, and each participating institution will use that document. The Protocol Chair is responsible for assuring that all participating institutions are using the correct version of the protocol.
- The Protocol Chair is responsible for the overall conduct of the study at all participating institutions and for monitoring its progress. All reporting requirements to CTEP are the responsibility of the Protocol Chair.
- The Protocol Chair is responsible for the timely review of Adverse Events (AE) to assure safety of the patients.
- The Protocol Chair will be responsible for the review of and timely submission of data for study analysis.

Responsibilities of the Coordinating Center

- Each participating institution will have an appropriate assurance on file with the Office for Human Research Protection (OHRP), NIH. 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 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 is responsible for the preparation of all submitted data for review by the Protocol Chair.
- The Coordinating Center will maintain documentation of AE reports. There are two options for AE reporting: (1) participating institutions may report directly to CTEP with a copy to the Coordinating Center, or (2) participating institutions report to the Coordinating Center who in turn report to CTEP. The Coordinating Center will submit AE reports to the Protocol Chair for timely review.
- Audits may be accomplished in one of two ways: (1) source documents and research records for selected patients are brought from participating sites to the Coordinating Center for audit, or (2) selected patient records may be audited on-site at participating sites. If the NCI chooses to have an audit at the Coordinating Center, then the Coordinating Center is responsible for having all source documents, research records, all IRB approval documents, NCI Drug Accountability Record forms, patient registration lists, response assessments scans, x-rays, etc. available for the audit.

17 APPENDIX H: Model Consent

Description of Research Study

You have been asked to take part in this research because you have prostate cancer that has worsened with a rising serum PSA level despite treatments with initial hormone therapy. Most patients who have rising serum PSA level will initially respond to lowering of the body's level of a hormone called testosterone. This can either be done through surgery (orchiectomy) or by medication (leuprolide or goserelin). If this treatment stops working, patients will often times begin an additional hormone medication called an antiandrogen. There are 3 FDA approved antiandrogens for the treatment of prostate cancer (bicalutamide, nilutamide, and flutamide). Bicalutamide is the most commonly prescribed antiandrogen used in prostate cancer. However, there is no standard treatment for prostate cancer patients who have failed a first line antiandrogen treatment and have a rising serum PSA level but no measurable disease on scans. Many patients will receive a second line antiandrogen therapy, but the response to this treatment generally lasts a few months (approximately 8 to 20 months before measurable disease develops on scans) and not all patients will respond to this therapy. This trial will use the antiandrogen drug flutamide alone or in combination with a cancer vaccine to determine if the addition of the vaccine increases the time to disease progression. This study will determine if adding the vaccine to flutamide will prolong the time to your disease progressing compared to flutamide alone. The vaccine used in this trial is a vaccine aimed at your prostate cancer called PROSTVAC-TRICOM.

PROSTVAC-TRICOM Vaccine
Studies in the laboratory using human tumor cells grown in culture dishes and studies on animals have shown that vaccines may be effective in killing cancer cells. One way in which your body can fight disease is with its own immune system. Your immune system can recognize certain proteins such as bacteria or viruses as foreign and remove them from your body. For unknown reasons however, the immune system fails to fully recognize proteins made by cancer cells. This may be one reason why cancer may grow or spread. A certain protein called prostate specific antigen (PSA) is produced by prostate cancer cells. PSA, thus, is a target for your immune system to attack your prostate cancer. We have developed an experimental vaccine in which we place the genes for PSA inside a virus. This technique can trigger the immune system to make immune cells that may be capable of recognizing and attacking cells that make PSA. This is a vaccination, in concept, like other vaccinations you have probably received, except that it is attempting to increase your body's ability to specifically reject your cancer cells. In this study, you will be vaccinated against the PSA protein that your tumor carries as well as receive other drugs that help the immune system. All of this is an attempt to help your immune system fight the cancer.

The vaccines used in this study consist of 2 parts. The first is a modified vaccinia virus termed PROSTVAC-V/TRICOM. Vaccinia is the same virus that has been used for many years to vaccinate against smallpox. We will refer to PROSTVAC-V/TRICOM as the "priming vaccination." This priming vaccine contains two kinds of human genetic material (DNA) that have been put inside the vaccinia virus. One type of DNA produces the PSA protein and is designed to focus the body's immune response on PSA found in your tumor. The next type of

DNA produces three other proteins (B7-1, ICAM-1, LFA-3), which help increase an immune cells ability to destroy its target. You will receive this priming vaccination only once at the start of the study. It will be given as an injection under the skin, usually in the thigh. Blood samples will be collected for research purposes prior to each vaccination (prior to your first vaccination, then approximately every 4 weeks, prior to each additional vaccine you receive).

The second vaccine is a virus similar to the vaccinia virus in this study is called fowlpox virus. The fowlpox virus contains the same DNA material as the vaccinia virus and is termed PROSTVAC-F/TRICOM. We will refer to this as the "boosting vaccination." You will receive the boosting vaccination 4 weeks after the priming vaccination. You will continue to receive the boosting vaccination every 4 weeks until either your disease worsens (either a rising PSA level or development of disease on scans) or you develop side effects requiring stopping the treatment. It will be given as an injection under the skin, usually in the thigh. These 2 vaccines, the priming and boosting vaccinations, are experimental agents.

Flutamide

Flutamide is a pill that blocks the affects of testosterone on the androgen receptor of the prostate cancer cell and may slow the progression of your prostate cancer. The drug is approved by the FDA for the treatment of prostate cancer. Two capsules are taken by mouth three times a day (a total of 6 pills a day are taken). The most common side effects are usually nausea and diarrhea.

Study Design

It is anticipated that 62 patients will be enrolled onto this protocol. The purpose of this study is to determine if combining the vaccine with flutamide (hormone) treatment will keep your disease from getting worse for a longer period of time than the flutamide alone. Patients must have a rising serum PSA level despite having their testosterone hormone levels lowered (either by surgery (Orchiectomy) or medication (leuprolide or goserelin) and have progressed on a prior antiandrogen pill (either bicalutamide or nilutamide). Patients cannot have metastatic prostate cancer (cancer that has spread to other parts of your body) on x-rays. There are 2 planned treatment groups. Each group will have 31 patients. Patients will be randomized (similar to a "flip of a coin") to receive either arm A, flutamide (hormone) treatment alone, or arm B flutamide treatment with the vaccine.

On day 1 of the study, all patients in both arms A and B will begin to take the flutamide by mouth. You will take two capsules by mouth, three times each day. In addition, patients randomized to arm B (flutamide and vaccine) will also be given the priming vaccination. You will receive this vaccination as an injection under your skin, usually the thigh. You will return in 4 weeks to receive a different vaccine, the boosting vaccine. This vaccine will also be given as an injection under the skin. You will return every 4 weeks to receive boosting vaccinations. Booster vaccines will be given approximately on day 29 and monthly thereafter while on study.
You will be seen in clinic every 4 weeks and will be followed for any clinical response to therapy (blood tests for PSA levels) as well as any toxicity to therapy. Prior to beginning treatment you will undergo a bone scan and CT scan to look for development of metastatic disease. If your PSA levels decline, no additional CT and bone scans will be done unless you

develop symptoms consistent with metastatic disease. If 2 consecutive PSA rises are seen from the last imaging scans, CT and bone scans will be done at your next scheduled visit. (Therefore, restaging scans would be done at an interval no less than 3 months.) You will then have CT and bone scans at 3 month intervals as long as PSA continues to rise. If your PSA levels decline or remain stable, you may continue to receive the treatment you were randomized. If you develop clinical progression (i.e. evidence of metastatic disease on scans), you will be taken off study.

If you do not develop evidence of metastatic disease on scans, but develop biochemical recurrence (i.e rising serum PSA levels), you will undergo the following:

- If randomized to Arm A, you will have flutamide discontinued. Twenty eight days later, we will recheck your serum PSA level and if it continues to rise, you may begin to receive the vaccine as described above. If your PSA level declines 28 days following discontinuation of flutamide, we will continue to check your serum PSA levels on a 28 day basis and if it begins to rise again and your scans show no evidence of metastatic disease, then you may begin to receive the vaccine as described above.
- If randomized to Arm B, you will have flutamide discontinued, but may continue to receive vaccine treatment.

If your PSA level continues to rise following the above treatment changes, you will come off-treatment, but can remain on study without treatment until the development of metastatic disease on scans.

Evaluation Tests

Information regarding your cancer will be requested from your oncologist and you will need to provide a blood sample for special test called HLA (human leukocyte antigen). This is the kind of blood test that is done when people are tested to see if they are a match for organ donation and is similar to finding out a person's blood type. We have the ability to do blood tests on those patients who are HLA-A2 positive to see if the vaccine is having a positive influence on their immune cells. A person's tissue type or HLA can influence how the immune system recognizes certain targets. Thirty to 50% of people are a type called HLA-A2. As part of your initial evaluation, your blood will be tested for your tissue type. The HLA-A2 test will be done at the hospital. There is no evidence that being HLA-A2 positive or negative has any impact on your prostate cancer. You are not required to have this marker to be part of this study.

During your initial visit, you will be examined in the outpatient clinic to see if you fully qualify. You will undergo a series of tests that may include a physical exam, blood tests, a bone scan and a CT scan. The overall evaluation time may take several days, depending on your situation. To find the extent of your prostate cancer, scans will be done within 4 weeks of the start of the study. Additionally, the Pathology Department at the hospital may review your prostate biopsy to confirm the diagnosis of prostate cancer. The level of PSA produced by your cancer will also be checked.

To qualify for this study, you must not have a history of allergy to eggs or to egg products. You must not have had your spleen removed for any reason. An electrocardiogram (EKG) will be done on all patients and possibly further heart studies may be done as needed to determine heart function. Blood tests will be done to be sure that your immune system is normal. The immune

system is made up of certain blood cells, lymph nodes (glands), the spleen and other parts of the body and protects the body against foreign substances like bacteria and possibly certain tumor cells. One of the tests of your immune system will be a blood test for the human immunodeficiency virus (HIV), the cause of acquired immunodeficiency syndrome (AIDS). For the HIV blood test, you will need to sign a separate consent form, which explains how you will be notified if the HIV test is positive. Another test that will be performed is to look for possible infection with Hepatitis B and C. If any of these tests is positive you will not be eligible to participate in this study because of the potential harm the vaccine may cause in patients who test positive for HIV and Hepatitis B and C. Additional blood tests will check to see if your liver, kidneys, and other organs are working well enough to start the treatment. Altogether these initial tests will require about 100 cc (6-7 tablespoonfuls) of blood.

You will have blood drawn from a vein at almost every clinic visit. The amount of blood drawn may vary, but will usually be approximately 4-7 tablespoons. The blood tests will be drawn to monitor your liver, kidneys, and other organs (as previously mentioned above).

Apheresis

In order to measure your immune response, sufficient amount of immune cells called lymphocytes will be needed. Patients who are HLA-A2 positive and are receiving the vaccine (arm B or patients who have crossed over to vaccine from arm A) will be asked to undergo a procedure called apheresis in order to obtain the quantity of lymphocytes needed to measure the immune response to the vaccine. This testing will provide no benefit to you and is part of the experimental portion of this research therapy. You will undergo this procedure 3 times. The first apheresis will be done before starting receiving your first vaccine, the second around day 85 from starting vaccine and the final around day 170 from starting the vaccine while on study. Apheresis is a procedure in which blood will be taken from you and processed by a machine that will take out lymphocytes and then return the rest of your blood to you. The procedure will take from 90 min to 2 hours during which time you will have to remain in a bed or a reclining chair. Individuals with bleeding disorders may be harmed by this procedure. You will be evaluated for such a condition before apheresis is done. All attempts will be made to protect you from any complications. Patients do not need to be hospitalized for the procedure.

Risk or Discomforts of Participation

Vaccinia (Priming Vaccination)

Many of the potential side effects from the vaccination are related to allergic responses to vaccinia or to an abnormal immune system. If you previously have had a smallpox (vaccinia) vaccination, you must have never had an allergic or severe reaction to such a vaccination. You must not have an allergy to eggs or egg products. You must have no skin diseases or open wounds. You must not have any other history of altered immune function, such as HIV. You must not have eczema or a history of eczema or other eczematoid skin disorders. You must also not be immunosuppressed (by disease or therapy), including HIV infection, atopic dermatitis, or have active autoimmune disease (autoimmune neutropenia, thrombocytopenia, or hemolytic anemia; systemic lupus erythematous, Sjogren syndrome, or scleroderma; myasthenia gravis; Goodpasture syndrome; Addison's disease, Hashimoto's thyroiditis, or active Graves' disease). There is a good chance that if you had any of these diagnoses in your medical history, you would

know it and you would recognize these medical terms. You must not have had your spleen removed. You must not have a history of seizures, encephalitis (brain infection) or multiple sclerosis ("MS").

Because you may "shed" live virus through your lesion for several days after vaccination, you must be able to avoid close contact with certain individuals for at least three weeks after the vaccination. Pregnant and breast-feeding women, young children, patients with immune system disorders, and individuals with skin disorders are not eligible for this study and contact with these individuals must be avoided. These individuals include:

- children under 3 years of age
- individuals with active or a history of eczema or other eczematoid skin disorders such as active cases of extensive psoriasis, exfoliative skin diseases, 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 HIV positive blood test, or those receiving immunosuppressive treatment.

"Close contact" means that these people share your house, you have repeated bodily contact with them, and/or you take care of them and touch them with your hands. You must not start treatment if you have any healing scars or skin rashes (for example, a burn or poison ivy), until the skin condition has healed. If you have any questions about this list of precautions or any of these medical terms and diagnoses, you should ask about them before starting treatment. It is very important that you tell us if you have any concerns about these precautions for your own safety and the safety of those you may come in contact with. Furthermore, due to the unknown risk to the fetus, you are advised to avoid fathering a child by practicing effective birth control during and four months following the last injection.

Some side effects could be due to an immune response to the PSA protein that may be caused by the vaccine. Some normal human cells including prostate and salivary gland cells have PSA. If the vaccine causes an immune (or "allergic") reaction against these normal cells, you could develop inflammation of these tissues. This may cause pain or tenderness at these sites. If these symptoms are caused by an immune reaction to your own normal tissues, the effect could be prolonged and difficult to reverse. It is also possible that you may develop a very active antibody (immune) reaction to PSA after the vaccination, which could cause fevers, rashes, joint pains, and, less commonly, kidney failure and severe allergic reaction inside blood vessels (vasculitis) of any part of your body. Care will be taken to minimize the side effects, but other unknown or unexpected side effects that could be severe or fatal are possible. While on this study you will be monitored for side effects and will be treated with appropriate medical care if they occur.

Vaccinia vaccinations have been given to over a billion people to immunize against smallpox. These vaccinations rarely have resulted in serious or fatal (deadly) complications (widespread infection of the virus in the skin, or infections of the eyes, or the brain).

1. On average, vaccinia stays active in your body for approximately 13-14 days. Therefore, prior to receiving your next vaccine, you will be evaluated for evidence of pyoderma (pus filled lesions seen on your skin at or around your vaccine site) or evidence of persistent vaccinia

infection. Physical evidence of persistent viral replication (which would be evidenced by the skin lesions, swelling of lymph nodes, and or fever) would require an evaluation prior to next vaccine administration that might include a skin or lymph node biopsy and may delay the next vaccine.

2. When vaccinia is given to protect against smallpox, it is usually scratched into the outer layers of the skin (scarification) with a two-pronged needle. You will be receiving your vaccine under the skin. A normal reaction after the "scarification" administration in a person who has been previously vaccinated with vaccinia includes appearance of a small bump (papule) in 3 days, a small blister or cluster of blisters in 5-7 days, and healing with little scarring within 2 to 3 weeks. Swollen lymph nodes ("swollen glands") and/or fever are infrequent. In individuals who have not previously received vaccinia, a red bump appearing on the third to fifth day is followed by a blister on day 5 to 6 and then by a pustule or "boil" 1-2 inches in diameter on day 9 to 11. A large area of redness may surround the blister and boil. A crusted scab usually forms by the second week and falls off by the third week leaving a vaccination scar roughly one inch in diameter. Fever and feeling like the flu (malaise) may occur during the blister and boil phases. Enlarged lymph nodes may develop and persist for months. Because you will receive your vaccine by a shot under the skin rather than by scratching it onto the skin the way a smallpox vaccination is given, you may have less of a skin reaction than is described above.

Side effects from the vaccinia vaccine are most common in young children, patients with disorders of the immune system, and individuals with skin disorders. That is why precautions are taken to exclude such individuals from exposure. It is important that you not touch the vaccination site and then touch other parts of your body. This is because the vaccinia virus may be transferred to other sites including the eye, the mucus membrane of the nose or mouth, or other area by rubbing the vaccination site and subsequently rubbing the eye or an open skin area. Spreading the virus in this way is known as autoinoculation. Healing usually occurs in 5-7 days. Blindness can result if vaccinia gets into the eye. A dressing will be placed over the vaccination site to reduce this risk. Generalized vaccinia may be characterized by several small blisters around the vaccination site or by widely distributed lesions developing 7-12 days after immunization. This is also known as a disseminated vaccinia infection. These lesions tend to follow a course of healing similar to that of the inoculation site. An allergic reaction to the vaccine with a rash or hives may occur within 7-10 days of vaccination and usually goes away within 2-4 days. Rarely, a serious allergic reaction requiring hospitalization may occur. The most serious reactions include post-vaccinia encephalomyelitis ("brain infection"), which can lead to coma and death and vaccinia gangrenosum which leads to a large non-healing sore and may lead to death. They occur almost exclusively in very young children who are exposed to vaccinia for the first time or in patients with impaired immunity; such individuals are not eligible for this study and those that must be avoided after vaccinia vaccination. The death rate for people receiving revaccination with vaccinia for smallpox is about 0.1 per million. Vaccinia Immune Globulin (VIG) has been successful as a therapy for some but not all of these complications. VIG is an injectable antibody preparation made from the plasma of people vaccinated with the vaccinia vaccine. VIG is not a commercially available drug and is considered an experimental agent. If symptoms develop suggestive of one of the previously described vaccinia complications, or a close contact occurs between a recently vaccinia-vaccinated patient and a susceptible person with one of the pre-existing medical conditions described above, the patient should report the findings immediately to the protocol investigator

or other established contact, for consideration for VIG therapy, since VIG may work better if given early. There is currently no other known effective treatment for these complications.

During the reintroduction of smallpox vaccination, several individuals thought to be at risk for heart disease experience heart attacks or angina. It is not known if the vaccinations had any connection to these events. However, in March 2003, the Centers for Disease Control and Prevention (CDC) recommended that patients with heart disease or at risk for heart disease, at least temporarily delay smallpox vaccination. A few individuals who had not been previously vaccinated developed inflammation in or around the heart. Although the incidence was very low, if you have poor heart function requiring treatment, you would not be eligible for this study.

Fowlpox (Boosting Vaccination)
The fowlpox virus does not grow in human cells and serious side effects from fowlpox have not been reported in humans. With any experimental compound, however, there is the risk of unexpected and serious or deadly complications, even if they have not been seen previously.

Some side effects could be due to an immune response to the PSA protein that may be caused by the vaccine. Some normal human cells including prostate and salivary gland cells have PSA. If the vaccine causes an immune (or "allergic") reaction against these normal cells, you could develop inflammation of these tissues. This may cause pain or tenderness at these sites. If these symptoms are caused by an immune reaction to your own normal tissues, the effect could be prolonged and difficult to reverse. It is also possible that you may develop a very active antibody (immune) reaction to PSA after the vaccination, which could cause fevers, rashes, joint pains, and, less commonly, kidney failure and severe allergic reaction inside blood vessels (vasculitis) of any part of your body. You may also experience fatigue, anemia (low red blood cell count), and leukopenia (low white blood cell count). Care will be taken to minimize the side effects, but other unknown or unexpected side effects that could be severe or fatal are possible. While on this study you will be monitored for side effects and will be treated with appropriate medical care if they occur.

Additional risks and side effects related to the vaccine therapy with PROSTVAC-V/TRICOM (priming vaccination) and PROSVTAC-F/TRICOM (boosting vaccination):

Possible:

- Swelling of the lymph nodes
- Constipation
- Diarrhea
- Nausea or the urge to vomit
- Chills
- Swelling of the arms and/or legs
- Fatigue or tiredness
- Fever
- Inflammation (swelling and redness) or damage to the tissue surrounding where a drug was injected
- Pain
- Joint pain
- Dizziness (or sensation of lightheadedness, unsteadiness, or giddiness)

- Itching

Patients receiving fowlpox vaccines should avoid direct contact with pet birds for at least 72 hours after vaccination or while there are any visible lesions at the injection site.

One patient treated with this vaccine developed thrombotic thrombocytopenic purpura (TTP). This is a serious disease that is associated with low blood counts (both red blood cells that carry oxygen and platelets that help your blood clot), bleeding, fever, neurologic symptoms (such as changes in level of alertness including coma, headache, difficulty speaking confusion or paralysis) and kidney dysfunction. The symptoms are due to the formation of clots that form or spread to many organs. This can usually be treated with exchange plasmapheresis, a therapy that removes and replaces plasma the protein containing fluid from a patient's blood. Should you go on this trial, we will follow you closely for any signs or symptoms of this disease.

Flutamide
Diarrhea, nausea, and vomiting are the most common gastrointestinal (GI) side effects, occurring in 11%–12% of patients. Other GI side effects that are not very common (approximately 1 in 20 patients taking flutamide) are increased appetite, upset stomach, constipation, decreased appetite, and indigestion. Gynecomastia (breast enlargement) and breast tenderness can occasionally occur. Abnormalities in your liver enzymes can occasionally occur. Damage to your liver (hepatitis) can also occur, but is uncommon. In very rare circumstances this has resulted in death. Other side effects include changes in your blood count resulting in decrease in your red blood cells (anemia 6%), decrease in your white blood cells (leucopenia and neutropenia – 1-3%). Additional side effects that can occur are hot flashes, intense amber to yellow-green urine discoloration, transiently mild declines in your kidney function. The use of flutamide may decrease your desire for sex and your ability to have an erection. Drowsiness, confusion, nervousness, headache, dizziness, insomnia, weakness, depression, anxiety, have been reported rarely (< 1%) in patients during flutamide administration. Myocardial infarction and myocardial ischemia have occurred rarely during flutamide administration. Rare cases of hypertension (1% of 294 patients) have been reported during flutamide treatment among patients who received a LHRH agonist concomitantly. Blurred vision has been rarely reported with flutamide therapy. Photosensitivity after sun exposure, with development of a skin rash may occur. You should apply topical sunscreens to sun-exposed skin and use protective clothing against exposure to excessive sunlight

Apheresis
The potential side effects of apheresis may be pain and bruising at needle sites, lightheadedness and rarely, fainting due to brief lowering of blood pressure. There is a very small chance of introducing infection at the site of the needle. Blood infections from contamination of the apheresis machine are a remote possibility. A tingling sensation around the mouth, fingers, or toes, and mild muscle cramps may occasionally occur as a side effect of a blood thinner used during the procedure. These symptoms are brief and may often be stopped by slowing the procedure.

Duration of Treatment

Your active participation in this research project will be for a period of approximately 6-9 months. It is important to emphasize that the supply of PROSTVAC-V/TRICOM (vaccinia) and PROSTVAC-F/TRICOM (fowlpox) may be limited and continued availability of the two agents cannot be guaranteed. Thus, even if there is improvement in your case, there may not be drug available for re-treatment. You may be treated much longer if your disease responds to the study drugs and you do not experience severe side effects. However, this period may be shorter depending on if your disease gets worse or if you experience serious side effects from the treatment. Your participation may be ended by the investigator, the National Cancer Institute (NCI), or by the Food and Drug Administration (FDA) for reasons that would be explained to you. For example, the investigator may stop your treatment for medical or safety reasons. New information developed during the course of this study that may affect your willingness to continue in this research project will be given to you as it becomes available.

Potential Benefit for Participation

The major purpose of this study is to first learn whether or not this combination of flutamide with vaccine therapy will improve your clinical response compared to flutamide treatment alone. We will also look at the safety of combining these agents. The information obtained from this study will allow us to design more effective treatments in the future. This is an investigational study; therefore, no benefit is known or guaranteed. You will be monitored closely while you are receiving this experimental treatment for any signs that might signal the earliest stage of toxicity so that appropriate intervention can be done.

You will be informed of any significant findings resulting from the study including any new information about the experimental procedure, the harms and benefits experienced by any individuals involved in the study, and long term side effects that had been observed.

Alternative Treatments

Chemotherapy with docetaxel has been shown to prolong life of patients with metastatic prostate cancer who have rising PSA despite hormonal therapy but currently there is no standard of care for patients who have not developed metastatic disease. To be eligible for this study you must have lowered serum levels of testosterone in your body (surgically lowered by orchiectomy or take medications such as leuprolide or goserelin) and you must have a rising PSA level on at least one antiandrogen drug (either bicalutamide or nilutamide). You must not have evidence of metastatic disease on bone scan or CT scan. You may or may not benefit from participation in this study. Participation in this study is purely voluntary. Choosing not to participate (or withdrawing at any point) from this study will in no way penalize your medical care or your relationship with you physicians. If you choose not to participate in this study, there are several alternative treatments/therapy for your cancer. They include:

Treatment with other hormone drugs including flutamide, nilutamide, or bicalutamide
Experimental treatments such as other types of immune therapy or chemotherapy (which is currently not considered standard of care for patients without metastatic disease)

No current treatment but watchful waiting

Each of these alternative therapies has a unique set of benefits and risks. Your physician will discuss these options with you. Furthermore, other centers are performing clinical trials with investigational agents as well as standard chemotherapy that you may consider as an alternative to this proposed trial.

Communication

The physicians involved in your care are available to answer all of your questions concerning this protocol. If you have any concerning or questions, you may contact Dr. (*insert physician name)*, the Principal Investigator at (*insert telephone number*).

Confidentiality of Your Own Records

You will receive a copy of this informed consent for your own records. Your records will be kept confidential, with the exception that personnel or representatives from the NCI, FDA, NIH Office of Biotechnology Activities and the drug companies that manufacture the investigational study drugs may have access to your medical record.

Research Subject's Rights

Your participation in this study is entirely voluntary, and you may refuse to participate, or withdraw from this protocol at any time and receive care from a physician of your choice. Your participation in this study may be ended by the Principal Investigator or an Associate Investigator without your consent if they feel termination is medically indicated.

Completion of Study

Because you are a study participant, investigators will ask your family for permission to do an autopsy when you die, even though this may be years after the study. This may help investigators learn about the effects of this experimental vaccine. By signing this consent form, you are not forcing your family to agree to this. You should talk about this request with your family and advise them of your wishes.

Your signature on this form indicates that you agree to participate in this medical research study under the direction of the principal investigator as listed above.

Disclosure

The Laboratory of Tumor Immunology and Biology, National Cancer Institute had an initial agreement with Therion Biologics Corporation, the manufacturer of this vaccine. The NCI now has transferred this agreement to BN Immunotherapeutics. Under this agreement, the manufacturer provides vaccine and some money for pre-clinical (laboratory) studies, as well as vaccine for clinical trials such as this one.

The National Institute of Health and members of the research team have developed a drug/device/product which is being used in this research. This means that it is possible that the results of this study could lead to payments to NIH scientists and to the National Institute of Health. By law, government scientists are required to receive such payment for their inventions. You will not receive any money from development of the drug/device/product.

Cost

You and/or your health plan/ insurance company will need to pay for some or all of the costs of treating your cancer in this study. Some health plans will not pay these costs for people taking part in studies. Check with your health plan or insurance company to find out what they will pay for. Taking part in this study may or may not cost your insurance company more than the cost of getting regular cancer treatment.

The NCI will supply the PROSTVAC-V/TRICOM and PROSTVAC-F/TRICOM vaccines at no charge while you take part in this study. The NCI does not cover the cost of getting the vaccines ready and giving it to you, so you or your insurance company may have to pay for this.

Even though it probably won't happen, it is possible that the manufacturer may not continue to provide the PROSTVAC-V/TRICOM and PROSTVAC-F/TRICOM to the NCI for some reason. If this would occur, other possible options are:

- You might be able to get the PROSTVAC-V/TRICOM and PROSTVAC-F/TRICOM from the manufacturer or your pharmacy but you or your insurance company may have to pay for it.

- If there is no PROSTVAC-V/TRICOM and PROSTVAC-F/TRICOM available at all, no one will be able to get more and the study would close.

If a problem with getting PROSTVAC-V/TRICOM and PROSTVAC-F/TRICOM occurs, your study doctor will talk to you about these options.

You will not be paid for taking part in this study.

For more information on clinical trials and insurance coverage, you can visit the National Cancer Institute's Web site at http://cancer.gov/clinicaltrials/understanding/insurance-coverage. You can print a copy of the "Clinical Trials and Insurance Coverage" information from this Web site.

Another way to get the information is to call 1-800-4-CANCER (1-800-422-6237) and ask them to send you a free copy.

OTHER PERTINENT INFORMATION

1. Confidentiality. When results of an NIH research study are reported in medical journals or at scientific meetings, the people who take part are not named and identified. In most cases, the NIH will not release any information about your research involvement without your written

permission. However, if you sign a release of information form, for example, for an insurance company, the NIH will give the insurance company information from your medical record. This information might affect (either favorably or unfavorably) the willingness of the insurance company to sell you insurance.

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

2. Problems or Questions. If you have any problems or questions about this study, or about your rights as a research participant, or about any research-related injury, contact the Principal Investigator, (*insert investigator name*), M.D., (*insert investigator address*), Telephone: (*insert investigator telephone number*).

Adult Patient's Consent

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

_____

Signature of Adult Patient/Legal Representative Date

_____

Print Name

_____

Signature of Investigator Date

_____

Print Name

INSTITUTE: National Cancer Institute

STUDY NUMBER: 07-C-0107 PRINCIPAL INVESTIGATOR: Ravi Madan, MD

STUDY TITLE: A Randomized Phase II Trial Combining Vaccine Therapy with PROSTVAC /TRICOM and Flutamide, vs. Flutamide Alone in Men with Androgen Insensitive, Non Metastatic (D0.5) Prostate Cancer

Continuing Review Approved by the IRB on 06/27/16
Amendment Approved by the IRB on 04/05/17 (P) Date Posted to Web: 04/18/17
Standard

INTRODUCTION

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

First, we want you to know that:

Taking part in NIH research is entirely voluntary.

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

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

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

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

Revision Copy

Description of Research Study

You have been asked to take part in this research because you have prostate cancer that has worsened with a rising serum PSA level despite treatments with initial hormone therapy. Most patients who have rising serum PSA level will initially respond to lowering of the body's level of a hormone called testosterone. This can either be done through surgery (orchiectomy) or by medication (leuprolide or goserelin). If this treatment stops working, patients will often times begin an additional hormone medication called an antiandrogen. There are 3 FDA approved antiandrogens for the treatment of prostate cancer (bicalutamide, nilutamide, and flutamide). Bicalutamide is the most commonly prescribed antiandrogen used in prostate cancer. However, there is no standard treatment for prostate cancer patients who have failed a first line antiandrogen treatment and have a rising serum PSA level but no measurable disease on scans. Many patients will receive a second line antiandrogen therapy, but the response to this treatment generally lasts a few months (approximately 8 to 20 months before measurable disease develops on scans) and not all patients will respond to this therapy. This trial will use the antiandrogen drug flutamide alone or in combination with a cancer vaccine to determine if the addition of the vaccine increases the time to disease progression. This study will determine if adding the vaccine to flutamide will prolong the time to your disease progressing compared to flutamide alone. The vaccine used in this trial is a vaccine aimed at your prostate cancer called PROSTVAC-TRICOM.

PROSTVAC-TRICOM Vaccine

Studies in the laboratory using human tumor cells grown in culture dishes and studies on animals have shown that vaccines may be effective in killing cancer cells. One way in which your body can fight disease is with its own immune system. Your immune system can recognize certain proteins such as bacteria or viruses as foreign and remove them from your body. For unknown reasons however, the immune system fails to fully recognize proteins made by cancer cells. This may be one reason why cancer may grow or spread. A certain protein called prostate specific antigen (PSA) is produced by prostate cancer cells. PSA, thus, is a target for your immune system to attack your prostate cancer. We have developed an experimental vaccine in which we place the genes for PSA inside a virus. This technique can trigger the immune system to make immune cells that may be capable of recognizing and attacking cells that make PSA. This is a vaccination, in concept, like other vaccinations you have probably received, except that it is attempting to increase your body's ability to specifically reject your cancer cells. In this study, you will be vaccinated against the PSA protein that your tumor carries as well as receive other drugs that help the immune system. All of this is an attempt to help your immune system fight the cancer.

The vaccines used in this study consist of 2 parts. The first is a modified vaccinia virus termed PROSTVAC-V/TRICOM. Vaccinia is the same virus that has been used for many years to vaccinate against smallpox. We will refer to PROSTVAC-V/TRICOM as the "priming vaccination." This priming vaccine contains two kinds of human genetic material (DNA) that

have been put inside the vaccinia virus. One type of DNA produces the PSA protein and is designed to focus the body's immune response on PSA found in your tumor. The next type of DNA produces three other proteins (B7-1, ICAM-1, LFA-3), which help increase an immune cells ability to destroy its target. You will receive this priming vaccination only once at the start of the study. It will be given as an injection under the skin, usually in the thigh. Blood samples will be collected for research purposes prior to each vaccination (prior to your first vaccination, then approximately every 4 weeks, prior to each additional vaccine you receive).

The second vaccine is a virus similar to the vaccinia virus in this study is called fowlpox virus. The fowlpox virus contains the same DNA material as the vaccinia virus and is termed PROSTVAC-F/TRICOM. We will refer to this as the "boosting vaccination." You will receive the boosting vaccination 4 weeks after the priming vaccination. You will continue to receive the boosting vaccination every 4 weeks until either your disease worsens (either a rising PSA level or development of disease on scans) or you develop side effects requiring stopping the treatment. It will be given as an injection under the skin, usually in the thigh. These 2 vaccines, the priming and boosting vaccinations, are experimental agents.

Flutamide

Flutamide is a pill that blocks the affects of testosterone on the androgen receptor of the prostate cancer cell and may slow the progression of your prostate cancer. The drug is approved by the FDA for the treatment of prostate cancer. Two capsules are taken by mouth three times a day (a total of 6 pills a day are taken). The most common side effects are usually nausea and diarrhea.

Study Design

It is anticipated that 62 patients will be enrolled onto this protocol. The purpose of this study is to determine if combining the vaccine with flutamide (hormone) treatment will keep your disease from getting worse for a longer period of time than the flutamide alone. Patients must have a rising serum PSA level despite having their testosterone hormone levels lowered (either by surgery (Orchiectomy) or medication (leuprolide or goserelin) and have progressed on a prior antiandrogen pill (either bicalutamide or nilutamide). Patients cannot have metastatic prostate cancer (cancer that has spread to other parts of your body) on x-rays. There are 2 planned treatment groups. Each group will have 31 patients. Patients will be randomized (similar to a "flip of a coin") to receive either arm A, flutamide (hormone) treatment alone, or arm B flutamide treatment with the vaccine.

On day 1 of the study, all patients in both arms A and B will begin to take the flutamide by mouth. You will take two capsules by mouth, three times each day. In addition, patients randomized to arm B (flutamide and vaccine) will also be given the priming vaccination. You will receive this vaccination as an injection under your skin, usually the thigh. You will return in 4 weeks to receive a different vaccine, the boosting vaccine. This vaccine will also be given as an injection

under the skin. You will return every 4 weeks to receive boosting vaccinations. Booster vaccines will be given approximately on day 29 and monthly thereafter while on study.
You will be seen in clinic every 4 weeks and will be followed for any clinical response to therapy (blood tests for PSA levels) as well as any toxicity to therapy. Prior to beginning treatment you will undergo a bone scan and CT scan to look for development of metastatic disease. If your PSA levels decline no restaging (CT and bone scans) will be done unless you develop symptoms consistent with metastatic disease. If 2 consecutive PSA rises are seen from the last imaging studies, CT and bone scans will be done at your next scheduled visit. (Therefore, restaging scans would be done at an interval no less than 3 months.) You will then have CT and bone scans at 3 month intervals as long as PSA continues to rise. If your PSA levels decline or remain stable, you may continue to receive the treatment you were randomized. If you develop clinical progression (i.e. evidence of metastatic disease on scans), you will be taken off study.

If you do not develop evidence of metastatic disease on scans, but develop biochemical recurrence (i.e. rising serum PSA levels), you will undergo the following:

- If randomized to Arm A, you will have flutamide discontinued. Twenty eight days later, we will recheck your serum PSA level and if it continues to rise, you may begin to receive the vaccine as described above. If your PSA level declines 28 days following discontinuation of flutamide, we will continue to check your serum PSA levels on a 28 day basis and if it begins to rise again and your scans show no evidence of metastatic disease, then you may begin to receive the vaccine as described above.
- If randomized to Arm B, you will have flutamide discontinued, but may continue to receive vaccine treatment.

If your PSA level continues to rise following the above treatment changes, you will come off-treatment, but can remain on study without treatment until the development of metastatic disease on scans.

Evaluation Tests

Information regarding your cancer will be requested from your oncologist and you will need to provide a blood sample for special test called HLA (human leukocyte antigen). This is the kind of blood test that is done when people are tested to see if they are a match for organ donation and is similar to finding out a person's blood type. We have the ability to do blood tests on those patients who are HLA-A2 positive to see if the vaccine is having a positive influence on their immune cells. A person's tissue type or HLA can influence how the immune system recognizes certain targets. Thirty to 50% of people are a type called HLA-A2. As part of your initial evaluation, your blood will be tested for your tissue type. The HLA-A2 test will be done at the NIH. There is no evidence that being HLA-A2 positive or negative has any impact on your prostate cancer. You are not required to have this marker to be part of this study.

During your initial visit, you will be examined in the outpatient clinic at the NIH to see if you fully qualify. You will undergo a series of tests that may include a physical exam, blood tests, a bone

scan and a CT scan. The overall evaluation time may take several days, depending on your situation. To find the extent of your prostate cancer, scans will be done within 4 weeks of the start of the study. Additionally, the Pathology Department at the NIH may review your prostate biopsy to confirm the diagnosis of prostate cancer. The level of PSA produced by your cancer will also be checked.

To qualify for this study, you must not have a history of allergy to eggs or to egg products. You must not have had your spleen removed for any reason. An electrocardiogram (EKG) will be done on all patients and possibly further heart studies may be done as needed to determine heart function. Blood tests will be done to be sure that your immune system is normal. The immune system is made up of certain blood cells, lymph nodes (glands), the spleen and other parts of the body and protects the body against foreign substances like bacteria and possibly certain tumor cells. One of the tests of your immune system will be a blood test for the human immunodeficiency virus (HIV), the cause of acquired immunodeficiency syndrome (AIDS). For the HIV blood test, you will need to sign a separate consent form, which explains how you will be notified if the HIV test is positive. Another test that will be performed is to look for possible infection with Hepatitis B and C. If any of these tests is positive you will not be eligible to participate in this study because of the potential harm the vaccine may cause in patients who test positive for HIV and Hepatitis B and C. Additional blood tests will check to see if your liver, kidneys, and other organs are working well enough to start the treatment. Altogether these initial tests will require about 100 cc (6-7 tablespoonfuls) of blood.

You will have blood drawn from a vein at almost every clinic visit. The amount of blood drawn may vary, but will usually be approximately 4-7 tablespoons. The blood tests will be drawn to monitor your liver, kidneys, and other organs (as previously mentioned above).

Apheresis

In order to measure your immune response, sufficient amount of immune cells called lymphocytes will be needed. Patients who are HLA-A2 positive and are receiving the vaccine (arm B or patients who have crossed over to vaccine from arm A) will be asked to undergo a procedure called apheresis in order to obtain the quantity of lymphocytes needed to measure the immune response to the vaccine. This testing will provide no benefit to you and is part of the experimental portion of this research therapy. You will undergo this procedure 3 times. The first apheresis will be done before starting receiving your first vaccine, the second around day 85 from starting vaccine and the final around day 170 from starting the vaccine while on study. Apheresis is a procedure in which blood will be taken from you and processed by a machine that will take out lymphocytes and then return the rest of your blood to you. The procedure will take from 90 min to 2 hours during which time you will have to remain in a bed or a reclining chair. Individuals with bleeding disorders may be harmed by this procedure. You will be evaluated for such a condition before apheresis is done. All attempts will be made to protect you from any complications. Patients do not need to be hospitalized for the procedure. The apheresis

procedure will be done at the Department of Transfusion Medicine (Blood Bank) in the NIH Clinical Center and is supervised by Blood Bank physicians.

Risk or Discomforts of Participation

Vaccinia (Priming Vaccination)

Many of the potential side effects from the vaccination are related to allergic responses to vaccinia or to an abnormal immune system. If you previously have had a smallpox (vaccinia) vaccination, you must have never had an allergic or severe reaction to such a vaccination. You must not have an allergy to eggs or egg products. You must have no skin diseases or open wounds. You must not have any other history of altered immune function, such as HIV. You must not have eczema or a history of eczema or other eczematoid skin disorders. You must also not be immunosuppressed (by disease or therapy), including HIV infection, atopic dermatitis, or have active autoimmune disease (autoimmune neutropenia, thrombocytopenia, or hemolytic anemia; systemic lupus erythematous, Sjogren syndrome, or scleroderma; myasthenia gravis; Goodpasture syndrome; Addison's disease, Hashimoto's thyroiditis, or active Graves' disease). There is a good chance that if you had any of these diagnoses in your medical history, you would know it and you would recognize these medical terms. You must not have had your spleen removed. You must not have a history of seizures, encephalitis (brain infection) or multiple sclerosis ("MS").

Because you may "shed" live virus through your lesion for several days after vaccination, you must be able to avoid close contact with certain individuals for at least three weeks after the vaccination. Pregnant and breast-feeding women, young children, patients with immune system disorders, and individuals with skin disorders are not eligible for this study and contact with these individuals must be avoided. These individuals include:

- children under 3 years of age
- individuals with active or a history of eczema or other eczematoid skin disorders such as active cases of extensive psoriasis, exfoliative skin diseases, 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 HIV positive blood test, or those receiving immunosuppressive treatment.

"Close contact" means that these people share your house, you have repeated bodily contact with them, and/or you take care of them and touch them with your hands. You must not start treatment if you have any healing scars or skin rashes (for example, a burn or poison ivy), until the skin condition has healed. If you have any questions about this list of precautions or any of these medical terms and diagnoses, you should ask about them before starting treatment. It is very important that you tell us if you have any concerns about these precautions for your own safety and the safety of those you may come in contact with. Furthermore, due to the unknown risk to the fetus, you are advised to avoid fathering a child by practicing effective birth control during and four months following the last injection.

Some side effects could be due to an immune response to the PSA protein that may be caused by the vaccine. Some normal human cells including prostate and salivary gland cells have PSA. If the vaccine causes an immune (or "allergic") reaction against these normal cells, you could develop inflammation of these tissues. This may cause pain or tenderness at these sites. If these symptoms are caused by an immune reaction to your own normal tissues, the effect could be prolonged and difficult to reverse. It is also possible that you may develop a very active antibody (immune) reaction to PSA after the vaccination, which could cause fevers, rashes, joint pains, and, less commonly, kidney failure and severe allergic reaction inside blood vessels (vasculitis) of any part of your body. Care will be taken to minimize the side effects, but other unknown or unexpected side effects that could be severe or fatal are possible. While on this study you will be monitored for side effects and will be treated with appropriate medical care if they occur.

Vaccinia vaccinations have been given to over a billion people to immunize against smallpox. These vaccinations rarely have resulted in serious or fatal (deadly) complications (widespread infection of the virus in the skin, or infections of the eyes, or the brain).

1. On average, vaccinia stays active in your body for approximately 13-14 days. Therefore, prior to receiving your next vaccine, you will be evaluated for evidence of pyoderma (pus filled lesions seen on your skin at or around your vaccine site) or evidence of persistent vaccinia infection. Physical evidence of persistent viral replication (which would be evidenced by the skin lesions, swelling of lymph nodes, and or fever) would require an evaluation prior to next vaccine administration that might include a skin or lymph node biopsy and may delay the next vaccine.
2. When vaccinia is given to protect against smallpox, it is usually scratched into the outer layers of the skin (scarification) with a two-pronged needle. You will be receiving your vaccine under the skin. A normal reaction after the "scarification" administration in a person who has been previously vaccinated with vaccinia includes appearance of a small bump (papule) in 3 days, a small blister or cluster of blisters in 5-7 days, and healing with little scarring within 2 to 3 weeks. Swollen lymph nodes ("swollen glands") and/or fever are infrequent. In individuals who have not previously received vaccinia, a red bump appearing on the third to fifth day is followed by a blister on day 5 to 6 and then by a pustule or "boil" 1-2 inches in diameter on day 9 to 11. A large area of redness may surround the blister and boil. A crusted scab usually forms by the second week and falls off by the third week leaving a vaccination scar roughly one inch in diameter. Fever and feeling like the flu (malaise) may occur during the blister and boil phases. Enlarged lymph nodes may develop and persist for months. Because you will receive your vaccine by a shot under the skin rather than by scratching it onto the skin the way a smallpox vaccination is given, you may have less of a skin reaction than is described above.

Side effects from the vaccinia vaccine are most common in young children, patients with disorders of the immune system, and individuals with skin disorders. That is why precautions are taken to exclude such individuals from exposure. It is important that you not touch the vaccination site and then touch other parts of your body. This is because the vaccinia virus may be transferred to other sites including the eye, the mucus membrane of the nose or mouth, or other area by rubbing

the vaccination site and subsequently rubbing the eye or an open skin area. Spreading the virus in this way is known as autoinoculation. Healing usually occurs in 5-7 days. Blindness can result if vaccinia gets into the eye. A dressing will be placed over the vaccination site to reduce this risk. Generalized vaccinia may be characterized by several small blisters around the vaccination site or by widely distributed lesions developing 7-12 days after immunization. This is also known as a disseminated vaccinia infection. These lesions tend to follow a course of healing similar to that of the inoculation site. An allergic reaction to the vaccine with a rash or hives may occur within 7-10 days of vaccination and usually goes away within 2-4 days. Rarely, a serious allergic reaction requiring hospitalization may occur. The most serious reactions include post-vaccinia encephalomyelitis ("brain infection"), which can lead to coma and death and vaccinia gangrenosum which leads to a large non-healing sore and may lead to death. They occur almost exclusively in very young children who are exposed to vaccinia for the first time or in patients with impaired immunity; such individuals are not eligible for this study and those that must be avoided after vaccinia vaccination. The death rate for people receiving revaccination with vaccinia for smallpox is about 0.1 per million. Vaccinia Immune Globulin (VIG) has been successful as a therapy for some but not all of these complications. VIG is an injectable antibody preparation made from the plasma of people vaccinated with the vaccinia vaccine. VIG is not a commercially available drug and is considered an experimental agent. If symptoms develop suggestive of one of the previously described vaccinia complications, or a close contact occurs between a recently vaccinia-vaccinated patient and a susceptible person with one of the pre-existing medical conditions described above, the patient should report the findings immediately to the protocol investigator or other established contact, for consideration for VIG therapy, since VIG may work better if given early. There is currently no other known effective treatment for these complications.

During the reintroduction of smallpox vaccination, several individuals thought to be at risk for heart disease experience heart attacks or angina. It is not known if the vaccinations had any connection to these events. However, in March 2003, the Centers for Disease Control and Prevention (CDC) recommended that patients with heart disease or at risk for heart disease, at least temporarily delay smallpox vaccination. A few individuals who had not been previously vaccinated developed inflammation in or around the heart. Although the incidence was very low, if you have poor heart function requiring treatment, you would not be eligible for this study.

Fowlpox (Boosting Vaccination)

The fowlpox virus does not grow in human cells and serious side effects from fowlpox have not been reported in humans. With any experimental compound, however, there is the risk of unexpected and serious or deadly complications, even if they have not been seen previously.

Some side effects could be due to an immune response to the PSA protein that may be caused by the vaccine. Some normal human cells including prostate and salivary gland cells have PSA. If the vaccine causes an immune (or "allergic") reaction against these normal cells, you could develop inflammation of these tissues. This may cause pain or tenderness at these sites. If these

symptoms are caused by an immune reaction to your own normal tissues, the effect could be prolonged and difficult to reverse. It is also possible that you may develop a very active antibody (immune) reaction to PSA after the vaccination, which could cause fevers, rashes, joint pains, and, less commonly, kidney failure and severe allergic reaction inside blood vessels (vasculitis) of any part of your body. You may also experience fatigue, anemia (low red blood cell count), and leukopenia (low white blood cell count). Care will be taken to minimize the side effects, but other unknown or unexpected side effects that could be severe or fatal are possible. While on this study you will be monitored for side effects and will be treated with appropriate medical care if they occur.

Additional risks and side effects related to the vaccine therapy with PROSTVAC-V/TRICOM (priming vaccination) and PROSVTAC-F/TRICOM (boosting vaccination):
Possible:

- Swelling of the lymph nodes
- Constipation
- Diarrhea
- Nausea or the urge to vomit
- Chills
- Swelling of the arms and/or legs
- Fatigue or tiredness
- Fever
- Inflammation (swelling and redness) or damage to the tissue surrounding where a drug was injected
- Pain
- Joint pain
- Dizziness (or sensation of lightheadedness, unsteadiness, or giddiness)
- Itching

Patients receiving fowlpox vaccines should avoid direct contact with pet birds for at least 72 hours after vaccination or while there are any visible lesions at the injection site.

One patient treated with this vaccine developed thrombotic thrombocytopenic purpura (TTP). This is a serious disease that is associated with low blood counts (both red blood cells that carry oxygen and platelets that help your blood clot), bleeding, fever, neurologic symptoms (such as changes in level of alertness including coma, headache, difficulty speaking confusion or paralysis) and kidney dysfunction. The symptoms are due to the formation of clots that form or spread to many organs. This can usually be treated with exchange plasmapheresis, a therapy that removes and replaces plasma the protein containing fluid from a patient's blood. Should you go on this trial, we will follow you closely for any signs or symptoms of this disease.

Flutamide

Diarrhea, nausea, and vomiting are the most common gastrointestinal (GI) side effects, occurring in 11%–12% of patients. Other GI side effects that are not very common (approximately 1 in 20 patients taking flutamide) are increased appetite, upset stomach, constipation, decreased appetite, and indigestion. Gynecomastia (breast enlargement) and breast tenderness can occasionally occur. Abnormalities in your liver enzymes can occasionally occur. Damage to your liver (hepatitis) can also occur, but is uncommon. In very rare circumstances this has resulted in death. Other side effects include changes in your blood count resulting in decrease in your red blood cells (anemia 6%), decrease in your white blood cells (leucopenia and neutropenia – 1-3%). Additional side effects that can occur are hot flashes, intense amber to yellow-green urine discoloration, transiently mild declines in your kidney function. The use of flutamide may decrease your desire for sex and your ability to have an erection. Drowsiness, confusion, nervousness, headache, dizziness, insomnia, weakness, depression, anxiety, have been reported rarely (< 1%) in patients during flutamide administration. Myocardial infarction and myocardial ischemia have occurred rarely during flutamide administration. Rare cases of hypertension (1% of 294 patients) have been reported during flutamide treatment among patients who received a LHRH agonist concomitantly. Blurred vision has been rarely reported with flutamide therapy. Photosensitivity after sun exposure, with development of a skin rash may occur. You should apply topical sunscreens to sun-exposed skin and use protective clothing against exposure to excessive sunlight

Apheresis

The potential side effects of apheresis may be pain and bruising at needle sites, lightheadedness and rarely, fainting due to brief lowering of blood pressure. There is a very small chance of introducing infection at the site of the needle. Blood infections from contamination of the apheresis machine are a remote possibility, but this has not occurred at the NIH. A tingling sensation around the mouth, fingers, or toes, and mild muscle cramps may occasionally occur as a side effect of a blood thinner used during the procedure. These symptoms are brief and may often be stopped by slowing the procedure.

Duration of Treatment

Your active participation in this research project will be for a period of approximately 6-9 months. It is important to emphasize that the supply of PROSTVAC-V/TRICOM (vaccinia) and PROSTVAC-F/TRICOM (fowlpox) may be limited and continued availability of the two agents cannot be guaranteed. Thus, even if there is improvement in your case, there may not be drug available for re-treatment. You may be treated much longer if your disease responds to the study drugs and you do not experience severe side effects. However, this period may be shorter depending on if your disease gets worse or if you experience serious side effects from the treatment. Your participation may be ended by the investigator, the National Cancer Institute, or by the Food and Drug Administration (FDA) for reasons that would be explained to you. For example, the investigator may stop your treatment for medical or safety reasons. New

information developed during the course of this study that may affect your willingness to continue in this research project will be given to you as it becomes available.

Potential Benefit for Participation

The major purpose of this study is to first learn whether or not this combination of flutamide with vaccine therapy will improve your clinical response compared to flutamide treatment alone. We will also look at the safety of combining these agents. The information obtained from this study will allow us to design more effective treatments in the future. This is an investigational study; therefore, no benefit is known or guaranteed. You will be monitored closely while you are receiving this experimental treatment for any signs that might signal the earliest stage of toxicity so that appropriate intervention can be done.

You will be informed of any significant findings resulting from the study including any new information about the experimental procedure, the harms and benefits experienced by any individuals involved in the study, and long term side effects that had been observed.

Cost and Reimbursement

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

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

Communication

The NCI physicians involved in your care are available to answer all of your questions concerning this protocol. If you have any concerning or questions, you may contact Dr. Ravi Madan, the Principal Investigator at 301-480-7168. If you have any complications when you are not in the

Clinical Center (e.g., at home or in a local hotel), you must call the page operator at (301) 496-1211 and ask for the NCI medical oncology branch physician on-call or the NIH Patients' Rights Representative who will be available to answer questions concerning your involvement in this study or your rights as a research subject. The representative is not directly associated with this study and can be contacted at (301) 496-2626.

Confidentiality of Your Own Records

You will receive a copy of this informed consent for your own records. In addition, a copy of the informed consent is on file at the National Cancer Institute and a copy will be made available to you whenever you want to see it. Your records will be kept confidential, with the exception that personnel or representatives from the NCI, FDA, NIH Office of Science Policy and the drug companies that manufacture the investigational study drugs may have access to your medical record.
A description of this clinical trial will be available on http://www.ClinicalTrials.gov, as required by U.S. Law. This Web site will not include information that can identify you. At most, the Web site will include a summary of study results. You can search this Web site at any time.

Alternative Treatments

Chemotherapy with docetaxel has been shown to prolong life of patients with metastatic prostate cancer who have rising PSA despite hormonal therapy but currently there is no standard of care for patients who have not developed metastatic disease. To be eligible for this study you must have lowered serum levels of testosterone in your body (surgically lowered by orchiectomy or take medications such as leuprolide or goserelin) and you must have a rising PSA level on at least one antiandrogen drug (either bicalutamide or nilutamide). You must not have evidence of metastatic disease on bone scan or CT scan. You may or may not benefit from participation in this study. Participation in this study is purely voluntary. Choosing not to participate (or withdrawing at any point) from this study will in no way penalize your medical care or your relationship with you physicians. If you choose not to participate in this study, there are several alternative treatments/therapy for your cancer. They include:

- Treatment with other hormone drugs including flutamide, nilutamide, or bicalutamide
- Experimental treatments such as other types of immune therapy or chemotherapy (which is currently not considered standard of care for patients without metastatic disease)
- No current treatment but watchful waiting

Each of these alternative therapies has a unique set of benefits and risks. Your physician will discuss these options with you. Furthermore, other centers are performing clinical trials with investigational agents as well as standard chemotherapy that you may consider as an alternative to this proposed trial.

Research Subject's Rights

Your participation in this study is entirely voluntary, and you may refuse to participate, or withdraw from this protocol at any time and receive care from a physician of your choice. Your participation in this study may be ended by the Principal Investigator or an Associate Investigator without your consent if they feel termination is medically indicated.

Completion of Study

Upon completing this study, you may be given the choice of taking part in other research protocols that may be appropriate for you. Otherwise, you will be returned to the care of your referring physician. It is important to stress that your participation in this study does not constitute a promise of long term care at the NIH Clinical Center. If there is no research study that can help you, you will be returned to the care of your private doctor. It is important to remember that if you do take part in this study, it is possible that you may be ineligible for certain future clinical trials because you would have received a form of gene or viral therapy. You may decide now not to receive treatment in this protocol, or you may choose at any time to stop the drug and withdraw from the protocol. In either case, you would be returned to the care of your referring physician.

Because you are a study participant, investigators will ask your family for permission to do an autopsy when you die, even though this may be years after the study. This may help investigators learn about the effects of this experimental vaccine. By signing this consent form, you are not forcing your family to agree to this. You should talk about this request with your family and advise them of your wishes.

Your signature on this form indicates that you agree to participate in this medical research study under the direction of the principal investigator as listed above.

Conflict of Interest

The National Institutes of Health (NIH) reviews NIH staff researchers at least yearly for conflicts of interest. This process is detailed in a Protocol Review Guide. You may ask your research team for a copy of the Protocol Review Guide or for more information. Members of the research team who do not work for NIH are expected to follow these guidelines but they do not need to report their personal finances to the NIH.
Members of the research team working on this study may have up to $15,000 of stock in the companies that make products used in this study. This is allowed under federal rules and is not a conflict of interest.

The Laboratory of Tumor Immunology and Biology, National Cancer Institute had an initial agreement with Therion Biologics Corporation, the manufacturer of this vaccine. The NCI now has transferred this agreement to BN Immunotherapeutics. Under this agreement, the

manufacturer provides vaccine and some money for pre-clinical (laboratory) studies, as well as vaccine for clinical trials such as this one.

The National Institute of Health and members of the research team have developed a drug which is being used in this research. This means that it is possible that the results of this study could lead to payments to NIH scientists and to the National Institute of Health. By law, government scientists are required to receive such payment for their inventions. You will not receive any money from development of the drug.

Use of Specimens and Data for Future Research

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

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

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

OTHER PERTINENT INFORMATION

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

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

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

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

4. Problems or Questions. If you have any problems or questions about this study, or about your rights as a research participant, or about any research-related injury, contact the Principal Investigator, Ravi Madan, M.D., Building 10, Room 12N226, Telephone: 301-480-7168.

If you have any questions about the use of your blood specimens or data for future research studies, please contact the Office of the Clinical Director, Telephone: 240-760-6070.

You may also call the Clinical Center Patient Representative at 301-496-2626.

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

COMPLETE APPROPRIATE ITEM(S) BELOW:

A. Adult Patient's Consent

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

Signature of Adult Patient/ Legal Representative _____ Date _____

Print Name _____

B. Parent's Permission for Minor Patient.

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

Signature of Parent(s)/ Guardian _____ Date _____

Print Name _____

C. Child's Verbal Assent (If Applicable)

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

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

THIS CONSENT DOCUMENT HAS BEEN APPROVED FOR USE FROM JUNE 27, 2016 THROUGH JUNE 26, 2017.

Signature of Investigator _____ Date _____

Print Name _____

Signature of Witness _____ Date _____

Print Name _____

INSTITUTE: National Cancer Institute

STUDY NUMBER: 07-C-0107 PRINCIPAL INVESTIGATOR: Ravi Madan, M.D.

STUDY TITLE: A Randomized Phase II Trial Combining Vaccine Therapy with PROSTVAC /TRICOM and Flutamide, vs. Flutamide Alone in Men with Androgen Insensitive, Non Metastatic (D0.5) Prostate Cancer

Continuing Review Approved by the IRB on 06/27/16
Amendment Approved by the IRB on 04/05/17 (P) Date Posted to Web: 04/19/17
Screening for HLA Typing

INTRODUCTION

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

First, we want you to know that:

Taking part in NIH research is entirely voluntary.

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

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

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

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

This consent is to allow us to conduct tests on your white blood cells in order to determine how your body's immune cells respond to the vaccine given in this investigational treatment protocol, entitled "A Randomized Phase II Trial Combining Vaccine Therapy with PROSTVAC/TRICOM

Revision Copy

and Flutamide vs. Flutamide Alone in Patients with Androgen Insensitive, non-Metastatic (D0.5) Prostate Cancer." This trial will enroll people with progression of their prostate cancer. Certain people have a certain marker on their normal blood cells, called HLA-A2. This marker is involved in immune cell recognition and will allow us to perform tests in the laboratory to determine response to vaccinations. *The blood sample can be drawn at your doctor's office or at the NIH.*

It is important that we test your blood to determine if you have the HLA-A2 marker on your cells. Accordingly, if you are interested in being considered for this study, we ask you to sign this consent form to allow us to obtain 10 cc (2 teaspoonfuls) of blood to conduct this test. *You may instead choose to have this screening test performed at another facility and have the results forwarded to us.*

Sending the blood to the NIH does not put you under any obligation to follow through with being treated on the protocol or even continuing the determination of your eligibility. The blood sample will not be used for any other purpose.

These tests may take up to 2 weeks. You and your physician will be informed of your eligibility for the trial as soon as the results are ready.

Please fax this form to (301) 480-1779, attention Sheri McMahon, R.N.

OTHER PERTINENT INFORMATION

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

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

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4. Problems or Questions. If you have any problems or questions about this study, or about your rights as a research participant, or about any research-related injury, contact the Principal Investigator, Ravi Madan, M.D., Building 10, Room 12N226, Telephone: 301-480-7168. Another researcher you may call is: Sheri McMahon, R.N. 240-760-6085. If you have any questions about the use of your tissue for future research studies, please contact the Office of the Clinical Director, Telephone: 240-760-6070.

You may also call the Clinical Center Patient Representative at 301-496-2626.

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B. Parent's Permission for Minor Patient.

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

Signature of Parent(s)/ Guardian _____ Date _____

Print Name _____

C. Child's Verbal Assent (If Applicable)

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

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

THIS CONSENT DOCUMENT HAS BEEN APPROVED FOR USE FROM JUNE 27, 2016 THROUGH JUNE 26, 2017.

Signature of Investigator _____ Date _____ Signature of Witness _____ Date _____

Print Name _____ Print Name _____