Abbreviated Title: Enzalutamide in non-met CSPC

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A Phase II Trial of Enzalutamide in Combination with PSA-TRICOM in Patients with Non-Metastatic Castration Sensitive Prostate Cancer

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Investigational Agents:

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

PRÉCIS

Background

- Androgen deprivation therapy (ADT) and surveillance are standard therapy options for prostate cancer patients with biochemical progression after localized therapy (or non-metastatic castration sensitive prostate cancer; nmCSPC also known as D0 Prostate Cancer). These patients cannot be cured of prostate cancer and the primary therapeutic goal is to contain the disease with anti-androgen therapy.
- ADT can be administered intermittently consisting of multiple short courses or continuously with similar long-term clinical outcomes.
- Previous studies with high dose bicalutamide (androgen receptor antagonist, ARA) have shown significant biochemical control in nmCSPC.
- Enzalutamide is a modern ARA with greater androgen receptor affinity than bicalutamide and further impairs downstream effects of androgen receptor activation. This agent is FDA approved for the treatment of chemotherapy refractory metastatic castration resistant prostate cancer.
- Given its favorable side effect profile, there is strong interest in using enzalutamide to treat patients with earlier stages of prostate cancer including nmCSPC.
- PSA-TRICOM (Prostvac™; developed by the National Cancer Institute [NCI] and licensed to Bavarian Nordic, Mountain View, CA) is a novel candidate prostate cancer immunotherapy for the treatment of prostate cancer. It is a viral vector based therapeutic cancer vaccine that is administered via subcutaneous injections. In a randomized controlled Phase 2 trial, PSA-TRICOM therapy was associated with a prolongation of survival in men with metastatic castrate-resistant prostate cancer. A phase III trial is currently enrolling patients in this same population.
- There is also rationale to use therapeutic cancer vaccines such as PSA-TRICOM in earlier stage prostate cancer patients to maximize the potential therapeutic effect of immune stimulating therapy.
- An ongoing NCI clinical trial that combined PSA-TRICOM with flutamide (an older FDA approved ARA) in nonmetastatic castration resistant prostate cancer has demonstrated safety and suggested the potential to improve time to progression.
- Analysis of previous trials using therapeutic cancer vaccines alone and in combination suggests that such therapies may alter tumor growth rate. If this hypothesis is correct, a therapeutic cancer vaccine may alter tumor regrowth rate/recovery after a cytoreductive therapy such as enzalutamide is discontinued.
- If PSA-TRICOM with enzalutamide can result in a reduced tumor regrowth rate as measured by PSA after a short course of enzalutamide therapy, it would provide an important proof of concept and potentially define it more clear role for therapeutic cancer vaccines in prostate cancer and potentially other cancers.
- To prospectively evaluate this hypothesis, all patients will be treated with enzalutamide in a manner similar to how short course ADT is used in common clinical practice. Half the patients will also be given PSA-TRICOM and PSA recovery after enzalutamide therapy will be compared between patients who received vaccine and those who did not.
- Preliminary data from the first cohort of randomized patients suggests that enzalutamide alone can induce an immunologic response. A second cohort of 15 patients will explore a

lower dose of enzalutamide at 80 mg to determine if similar immunologic responses can also be seen at a lower dose, where toxicity is less likely.

Objectives

Primary Endpoint:

- Determine if PSA-TRICOM combined with the novel androgen receptor antagonist enzalutamide will result in a decrease in PSA growth kinestics (tumor re-growth rate) after enzalutamide discontinuation in patients with non-metastatic, castration sensitive prostate cancer (i.e. patients with normal testosterone).

Eligibility

- Patients with nonmetastatic castration sensitive prostate cancer and a PSA over 2.0 ng/ml
- Patients with normal testosterone levels.
- Histologically confirmed adenocarcinoma.
- Patients with a PSA doubling time of 12 months or less.
- ECOG 0-1.

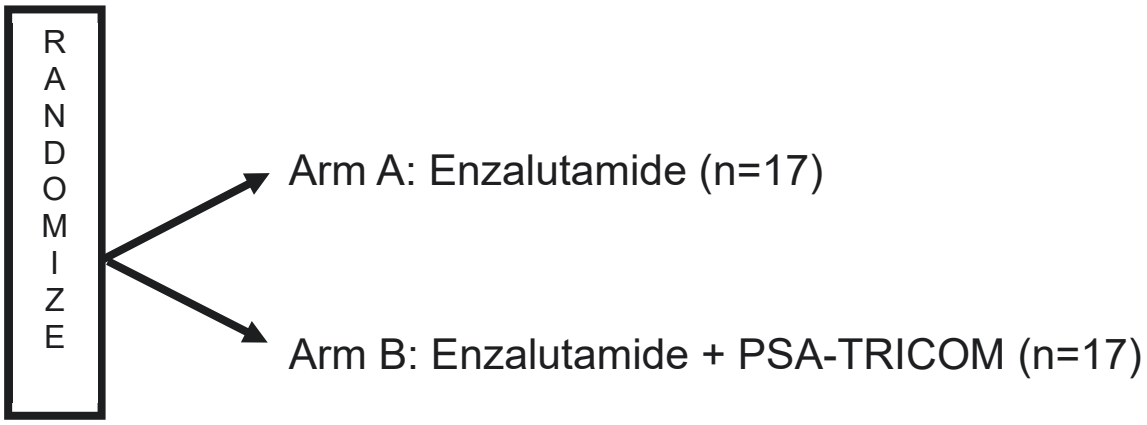
Design

- Randomized pilot study
- Cohort 1:
  Thirty-four patients to be enrolled and randomized 1:1 to
  - Arm A: Enzalutamide for 3 months.
  - Arm B: Enzalutamide 3 months + PSA-TRICOM on weeks 1, 3,5,9,13,17 and 21.

Cohort 1

Arm A: Enzalutamide (n=17)

Arm B: Enzalutamide + PSA-TRICOM (n=17)



- Enzalutamide will be given at the standard dose of 160 mg daily for 3 months. PSA-TRICOM (Prostvac-V/F) will consist of a single subcutaneous (sc) immunization of Prostvac-V in Week 1, followed by 6 Prostvac-F immunizations administered in Weeks 3, 5, 9, 13, 17, and 21. Patients will be retreated with a 3-month course of enzalutamide after PSA has returned to baseline values at study entry or higher. Patients will have had to be on study for at least 7 months or longer in order to be retreated with an additional course of enzalutamide therapy. Patients will be followed for PSA recovery after enzalutamide has been discontinued. Patients who do not develop a 25% decline in PSA after 3 months will not be evaluated for tumor re-growth and additional patients will be enrolled to evaluate for that endpoint. Patients will be stratified based on a doubling time of greater than or less than 6 months.

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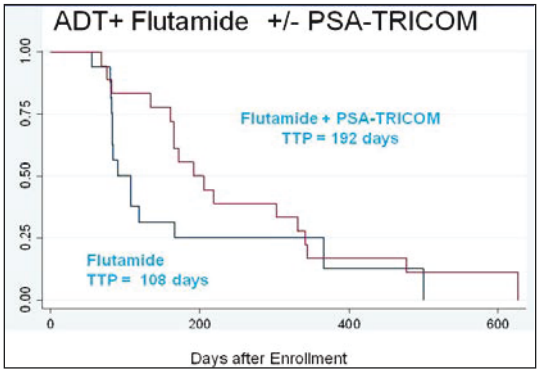

1 INTRODUCTION

1.1 STUDY OBJECTIVES

1.1.1 Primary

1.1.1.1 Determine if PROSTVAC-V/F combined with the novel ARA enzalutamide will result in a decrease in PSA growth kinetics after enzalutamide discontinuation in patients with non-metastatic, castration sensitive prostate cancer (i.e. patients with normal testosterone).

1.1.2 Secondary

1.1.2.1 Determine the impact of enzalutamide on PSA as a single agent in patients with normal testosterone and non-metastatic, castration sensitive prostate cancer

1.1.2.2 Evaluate the immune response in patients treated with both the combination of PSA-TRICOM and enzalutamide compared to enzalutamide alone. Immune response evaluation will include CD4 cells, CD 8 cells, PSA-specific T-cells, natural killer cells, Regulatory T-cells, cytokines, anti-glycan antibodies and naïve thymic emigrants.

1.1.2.3 Associate immunologic outcomes with PSA responses.

1.1.2.4 Evaluate the toxicity of enzalutamide and PSA-TRICOM combination in Arm B and compare to enzalutamide alone in Arm A.

1.1.2.5 Evaluate PSA responses to a second 3-month course of enzalutamide in patients eligible for re-treatment based on the protocol.

1.1.2.6 Evaluate changes in testosterone and related hormone levels and each follow up visit, and pre-Enzalutamide administration, and 1 hour post, 24 hours post, 48 hours post, and 72 hours post administration, when logistically feasible.

1.1.2.7 Evaluate plasma VEGF levels at the start and end of each patient's second 12-week course of Enzalutamide.

1.1.2.8 Evaluate the immune response in patients treated with enzalutamide at 80 mg/day. Immune response evaluation will include CD4 cells, CD 8 cells, PSA-specific T-cells, natural killer cells, Regulatory T-cells, cytokines, anti-glycan antibodies and naïve thymic emigrants.

1.2 BACKGROUND AND RATIONALE

1.2.1 Enzalutamide

Enzalutamide is a modern update of the original androgen receptor antagonists (ARAs), the last of which was developed over 2 decades ago. Since then, the prevailing focus shifted to chemotherapy and chemotherapy combinations (1). In addition to binding to the androgen receptor with greater affinity than standard ARAs, enzalutamide prevents downstream effects including nuclear translocation, DNA binding, and signaling to co-activators (2). Furthermore, enzalutamide has not demonstrated any agonist properties unlike previous ARAs which showed agonist properties in approximately 15%-20% of patients (3, 4). (Figure 1). Another important

characteristic is that unlike the modern androgen-biosynthesis inhibitor abiraterone, enzalutamide does not require daily prednisone. (Long-term prednisone could have additional side effects and could impact the immune response.) The U.S. Food and Drug Administration (FDA) approved enzalutamide for metastatic prostate cancer in docetaxel-refractory patients in August, 2012 (5).

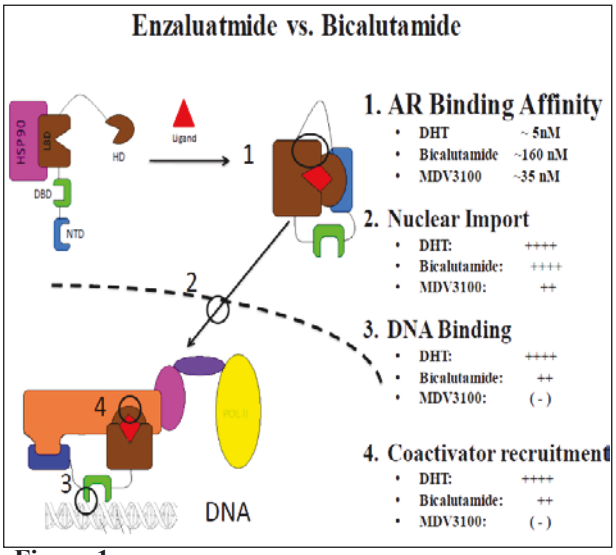

Figure 1

A Phase I/II study of enzalutamide demonstrated safety and suggested efficacy in both chemotherapy-naïve and chemotherapy-treated castration-resistant prostate cancer (CRPC) patients, the vast majority of whom had metastatic disease (6). Two phase III studies were then launched in metastatic CRPC (mCRPC). One study enrolled chemotherapy-naïve patients; the other (AFFIRM) evaluated patients who had progressive disease on docetaxel. AFFIRM enrolled 1199 patients with progressive mCRPC on docetaxel and randomized them 2:1 to enzalutamide 160 mg/day (n=800) or placebo (n=399). The overall survival favored patients randomized to the enzalutamide arm 18.4 to 13.6 months. This 4.8 months improvement in survival represented a 37% risk reduction in death (hazard ratio 0.63; 95% CI: 0.53 to 0.75; P<0.001) the largest relative and absolute improvement in overall survival in an appropriately powered phase III study in prostate cancer. Median TTP based on radiographic findings was 8.3 vs. 2.9 months; HR: 0.40; P<0.001). (Figure 2) Modest increases in fatigue, hot flashes, diarrhea, musculoskeletal pain, and headaches reported in the enzalutamide group, but this was possibly related to the substantially longer monitoring time for patients on this treatment compared to the placebo group. Therefore, there were no significant concerns about the side effect profile of enzalutamide compared to placebo (7). The U.S. FDA approved enzalutamide for chemotherapy-refractory mCRPC patients in August of 2012 (5). The second phase III trial in chemotherapy naïve patients is ongoing.

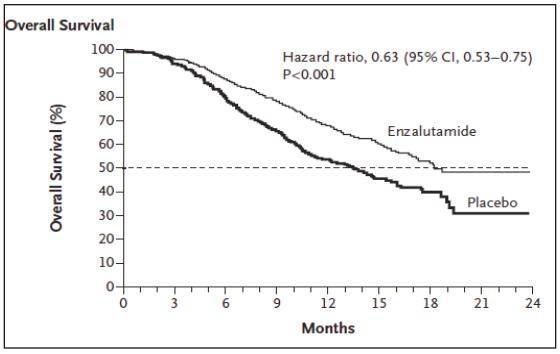

Figure 2. Enzalutamide demonstrates improved overall survival in mCRPC patients previously treated with docetaxel (7).

The ultimate role of enzalutamide in the treatment of prostate cancer will likely be in earlier-stage disease. The minimal side effects of enzalutamide in patients with advanced disease will create strong interest in providing this therapy for chemotherapy- naïve mCRPC and even patients with non-metastatic disease, thus supplanting the use of older ARAs such as bicalutamide which are commonly used in non-metastatic patients (8). Also, unlike the modern androgen-biosynthesis inhibitor abiraterone, enzalutamide does not require daily prednisone. Enzalutamide therapy will thus avoid prednisone's potential long-term side effects in earlier stage disease, an important consideration for future treatment strategies in this population. Combination with immune based therapies is attractive because unlike abiraterone, this treatment does not require prednisone which can be immunosuppressive. In addition, given the data which suggest that hormonal therapies can enhance immune response, it would be valuable to gain a better understanding of the immune impact of enzalutamide given the emerging role of immunotherapy in prostate cancer (9).

At this time, there is no data for the role of enzalutamide in non-metastatic, castration-sensitive prostate cancer (nmCSPC) patients with normal testosterone. (Hereafter in this document, nmCSPC will also refer to patients with a normal testosterone and thus presumed sensitivity to castration.) Previously, the first-generation ARA, bicalutamide, has been evaluated in the nmCSPC setting as patients and clinicians seek ways to avoid/delay the symptoms associated with ADT. Forty-one patients with nmCSPC were treated with high-dose (150 mg) bicalutamide, with a secondary addition of finasteride but no androgen suppression therapy. Thirty-four of 36 evaluable patients reached a PSA nadir that represented a decline of PSA by 95.5%. The median time to treatment failure was 21.3 months (10). This time frame was consistent with other trials that evaluated high dose bicalutamide in castration-sensitive metastatic disease (11). Based on these findings, the use of single agent enzalutamide in this population would be interesting to evaluate given the limited favorable side effect profile and the possibility that enzalutamide is more efficacious than bicalutamide.

1.2.2 Therapeutic Cancer Vaccines in Prostate Cancer

The goal of therapeutic cancer vaccines is to generate a targeted immune response leading to immune-mediated anti-tumor activity. Sipuleucel-T is a therapeutic cancer vaccine generated from peripheral blood mononuclear cells obtained from individual patients via leukapheresis. This vaccine is generated after a patient's peripheral immune cells are collected via

leukapheresis, transported to a regional processing center where they are exposed in vitro to a PAP/GM-CSF fusion protein. At the end of this process, the activated cellular product is re-infused into the patient. A full course of therapy repeats this process 3 times every 2 weeks for 1 month (12, 13). A phase III trial (n = 512) demonstrated an overall survival benefit for the vaccine (25.8 months vs. 21.7 months; P = 0.032) (14). Based on these overall survival findings, the FDA approved sipuleucel-T for the treatment of asymptomatic or minimally symptomatic mCRPC, making it the first FDA-approved therapeutic cancer vaccine for the treatment of any malignancy.

1.2.3 PSA-TRICOM

PSA-TRICOM (Prostvac™; developed by the National Cancer Institute [NCI] and licensed to Bavarian Nordic, Mountain View, CA), an off-the-shelf therapeutic cancer vaccine, offers an alternative strategy (15, 16). (The LTIB and Bavarian Nordichave an ongoing CRADA for the preclinical and clinical development of PSA-TRICOM.) To target prostate-specific antigen (PSA), PSA-TRICOM vaccine employs genetically altered poxviruses to deliver targeting information to immune cells and generate an immune response. Administered subcutaneously, the poxviruses deliver the transgenes for the tumor associated antigens (PSA) to antigen presenting cells through cellular infection. Once these pox viruses are within the cellular cytoplasm, the transgenes are processed. The end result is an antigen presenting cell expressing a PSA peptide within the major histocompatibility complex, resulting in PSA-specific cytolytic T lymphocytes activation (17, 18). (Figure 4) This approach does not require expensive, labor-intensive *ex vivo* preparation of patients' peripheral blood. PSA-TRICOM is thus potentially more logistically and financially feasible over the long-term than sipuleucel-T (19).

PSA-TRICOM has been investigated in 2 phase II trials in mCRPC, both of which administered the vaccine at monthly intervals until disease progression. An industry-sponsored, placebo-controlled, multicenter trial in 125 mCRPC patients randomized them 2:1 in favor of PSA-TRICOM; the placebo was an empty poxviral vector containing no transgenes. As was seen in the sipuleucel-T studies, patients receiving vaccine showed no change in TTP, yet had an overall survival benefit (25.1 months with PSA-TRICOM vs. 16.6 months with placebo; P = 0.0061) (20). (Figure 3) A second phase II study of PSA-TRICOM of 32 mCRPC patients at the NCI demonstrated that the vaccine was able to generate a T-cell specific immune response and patients with the greatest magnitude of this response had superior outcomes (21). Based on the findings in these trials, a phase III trial of PSA-TRICOM in mCRPC is currently underway (22).

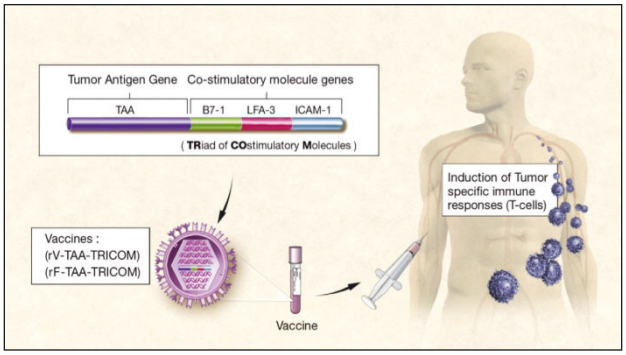

Figure 3. Poxviral vaccine strategy: Modified poxvirus contains transgenes for the tumor-associated antigen PSA and 3 T-cell costimulatory molecules (16)

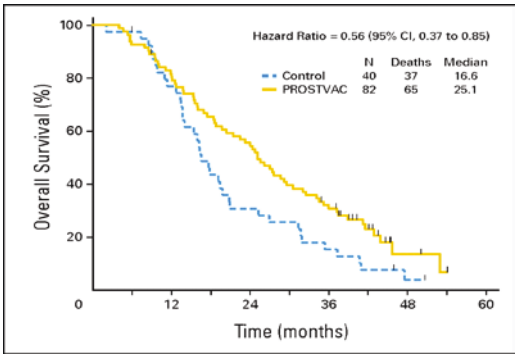

Figure 4. PSA-TRICOM improved survival in mCRPC patients in a randomized multi center phase II. (20)

Like enzalutamide, PSA-TRICOM is very well-tolerated, with common side effects of grade 1 injection-site reactions or flu-like symptoms (20, 21). Also like enzalutamide, favorable side effect profile and the potential ability to induce a sustained antitumor immune response, clinical trials are ongoing evaluating strategies to use PSA-TRICOM in earlier disease patients.

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

1.2.4 Rationale for Combining Enzalutamide with PSA-TRICOM

Accumulating data demonstrate that androgen-deprivation therapy (ADT) affects not only prostate cancer growth, but also the immune system and suggest that ADT in prostate cancer can augment the immune response by increasing T-cell infiltration into the prostate (9). The impact of this T-cell trafficking would be even greater if T cells were primed by a vaccine prior to ADT (23). Furthermore, ADT has been shown to decrease immune tolerance of self antigens that are over-expressed in many cancers, increase the production of new T-cells from

the thymus (Table 1), and enhance the Cytotoxic T-cell repertoire (9, 24-26). Of the two modern anti-androgen therapies for prostate cancer that have emerged in the last few years, enzalutamide is preferred over abiraterone, because abiraterone requires prednisone which may influence the immune response in a vaccine-based combination regimen.

	Baseline	After ADT	
Naïve CD4 cells	3.25% of CD3+ cells	3.95% of CD3+ cells	p = 0.0060
T-Cell Receptor Excision Circles	93 per 100,000 cells	147 per 100,000 cells	p = 0.0025

Table 1. ADT Increases Naïve T-cell Emigrants from the Thymus. A previous NCI trial (NCT00514072) evaluated naïve T-cell emigrants from the thymus after 3 months of ADT. T-cell receptor excision circles are detectable byproducts of thymic generation of new T-cells and provide a way to quantify such cells. Naïve CD4 cells are defined by flow cytometry as described in the correlative studies section below. By both measures there was a significant increase in the number of naïve T-cells which potentially can be activated by vaccine. (unpublished)

1.2.5 Combining Androgen Receptor Antagonists with PSA-TRICOM, clinical evidence

An ongoing clinical trial at the NCI is combining PSA-TRICOM with flutamide, an FDA approved ARA, in men with non-metastatic CRPC (NCT00450463) . Patients are randomized to either PSA-TRICOM with flutamide or flutamide alone. The primary endpoint is TTP as determined by PSA (Bubley criteria) or development of metastatic disease (28). An interim analysis with about half the patients accrued demonstrated that these 2 agents can be safely combined with minimal toxicity. The interim TTP analysis favored the combination of PSA-TRICOM and flutamide compared to flutamide alone (192 days vs. 108 days) (29). (Figure 5) The study is completing accrual at the NCI and the Cancer Institute of New Jersey, but the interim analysis provides a preliminary clinical rationale for the combination of PSA-TRICOM with a modern ARA in chemotherapy-naïve patients with mCRPC. The proposed trial in mCRPC will use metastatic progression as an endpoint, which is a more established therapeutic measure than criteria currently established for nonmetastatic disease (30).

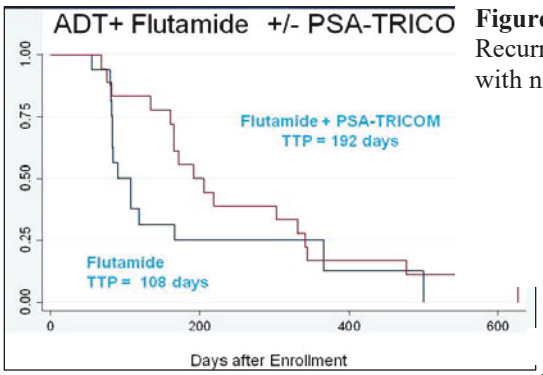

Figure 5. Patient Population: "Biochemical Recurrence" or Rising PSA after surgery or RT with normal testosterone (nmCSPC)

1.2.6 Enzalutamide's Immune Effects in Preclinical

Models

Enzalutamide has been evaluated preclinically by Dr. James Hodge of the Laboratory of Tumor Immunology and Biology (LTIB) at the NCI. The first objective was to determine the appropriate dose to use in evaluation of the male C57BL/6 mice. Doses at 1, 10, 50 and 100 mg/day were evaluated and the 10 mg/day dose was found to be most appropriate given that it achieved a serum concentration of 20 ug/ml, consistent with serum levels in humans. Therefore, that was the dose evaluated in male C57BL/6 mice. (Figure 6A) It was confirmed that enzalutamide at 10 mg/day had a significant decrease in the weight of the genitourinary organs after 14 days of dosing, demonstrating the physiologic effects of enzalutamide at 10 mg/day dose level. Figure 6B)

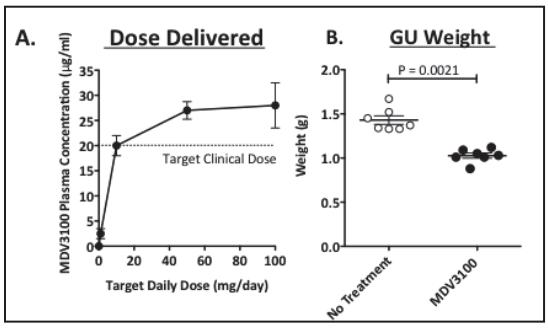

Figure 6. **Enzalutamide mediates a reduction in genitourinary (GU) weight. (A)** Concentration of Enzalutamide in plasma of mice on Enzalutamide daily diet. Male C57BL/6 mice (n=3) were treated with Enzalutamide at different calculated target daily doses (0, 1, 10, 50, and 100mg) for 14 days. On day 15, blood was collected and Enzalutamide concentration in the plasma was determined. **(B)** Exposure to Enzalutamide causes reduction of GU weight. Male C57BL/6 mice (n=7) were not treated (open circles) or treated with Enzalutamide (closed circles) for 14 days. On day 15, mice were sacrificed and their GU and were harvested and weighed. All experiments were done three times with similar results. Statistical analyses were done by Student's t-test

From an immunologic standpoint, complete blood counts and Fluorescence-activated cell sorting analysis of immune cell subpopulations were found to be unchanged. Furthermore, functional analysis based on mixed lymphocyte response and CD3-induced proliferation assays of CD4 cells demonstrated no significant differences. In addition, similar to previous studies with hormonal therapies, enzalutamide treatment was associated with increased thymic weights and increased T-cell excision circles, consistent with the production of new T-cells from the thymus. (Figure 7) This series of experiments (publication pending) provides evidence that enzalutamide likely will not diminish the quantity or functionality of immune cells and may even enhance thymic production of naïve T-cells, allowing for potential immune stimulation by a therapeutic cancer vaccine such as PSA-TRICOM.

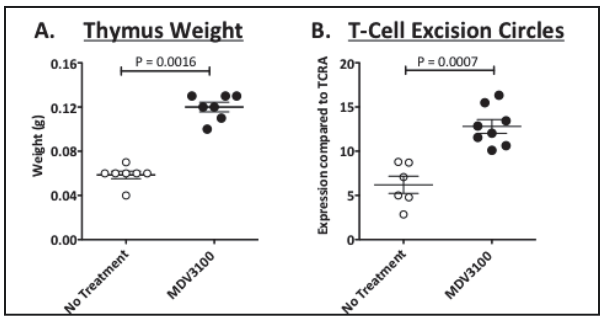

Figure 7**. A**. Enzalutamide mediates an enlargement of thymus and an increase in T-cell Receptor Excision Circles (TREC) levels. **(A)**. Male C57BL/6 mice (n=7) were not treated (open circles) or treated with Enzalutamide (closed circles) for 14 days. On day 15, mice were sacrificed, and their thymi harvested and weighed. **(B)**. Enzalutamide significantly increases T-cell Excision Circle (TREC) levels in male mice. Male C57BL/6 mice (n=7) were not treated (open circles) or treated with Enzalutamide (closed circles) for 14 days. On day 15, 100ng of DNA from blood was collected and TREC levels were quantified by RT-PCR in triplicate. Results were normalized against the constant gene segment of TCRA, which serves as endogenous reference gene. All experiments were done three times with similar results. Statistical analyses were done by Student's t-test

1.2.7 Rationale for conducting the study in nmCSPC patients

The standard of care for patients with nmCSPC includes ADT and active surveillance. Generally, these patients can be followed without therapy until their PSA doubling time escalates at which time ADT is often started. All patients in the proposed trial will receive enzalutamide. Based on previous trials with high dose bicalutamide, 34 of 36 evaluable patients had PSA declines by a median of 96.5% and these nadirs were achieved within the first 4 weeks of treatment (10). We would expect similar if not better results with enzalutamide. Therefore, a clinical trial in this population is ethical and appropriate. The NCI has conducted several trials in this population previously.

This population will also offer unique opportunity to evaluate the independent impact (in the absence of ADT) of enzalutamide on both PSA as well as the immune system. Much like previous studies with high-dose bicalutamide in this population, similar trials are likely to be done to see if disease control with enzalutamide is possible in nmCSPC, with less side effects relative to ADT (10).

Given the previous experience with high dose bicalutamide in this population, a study with time to progression as an end point with continuous enzalutamide would be expected to take well over 2 years (10). We feel that using a short course of enzalutamide therapy of 3 months we can assess the immunologic impact and determine the impact of vaccine on tumor re-growth rates. Therefore, for practical considerations (number of patients required and length of time to conduct the trial with a clinical progression endpoint) this trial will be conducted over a minimum of 6-12 months, including a 3 month course of therapy of enzalutamide.

This proposed brief 3 month enzalutamide course of therapy is consistent with a standard approach of intermittent ADT (36, 37). In the intermittent ADT approach in nmCSPC, one or two doses of ADT are given and then PSA is followed for recovery at which time subsequent ADT dosing is considered. The same intermittent strategy will be done in this trial, substituting enzalutamide for ADT. Furthermore, this population allows for the unique opportunity to follow PSA/tumor re-growth rate/recovery once a standard therapy is discontinued. Consistent with common clinical practice no immediate re-treatment will be required in the months following discontinuation of enzalutamide. Patients would be started on ADT as clinically appropriate, as suggested by their PSA doubling time.

1.2.8 The Importance of Evaluating the Immunologic Response

Our group at in the LTIB has previously evaluated immunologic parameters in clinical trials with PSA-TRICOM among several immunotherapy studies. A previous trial in mCRPC patients with PSA-TRICOM alone suggested that patients with greatest magnitude of T-cell-specific response against PSA had favorable clinical outcomes (21). That same trial also suggested that changes in regulatory T-cell function were also associated with improved clinical outcomes (38). While the these findings are not surrogate markers of response, they have improved our knowledge of a vaccine-generated immune response and provided a better understanding of what factors are potentially important in mounting a sufficient anti-tumor immune response that could be associated with improved clinical outcomes. These and other data have allowed us to optimize vaccines in subsequent clinical trials.

Similarly, immunologic parameters will be valuable in understanding the benefits of PSA-TRICOM with enzalutamide. Are the same associations seen or other changes in natural killer cells or cytokines of greater importance in this combination? Furthermore, baseline

immune characteristics will be evaluated to determine if they have the potential to predict responders or likely non-responders to immune combinations. While this hypothesis generating data would have to be prospectively evaluated in future clinical trials, it may improve our understanding about how best to deploy vaccines in combination with enzalutamide and other forms of hormonal therapy. If enzalutamide increases naïve T-cell production from the thymus as suggested by the pre-clinical data, this may have important ramifications in the timing of vaccine in future clinical trials. This and other data may also provide information early on in the therapeutic regimen about which patients would benefit from continued vaccine in combination with enzalutamide.

It will also be of great importance to understand what the immunologic impact is of enzalutamide alone. New data from commonly used cytotoxic agents has suggested that some chemotherapy agents can enhance anti-tumor immune responses (34, 39). With this new understanding, clinical trials are being design to exploit this aspect of these respective therapies. Enzalutamide and other modern androgen receptor-targeting agents will be the mainstay of prostate cancer therapy for the foreseeable future. Future trials are likely to evaluate enzalutamide monotherapy in men with nmCSPC and this trial will be able to evaluate the specific immunologic impact of enzalutamide in men with normal testosterone levels. With sipuleucel-T already approved and ipilimumab and PSA-TRICOM in phase III testing, it is likely that immunologic optimization will be important in the future treatments of prostate cancer. Few groups are as well positioned and have the experience as the LTIB to conduct this rigorous immune testing from clinical samples of patients treated with enzalutamide to gain a better understanding about its immune properties.

1.2.9 Change in regrowth rate as endpoint

Although vaccines have improved overall survival in mCRPC, they have not been shown to change short term TTP when used as monotherapy. Interestingly, similar findings were also seen with ipilumimab in melanoma (40). Although this is not what is customarily seen with cancer treatments, it may be a characteristic of modern immune therapeutics (41). An emerging mechanism to evaluate tumor growth rates using mathematical models may provide additional insight into this phenomenon(42, 43). In a recent review of 5 NCI mCRPC trials including a vaccine trial, there appeared to be a sustained decrease in tumor growth rate in vaccine patients as compared to those treated with chemotherapy, who had transient reductions in tumor size, but then growth rate resumed at the same pre-treatment rate once cytotoxic therapy was discontinued. (44) This data suggests that a vaccine induced immune response may not decrease tumor size (or change TTP) but may lead to altered tumor growth rates which could have more substantial impact on overall survival (41). (See Figure 9)

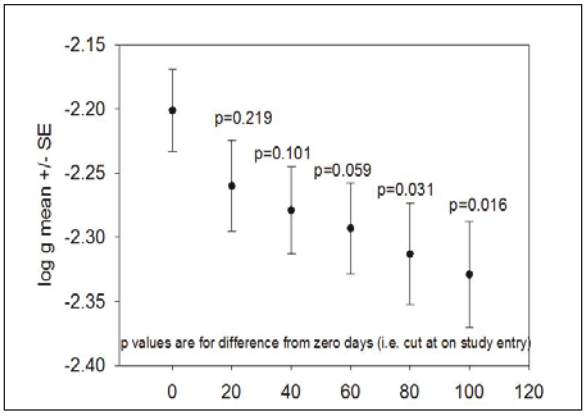

Figure 8. **PSA-TRICOM can affect growth rate in 80 days.** An analysis of the ECOG trial 9802, which treated castration-sensitive non-metastatic prostate cancer patients (n=29) suggests PSA-TRICOM alters tumor growth rate, as measured by PSA *(log g (growth rate) +/- SE (standard error)),* within 80 to100 days.(45) This retrospective analysis used the tumor growth rate equation (44) determined that by day 80 after PSA-TRICOM therapy was initiated a statistically significant change in tumor growth rate was detectable compared to baseline. This altered growth rate was sustained at day 100 as well. These data supports the hypothesis that vaccines exert their therapeutic effect not by reducing tumor burden, but by altering tumor growth rate.

Furthermore, unpublished data from an ECOG PSA-TRICOM trial in non-metastatic prostate cancer demonstrated a reduced growth rate within 100 days of vaccine initiation (45). (Figure 8) Similarly, Sipuleucel-T has significantly prolonged PSA doubling time (another way to measure growth rate) after initial hormonal therapy in the same patient population (46). Together, these data suggest that vaccine can impact growth rate in the nmCSPC population within 80 to 100 days. Furthermore, for patients getting combination therapy, disease can be controlled by standard therapies in the short term, while vaccines may impact tumor re-growth rates in the long term, ultimately resulting in prolonged TTP compared to standard therapy alone, (Figure 10).

This trial will provide an opportunity to prospectively define the mechanism by which vaccines in combination with standard therapies can result in TTP benefit compared to that same standard therapy alone. By discontinuing the enzalutamide after 3 months and allowing PSA values to recover to baseline, we will have the opportunity to determine if a vaccine-mediated immune response can alter that tumor re-growth rate. As demonstrated by Drs. Fojo and Stein previously, 80-100 days is sufficient time to calculate a meaningful change in growth rate in patients with nmCSPC. (Figure 8)

If this study can prospectively demonstrate a difference, measured by one standard deviation in tumor re-growth rate, it would provide an important proof of concept for the mechanism by which vaccines may improve standard therapies. Furthermore, it would better define the potential role of vaccines (as part of a therapeutic combination) in the treatment of prostate cancer and other malignancies.

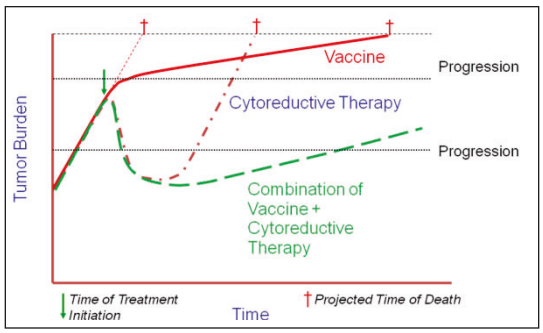

Figure 9. **Enhanced Efficacy with Enzalutamide.** Enzalutamide has greater androgen receptor (AR) binding affinity and a greater negative impact on AR nuclear import than bicalutamide with Dihydrotestosterone (DHT) as a control. In addition with enzalutamide there is no AR binding with the DNA and coactivatior receruitment. (2)

1.2.10 Tumor Growth Kinetics

1.2.10.1 The regression-growth equation

Drs. Stein and Fojo at the NCI have developed an equation based on the assumption that the change of a tumor's quantity during therapy results from 2 independent component processes: an exponential (first-order kinetics) decrease/regression and an exponential regrowth of the tumor. (44) The equation is $f(t) = exp(-d * t)+exp(g * t) - 1$ (A) where exp is the base of the natural logarithm, $e = 2.7182$, and $f(t)$ is the tumor (or in MTC calcitonin) measurement at time t in days, normalized to (divided by) the tumor measurement at day 0, the time at which treatment is commenced. Rate constant d (**d**ecay, in days^{-1}) represents the exponential decrease/regression of the serum tumor marker (i.e. PSA) signal during therapy. Rate constant g (**g**rowth, also in days^{-1}) represents the exponential growth/regrowth of the tumor during treatment. These rate constants may be expressed in terms of half-lives and doubling times. Thus, d equals $\ln_2$ (0.693) divided by the time it takes for the regressing part to shrink by half, whereas g equals $\ln_2$ divided by the time for the growing component to double. (44)

Two earlier papers depict theoretical curves depicting the separate components of Equation (A) and how these combine together to give the time dependence of the tumor size, f. (44) When the data showed a continuous decrease from the time of treatment start, so that only the regression parameter d was found to differ significantly from zero with $P < 0.05$, Equation (A) was replaced by the following reduced form, with the growth rate constant eliminated: $f(t) = exp(-d * t)$ (B) When tumor measurements showed a continuous increase, so that only the growth parameter g was found to differ significantly from zero with $P < 0.05$, Equation (A) was replaced by the following reduced form, with the decay constant eliminated: f(t) = exp(g *t) .

1.2.10.2 Data Analysis

For each patient an attempt to fit Equation (A) to each data set for which more than one data point is available. Curve fitting will be performed using Sigmaplot (Systat Software), or by using the Solver routine in an Excel spreadsheet. We will extract parameters g and d with their associated Student's t and P values. (44) Data will be analyzed in Excel (Microsoft) and in Sigmaplot 9.0. Linear regressions to evaluate the relationship between the growth rate constant, g, or other parameters will be implemented using the polynomial linear routine of Sigmaplot 9.0. Sample comparisons were performed by Student's t-test, using SigmaStat 3.5 (Systat Software), with P set at 0.05 for significance. (44)

1.2.11 Rationale for Re-Treatment of Patients with a Second Course of Enzalutamide

As previously stated, there is no standard of care for patients with nmCSPC. Given that 20-40% of patients diagnosed each year with prostate cancer (over 250,000 men in the US alone) have recurrent disease after definitive therapy, this makes up a large population of patients. The standard of care for patients with nmCSPC includes ADT and active surveillance. Generally, these patients can be followed without therapy until their PSA doubling time escalates at which time ADT is often started. ADT can be given continuously or intermittently, i.e. 2-3 doses of ADT to suppress PSA, and then allow for PSA recovery. Once PSA has recovered ADT can be resumed for an intermittent basis based on PSA metrics and continued castration sensitive disease. A large study of intermittent vs. continuous ADT in this population of patients has demonstrated that intermittent ADT was non-inferior to continuous ADT in terms of overall survival. Also there were improvements in quality of life factors in patients treated with intermittent ADT. [49]

Given that this trial will explore the short term benefits of a brief course of enzalutamide for 3 months, it may be prudent to evaluate if this treatment could be given for a second course and induce a second, sustained PSA decline. From a broad prostate cancer perspective, this would be valuable information moving forward if future larger trials would be considered to evaluate enzalutamide as possible alternative form of intermittent anti-androgen therapy. Such a therapy may be preferred over (intermittent) ADT from a quality of life perspective.

1.2.12 Rationale for Evaluating Changes in Testosterone and Related Hormone Levels

Enzalutamide, an androgen receptor inhibitor, differs from traditional androgen deprivation therapy in its ability to inhibit the androgen receptor without lowering testosterone and is associated with an overall increase in testosterone levels. Consequently, enzalutamide and other non-steroidal antiandrogens have a different side effect profile. A recent European study evaluated Enzalutamide monotherapy in hormone-naïve prostate cancer, and examined a series of hormone levels at various time points in administration to determine changes in testosterone and testosterone related hormones.[50] While they found increased levels of testosterone, estradiol, and luteinizing hormone, levels were checked until the 25 week time point and only once thereafter.

We seek to further elucidate this mechanism of action by obtaining hormone levels at each follow up visit, as well as pre-Enzalutamide, 1 hour post, 24 hours post, 48 hours post, and 72 hours post administration, when logistically feasible. Dr. Doug Figg's lab is currently evaluating a murine model to demonstrate the presence of androgen receptors in the hypothalamus which may influence the levels of testosterone and related hormones in the serum. This pre-clinical data can also be correlated with clinical informatics that will be obtained with these serial hormonal levels.

1.2.12.1 Evaluating Immunologic Effects of Low Dose Enzalutamide

Patients from the arm receiving enzalutamide alone (160 mg daily for 84 days, without ADT) have been assessed for immunologic impact from cohort 1. Peripheral blood mononuclear cells

(PBMCs) collected from 12 patients pre and post enzalutamide (days 14, 28, 84, and 100) were analyzed by flow cytometry to identify 123 immune cell subsets, including 9 standard subsets (CD4+ and CD8+ T cells, T-regulatory cells (Treg), B cells, conventional and plasmacytoid dendritic cells (cDC, pDC), natural killer cells (NK), natural killer T cells (NKT), and myeloid derived suppressor cell (MDSC)), and 114 subsets relating to maturation and function. PBMCs were also assessed for TREC to identify recent thymic emigrants, and by microarray to determine changes in global gene expression.

Treatment with enzalutamide induced several notable alterations in peripheral immune cells, suggesting that it has potential immune activating properties. These changes occurred early following treatment, and included an increase of NK cells, decreased frequencies of MDSCs with a suppressive phenotype (e.g. PD-L1+ MDSC, gMDSC, and CD16+ MDSC), and decreased frequencies of both CD4+ and CD8+ T-lymphocytes expressing the immune inhibitory checkpoint molecule CTLA4.

Additionally, treatment with enzalutamide increased TREC levels by >75% in 7 out of 12 patients compared to pre-therapy levels (p=0.012); naïve CD4+and CD8+ T-lymphocytes were also elevated by >25% in those patients demonstrating the greatest increase in TREC. Gene expression analysis of PBMCs corroborated these findings, showing that enzalutamide increased activation of interferon-gamma signalingand related immune activating pathways.

These preliminary findings demonstrate that short-course enzalutamide has immune activating properties in cancer patients, and support the combination of enzalutamide with immunotherapy. Since the immunologic effects are likely separate from just blocking AR (based on preclinical data serving as the rationale for this study) it would be prudent to explore the immunologic impact of lower doses of enzalutamide (i.e. 80 mg) to determine if the immune impact is preserved, while also limiting the toxicity seen with enzalutamide alone at 160 mg/day. Although enzalutamide is well tolerated, fatigue and breast tenderness in men were frequently seen and could limit its use (independent of ADT) as an immune adjuvant. It is likely these side effects can be minimized at a lower dose. If this can be accomplished at a lower dose, it is more likely to lead to the use of enzalutamide as a possible immunologic adjuvant in other diseases where immune therapies are now approved including lung cancer, bladder cancer, melanoma, and renal cancer.

We planned a study to explore the immunologic effects in biochemically recurrent prostate cancer of enzalutamide 80 mg/day for 3 months. The data were to have been compared against the existing immune data described above with enzalutamide at 160 mg/day for 3 months, however this cohort was closed before enrollment due to lack of patient interest in participation

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 National Institutes of Health (NIH) Clinical

Center, or Walter Reed National Military Medical Center prior to enrollment. If no pathologic specimen is available, patients may enroll with a pathologist's report showing a histologic diagnosis of prostate cancer and a clinical course consistent with the disease.

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

C. ECOG performance status of 0–1 (Karnofsky ≥80%, see Appendix A).
D. Patients must have a PSA doubling time of 12 months or less.
E. Patients must have a rising PSA as confirmed by 3 values done at least 1 week apart and over no less than 1 month.
F. Recovery from acute toxicity related to prior therapy, including surgery and radiation, or no toxicity ≥ grade 2.
G. Negative CT scan/MRI and bone scan for metastatic prostate cancer.
H. Hematological eligibility parameters (within 16 days before starting therapy; see Appendix D):
   - Granulocyte count ≥ 1000/mm^3
   - Platelet count ≥ 100 000/mm^3
   - Hgb ≥ 10 g/dL

I. Biochemical eligibility parameters (within 16 days before starting therapy):
   - Hepatic function: bilirubin ≤1.5 mg/dL (OR in patients with Gilbert's syndrome, a total bilirubin ≤ 3.0), AST and ALT ≤ 2.5 times upper limit of normal.

J. No other active malignancies within the past 36 months (with the exception of nonmelanoma skin cancers or carcinoma in situ of the bladder) or life-threatening illnesses
K. Willing to travel to the NIH for follow-up visits.
L. 18 years of age or older.
M. Able to understand and sign informed consent.
N. Baseline testosterone ≥ lower limit of normal.
O. PSA ≤ 20 ng/mL.
P. The effects of enzalutamide, PSA-TRICOM or the combination on the developing human fetus are unknown. For this reason, men must agree to use adequate contraception (hormonal or barrier method of birth control; abstinence) prior to study entry and for the duration of study participation. Should a woman become pregnant or suspect she is pregnant while her partner is participating in this study, she should inform her treating physician immediately.

2.1.2 Exclusion Criteria

A. Immunocompromised status due to:
   - Human immunodeficiency virus (HIV) positivity.

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

B. Chronic administration (defined as daily or every other day for continued use > 14 days) of corticosteroids deemed systemic by investigator within 28 days before the first planned dose of PSA-TRICOM. Use of inhaled steroids, nasal sprays, and topical creams for small body areas is allowed.
C. Serious intercurrent medical illness that, in the judgment of the investigator, would interfere with patient's ability to carry out the treatment program.
D. History of seizure, including any febrile seizure, loss of consciousness, or transient ischemic attack, or any condition that may pre-dispose to seizure (e.g., prior stroke, brain arteriovenous malformation, head trauma with loss of consciousness requiring hospitalization).
E. Other medications used for urinary symptoms including 5-alpha reductase inhibitors (finasteride and dutasteride) and alternative medications known to alter PSA (eg phytoestrogens and saw palmetto)
F. History of prior chemotherapy
G. History of prior immunotherapy within the last 3 years
H. Major surgery within 4 weeks prior to enrollment (Day 1 visit).
I. History of allergic reactions attributed to compounds of similar chemical or biologic composition to *enzalutamide* or poxviral vaccines (e.g., vaccinia vaccine)
J. Known allergy to eggs, egg products, aminoglycoside antibiotics (for example, gentamicin or tobramycin).
K. History of atopic dermatitis or active skin condition (acute, chronic, exfoliative) that disrupts the epidermis
L. Previous serious adverse reactions to smallpox vaccination
M. Unable to avoid close contact or household contact with the following high-risk individuals for three weeks after the Day 1 vaccination: (a) children ≤ 3 years of age, (b) pregnant or nursing women, (c) individuals with prior or concurrent extensive eczema or other eczemoid skin disorders, or (d) immunocompromised individuals, such as those with HIV.
N. Receipt of an investigational agent within 30 days (or 60 days for an antibody-based therapy) before the first planned dose of study drugs.
O. Patients who test positive for HBV or HCV
P. Use of herbal products that may decrease PSA levels (e.g. saw palmetto)
Q. Any gastrointestinal disease that could hinder the absorption of enzalutamide
R. Uncontrolled hypertension (SBP>170/ DBP>105)

2.1.3 Recruitment Strategies

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

2.2 SCREENING EVALUATION

A. Pathological confirmation of diagnosis by the Laboratory of Pathology, CC, NIH, or Walter Reed National Military Medical Center (if specimen is available) may be obtained anytime prior to enrollment.
B. The following parameters will be obtained within 8 weeks prior to start of enrollment:
    1. HIV test
    2. Hepatitis B and C
    3. Tc-99 whole-body scintigraphy
    4. CT (or MRI may be substituted at investigator's discretion) of chest, abdomen and pelvis

2.3 REGISTRATION PROCEDURES

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

2.4 TREATMENT ASSIGNMENT AND RANDOMIZATION/STRATIFICATION PROCEDURES

Cohorts

Number	Name	Description
1	*Cohort 1*	*Patients with nonmetastatic castration sensitive prostate cancer*

Arms

Letter	Name	Description
A	*Standard dose enzalutamide alone*	*Enzalutamide alone administered at the standard dose (160 mg daily)*
B	*Enzalutamide with PSA-TRICOM*	*Standard dose enzalutamide with concomitant vaccinia-PSA-TRICOM and then fowlpox-vaccine*

Stratifications

Name	Distinct Options	Notes
PSA doubling time less than 6 months	*Yes* *No*	

Randomization and Arm Assignment
After confirmation of eligibility at Central Registration Office (CRO), patients in cohort 1 will be randomized on a 1:1 basis between arms A and B, stratifying for PSA doubling time using a computerized randomization process.

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

2.5 BASELINE EVALUATION

A. Baseline electrocardiogram (EKG) on all patients, and appropriate cardiologic evaluation, as clinically indicated, to provide baseline function and identify any patients who should be monitored closely for cardiac risks associated with vaccinia vaccination
B. Apheresis (cohort 1 only)
C. 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
        - Serum PSA
        - CBC/differential, with platelet count
        - Serum testosterone level
        - Serum chemistries (Na^+, K^+, Cl^-, CO_2, glucose, BUN, creatinine, albumin, calcium, magnesium, phosphorus, alkaline phosphatase, ALT, AST, total bilirubin, LDH, CK, uric acid, total protein)
        - Lymphocyte phenotyping CD3/CD4/CD8

3 STUDY IMPLEMENTATION

3.1 STUDY DESIGN

- This is a randomized Pilot trial of enzalutamide vs. enzalutamide and PSA-TRICOM in patients with nmCSPC prostate cancer. Thirty-four patients will be enrolled.
- Enzalutamide will be given at the standard dose of 160 mg daily for 3 months (7). PSA-TRICOM will be administered identical to the Phase III dosing with vaccine given week 1 (vaccinia-PSA-TRICOM, $2x10^8$ infectious units subcutaneously) and then fowlpox-vaccine ($1x10^9$ infectious units subcutaneously) on weeks 3 and 5

followed by monthly fowlpox-vaccine ($1x10^9$ infectious units subcutaneously) for a total of 5 months (including vaccinia, vaccine is administered over 6 months).

- Patients will be followed for PSA recovery after enzalutamide has been discontinued. Patients who do not develop a 25% decline in PSA after 3 months will not be evaluated for tumor re-growth and additional patients will be enrolled to evaluate for that endpoint.
- Patients who have been on study for at least 7 months without PSA equaling or exceeding the value at study entry, and whose PSA subsequently (beyond 7 months) returns to or exceeds the value at study entry, may be retreated with a 3-month course of Enzalutamide.
- Patients who continue to be followed for rising PSA after completing either one or 2 courses of enzalutamide but have not required ADT may be followed after treatment. Such visits may occur at 4 week intervals based on logistics and clinical parameters (i.e. PSA velocity) once patients have exceeded their baseline PSA value. This approach is consistent with the clinical management of such non-metastatic patients (rising PSA and not on ADT.)
- The primary endpoint of this trial will be a comparison of the tumor re-growth rates at 7 months. This time frame should be sufficient to evaluate immune effect on the re-growth rate given that the ECOG 9802 analysis demonstrated a significant change in tumor growth rate after starting PSA-TRICOM.
  Secondary endpoints include growth rate at points other than 7 months, toxicity, immunologic responses to vaccine with enzalutamide and to enzalutamide alone in men with normal testosterone, and response to second course of enzalutamide. When possible, patients will be followed for TTP. End of study period will be 12 months in each arm (24 months if patients are eligible and elect to receive a 2^{nd} course of enzalutamide).

- Preliminary data from the first cohort of randomized patients suggests that enzalutamide alone can induce an immunologic response. A second cohort of 15 patients will explore a lower dose of enzalutamide at 80 mg to determine if similar immunologic responses can also be seen at a lower dose, where toxicity is less likely.

3.1.1 Protocol Stopping Rules

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

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

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

All patients will receive vaccine treatments at the NIH Clinical Center, patients will receive enzalutamide primarily in the NCI outpatient setting.

We estimate an accrual of approximately 2 patients per month, with full accrual of 34 patients within 18 months.

3.2 DRUG ADMINISTRATION

All patients in cohort 1 (n=34) will receive enzalutamide 160 mg daily orally for 3 months. Patients randomized to vaccine arm (Arm B) will receive PSA-TRICOM identical to Phase III dosing, which consists of a single immunization of PROSTVAC-V (vaccinia-PSA-TRICOM, $2x10^8$ infectious units subcutaneously) in Week 1, followed by immunizations of PROSTVAC-F (fowlpox-PSA-TRICOM $1x10^9$ infectious units subcutaneously) administered in Weeks 3, 5, 9, 13, 17, and 21.

3.3 TREATMENT MODIFICATIONS

3.3.1 Vaccine

3.3.1.1 Dosing delay

- Patients must have recovered to ≤ grade 1 toxicity related to vaccine for the parameters used to assess levels of organ function required for eligibility (see Section 2.1.1) after each vaccination in order to receive a subsequent vaccination.
- Patients with ≤ grade 2 toxicity not related to vaccine treatment (i.e. attributed to enzalutamide) will remain eligible to receive vaccine.
- Patients that recover from > grade 2 toxicity unrelated to vaccine treatment (i.e. related to enzalutamide) may remain eligible for the vaccine.
- If ≥ grade 2 nonautoimmune toxicity attributable to the vaccine persists for > 42 days, the patient will not receive further vaccine inoculations and will be removed from the protocol treatment and followed for resolution of toxicity and immune endpoints.
- Patients who develop grade 3 injection site reactions will have their vaccine held until injection site reaction resolves to grade 2 or less.
- Patients who develop ≥ a grade 2 allergic or autoimmune disease that threatens vital organ function or any ≥ grade 3 autoimmunity, not related to a therapeutic response, will be removed from the protocol treatment and followed for resolution of toxicity and immune endpoints.
- Patients who develop any grade 4 toxicity attributable to the study drug(s) will be removed from the protocol treatment and followed for resolution of toxicity and immune endpoints.
- If a scheduled vaccine dose is missed due to scheduling or logistical issues, the vaccine may be given within 7 days of the appointed time

3.3.1.2 Dose modifications

No dose modifications are allowed with this vaccine.

3.3.2 Enzalutamide

If a patient experiences a ≥ Grade 3 non-hematologic toxicity attributable to enzalutamide or an intolerable side effect attributable to enzalutamide, withhold dosing for one week or until symptoms improve to ≤ Grade 2, then resume at the same dose if clinically appropriate. If toxicity recurs enzaluatmide will be held again using the above parameters or discontinued at the discretion of the investigator.

Patients who develop any grade 4 toxicity attributable to the study drug(s) will be removed from the protocol treatment and followed for resolution of toxicity and immune endpoints.

3.4 PROTOCOL EVALUATION (SEE APPENDIX C)

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

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

3.4.3 Laboratory Studies

(See also Appendix C)

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

- The following serum hormone levels will be drawn at each follow up visit after enrollment and pre-Enzalutamide administration, and 1 hour post, 24 hours post, 48 hours post, and 72 hours post administration, when logistically feasible.
  - testosterone
  - dihydrotestosterone (DHT)
  - sex hormone-binding globulin (SHBG)
  - dehydroepiandrosterone (DHEA)
  - luteinizing hormone (LH)
  - follicle-stimulating hormone (FSH)
  - androstenedione
  - prolactin
  - estradiol
- Plasma VEGF levels will be drawn at the start and at the end of each patient's second 12-week course of Enzalutamide.

3.4.4 Radiographic assessment

Patients will not undergo scheduled radiographic assessment for metastatic disease unless clinically indicated for increasing PSA or symptoms. Radiologic studies consisting of bone scan, CT scan of chest, and CT scan of abdomen/pelvis will be performed at baseline. MRI may be substituted for CT scan at the discretion of the investigator.

Patients will undergo a radiographic assessment with CT scan of chest, abdomen, and pelvis and bone scan once PSA returns to baseline at study entry or higher. These scans will be obtained prior to retreatment with a 3-month course of enzalutamide.

For patients who are eligible and elect to receive a second course of enzalutamide therapy for 3 months, re-staging CT scan of chest, abdomen, and pelvis and bone scan will again be done when PSA reaches baseline.

3.4.5 Assessment for PSA-TRICOM administration (Arm B)

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

3.5 CONCURRENT THERAPIES

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

3.6 CRITERIA FOR REMOVAL FROM PROTOCOL THERAPY AND OFF STUDY CRITERIA

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

3.6.1 Criteria for removal from protocol therapy

Patients will be removed from treatment for the following:

- Clinical or radiographic progression of disease.
- Grade 3 or greater toxicity attributed to treatment that does not resolve to grade 1 within 42 days from time of scheduled treatment.
- Grade 3 or greater toxicity not related to treatment that does not resolve to grade 2 within 42 days from time of scheduled treatment.
- Grade 2 or greater autoimmune disease that threatens vitals organs.

- Any Grade 4 toxicity that is possibly, probably or definitely related to the protocol treatment will require a patient to be off-treatment
- Intercurrent illness or medical circumstances. If at any time the constraints of this protocol are detrimental to the patient's health, the patient may be removed from protocol therapy and reasons for withdrawal will be documented.
- Any grade of seizure will require a patient to be off-treatment.
- Requirement for androgen deprivation therapy
- Completion of protocol therapy

3.6.2 Off-Study Criteria

- Patient is off-treatment and has agreed to be followed on a long term therapy protocol as outlined in section **3.7**
- Patient requests to be taken off study. Reasons for withdrawal will be documented.
- Noncompliance with protocol guidelines (patient removed at discretion of Principal Investigator).
- Death

3.6.3 Off Protocol Therapy and Off-Study Procedure

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

3.7 POST-STUDY EVALUATION (FOLLOW-UP)

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

4 BIOSPECIMEN COLLECTION

4.1 CORRELATIVE STUDIES FOR RESEARCH

4.1.1 Immunologic Parameters

- IFN-gamma ELISPOT assay for PSA-specific T lymphocytes (HLA-A2 patients only)
- Antibodies to PSA, vaccinia, fowlpox may be tested
- Leukocyte CD3, CD4, CD8 subsets; CD4:CD8 ratio will be drawn at baseline and monthly prior to vaccination while the patient remains on trial.
- The results of the HIV antibody need to be available before treatment to determine eligibility

- HLA class I expression (HLA typing) with A2 subtyping (obtain anytime prior to enrollment). Patients may refuse or test may be omitted for logistical purposes at the discretion of the investigator.
- Additional studies will include but are not limited to quantitative and qualitative assessments of regulatory T-cells, Natural Killers cells, Myeloid Derived Suppressor Cells, anti-glycan antibodies and Naïve T-cell/new thymic emigrants.
- Assessment of levels of cytokines.
- Immunologic studies will be repeated more frequently if clinically indicated, and any abnormalities potentially related to treatment will be followed until they have resolved, or have been determined not to be treatment-related.

4.1.2 Collection of Research (Immunologic) Blood Samples

Immunologic blood samples will be obtained via apheresis when logistically feasible at Baseline (prior to treatment), after 3 months and after 6 months in all HLA-A2 patients. (Additional apheresis could be considered at the discretion of the investigator and with the consent of the patient.) We plan to examine the immune response in selected patients (HLA-A2 positive) when feasible and if patients consent. (The HLA-A2 requirement is based on the ability to assess immune response in these patients using immunologic response techniques (ELISPOT). This analysis is not limited to these patients due to any reason to believe that non-HLA-A2 will not have an immune response, but rather those immune responses can not be detected by our current ELISPOT methods.)

In all patients undergoing apheresis, 5×10^8 to 2×10^9 mononuclear cells will be obtained by a single-access (single venipuncture) 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 allow us to obtain sufficient PBMC samples from patients to be able to perform 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 is not obtained, we will obtain 6 green top (10ml) tubes. These will be used for ELISPOT assays (as described in Appendix D). In addition, 2 SST (tiger) top tubes will be drawn for antibody testing (as described in Appendix D). If apheresis is not done, research samples will be obtained per Appendix C

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 Jen Bangh at banghj@mail.nih.gov and Theresa Burks burkst@mail.nih.gov.

4.1.3 Immunologic Assays

4.1.3.1 IFN-gamma ELISPOT

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 IFN-gamma production, is used to determine CTL precursor frequency to peptides from PSA, PSMA, MUC-1, PSCA, CEA, and PAP in both pre- and post-vaccination peripheral blood mononuclear cells, as previously described (39-40). Briefly, 96-well mL HA plates (Millipore Corporation, Bedford, MA) are coated with 100 µl/well of capture MAb against human IFN-gamma 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 (41). We also perform each sample with 6 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 24 h and lysed with phosphate-buffered saline (PBS)-Tween (.05%). Biotinylated anti IFN-gamma 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 2 separate investigators in a *blinded manner*, and the frequency of responding cells will be determined.

4.1.3.2 CD4 T Cell Proliferation Assay

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

4.1.3.3 Sera Antibody Analysis

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

4.1.3.4 Flow cytometry analysis of thymic emigrant

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

4.1.3.5 Natural Killer (NK) CELLS

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

4.1.3.6 Regulatory T Cells

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

4.1.4 Additional Assays

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

All samples will be labeled with the following identifier system.

- Patient's enrollment #
- Trial number
- Patient's initials
  Example: 01-ABC

These labels are used only to send the samples from the NIH Clinical Center to the NCI Frederick Central Repository. The NCI Repository will process all samples, appropriately discard the label on the blood tube, and then store the samples with unique identifiers, to which only NCI study personnel will have the code to link to patient specific clinical information. Samples will be tracked according to Section **4.2**.

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

4.1.5 Plasma VEGF levels

Serum samples will be collected to measure vascular endothelial growth factor (VEGF) levels. For this purpose, one 6mL EDTA tube will be obtained at baseline (prior to treatment), 1 month, 3 months, 3.5 months, 4 months, and 6 months. The analysis will be done with assays developed on electrochemiluminescence platform that provides ultra-high sensitivity and very large signal dynamic range. These studies will be done by the Molecular Pharmacology Program, under the direction of Dr. Doug Figg.

Write the collection time on the vacutainer tube and place the sample on wet ice immediately after collection. The sample should be picked up within 1 hour of collection.

Please e-mail Julie Barnes at Julie.barnes@nih.gov and Paula Carter pcartera@mail.nih.gov at least 24 hours before transporting samples (the Friday before is preferred).
For sample pickup, page 102-11964.
For immediate help, call 240-760-6180 (main blood processing core number) or, if no answer, 240-760-6190 (main clinical pharmacology lab number).
For questions regarding sample processing, contact Julie Barnes by e-mail or at 240-760-6044.

4.1.6 Serum Analysis

Of the sample collected in **4.1.2**, de-identified serum may be sent for analysis of pre-treatment, on-treatment, on progression and post-treatment absolute concentrations of steroids and ratios of 11BHSD substrates and products with presence of treatment and the nature of the clinical response to enzalutamide. The serum samples may be sent under an MTA to the laboratory of Dr. Nima Sharifi, of the Cleveland Clinic Foundation, at the following address:

Michael P. Berk
Cleveland Clinic Foundation
Lerner Research Institute
Cancer Biology, NB4-15
2111 East 96th Street
Cleveland, OH 44106
Tel. = 216.445.9752
Email = berkm@ccf.org

4.2 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 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, backup, 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 or 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 II (BSI). This inventory tracking system is used to manage the storage and retrieval of specimens, as well as to maintain specimen data. BSI is designed for controlled, concurrent access. It provides a real-time, multi-user environment for tracking millions of specimens. The system controls how and in what order database updates and searches are performed. This control prevents deadlocks and race conditions. For security, BSI has user password access, 3 types of user access levels, and 36 user permissions (levels of access) that can be set to control access to the system functions. BSI provides audit tracking for processes that are done to specimens including shipping, returning to inventory, aliquoting, thawing, additives, and other processes. BSI tracks the ancestry of specimens as they are aliquoted, as well as discrepancies and discrepancy resolution for specimens received by the repository. If a specimen goes out of the inventory, the system maintains data associated with the withdraw request. Vials are labeled with a unique BSI ID which is printed in both eye-readable and bar-coded format. No patient-specific information is encoded in this ID.

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

Samples will be used for research analysis, including immunologic monitoring as outlined in section **4.1.3**. 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.

4.2.1 Protocol Completion/Sample Destruction

Once primary research objectives for the protocol are achieved, intramural researchers can request access to remaining samples, provided they have an IRB-approved protocol and patient consent.
Samples and associated data will be stored permanently unless the patient withdraws consent. If the patient withdraws consent the participant's data will be excluded from future distributions, but data that have already been distributed for approved research use will not be able to be retrieved. The PI will report destroyed samples to the IRB if samples become unsalvageable or destroyed by environmental conditions (ex. broken freezer or lack of dry ice in shipping container) or if a patient chooses to withdraw his/her consent. Samples will also be reported as lost if they are lost in transit or misplaced by a researcher. Freezer problems, lost samples or other problems associated with samples will also be reported to the IRB, the NCI Clinical Director, and the office of the CCR, NCI.

5 SUPPORTIVE CARE

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

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

Symptomatic anemia should be treated with appropriate red blood cell or erythropoietin support. Thrombocytopenia should be treated conservatively. In the absence of bleeding or a planned invasive procedure, platelet transfusions should be given for a platelet count below 10,000/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.

5.1 EXCLUDED MEDICATIONS

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

- Concurrent chemotherapy
- Concurrent radiation therapy
- Concurrent immunotherapy
- Concurrent anti-cancer radionuclides
- Concurrent systemic corticosteroid use (daily or every other day for continued use > **14 days**; See section **2.1.2**)
- Concomitant use of secondary hormonal treatments

5.2 TREATMENT OF VACCINIA VACCINATION COMPLICATION

5.2.1 Vaccinia Immune Globulin:

First-line treatment of some of the complications of vaccinia caused by dissemination of vaccinia virus (severe cases of inadvertent inoculation involving extensive lesions or if comorbid conditions exist, severe cases of generalized vaccinia in patients that are systemically ill and whose condition might be toxic or who have serious underlying immunosuppressive illnesses, eczema vaccinatum, and progressive vaccinia) is with VIG.

VIG is contraindicated, however, for the treatment of isolated vaccinial keratitis. VIG is a sterile solution of the immunoglobulin fraction of pooled plasma from individuals inoculated with vaccinia vaccine. VIG is an investigational agent available through the CDC's Strategic National Pharmaceutical Stockpile under an IND protocol by contacting the CDC's Smallpox Vaccine Adverse Events Clinician Information Line at 1-877-554-4625. Upon receipt of a call from a patient or upon direct observation of a patient or contact who manifests signs and symptoms of any of the above conditions, the investigator should place a call to the CDC as soon as possible:
1) to initiate review of the clinical case,
2) to seek consultation on the appropriateness of VIG therapy,
3) to determine the appropriate VIG dose and dosing method for administration, if VIG therapy is required, and
4) to determine how to access and have the appropriate doses of VIG delivered.

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

5.2.2 Cidofovir (Vistide®, Gilead Sciences):

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

6 DATA COLLECTION AND EVALUATION

6.1 DATA COLLECTION

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.

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

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

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

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

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

6.1.6 Drug Accountability Records are kept for each patient.

6.1.7 We will not report Grade 1 adverse events.

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

An abnormal laboratory value will be recorded in the database as an AE **only** if the laboratory abnormality is characterized by any of the following:

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

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

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

6.2 RESPONSE CRITERIA

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

6.2.1 Disease Progression

6.2.1.1 PSA will not be used to measure progression of disease given the intermittent use of enzalutamide. It is expected that PSA will rise after a 3 month course of enzalutamide is completed and the treatment is discontinued. Patients will be offered androgen deprivation therapy (ADT) as clinically indicated or a second course of enzalutamide as indicated in the protocol. Starting ADT will be done at the discretion of the investigator, although this will result in treatment termination and removal from the clinical trial. (Conventionally this is when PSA has a doubling time of less than 3 months, but patients may refuse treatment in favor of surveillance which is also clinically appropriate. Conversely, patients may go on ADT at any time, although this

may result in removal from the trial.) PSA will be used to calculate PSA kinetics/tumor growth kinetics.

6.2.1.2 Development of a new bone lesion.

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

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

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

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

6.3 TOXICITY CRITERIA

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

7 SAFETY REPORTING REQUIREMENTS/DATA AND SAFETY MONITORING PLAN

7.1 DEFINITIONS

7.1.1 Adverse Event

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

7.1.2 Suspected adverse reaction

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

7.1.3 Unexpected adverse reaction

An adverse event or suspected adverse reaction is considered "unexpected" if it is not listed in the investigator brochure or is not listed at the specificity or severity that has been observed; or, if an investigator brochure is not required or available, is not consistent with the risk information described in the general investigational plan or elsewhere in the current application.

"Unexpected", also refers to adverse events or suspected adverse reactions that are mentioned in the investigator brochure as occurring with a class of drugs or as anticipated from the pharmacological properties of the drug, but are not specifically mentioned as occurring with the particular drug under investigation.

7.1.4 Serious

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

7.1.5 Serious Adverse Event

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

- Death,
- A life-threatening adverse drug experience
- Inpatient hospitalization or prolongation of existing hospitalization
- Persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- A congenital anomaly/birth defect.
- Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered a serious adverse drug experience when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

7.1.6 Disability

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

7.1.7 Life-threatening adverse drug experience

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

7.1.8 Protocol Deviation (NIH Definition)

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

7.1.9 Non-compliance (NIH Definition)

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

7.1.10 Unanticipated Problem

Any incident, experience, or outcome that:

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

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

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

- Is related or possibly related to participation in the research; **AND**
- Suggests that the research places subjects or others at a *greater risk of harm* (including physical, psychological, economic, or social harm) than was previously known or recognized.

7.2 NCI-IRB AND CLINICAL DIRECTOR REPORTING

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

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

7.2.2 NCI-IRB Requirements for PI Reporting at Continuing Review

The protocol PI will report to the NCI-IRB:

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

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

7.2.3 NCI-IRB Reporting of IND Safety Reports

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

7.3 IND SPONSOR REPORTING CRITERIA

During the first 30 days after the subject receives investigational agent/intervention, the investigator must immediately report to the sponsor, using the mandatory MedWatch form 3500a, any serious adverse event, whether or not considered drug related, including those listed in the protocol or investigator brochure and must include an assessment of whether there is a reasonable possibility that the drug caused the event. For serious adverse events that occur more than 30 days after the last administration of investigational agent/intervention, only report those that have an attribution of at least possibly related to the agent/intervention.

Required timing for reporting per the above guideline:

- Deaths (except death due to progressive disease) must be reported via email within 24 hours. A complete report must be submitted within one business day.
- Other serious adverse events as well as deaths due to progressive disease must be reported within one business day

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

7.3.1 Reporting Pregnancy

7.3.1.1 Paternal exposure

Male patients should refrain from fathering a child or donating sperm during the study and for 3 months after the last dose of study treatment. Pregnancy of the patient's partner is not considered to be an AE. However, the outcome of all pregnancies (spontaneous miscarriage, elective termination, ectopic pregnancy, normal birth, or congenital abnormality) occurring from the date of the first dose until 3 months after the last dose should, if possible, be followed up and documented.

7.4 SAFETY REPORTING CRITERIA TO THE PHARMACEUTICAL COLLABORATORS

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

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

7.4.1 Reporting to Astellas

The Investigator should complete and submit an SAE Medwatch 3500 Form, containing all information that is required by the Regulatory Authorities, to Astellas by either e-mail or fax within 24 hours of awareness whether or not related to the study drug. If submission of this SAE by email or fax or is not possible within 24 hours, the local drug safety contact (IRB, Investigator, etc.) should be informed by phone.

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

Astellas Pharma Global Development – United States

Email: Safety-us@us.astellas.com
Fax number: (847) 317-1241

The following minimum information is required:

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

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

7.4.2 Reporting to Bavarian Nordic, Inc.

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

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

7.5 INSTITUTIONAL BIOSAFETY COMMITTEE (IBC) REPORTING CRITERIA

7.5.1 Serious Adverse Event Reports to OSP/IBC

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

7.5.2 Annual Reports to IBC

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

7.5.2.1 Clinical Trial Information

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

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

7.5.2.2 Progress Report and Data Analysis

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

- a narrative or tabular summary showing the most frequent and most serious adverse experiences by body system
- a summary of all serious adverse events submitted during the past year
- a summary of serious adverse events that were expected or considered to have causes not associated with the use of the gene transfer product such as disease progression or concurrent medications
- if any deaths have occurred, the number of participants who died during participation in the investigation and causes of death
- a brief description of any information obtained that is pertinent to an understanding of the gene transfer product's actions, including, for example, information about dose-response, information from controlled trials, and information about bioavailability.

7.6 DATA AND SAFETY MONITORING PLAN

7.6.1 Principal Investigator/Research Team

The Principal Investigator, lead associate investigator and the research nurse will meet weekly at each clinic to review all adverse events for each subject in this trial and to determine dose limiting toxicities and escalation rules. Unexpected adverse events and/or serious adverse events will be reported to the NCI's Institutional Review Board (IRB) and sponsor/FDA as outlined above. If trends are noted and/or risks warrant it, accrual will be interrupted, dose levels expanded and/or the protocol and/or consent will be modified accordingly.

All data will be collected in a timely manner and reviewed by the principal investigator or a lead associate investigator. Adverse events will be reported as required above. Any safety concerns,

new information that might affect either the ethical and or scientific conduct of the trial, or protocol deviations will be immediately reported to the IRB using iRIS and to the Sponsor.

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

7.6.2 Sponsor Monitoring Plan

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

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

The monitoring program also extends to multi-site research when the CCR is the coordinating center.
This trial will be monitored by personnel employed by an NCI contractor. Monitors are qualified by training and experience to monitor the progress of clinical trials. Personnel monitoring this study will not be affiliated in any way with the trial conduct. Safety Monitoring Committee (SMC)

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

8 STATISTICAL CONSIDERATIONS

8.1.1 Cohort 1

The primary objective of this study is to compare the change in the growth rate in the two arms from the time of discontinuation of enzalutamide (3 months after enrollment) to 4 months after completing enzalutamide therapy +/- PSA-TRICOM (7 months after enrollment). With growth rate changes obtained on all patients on both arms, and if the growth rate changes in the two arms had the same standard deviation, with 17 patients per arm, there would be 80% power to detect a difference between the arms equal to 1 SD of the difference from baseline to 7 months with a two-tailed 0.05 significance level two-group t-test**.** Thus, a total of 34 evaluable patients

are required for analysis. To allow for a small number of patients who do not develop a 25% decline in PSA after 3 months, additional patients will be randomized if necessary to obtain a minimum of 17 evaluable patients on each arm. In order to allow for a small number of such patients, the accrual ceiling will be 38.

Patients will be stratified based a doubling time of greater than or less than 6 months.

Secondary endpoints will be compared between the two arms using appropriate non-parametric tests, such as a Wilcoxon rank sum test. Results will be presented without formal adjustment for multiple comparisons but reported in the context of secondary evaluation of a potentially large number of endpoints. The significance of paired hormone level results within arms will be determined by a paired t-test or Wilcoxon signed rank test as appropriate.

8.1.2 Halting Rules

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

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

8.1.3 The regression-growth equation

The equation is $f(t) = exp(-d * t)+exp(g * t) - 1$ (A) where exp is the base of the natural logarithm, $e = 2.7182$, and $f(t)$ is the tumor (or in MTC calcitonin) measurement at time t in days, normalized to (divided by) the tumor measurement at day 0, the time at which treatment is commenced. Rate constant d (**d**ecay, in $days^{-1}$) represents the exponential decrease/regression of the a serum tumor marker (i.e. PSA) signal during therapy. Rate constant g (**g**rowth, also in $days^{-1}$) represents the exponential growth/regrowth of the tumor during treatment. These rate constants may be expressed in terms of half-lives and doubling times. Thus, d equals ln_2 (0.693) divided by the time it takes for the regressing part to shrink by half, whereas g equals ln_2 divided by the time for the growing component to double. (44)

Two earlier papers depict theoretical curves depicting the separate components of Equation (A) and how these combine together to give the time dependence of the tumor size, *f*. (44) When the data showed a continuous decrease from the time of treatment start, so that only the regression parameter d was found to differ significantly from zero with $P < 0.05$, Equation (A) was replaced by the following reduced form, with the growth rate constant eliminated: $f(t) = exp(-d * t)$ (B) When tumor measurements showed a continuous increase, so that only the growth parameter g was found to differ significantly from zero with $P < 0.05$, Equation (A) was replaced by the following reduced form, with the decay constant eliminated: f(t) = exp(g *t) .

8.1.4 Data Analysis

For each patient an attempt to fit Equation (A) to each data set for which more than one data point is available. Curve fittingbo will be performed using Sigmaplot (Systat Software), or by

using the Solver routine in an Excel spreadsheet. We will extract parameters g and d with their associated Student's t and P values. (44) Data will be analyzed in Excel (Microsoft) and in Sigmaplot 9.0. Linear regressions to evaluate the relationship between the growth rate constant, *g*, or other parameters will be implemented using the polynomial linear routine of Sigmaplot 9.0. Sample comparisons were performed by Student's *t*-test, using SigmaStat 3.5 (Systat Software), with *P* set at 0.05 for significance. (44)

9 COLLABORATIVE AGREEMENTS

9.1 AGREEMENT TYPE

9.1.1 Cooperative Research and Development Agreement (CRADA)

- A CRADA (#02859) is in place with Medivation and Astellas to provide enzalutamide.
- A CRADA (#02377) is in place with Bavarian Nordic to provide PSA-TRICOM.

9.1.2 Material Transfer Agreement (MTA)

- A MTA is in place with The Cleveland Clinic Foundation for the studies discussed in section 4.1.6 (MTA#4122006).

10 HUMAN SUBJECT PROTECTIONS

10.1 RATIONALE FOR SUBJECT SELECTION

10.1.1 Selection Based on Gender, Ethnicity, and Race

Subjects from all racial/ethnic groups are eligible for this study if they meet the eligibility criteria. To date, there is no information that suggests that differences in drug metabolism or disease response would be expected in one group compared with another. Efforts will be made to extend accrual to a representative population, but in this preliminary study, a balance must be struck between patient safety considerations and limitations on the number of individuals exposed to potentially toxic and/or ineffective treatments on one hand and the need to explore ethnic aspects of clinical research on the other hand. If differences in outcome that correlate with ethnic identity are noted, accrual may be expanded or a follow-up study may be written to investigate those differences more fully. Women are not eligible for this study as this diease occurs only in men.

10.1.2 Strategies/Procedures for Recruitment

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

10.1.3 Justification for Exclusions

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

10.2 PARTICIPATION OF CHILDREN

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

10.3 PARTICIPATION OF NIH SUBJECTS UNABLE TO GIVE CONSENT

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 **7.5**), all subjects will be offered the opportunity to fill in their wishes for research and care, and assign a substitute decision maker on the "NIH Advance Directive for Health Care and Medical Research Participation" form so that another person can make decisions about their medical care in the event that they become incapacitated or cognitively impaired during the course of the study. Note: The PI or AI will contact the NIH Ability to Consent Assessment Team for evaluation. For those subjects that become incapacitated and do not have pre-determined substitute decision maker, the procedures described in MEC Policy 87-4 for appointing a surrogate decision maker for adult subjects who are (a) decisionally impaired, and (b) who do not have a legal guardian or durable power of attorney, will be followed.

10.4 Evaluation of Benefits/Risks/Discomforts

There is no standard therapy for patients with prostate cancer and rising PSA following local definitive therapy. Potential risks of vaccine and enzalutamide therapy in this patient population include the range of side effects outlined in section 9. Both drugs have been well tolerated in previous large trials and there are no anticipated overlapping toxicities for patients getting both drugs.

10.4.1 Alternative Approaches or Treatments

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

10.4.2 Procedures to Eliminate or Minimize Potential Risks

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

10.4.3 Provisions for Monitoring Data Collection to Ensure Subject Safety

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

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

10.5 Risks/Benefits Analysis

This study involves clinical research with an experimental vaccine designed to generate an immune response against antigens found in prostate cancer. Patients will undergo multiple vaccinations. Side effects of the vaccine are outlined elsewhere (see section 11). Whether the vaccine will have any clinical effect is unknown; therefore, benefit cannot be promised, nor the chance of benefit accurately predicted. Enzalutamide is an FDA-approved therapy with a known and minimal toxicity profile (section 11). Participation in this study is voluntary, and refusal will not result in penalty or loss of benefit to which the patient is otherwise entitled.

The standard of care for patients with nmCSPC includes ADT and active surveillance. Generally, these patients can be followed without therapy until their PSA doubling time escalates at which time ADT is often started. All patients in the proposed trial will receive enzalutamide. Based on previous trials with high dose bicalutamide, 34 of 36 evaluable patients had PSA declines by a median of 96.5% and these nadirs were achieved within the first 4 weeks of treatment (10). We would expect similar if not better results with enzalutamide. Therefore, a clinical trial in this population is ethical and appropriate. The NCI has conducted several trials in this population previously.

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

10.6 Consent and Assent Process and Documentation

The investigational nature and objectives of this trial, the procedures involved, and their attendant risks and discomforts, potential benefits, and potential alternative therapies will be explained to the patient and a signed informed consent obtained. Any experimental invasive procedure will require a separate consent form. All Associate Investigators listed in this protocol who have clinical privileges are permitted to obtain informed consent. For the apheresis for research in the protocol, the patient will consent at the time of the procedures. If the patient refuses the apheresis at that time, the refusal will be documented in the medical record and in the research record.

10.6.1 Telephone Re-Consent Procedure

Re-consent 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.

10.6.2 Informed Consent of Non-English Speaking Subjects

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

given to the subject or their legally authorized representative and the signed original will be filed in the medical record.

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

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

11 PHARMACEUTICAL AND INVESTIGATIONAL DEVICE INFORMATION

11.1 RECOMBINANT FOWLPOX-PSA(L155)/TRICOM™

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

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

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

11.1.1 How Supplied

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

11.1.2 Preparation

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

11.1.3 Storage

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

11.1.4 Stability

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

11.1.5 Route of Administration

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

11.1.6 Special Handling

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

Preparation, Handling and Disposal Recommendations

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

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

11.2 RECOMBINANT VACCINIA-PSA(L155)/TRICOM™

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

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

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

11.2.1 How Supplied

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

11.2.2 Preparation

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

11.2.3 Storage

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

11.2.4 Stability

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

11.2.5 Route of Administration

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

11.2.6 Special Handling and Precautions

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

Preparation, Handling and Disposal Recommendations

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

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

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

19. Immediately report spills and accidents resulting in overt exposure to recombinant DNA molecules to the 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 $\geq$ 3 known risk factors for cardiac disease (e.g., hypertension, hypercholesterolemia, diabetes, first degree relative with onset of cardiac complications prior to age 50, smoker), not receive routine, non-emergency, prophylactic vaccination with the licensed vaccinia vaccine while a possible causal relationship between vaccination and cardiac events is being evaluated.

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

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

Recombinant Vaccinia Vaccine Patient Care Implications, Contraindications and Potential Complications

Patient Care Implications and Contraindications

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

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

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

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

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

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

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

Potential Complications Associated With Recombinant Vaccinia Vaccination

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

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

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

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

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

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

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

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

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

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

develops 6-9 days after vaccination. The lesions follow the same course as the vaccination site lesion. The lesions are hematogenously spread and may contain vaccinia virus. In immunocompetent individuals, the rash is generally self-limiting and requires supportive care therapy. VIG treatment can be used in severe cases for patients who are systemically ill and whose condition might be toxic or who have serious underlying immunosuppressive illnesses.

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

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

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

protocol eligibility criteria. Patients will be encouraged to minimize cardiovascular disease risks and encouraged to follow risk reduction according to standard medical practice. At this time the evidence for an association with myocarditis, pericarditis, or myopericarditis seems plausible, but a rare event. If not otherwise excluded, patients with known CHF or clinically significant cardiomyopathy requiring treatment should be excluded from protocol eligibility.

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

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.

11.3 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].

11.4 ENZALUTAMIDE

Please see FDA-approved packet insert for Enzalutamide for complete agent information.

11.4.1 Description and formulation

Enzalutamide (XTANDI ©) is an androgen receptor inhibitor. The chemical name is 4-{3-[4-cyano-3-(trifluoromethyl)phenyl]-5,5- dimethyl-4-oxo-2-sulfanylideneimidazolidin-1-yl}-2-fluoro-*N*-methylbenzamide. Enzalutamide is a white crystalline non-hygroscopic solid. It is practically insoluble in water. XTANDI is provided as liquid-filled soft gelatin capsules for oral administration. Each capsule contains 40 mg of enzalutamide as a solution in caprylocaproyl polyoxylglycerides. The inactive ingredients are caprylocaproyl polyoxylglycerides, butylated hydroxyanisole, butylated hydroxytoluene, gelatin, sorbitol sorbitan solution, glycerin, purified water, titanium dioxide, and black iron oxide.

11.4.2 Source

Medivation and Astellas Inc. have reviewed the proposed clinical trial design and have agreed to provide enzalutamide for the trial.

11.4.3 Storage

Store enzalutamide capsules at 20°C to 25°C (68°F to 77°F) in a dry place and keep the container tightly closed. Excursions permitted from 15°C to 30°C (59°F to 86°F).

11.4.4 Stability

The company providing the IND drug will also provide the expiration date for each lot allocated to this study.

11.4.5 Dosage and Administration

Enzalutamide 160 mg (four 40 mg capsules) administered orally once daily.
Swallow capsules whole. Enzalutamide can be taken with or without food.

11.4.6 Adverse Effects

Seizure
In the randomized clinical trial, 7 of 800 (0.9%) patients treated with enzalutamide 160 mg once daily experienced a seizure. No seizures occurred in patients treated with placebo. Seizures occurred from 31 to 603 days after initiation of enzalutamide. Patients experiencing seizure were permanently discontinued from therapy and all seizures resolved. There is no clinical trial experience re-administering enzalutamide to patients who experienced seizures. The safety of enzalutamide in patients with predisposing factors for seizure is not known because these patients were excluded from the trial. These exclusion criteria included a history of seizure, underlying brain injury with loss of consciousness, transient ischemic attack within the past 12 months, cerebral vascular accident, brain metastases, brain arteriovenous malformation or the use of concomitant medications that may lower the seizure threshold. Because of the risk of seizure associated with enzalutamide use, patients should be advised of the risk of engaging in any activity where sudden loss of consciousness could cause serious harm to themselves or others.

Other adverse events
The most common adverse drug reactions (≥ 5%) reported in patients receiving enzalutamide in the randomized clinical trial were asthenia/fatigue, back pain, diarrhea, arthralgia, hot flush, peripheral edema, musculoskeletal pain, headache, upper respiratory infection, muscular weakness, dizziness, insomnia, lower respiratory infection, spinal cord compression and cauda equina syndrome, hematuria, paresthesia, anxiety, and hypertension. Grade 3 and higher adverse reactions were reported among 47% of enzalutamide-treated patients and 53% of placebo-treated patients. Discontinuations due to adverse events were reported for 16% of enzalutamide-treated patients and 18% of placebo-treated patients. The most common adverse reaction leading to treatment discontinuation was seizure, which occurred in 0.9% of the enzalutamide-treated patients compared to none (0%) of the placebo-treated patients.

Laboratory Abnormalities
In the randomized clinical trial, Grade 1-4 neutropenia occurred in 15% of patients on enzalutamide (1% Grade 3-4) and in 6% of patients on placebo (no Grade 3-4). The incidence of Grade 1-4 thrombocytopenia was similar in both arms; 0.5% of patients on enzalutamide and 1% on placebo experienced Grade 3-4 thrombocytopenia. Grade 1-4 elevations in ALT occurred in 10% of patients on enzalutamide (0.3% Grade 3-4) and 18% of patients on placebo (0.5% Grade 3-4). Grade 1-4 elevations in bilirubin occurred in 3% of patients on enzalutamide and 2% of patients on placebo.

Infections
In the randomized clinical trial, 1.0% of patients treated with enzalutamide compared to 0.3% of patients on placebo died from infections or sepsis. Infection-related serious adverse events were reported in approximately 6% of the patients on both treatment arms.

Falls and Fall-related Injuries
In the randomized clinical trial, falls or injuries related to falls occurred in 4.6% of patients treated with enzalutamide compared to 1.3% of patients on placebo. Falls were not associated with loss of consciousness or seizure. Fall-related injuries were more severe in patients treated with enzalutamide and included non-pathologic fractures, joint injuries, and hematomas.

Hallucinations
In the randomized clinical trial, 1.6% of patients treated with enzalutamide were reported to have Grade 1 or 2 hallucinations compared to 0.3% of patients on placebo. Of the patients with hallucinations, the majority were on opioid-containing medications at the time of the event. Hallucinations were visual, tactile, or undefined.

11.4.7 Drug Interactions

Drugs that Inhibit or Induce CYP2C8
Co-administration of a strong CYP2C8 inhibitor (gemfibrozil) increased the composite area under the plasma concentration-time curve (AUC) of enzalutamide plus N-desmethyl enzalutamide in healthy volunteers. Co-administration of enzalutamide with strong CYP2C8 inhibitors should be avoided if possible. If co-administration of enzalutamide with a strong CYP2C8 inhibitor cannot be avoided, reduce the dose of enzalutamide.
The effects of CYP2C8 inducers on the pharmacokinetics of enzalutamide have not been evaluated *in vivo*.

Co-administration of enzalutamide with strong or moderate CYP2C8 inducers (e.g., rifampin) may alter the plasma exposure of enzalutamide and should be avoided if possible. Selection of a concomitant medication with no or minimal CYP2C8 induction potential is recommended.

Drugs that Inhibit or Induce CYP3A4
Co-administration of a strong CYP3A4 inhibitor (itraconazole) increased the composite AUC of enzalutamide plus Ndesmethyl enzalutamide by 1.3 fold in healthy volunteers
The effects of CYP3A4 inducers on the pharmacokinetics of enzalutamide have not been evaluated *in vivo*.

Co-administration of enzalutamide with strong CYP3A4 inducers (e.g., carbamazepine, phenobarbital, phenytoin, rifabutin, rifampin, rifapentine) may decrease the plasma exposure of enzalutamide and should be avoided if possible. Selection of a concomitant medication with no or minimal CYP3A4 induction potential is recommended. Moderate CYP3A4 inducers (e.g., bosentan, efavirenz, etravirine, modafinil, nafcillin) and St. John's Wort may also reduce the plasma exposure of enzalutamide and should be avoided if possible.

Effect of Enzalutamide on Drug Metabolizing Enzymes
Enzalutamide is a strong CYP3A4 inducer and a moderate CYP2C9 and CYP2C19 inducer in humans. At steady state, enzalutamide reduced the plasma exposure to midazolam (CYP3A4 substrate), warfarin (CYP2C9 substrate), and omeprazole (CYP2C19 substrate). Concomitant use of enzalutamide with narrow therapeutic index drugs that are metabolized by CYP3A4 (e.g., alfentanil, cyclosporine, dihydroergotamine, ergotamine, fentanyl, pimozide, quinidine, sirolimus

and tacrolimus), CYP2C9 (e.g., phenytoin, warfarin) and CYP2C19 (e.g., proton pump inhibitors (lansoprazole, omeprazole, pantoprazole, rabeprazole) and clopidogrel.) should be avoided, as enzalutamide may decrease their exposure. If co-administration with warfarin cannot be avoided, conduct additional INR monitoring.

12 REFERENCES

1. Furr BJ. "Casodex" (ICI 176,334)--a new, pure, peripherally-selective anti-androgen: preclinical studies. Horm Res. 1989;32 Suppl 1:69-76. Epub 1989/01/01. PubMed PMID: 2533159.
2. Chen Y, Clegg NJ, Scher HI. Anti-androgens and androgen-depleting therapies in prostate cancer: new agents for an established target. The lancet oncology. 2009;10(10):981-91. Epub 2009/10/03. doi: 10.1016/S1470-2045(09)70229-3. PubMed PMID: 19796750; PubMed Central PMCID: PMC2935850.
3. Sartor AO, Tangen CM, Hussain MH, Eisenberger MA, Parab M, Fontana JA, et al. Antiandrogen withdrawal in castrate-refractory prostate cancer: a Southwest Oncology Group trial (SWOG 9426). Cancer. 2008;112(11):2393-400. Epub 2008/04/03. doi: 10.1002/cncr.23473. PubMed PMID: 18383517.
4. Small EJ, Halabi S, Dawson NA, Stadler WM, Rini BI, Picus J, et al. Antiandrogen withdrawal alone or in combination with ketoconazole in androgen-independent prostate cancer patients: a phase III trial (CALGB 9583). J Clin Oncol. 2004;22(6):1025-33. Epub 2004/03/17. doi: 10.1200/JCO.2004.06.037 JCO.2004.06.037 [pii]. PubMed PMID: 15020604.
5. FDA. Enzalutamide (XTANDI Capsules) [September 4, 2012]. Available from: http://www.fda.gov/Drugs/InformationOnDrugs/ApprovedDrugs/ucm317997.htm?source=govdelivery.
6. Scher HI, Beer TM, Higano CS, Anand A, Taplin ME, Efstathiou E, et al. Antitumour activity of MDV3100 in castration-resistant prostate cancer: a phase 1-2 study. Lancet. 2010;375(9724):1437-46. Epub 2010/04/20. doi: S0140-6736(10)60172-9 [pii] 10.1016/S0140-6736(10)60172-9. PubMed PMID: 20398925; PubMed Central PMCID: PMC2948179.
7. Scher HI, Fizazi K, Saad F, Taplin ME, Sternberg CN, Miller MD, et al. Increased Survival with Enzalutamide in Prostate Cancer after Chemotherapy. N Engl J Med. 2012. Epub 2012/08/17. doi: 10.1056/NEJMoa1207506. PubMed PMID: 22894553.
8. Sharifi N, Gulley JL, Dahut WL. Androgen deprivation therapy for prostate cancer. JAMA : the journal of the American Medical Association. 2005;294(2):238-44. Epub 2005/07/15. doi: 10.1001/jama.294.2.238. PubMed PMID: 16014598.
9. Aragon-Ching JB, Williams KM, Gulley JL. Impact of androgen-deprivation therapy on the immune system: implications for combination therapy of prostate cancer. Front Biosci. 2007;12:4957-71. Epub 2007/06/16. doi: 2441 [pii]. PubMed PMID: 17569623.
10. Tay MH, Kaufman DS, Regan MM, Leibowitz SB, George DJ, Febbo PG, et al. Finasteride and bicalutamide as primary hormonal therapy in patients with advanced adenocarcinoma of the prostate. Annals of oncology : official journal of the European Society for Medical Oncology / ESMO. 2004;15(6):974-8. Epub 2004/05/21. PubMed PMID: 15151957.
11. Boccardo F, Rubagotti A, Barichello M, Battaglia M, Carmignani G, Comeri G, et al. Bicalutamide monotherapy versus flutamide plus goserelin in prostate cancer patients: results

of an Italian Prostate Cancer Project study. Journal of clinical oncology : official journal of the American Society of Clinical Oncology. 1999;17(7):2027-38. Epub 1999/11/24. PubMed PMID: 10561254.
12. Rini BI. Technology evaluation: APC-8015, Dendreon. Curr Opin Mol Ther. 2002;4(1):76-9. PubMed PMID: 11883698.
13. Patel PH, Kockler DR. Sipuleucel-T: a vaccine for metastatic, asymptomatic, androgen-independent prostate cancer. Ann Pharmacother. 2008;42(1):91-8. PubMed PMID: 18094343.
14. Kantoff PW, Higano CS, Shore ND, Berger ER, Small EJ, Penson DF, et al. Sipuleucel-T immunotherapy for castration-resistant prostate cancer. N Engl J Med. 2010;363(5):411-22. Epub 2010/09/08. doi: 10.1056/NEJMoa1001294. PubMed PMID: 20818862.
15. Madan RA, Arlen PM, Mohebtash M, Hodge JW, Gulley JL. Prostvac-VF: a vector-based vaccine targeting PSA in prostate cancer. Expert Opin Investig Drugs. 2009;18(7):1001-11. Epub 2009/06/25. doi: 10.1517/13543780902997928. PubMed PMID: 19548854.
16. Madan RA, Bilusic M, Heery C, Schlom J, Gulley JL. Clinical evaluation of TRICOM vector therapeutic cancer vaccines. Semin Oncol. 2012;39(3):296-304. Epub 2012/05/19. doi: S0093-7754(12)00051-6 [pii]
10.1053/j.seminoncol.2012.02.010. PubMed PMID: 22595052; PubMed Central PMCID: PMC3398615.
17. Arlen PM, Mohebtash M, Madan RA, Gulley JL. Promising novel immunotherapies and combinations for prostate cancer. Future Oncol. 2009;5(2):187-96. Epub 2009/03/17. doi: 10.2217/14796694.5.2.187. PubMed PMID: 19284377; PubMed Central PMCID: PMC2705770.
18. Essajee S, Kaufman HL. Poxvirus vaccines for cancer and HIV therapy. Expert Opin Biol Ther. 2004;4(4):575-88. PubMed PMID: 15102606.
19. Longo DL. New therapies for castration-resistant prostate cancer. The New England journal of medicine.363(5):479-81. PubMed PMID: 20818868.
20. Kantoff PW, Schuetz TJ, Blumenstein BA, Glode LM, Bilhartz DL, Wyand M, et al. Overall survival analysis of a phase II randomized controlled trial of a Poxviral-based PSA-targeted immunotherapy in metastatic castration-resistant prostate cancer. J Clin Oncol. 2010;28(7):1099-105. PubMed PMID: 20100959.
21. Gulley JL, Arlen PM, Madan RA, Tsang KY, Pazdur MP, Skarupa L, et al. Immunologic and prognostic factors associated with overall survival employing a poxviral-based PSA vaccine in metastatic castrate-resistant prostate cancer. Cancer Immunol Immunother. 2010;59(5):663-74. Epub 2009/11/06. doi: 10.1007/s00262-009-0782-8. PubMed PMID: 19890632; PubMed Central PMCID: PMC2832083.
22. clinicaltrials.gov. A Phase 3 Efficacy Study of a Recombinant Vaccinia Virus Vaccine to Treat Metastatic Prostate Cancer (Prospect) [cited 2012 August 1]. Available from: http://clinicaltrials.gov/ct2/show/NCT01322490?term=PROSPECT&rank=6.
23. Mercader M, Bodner BK, Moser MT, Kwon PS, Park ES, Manecke RG, et al. T cell infiltration of the prostate induced by androgen withdrawal in patients with prostate cancer. Proceedings of the National Academy of Sciences of the United States of America. 2001;98(25):14565-70. PubMed PMID: 11734652.
24. Goldberg GL, Sutherland JS, Hammet MV, Milton MK, Heng TS, Chidgey AP, et al. Sex steroid ablation enhances lymphoid recovery following autologous hematopoietic stem cell transplantation. Transplantation. 2005;80(11):1604-13. PubMed PMID: 16371932.

25. Drake CG, Doody AD, Mihalyo MA, Huang CT, Kelleher E, Ravi S, et al. Androgen ablation mitigates tolerance to a prostate/prostate cancer-restricted antigen. Cancer cell. 2005;7(3):239-49. PubMed PMID: 15766662.
26. Sutherland JS, Goldberg GL, Hammett MV, Uldrich AP, Berzins SP, Heng TS, et al. Activation of thymic regeneration in mice and humans following androgen blockade. J Immunol. 2005;175(4):2741-53. PubMed PMID: 16081852.
27. Vaccine Therapy With PROSTVAC/TRICOM and Flutamide Versus Flutamide Alone to Treat Prostate Cancer [cited 2012 August 5]. Available from: http://clinicaltrials.gov/ct2/show/NCT00450463.
28. Bubley GJ, Carducci M, Dahut W, Dawson N, Daliani D, Eisenberger M, et al. Eligibility and response guidelines for phase II clinical trials in androgen-independent prostate cancer: recommendations from the Prostate-Specific Antigen Working Group. J Clin Oncol. 1999;17(11):3461-7. Epub 1999/11/05. PubMed PMID: 10550143.
29. Bilusic M, Gulley JL, Heery C, Apolo AB, Arlen P, Rauckhorst M, et al. A randomized phase II study of flutamide with or without PSA-TRICOM in nonmetastatic castration-resistant prostate cancer. J Clin Oncol 29: 2011 (suppl 7; abstr 163).
30. Scher HI, Halabi S, Tannock I, Morris M, Sternberg CN, Carducci MA, et al. Design and end points of clinical trials for patients with progressive prostate cancer and castrate levels of testosterone: recommendations of the Prostate Cancer Clinical Trials Working Group. J Clin Oncol. 2008;26(7):1148-59. Epub 2008/03/04. doi: 26/7/1148 [pii] 10.1200/JCO.2007.12.4487. PubMed PMID: 18309951.
31. Hurwitz AA, Foster BA, Allison JP, Greenberg NM, Kwon ED. The TRAMP mouse as a model for prostate cancer. Curr Protoc Immunol. 2001;Chapter 20:Unit 20 5. Epub 2008/04/25. doi: 10.1002/0471142735.im2005s45. PubMed PMID: 18432778.
32. Farsaci B, Higgins JP, Hodge JW. Consequence of dose scheduling of sunitinib on host immune response elements and vaccine combination therapy. Int J Cancer. 2012;130(8):1948-59. Epub 2011/06/03. doi: 10.1002/ijc.26219. PubMed PMID: 21633954; PubMed Central PMCID: PMC3232304.
33. Garnett CT, Schlom J, Hodge JW. Combination of docetaxel and recombinant vaccine enhances T-cell responses and antitumor activity: effects of docetaxel on immune enhancement. Clin Cancer Res. 2008;14(11):3536-44. Epub 2008/06/04. doi: 14/11/3536 [pii] 10.1158/1078-0432.CCR-07-4025. PubMed PMID: 18519787; PubMed Central PMCID: PMC2682419.
34. Aymeric L, Apetoh L, Ghiringhelli F, Tesniere A, Martins I, Kroemer G, et al. Tumor cell death and ATP release prime dendritic cells and efficient anticancer immunity. Cancer Res. 2010;70(3):855-8. Epub 2010/01/21. doi: 0008-5472.CAN-09-3566 [pii] 10.1158/0008-5472.CAN-09-3566 [doi]. PubMed PMID: 20086177.
35. Ullrich E, Bonmort M, Mignot G, Kroemer G, Zitvogel L. Tumor stress, cell death and the ensuing immune response. Cell Death Differ. 2008;15(1):21-8. Epub 2007/11/10. doi: 4402266 [pii] 10.1038/sj.cdd.4402266. PubMed PMID: 17992190.
36. Crook JM, Szumacher E, Malone S, Huan S, Segal R. Intermittent androgen suppression in the management of prostate cancer. Urology. 1999;53(3):530-4. Epub 1999/03/30. PubMed PMID: 10096379.
37. Klotz L, O'Callaghan CJ, Ding K, Dearnaley DP, Higano CS, Horwitz EM, et al. A phase III randomized trial comparing intermittent versus continuous androgen suppression for patients

with PSA progression after radical therapy: NCIC CTG PR.7/SWOG JPR.7/CTSU JPR.7/UK Intercontinental Trial CRUKE/01/013. J Clin Oncol 29: 2011 (suppl 7; abstr 3)
38. Vergati M, Cereda V, Madan RA, Gulley JL, Huen NY, Rogers CJ, et al. Analysis of circulating regulatory T cells in patients with metastatic prostate cancer pre- versus post-vaccination. Cancer Immunol Immunother. 2011;60(2):197-206. Epub 2010/10/27. doi: 10.1007/s00262-010-0927-9. PubMed PMID: 20976449; PubMed Central PMCID: PMC3202216.
39. Tesniere A, Panaretakis T, Kepp O, Apetoh L, Ghiringhelli F, Zitvogel L, et al. Molecular characteristics of immunogenic cancer cell death. Cell Death Differ. 2008;15(1):3-12. Epub 2007/11/17. doi: 4402269 [pii]
10.1038/sj.cdd.4402269 [doi]. PubMed PMID: 18007663.
40. Hodi FS, O'Day SJ, McDermott DF, Weber RW, Sosman JA, Haanen JB, et al. Improved survival with ipilimumab in patients with metastatic melanoma. N Engl J Med. 2010;363(8):711-23. Epub 2010/06/08. doi: NEJMoa1003466 [pii]
10.1056/NEJMoa1003466. PubMed PMID: 20525992.
41. Madan RA, Gulley JL, Fojo T, Dahut WL. Therapeutic cancer vaccines in prostate cancer: the paradox of improved survival without changes in time to progression. Oncologist. 2010;15(9):969-75. Epub 2010/08/28. doi: theoncologist.2010-0129 [pii]
10.1634/theoncologist.2010-0129. PubMed PMID: 20798195; PubMed Central PMCID: PMC3228037.
42. Stein WD, Yang J, Bates SE, Fojo T. Bevacizumab reduces the growth rate constants of renal carcinomas: a novel algorithm suggests early discontinuation of bevacizumab resulted in a lack of survival advantage. The oncologist. 2008;13(10):1055-62. Epub 2008/10/02. doi: 10.1634/theoncologist.2008-0016. PubMed PMID: 18827177.
43. Stein WD, Wilkerson J, Kim ST, Huang X, Fojo AT, Motzer RJ, et al. Analyzing the pivotal trial that compared sunitinib and interferon alfa in renal cell carcinoma, using a method that assesses tumor regression and growth. Clin Cancer Res. 2012. Epub 2012/02/22. doi: 1078-0432.CCR-11-2275 [pii]
10.1158/1078-0432.CCR-11-2275. PubMed PMID: 22344231.
44. Stein WD, Gulley JL, Schlom J, Madan RA, Dahut W, Figg WD, et al. Tumor regression and growth rates determined in five intramural NCI prostate cancer trials: the growth rate constant as an indicator of therapeutic efficacy. Clinical cancer research: an official journal of the American Association for Cancer Research. 2011;17(4):907-17. Epub 2010/11/26. doi: 10.1158/1078-0432.CCR-10-1762. PubMed PMID: 21106727.
45. DiPaola RS, Chen Y, Bubley GJ, Hahn NM, Stein M, Schlom J, et al., editors. A phase II study of PROSTVAC-V (vaccinia)/TRICOM and PROSTVAC-F (fowlpox)/TRICOM with GM-CSF in patients with PSA progression after local therapy for prostate cancer: Results of ECOG 9802. Genitourinary Cancers Symposium 2009.
46. Beer TM, Bernstein GT, Corman JM, Glode LM, Hall SJ, Poll WL, et al. Randomized trial of autologous cellular immunotherapy with sipuleucel-T in androgen-dependent prostate cancer. Clin Cancer Res. 2011;17(13):4558-67. Epub 2011/05/12. doi: 1078-0432.CCR-10-3223 [pii]
10.1158/1078-0432.CCR-10-3223. PubMed PMID: 21558406.
47. Arlen PM, Gulley JL, Palena C, Marshall J, Schlom J, Tsang KY. A novel ELISPOT assay to enhance detection of antigen-specific T cells employing antigen-presenting cells expressing vector-driven human B7-1. J Immunol Methods. 2003;279(1-2):183-92. Epub 2003/09/13. doi: S0022175903001856 [pii]. PubMed PMID: 12969559.

48. 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.
49. Crook JM, O'Callaghan CJ, Duncan G, et al. Intermitten androgen suppression for rising PSA level after radiotherapy. N Engl J Med. 367: 895-903, 2012.
50. Tombal, B., et al., Enzalutamide monotherapy in hormone-naive prostate cancer: primary analysis of an open-label, single-arm, phase 2 study. Lancet Oncol, 2014. 15(6): p. 592-600.

13 APPENDIX A: PERFORMANCE STATUS CRITERIA

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

14 APPENDIX B: VACCINIA-PSA(L155)/TRICOM PATIENT INSTRUCTION SHEET

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

1. What vaccination site reactions can you expect?

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

In patients who have never received vaccinia or in some who received it a very long time ago, a red swelling is followed by 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
12th floor Oncology Clinic	(301) 496-4026*
Ravi Madan, MD	(301) 480-7168*
James Gulley, MD, PhD	(301) 480-7164*

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

15 APPENDIX C : ON STUDY AND FOLLOW-UP EVALUATIONS

COHORT 1

	Arm A (Enzalutamide only)	**Arm B (Enzalutamide + PSA-TRICOM)**
Prior to Enrollment	Screening for eligibility, Informed consent, NIH Advanced Directives Form[1]	Screening for eligibility, Informed consent, NIH Advanced Directives form[1]
Within 8 weeks of initiating treatment	***Apheresis**, Lab work (HIV, Hep B and C), baseline scans (MRI/CT and bone scans), EKG	***Apheresis**, Lab work (HIV, Hep B and C), baseline scans (MRI/CT and bone scans), EKG
Within 16 days of beginning treatment (day -16 to day 1)	Baseline history and physical exam, ECOG status, Research Blood, CBC, Acute, Hepatic, Mineral Panels, LDH, CK, Uric Acid, Total Protein, TBNK, PSA, testosterone, PAP (prostatic acid phosphatase), dihydrotestosterone (DHT), sex hormone-binding globulin (SHBG), dehydroepiandrosterone (DHEA), luteinizing hormone (LH), follicle-stimulating hormone (FSH), androstenedione prolactin, estradiol **Randomization**	Baseline history and physical exam, ECOG status, Research Blood, CBC, Acute, Hepatic, Mineral Panels, LDH, CK, Uric Acid, Total Protein, TBNK, PSA, testosterone, PAP (prostatic acid phosphatase), dihydrotestosterone (DHT), sex hormone-binding globulin (SHBG), dehydroepiandrosterone (DHEA), luteinizing hormone (LH), follicle-stimulating hormone (FSH), androstenedione prolactin, estradiol **Randomization**
Day 1	**Enzalutamide day 1 month 1 (daily dose of 160 mg)**	**Enzalutamide day 1 month 1 + PSA-TRICOM (Prostvac-V) dose 1**
Week 3	Physical examination Research Blood, CBC, Acute, Hepatic, Mineral Panels, LDH, CK, Uric Acid, Total Protein,	Physical examination Research Blood, CBC, Acute, Hepatic, Mineral Panels, LDH, CK, Uric Acid, Total Protein,

	Arm A (Enzalutamide only)	**Arm B (Enzalutamide + PSA-TRICOM)**
	TBNK, PSA, testosterone, PAP, dihydrotestosterone (DHT), sex hormone-binding globulin (SHBG), dehydroepiandrosterone (DHEA), luteinizing hormone (LH), follicle-stimulating hormone (FSH), androstenedione prolactin, estradiol	TBNK, PSA, testosterone, PAP, dihydrotestosterone (DHT), sex hormone-binding globulin (SHBG), dehydroepiandrosterone (DHEA), luteinizing hormone (LH), follicle-stimulating hormone (FSH), androstenedione prolactin, estradiol **PSA-TRICOM (Prostvac-F) dose 1**
Week 5	Physical examination Research Blood, CBC, Acute, Hepatic, Mineral Panels, LDH, CK, Uric Acid, Total Protein, TBNK, PSA, testosterone, PAP, dihydrotestosterone (DHT), sex hormone-binding globulin (SHBG), dehydroepiandrosterone (DHEA), luteinizing hormone (LH), follicle-stimulating hormone (FSH), androstenedione prolactin, estradiol **Enzalutamide day 1 month 2 (daily dose of 160 mg)**	Physical examination Research Blood, CBC, Acute, Hepatic, Mineral Panels, LDH, CK, Uric Acid, Total Protein, TBNK, PSA, testosterone, PAP, dihydrotestosterone (DHT), sex hormone-binding globulin (SHBG), dehydroepiandrosterone (DHEA), luteinizing hormone (LH), follicle-stimulating hormone (FSH), androstenedione prolactin, estradiol **Enzalutamide day 1 month 2 + PSA-TRICOM (Prostvac-F) dose 2**
Week 9	Physical examination , CBC, Acute, Hepatic, Mineral Panels, LDH, CK, Uric Acid, Total Protein, TBNK, PSA, testosterone, PAP, dihydrotestosterone (DHT), sex hormone-binding globulin (SHBG), dehydroepiandrosterone (DHEA), luteinizing hormone (LH), follicle-stimulating hormone (FSH), androstenedione	Physical examination , CBC, Acute, Hepatic, Mineral Panels, LDH, CK, Uric Acid, Total Protein, TBNK, PSA, testosterone, PAP, dihydrotestosterone (DHT), sex hormone-binding globulin (SHBG), dehydroepiandrosterone (DHEA), luteinizing hormone (LH), follicle-stimulating hormone (FSH), androstenedione prolactin, estradiol

	Arm A (Enzalutamide only)	**Arm B (Enzalutamide + PSA-TRICOM)**
	prolactin, estradiol **Enzalutamide day 1 month 3 (daily dose of 160 mg)**	**Enzalutamide day 1 month 3 + PSA-TRICOM (Prostvac-F) dose 3**
Week 13	Physical examination , CBC, Acute, Hepatic, Mineral Panels, LDH, CK, Uric Acid, Total Protein, TBNK, PSA, testosterone, PAP, dihydrotestosterone (DHT), sex hormone-binding globulin (SHBG), dehydroepiandrosterone (DHEA), luteinizing hormone (LH), follicle-stimulating hormone (FSH), androstenedione prolactin, estradiol ***Apheresis** ***Enzalutamide therapy is discontinued***	Physical examination , CBC, Acute, Hepatic, Mineral Panels, LDH, CK, Uric Acid, Total Protein, TBNK, PSA, testosterone, PAP, dihydrotestosterone (DHT), sex hormone-binding globulin (SHBG), dehydroepiandrosterone (DHEA), luteinizing hormone (LH), follicle-stimulating hormone (FSH), androstenedione prolactin, estradiol ***Apheresis** ***Enzalutamide therapy is discontinued*** **PSA-TRICOM (Prostvac-F) dose 4**
Week 15	, CBC, Acute, Hepatic, Mineral Panels, LDH, CK, Uric Acid, Total Protein, TBNK, PSA, testosterone, PAP, dihydrotestosterone (DHT), sex hormone-binding globulin (SHBG), dehydroepiandrosterone (DHEA), luteinizing hormone (LH), follicle-stimulating hormone (FSH), androstenedione prolactin, estradiol	, CBC, Acute, Hepatic, Mineral Panels, LDH, CK, Uric Acid, Total Protein, TBNK, PSA, testosterone, PAP, dihydrotestosterone (DHT), sex hormone-binding globulin (SHBG), dehydroepiandrosterone (DHEA), luteinizing hormone (LH), follicle-stimulating hormone (FSH), androstenedione prolactin, estradiol

	Arm A (Enzalutamide only)	**Arm B (Enzalutamide + PSA-TRICOM)**
Week 17	Physical examination Research Blood, CBC, Acute, Hepatic, Mineral Panels, LDH, CK, Uric Acid, Total Protein, TBNK, PSA, testosterone, PAP, dihydrotestosterone (DHT), sex hormone-binding globulin (SHBG), dehydroepiandrosterone (DHEA), luteinizing hormone (LH), follicle-stimulating hormone (FSH), androstenedione prolactin, estradiol	Physical examination Research Blood, CBC, Acute, Hepatic, Mineral Panels, LDH, CK, Uric Acid, Total Protein, TBNK, PSA, testosterone, PAP, dihydrotestosterone (DHT), sex hormone-binding globulin (SHBG), dehydroepiandrosterone (DHEA), luteinizing hormone (LH), follicle-stimulating hormone (FSH), androstenedione prolactin, estradiol **PSA-TRICOM (Prostvac-F) dose 5**
Week 21	Physical examination , CBC, Acute, Hepatic, Mineral Panels, LDH, CK, Uric Acid, Total Protein, TBNK, PSA, testosterone, PAP, dihydrotestosterone (DHT), sex hormone-binding globulin (SHBG), dehydroepiandrosterone (DHEA), luteinizing hormone (LH), follicle-stimulating hormone (FSH), androstenedione prolactin, estradiol	Physical examination , CBC, Acute, Hepatic, Mineral Panels, LDH, CK, Uric Acid, Total Protein, TBNK, PSA, testosterone, PAP dihydrotestosterone (DHT), sex hormone-binding globulin (SHBG), dehydroepiandrosterone (DHEA), luteinizing hormone (LH), follicle-stimulating hormone (FSH), androstenedione prolactin, estradiol **PSA-TRICOM (Prostvac-F) dose 6**
Week 25	Physical examination , CBC, Acute, Hepatic, Mineral Panels, LDH, CK, Uric Acid, Total Protein, TBNK, PSA, testosterone, PAP, dihydrotestosterone (DHT), sex hormone-binding globulin (SHBG), dehydroepiandrosterone	Physical examination , CBC, Acute, Hepatic, Mineral Panels, LDH, CK, Uric Acid, Total Protein, TBNK, PSA, testosterone, PAP, dihydrotestosterone (DHT), sex hormone-binding globulin (SHBG), dehydroepiandrosterone (DHEA), luteinizing hormone (LH),

	Arm A (Enzalutamide only)	**Arm B (Enzalutamide + PSA-TRICOM)**
	(DHEA), luteinizing hormone (LH), follicle-stimulating hormone (FSH), androstenedione prolactin, estradiol ***Apheresis**	follicle-stimulating hormone (FSH), androstenedione prolactin, estradiol ***Apheresis** ***PSA-TRICOM (Prostvac-F) is discontinued***
Week 29	Physical examination , CBC, Acute, Hepatic, Mineral Panels, LDH, CK, Uric Acid, Total Protein, TBNK, PSA, testosterone, PAP, dihydrotestosterone (DHT), sex hormone-binding globulin (SHBG), dehydroepiandrosterone (DHEA), luteinizing hormone (LH), follicle-stimulating hormone (FSH), androstenedione prolactin, estradiol	Physical examination , CBC, Acute, Hepatic, Mineral Panels, LDH, CK, Uric Acid, Total Protein, TBNK, PSA, testosterone, PAP, dihydrotestosterone (DHT), sex hormone-binding globulin (SHBG), dehydroepiandrosterone (DHEA), luteinizing hormone (LH), follicle-stimulating hormone (FSH), androstenedione prolactin, estradiol
Up to Week 52 Every 4 weeks **	Physical examination , CBC, Acute, Hepatic, Mineral Panels, LDH, CK, Uric Acid, Total Protein, TBNK, PSA, testosterone, PAP, dihydrotestosterone (DHT), sex hormone-binding globulin (SHBG), dehydroepiandrosterone (DHEA), luteinizing hormone (LH), follicle-stimulating hormone (FSH), androstenedione prolactin, estradiol Research Blood (week 52 only)	Physical examination , CBC, Acute, Hepatic, Mineral Panels, LDH, CK, Uric Acid, Total Protein, TBNK, PSA, testosterone, PAP, dihydrotestosterone (DHT), sex hormone-binding globulin (SHBG), dehydroepiandrosterone (DHEA), luteinizing hormone (LH), follicle-stimulating hormone (FSH), androstenedione prolactin, estradiol Research Blood (week 52 only)

	Arm A (Enzalutamide only)	**Arm B (Enzalutamide + PSA-TRICOM)**
Beyond Week 52 Every 8 weeks (+/- 7 days)**	Physical examination , CBC, Acute, Hepatic, Mineral Panels, LDH, CK, Uric Acid, Total Protein, TBNK, PSA, testosterone, PAP, dihydrotestosterone (DHT), sex hormone-binding globulin (SHBG), dehydroepiandrosterone (DHEA), luteinizing hormone (LH), follicle-stimulating hormone (FSH), androstenedione prolactin, estradiol Research Blood (every 16 weeks)	Physical examination , CBC, Acute, Hepatic, Mineral Panels, LDH, CK, Uric Acid, Total Protein, TBNK, PSA, testosterone, PAP, dihydrotestosterone (DHT), sex hormone-binding globulin (SHBG), dehydroepiandrosterone (DHEA), luteinizing hormone (LH), follicle-stimulating hormone (FSH), androstenedione prolactin, estradiol Research Blood (every 16 weeks)
Once PSA reaches baseline value	Restaging Scans (MRI/CT and bone scans)	Restaging scans (MRI/CT and bone scans)
Course 2, Week 1	Enzalutamide day 1 month 1, course 2 (daily dose of 160 mg) Research labs including plasma VEGF levels	Enzalutamide day 1 month 1, course 2 Research labs including plasma VEGF levels
Course 2,Week 5	Physical examination Research Blood, CBC, Acute, Hepatic, Mineral Panels, LDH, CK, Uric Acid, Total Protein, TBNK, PSA, testosterone, PAP, dihydrotestosterone (DHT), sex hormone-binding globulin (SHBG), dehydroepiandrosterone (DHEA), luteinizing hormone (LH), follicle-stimulating	Physical examination Research Blood, CBC, Acute, Hepatic, Mineral Panels, LDH, CK, Uric Acid, Total Protein, TBNK, PSA, testosterone, PAP, dihydrotestosterone (DHT), sex hormone-binding globulin (SHBG), dehydroepiandrosterone (DHEA), luteinizing hormone (LH), follicle-stimulating hormone (FSH), androstenedione prolactin, estradiol

	Arm A (Enzalutamide only)	**Arm B (Enzalutamide + PSA-TRICOM)**
	hormone (FSH)androstenedione prolactin, estradiol Enzalutamide day 1 month 2 course 2 (daily dose of 160 mg)	Enzalutamide day 1 month 2 course 2
Course 2, Week 9	Physical examination , CBC, Acute, Hepatic, Mineral Panels, LDH, CK, Uric Acid, Total Protein, TBNK, PSA, testosterone, PAP, dihydrotestosterone (DHT), sex hormone-binding globulin (SHBG), dehydroepiandrosterone (DHEA), luteinizing hormone (LH), follicle-stimulating hormone (FSH), androstenedione prolactin, estradiol Enzalutamide day 1 month 3 course 2 (daily dose of 160 mg)	Physical examination , CBC, Acute, Hepatic, Mineral Panels, LDH, CK, Uric Acid, Total Protein, TBNK, PSA, testosterone, PAP, dihydrotestosterone (DHT), sex hormone-binding globulin (SHBG), dehydroepiandrosterone (DHEA), luteinizing hormone (LH), follicle-stimulating hormone (FSH), androstenedione prolactin, estradiol Enzalutamide day 1 month 3 course 2
Course 2, Week 13	Physical examination Research Blood, CBC, Acute, Hepatic, Mineral Panels, LDH, CK, Uric Acid, Total Protein, TBNK, PSA, testosterone, PAP, dihydrotestosterone (DHT), sex hormone-binding globulin (SHBG), dehydroepiandrosterone (DHEA), luteinizing hormone (LH), follicle-stimulating hormone (FSH), androstenedione prolactin, estradiol	Physical examination Research Blood, CBC, Acute, Hepatic, Mineral Panels, LDH, CK, Uric Acid, Total Protein, TBNK, PSA, testosterone, PAP, dihydrotestosterone (DHT), sex hormone-binding globulin (SHBG), dehydroepiandrosterone (DHEA), luteinizing hormone (LH), follicle-stimulating hormone (FSH), androstenedione prolactin, estradiol Research labs including plasma VEGF levels

	Arm A (Enzalutamide only)	**Arm B (Enzalutamide + PSA-TRICOM)**
	Research labs including plasma VEGF levels *Apheresis Enzalutamide therapy is discontinued for course 2	*Apheresis Enzalutamide therapy is discontinued for course 2
Every 4 weeks thereafter	Physical examination , CBC, Acute, Hepatic, Mineral Panels, LDH, CK, Uric Acid, Total Protein, TBNK, PSA, testosterone, PAP, dihydrotestosterone (DHT), sex hormone-binding globulin (SHBG), dehydroepiandrosterone (DHEA), luteinizing hormone (LH), follicle-stimulating hormone (FSH), androstenedione prolactin, estradiol Research Blood (every 16 weeks)	Physical examination , CBC, Acute, Hepatic, Mineral Panels, LDH, CK, Uric Acid, Total Protein, TBNK, PSA, testosterone, PAP, dihydrotestosterone (DHT), sex hormone-binding globulin (SHBG), dehydroepiandrosterone (DHEA), luteinizing hormone (LH), follicle-stimulating hormone (FSH), androstenedione prolactin, estradiol Research Blood (every 16 weeks)

*Apheresis is not mandatory and will be scheduled based on availability
-Patients may come off study before week 52 as clinically indicated.
-All patient visits can be delayed by 1 week for logistical issues.
** Patients will be followed until PSA reaches baseline. Patients who continue to be followed for rising PSA but have not required ADT may be seen beyond the study period. The study period ends 52 weeks after enrollment unless patients receive a second course of enzalutamide, then study period ends 9 months after the end of the second course.

[1] As indicated in section **7.3**, all subjects will be offered the opportunity to complete an NIH advanced directives form. This should be done preferably at baseline but can be done at any time during the study as long as the capacity to do so is retained. The completion of the form is strongly recommended, but is not required.

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

Blood Studies	Blood Tube/Comments	Destination
CBC with differential	1 light lavender tube	CC Department of Laboratory Medicine (DLM)
Hepatic Panel, Mineral Panel, Acute Care Panel, LDH, CK, Uric Acid, Total Protein	1 4 mL SST	CC DLM
Anti-HIV-1/2	1 8 mL SST	CC TTV lab
Testosterone, total	1 red top tube	CC DLM
Prostate Specific Antigen	4 mL SST	CC DLM
Lymphocyte Phenotyping, TBNK	1 light lavender tube	CC DLM
Immunology Assays	6 10 mL Na Heparin tubes 2 7 ml SST tubes Apheresis Product	NCI-Frederick 1-301-846-5893
Serum VEGF	6mL EDTA tube	Dr. Figg's lab - Blood Processing Core (BPC)