Short Title: Enzalutamide Vaccine in mCRPC
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Title: A Randomized Phase II Trial Combining Vaccine Therapy with PROSTVAC /TRICOM and Enzalutamide vs. Enzalutamide Alone in Men with Metastatic Castration Resistant Prostate Cancer

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Investigational Agent:

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.

Commercial Agents: Cidofovir

PRÉCIS

Background:

- Enzalutamide is a well-tolerated, modern androgen receptor antagonist (ARA) with more enhanced anti-tumor activity compared to previous ARAs. Phase III trial has demonstrated a 4.8 month improvement in survival and a 37% risk reduction in death in metastatic castration resistant prostate cancer (mCRPC) patients who have had previous docetaxel.
- PROSTVAC™ is a therapeutic cancer vaccine which is designed to induce an anti-tumor immune response. In a randomized controlled Phase 2 trial, PROSTVAC therapy was associated with a prolongation of survival by 8.5 months in men with metastatic castrate-resistant prostate cancer. An international Phase 3 trial is on-going.
- Preclinical data has demonstrated that hormonal therapies such as ARAs can enhance the immune response through multiple mechanisms. Specifically, our group has shown that enzalutamide can increase thymic production of naïve T-cells, which could be activated by a cancer vaccine. Together, these data provide an important rationale to combine enzalutamide with PSA-TRICOM in mCRPC.
- Data from the clinical trials with these therapies suggest that they are very well tolerated and without overlapping toxicity.

Objective:

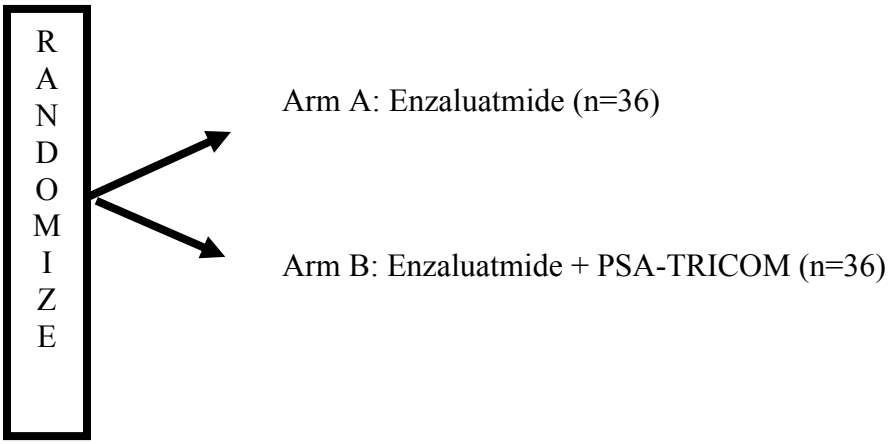
- Determine if PSA-TRICOM combined with enzalutamide will increase time to progression (as defined by Prostate Cancer Clinical Trials Working Group 2 criteria, incorporated in section 5.2) in chemotherapy-naïve metastatic castration resistant prostate cancer patients compared to enzalutamide alone.

Eligibility:

- mCRPC patients with rising PSA or progressive disease despite castration levels of testosterone.
- Chemotherapy-naïve with minimal or no symptoms related to prostate cancer.
- Patients with history of autoimmune disease, brain/leptomeningeal metastasis, a second malignancy within 3 years of enrollment, or a severe co-morbid condition will be excluded.
- Patients who have received abiraterone will be excluded
- Patients will be stratified based on previous immunotherapy used as cancer treatment.

Design:

Patient Population: chemotherapy-naïve, mCRPC



- The study will randomize chemotherapy-naïve, mCRPC patients to either enzalutamide alone or enzalutamide with PSA-TRICOM. Enzalutamide will be given at the standard dose of 160 mg daily.
- 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 week 3, 5 and then monthly fowlpox-vaccine ($1x10^9$ infectious units subcutaneously).
- After completing 6 months of vaccine, fowlpox-vaccine ($1x10^9$ infectious units subcutaneously will be administered every 3 months. Patients will be treated until radiographic progression on scans using Prostate Cancer Working Group Criteria.

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STATEMENT OF COMPLIANCE

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

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

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

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

1. INTRODUCTION

1.1 STUDY OBJECTIVES

1.1.1 Primary Objective

- Determine if PSA-TRICOM combined with enzalutamide will increase time to progression (as defined by Prostate Cancer Clinical Trials Working Group 2 criteria) in chemotherapy-naïve metastatic castration resistant prostate cancer patients compared to enzalutamide alone.

1.1.2 Secondary Objectives

- Determine if PSA-TRICOM combined with enzalutamide will increase overall survival in chemotherapy-naïve metastatic castration resistant prostate cancer patients compared to enzalutamide alone.
- Determine if PSA-TRICOM combined with enzalutamide will delay PSA progression in chemotherapy-naïve metastatic castration resistant prostate cancer patients compared to enzalutamide alone

1.1.3 Exploratory Objectives

- 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, Regulatory T-cell function, cytokines, anti-glycan antibodies, naïve thymic emigrants.
- Associate immunologic outcomes with clinical outcomes.

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 for the treatment of prostate cancer. 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**) The U.S. Food and Drug Administration (FDA) approved enzalutamide for metastatic prostate cancer in docetaxel-refractory patients in August, 2012 and in 2014 for docetaxel naive patients [5-7].

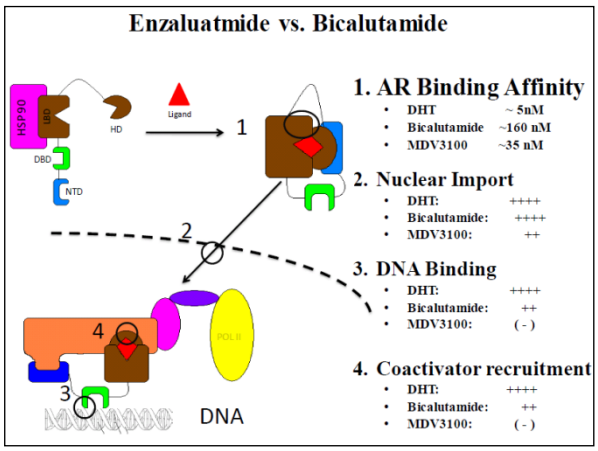


Figure 1. Enhanced Efficacy with Enzalutamide. Enzalutamide has greater androgen receptor (AR) binding affinity and a greater negative impact on AR nuclear import than bicalutamide with Dihydrotestosterone (DHT) as a control. In addition with enzalutamide there is no AR binding with the DNA and coactivatior recruitment.

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 [8]. Two phase III studies were then launched in metastatic CRPC (mCRPC). One study enrolled chemotherapy-naïve patients; the other enrolled 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 [5]. The U.S. FDA approved enzalutamide for chemotherapy-

refractory mCRPC patients in August of 2012 [6]. PREVAIL enrolled 1717 patient with mCRPC who progressed on ADT and randomized 1:1 to enzalutamide 160mg/day (n=872) or placebo (n=845). The enzalutamide arm had an 81% decreased risk of radiographic progression or death (HR: 0.186; 95% CI: 0.15 to 0.23; P<0.0001). The overall survival at 5 years favored patients randomized to the enzalutamide arm 35.5 to 31.4 months with a rate of 26% for the enzalutamide arm compared to 21% for the placebo arm.

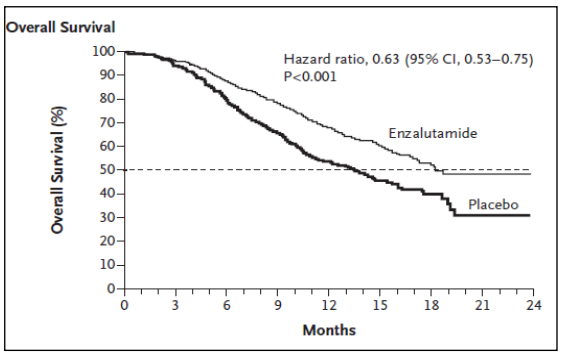


Figure 2. **Enzalutamide demonstrates improved overall survival in mCRPC patients previously treated with docetaxel** [5]

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. 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 do 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].

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 [10, 11]. A phase III trial (n = 512) demonstrated an overall survival benefit for the vaccine (25.8 months vs. 21.7 months; P = 0.032) [12]. 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 [13, 14]. (The LTIB and Bavarian Nordic have 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 [13, 15]. (**Figure *3***) 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 [16].

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; $P = 0.0061$) [17]. (**Figure 4**) 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 [18].

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.

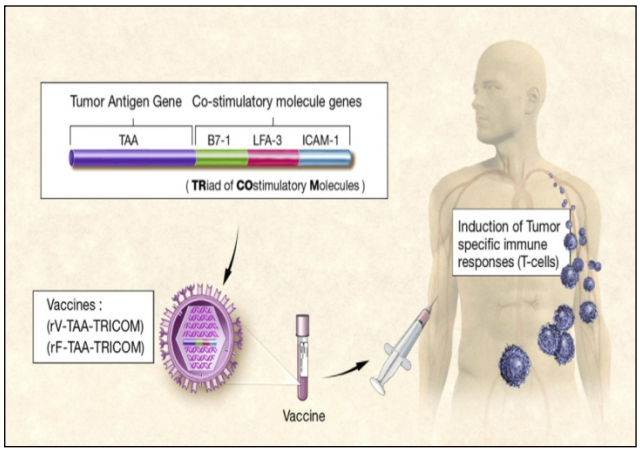


Figure 3. Poxviral vaccine strategy: Modified poxvirus contains transgenes for the tumor-associated antigen PSA and 3 T-cell costimulatory molecules [12]

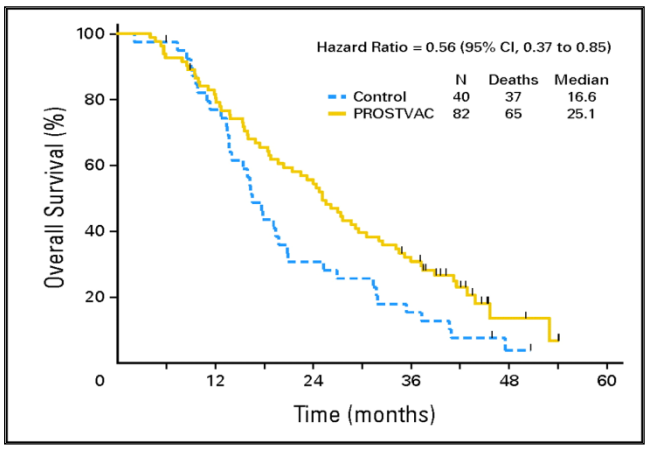


Figure 4. PSA-TRICOM improved survival in mCRPC patients in a randomized multicenter phase II trial. [13]

Like enzalutamide, PSA-TRICOM is very well-tolerated, with common side effects of grade 1 injection-site reactions or flu-like symptoms [17]. 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.

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 [19]. 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, 20, 21]. 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. (Thirty-three patients with biochemically recurrent prostate cancer received ADT on this trial.) 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

An ongoing clinical trial at the NCI is combining PSA-TRICOM with flutamide, an FDA approved ARA, flutamide 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 [22]. 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) [7]. (**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 non-metastatic disease [23].

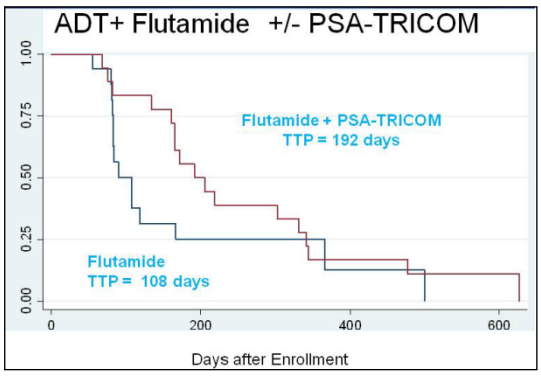


Figure 5. Vaccines may improve TTP in combination therapy. Preliminary data from an interim analysis of an ongoing trial of flutamide alone (n=17) vs. Flutamide + PSA-TRICOM (n=18) in patients with nonmetastatic CRPC (NCT00450463) suggest that adding vaccine to ADT + flutamide may delay disease progression. ([23])

1.2.5.1 Enzalutamide does not Diminish Immune Cell Numbers or Function 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 6**A) It was confirmed that enzalutamide at 10 mg/day had a significant decrease in the weight of the genitourinary organs of Male C57BL/6 mice after 14 days of dosing, demonstrating the physiologic effects of enzalutamide at 10 mg/day dose level. (**Figure 6**B)

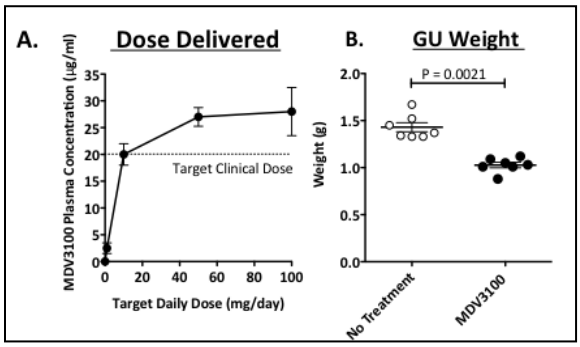


Figure 6. Enzalutamide mediates a reduction in genitourinary (GU) weight. (A). Concentration of Enzalutamide in plasma of mice on Enzalutamide daily diet. Male C57BL/6 mice (n=3) were treated with Enzalutamide at different calculated target daily doses (0, 1, 10, 50, and 100mg) for 14 days. On day 15, 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.

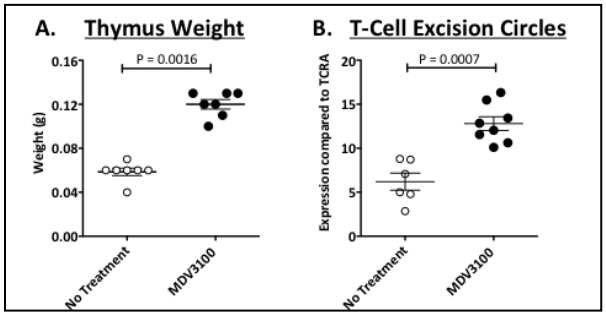


Figure 7. Enzalutamide mediates an enlargement of thymus and an increase in T-cell Receptor Excision Circles (TREC) levels. (A). Male C57BL/6 mice (n=7) were not treated (open circles) or treated with Enzalutamide (closed circles) for 14 days. On day 15, mice were sacrificed, and their thymus harvested and weighed. (7B). 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

1.2.6 The Importance of Evaluating the Immunologic Response

Our group at in the GMB and 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 [17]. That same trial also suggested that changes in regulatory T-cell function were also associated with improved clinical outcomes [24]. While these findings are not surrogate markers of response, they have greatly improved our knowledge of a vaccine-generated immune response and provided a better understanding of what factors are potentially important in mounting a sufficient anti-tumor immune response that could be associated with improved clinical outcomes. 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 these hypothesis generating data would have to 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 can enhance anti-tumor immune responses [25, 26]. 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. 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 GMB and LTIB to conduct this rigorous immune testing from clinical samples of patients treated with enzalutamide to gain a better understanding about its immune properties.

1.2.7 Rationale for Time to Progression as a Primary 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 ipilimumab in melanoma [27]. Although this is not what is customarily seen with cancer treatments, it may be a characteristic of modern immune therapeutics [28]. An emerging mechanism to evaluate tumor growth rates using mathematical models may provide additional insight into this phenomenon [29, 30]. 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. [31] 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 [32]. (See **Figure 8**).

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 [33]. Similarly, Sipuleucel-T has significantly prolonged PSA doubling time (another way to measure growth) after initial hormonal therapy in the same patient population [31]. Together, these data suggest that for patients getting combination therapy, disease can be controlled by standard therapies in the short term, while vaccines may impact tumor growth rates in the long term, ultimately resulting in prolonged TTP compared to standard therapy alone. (**Figure 8**)

If vaccines can alter growth rates and if cytoreductive therapies can reduce tumor burden, it would be reasonable to presume that vaccine combination therapies would potentially result in a TTP benefit.

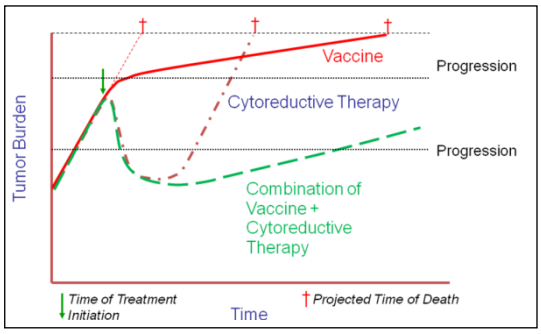


Figure 8. Vaccine as part of combination therapy may improve TTP. Although vaccine as monotherapy may have no impact on short-term TTP, vaccine in combination regimens may improve TTP by altering the rate of regrowth as the effects of the companion therapy begin to diminish. If the companion therapy is cytoreductive, tumor volume will decrease, but as the therapeutic benefits wane, regrowth will occur. If sufficient time has passed to generate an immune response, the anti-tumor effects of the immune system may result in a delay in TTP (due to a slower re-growth rate) relative to cytoreductive treatment alone. [13]

1.3 SUMMARY OF RATIONALE

1) Enzalutamide is a well-tolerated, modern androgen receptor antagonist. Phase III trial have demonstrated a 4.8 month improvement in survival in mCRPC patients who were previously treated with docetaxel.
2) PROSTVAC™ is a novel candidate for prostate cancer immunotherapy. In a randomized controlled Phase 2 trial, PROSTVAC therapy was associated with a prolongation of survival by 8.5 months in men with metastatic castrate-resistant prostate cancer. The Phase 3 efficacy trial is on-going.
3) Preclinical data has demonstrated that hormonal therapies such as ARAs can enhance the immune response through multiple mechanisms. Specifically, the LTIB has shown that enzalutamide can increase thymic production of naïve T-cells, which could be activated by a cancer vaccine.
4) Data from the clinical trials with these therapies suggest that they are very well tolerated and without overlapping toxicity.

5) Data suggest that for patients getting combination therapy, TTP may be a reasonable clinical endpoint and such a finding could be important in determining the ultimate clinical role of therapeutic cancer vaccines.

Based on the above rationale, we will evaluate whether combining enzalutamide and PSA-TRICOM vaccine prolongs time to disease progression compared with enzalutamide alone in chemotherapy-naïve mCRPC.

2 ELIGIBILITY ASSESSMENT AND ENROLLMENT

2.1 ELIGIBILITY CRITERIA

2.1.1 Inclusion Criteria

2.1.1.1 Patients must have histologically or cytologically confirmed prostate cancer confirmed by either the Laboratory of Pathology at the NIH Clinical Center, Walter Reed National Military Medical Center at Bethesda prior to starting this study. If no pathologic specimen is available, patients may enroll with a pathologist's report showing a histological diagnosis of prostate cancer and a clinical course consistent with the disease.

2.1.1.2 Castrate testosterone level (<50ng/dl or 1.7nmol /L)

2.1.1.3 Metastatic disease documented by one of the following:

- Metastatic bone disease on an imaging study, or
- Soft tissue disease documented by CT/MRI, or

2.1.1.4 Progressive disease at study entry defined as one or more of the following criteria occurring in the setting of castrate levels of testosterone:

i. Radiographic progression defined as **any new** or enlarging bone lesions or growing lymph node disease, consistent with prostate cancer

OR

ii. PSA progression defined by sequence of rising values separated by >1 week (2 separate increasing values over a minimum of 2ng/ml (PCWG2 PSA eligibility criteria). If patients had been on flutamide, PSA progression is documented 4 weeks or more after withdrawal. For patients on bicalutamide or nilutamide disease progression is documented 6 or more weeks after withdrawal. The requirement for a 4-6 week withdrawal period following discontinuation of flutamide, nilutamide or bicalutamide only applies to patients who have been on these drugs for at least the prior 6 months. For all other patients they must stop bicalutamide, nilutamide or flutamide the day prior to enrollment.

2.1.1.5 Asymptomatic or mildly symptomatic from prostate cancer; no use of regularly scheduled opiate analgesics for prostate cancer-related pain

2.1.1.6 Patients must agree to continue to continuation of androgen deprivation therapy (ADT) with a gonadotropin-releasing hormone analogue/antagonist or bilateral orchiectomy

2.1.1.7 Age ≥18 years.

2.1.1.8 ECOG performance status ≤1 (Karnofsky ≥80%, see (**APPENDIX A)**.

2.1.1.9 Patients must have normal organ and marrow function as defined below:

- absolute neutrophil count ≥1,500/mcL
- platelets ≥100,000/mcL
- total bilirubin within normal institutional limits; for patients with Gilbert's syndrome, total bilirubin ≤3.0mg/dL
- AST(SGOT)/ALT(SGPT) ≤2.5 X institutional upper limit of normal
- creatinine within 1.5 X normal institutional limits

OR

- creatinine clearance ≥60 mL/min/1.73 m^2 for patients with creatinine levels above institutional normal by Cockcroft-Gault Equation

$$\text{creatinine clearance (mL/min)} = \frac{(140 - \text{age [y]}) \times \text{weight [kg]}}{(72 \times \text{serum creatinine [mg/dL]})} \times \text{Sex} \times \frac{\text{BSA [m}^2\text{]}}{1.73\ \text{m}^2}$$

In the equation above, the factor for "Sex" equals 0.85 for females and 1 for males

2.1.1.10 The effects of enzalutamide alone or in combination with PSA-TRICOM on the developing human fetus are unknown. For this reason, men must agree to use adequate contraception (hormonal or barrier method of birth control; abstinence) prior to study entry, for the duration of study participation and for three (3) months after the last dose of enzalutamide. Should a woman become pregnant or suspect she is pregnant while her partner is participating in this study, she should inform her treating physician immediately.

2.1.1.11 Ability of subject to understand and the willingness to sign a written informed consent document.

2.1.2 Exclusion Criteria

2.1.2.1 Patients who are immunocompromised as listed as follows:

- Human immunodeficiency virus positivity due to the potential for decreased tolerance and may be at risk for severe side effects
- Chronic administration (defined as daily or every other day for continued use >14 days) of systemic corticosteroids (including steroid eye drops) or other immune suppressive drugs, within 28 days before the first planned dose of PSA-TRICOM. Nasal, or inhaled steroid, and topical steroid creams for small body areas are not excluded.
- Patients who have undergone allogeneic peripheral stem cell transplantation, or solid organ transplantation requiring immunosuppression
- History of splenectomy

2.1.2.2 History of, or active autoimmune disease (such as Autoimmune neutropenia, thrombocytopenia, or hemolytic anemia, systemic lupus erythematosis, Sjogrens syndrome, scleroderma, myasthenia gravis, Goodpasture's syndrome, Addisons disease, Hashimotos thyroiditis, Crohns or Graves disease). Patients with type 1 diabetes

mellitus or vitiligo are not excluded if the condition is well controlled.

2.1.2.3 Patients with a history of brain/leptomeningeal metastasis

2.1.2.4 Patients who have been treated with abiraterone will be excluded

2.1.2.5 Patients with history of seizure as an adult including febrile seizure or any condition that may predispose to seizure (e.g., prior stroke, brain arteriovenous malformation, head trauma with loss of consciousness requiring hospitalization). Also, current or prior treatment with anti-epileptic medications for the treatment of seizures or history of loss of consciousness. Also transient ischemic attack within 12 months prior to randomization will not be permitted.

2.1.2.6 Patients with second malignancy within 3 years of enrollment; Patients curatively treated non-melanoma skin cancers or carcinoma in situ of the bladder, are not excluded.

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

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

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

2.1.2.10 Previous adverse reactions to smallpox vaccination

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

2.1.2.12 Any condition which, in the opinion of the investigator, would prevent full participation in this trial (including the long-term follow-up), or would interfere with the evaluation of the trial endpoints.

2.1.2.13 Patients with prior chemotherapy for nonmetastatic prostate cancer within a year are excluded.

2.1.2.14 Receipt of an investigational agent within 28 days (or 56 days for an antibody-based therapy) before the first planned dose of study drugs. (Immune checkpoint inhibitors that are antibody-based will only require 28 days before enrollment)

2.1.2.15 Uncontrolled intercurrent illness including, but not limited to, ongoing or active infection, symptomatic congestive heart failure, unstable angina pectoris, cardiac arrhythmia, uncontrolled hypertension (SBP>170/ DBP>105) or psychiatric illness/social situations within 12 months that would limit compliance with study requirements.

2.1.2.16 Use of herbal products that may decrease PSA levels (e.g. saw palmetto)

2.1.2.17 Any gastrointestinal disease that could hinder the absorption of enzalutamide.

2.1.2.18 Patients who have had chemotherapy for metastatic castration-resistant prostate cancer. (Patients who have had docetaxel for metastatic castration sensitive disease per CHAARTED Data[34] may enroll as long as they did not have progressive disease

while on docetaxel and are 6 months removed from treatment, with all treatment related toxicities resolving to at least grade 1.)

2.1.2.19 Patients who have received radiation therapy, radionuclide therapy or undergone surgery within certain duration (4 weeks) of enrollment

2.1.3 Recruitment Strategies

This study will be listed on available websites (www.clinicaltrials.gov) and participants will be recruited from the current patient population at NIH and participating sites.

2.2 SCREENING EVALUATION

Note: Screening evaluation testing/procedures are conducted under the separate screening protocol, 01-C-0129 (Eligibility Screening and Tissue Procurement for the NIH Intramural Research Program Clinical Protocols).

1. The following tests may be obtained any time prior to enrollment:
   - Pathological confirmation of diagnosis and PSA expression in either the Laboratory of Pathology at NIH Clinical Center, Walter Reed National Military Medical Center at Bethesda. However, if no pathologic specimen is available, patients may enroll with a pathologist's report showing a histologic diagnosis of prostate cancer and a clinical course consistent with the disease.
2. The following parameters will be obtained within 8 weeks prior to enrollment:
   - HIV test
   - Hepatitis B and C
3. The following parameters will be obtained within 30 days prior to enrollment:
   - Tc-99 whole body scintigraphy
   - CT of chest/abdomen /pelvis (MRI may be substituted at investigator's discretion).
   - History and physical examination with ECOG
   - Serum PSA
   - Complete blood count plus differential and platelet count
   - Hepatic and Acute Care panels
   - Testosterone level

2.3 REGISTRATION PROCEDURES

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

2.3.1 Treatment Assignment and Randomization/Stratification Procedures

Cohorts

Number	Name	Description
1	*Prostate Cancer Patients*	*Patients with chemotherapy naïve metastatic castrate-resistant prostate cancer*

Arms

Number	Name	Description
1	*A*	*Enzalutamide alone*
2	*B*	*Enzalutamide with PSA-TRICOM*

Stratifications

Name	Distinct Options	Notes
Prior Immunotherapy	*Yes* *No*	

Randomization and Arm Assignment

Patients must be registered with Central Registration Information Services within 24 hours after signing the consent. All Patients on study will be directly assigned to cohort 1.

Patients in cohort 1 will be randomized within 24 hours after registration. Block randomization will be used to randomly assign patients in a 1:1 manner between arms A and B. Randomization will be stratified by prior immunotherapy used as cancer treatment.

For randomization, authorized staff should call 240-760-7968 between the hours of 8:30 a.m. and 5:00 p.m., Monday through Friday. A recorder is available during non-working hours.

2.4 BASELINE EVALUATION

The following parameters will be obtained within 16 days prior to start of treatment:

2.4.1 Clinical Evaluation

- History and physical examination
- ECOG performance status (see **APPENDIX A**)
- Height, Weight
- 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

2.4.2 Laboratory studies

- Complete blood count plus differential and platelet count
- Acute care panel
- Hepatic panel
- Mineral panel
- LDH, CK, Uric Acid, Total Protein
- urinalysis
- Serum PSA
- Serum CEA level

- Lymphocyte phenotyping CD4/CD8
- HLA class I expression (results will not be required prior to enrollment)

2.4.3 Leukapheresis

Optional for HLA-A2 positive patients.

3 STUDY IMPLEMENTATION

3.1 STUDY DESIGN

The study will randomize chemotherapy-naïve, mCRPC patients to either enzalutamide alone or enzalutamide with PSA-TRICOM.

Enzalutamide will be given at the standard dose of 160 mg daily [5].

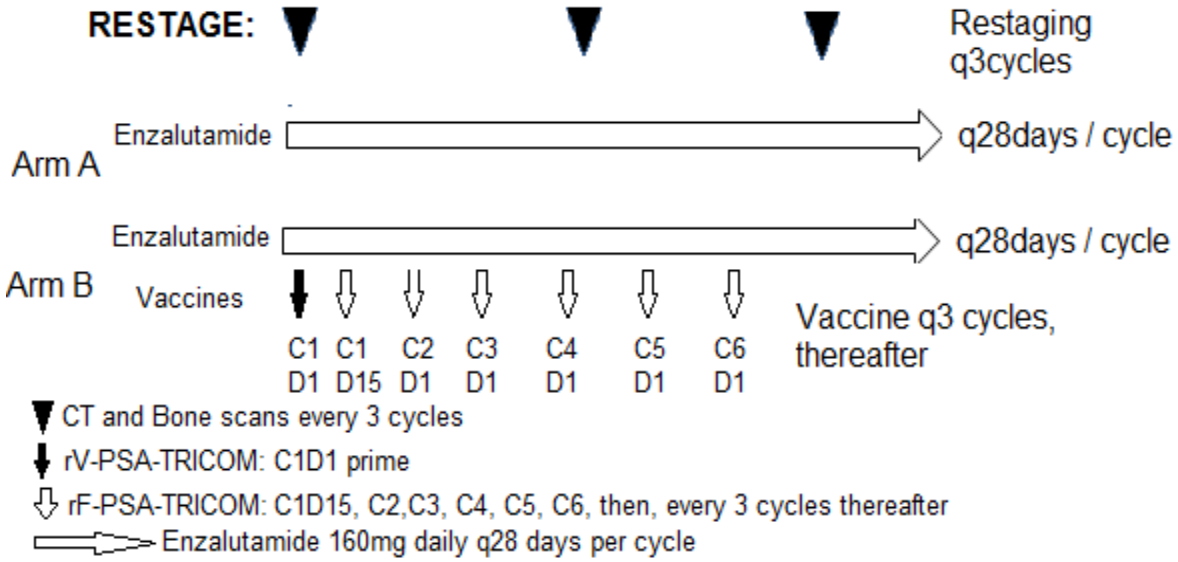
PSA-TRICOM will be administered identical to the Phase III dosing with vaccine given week 1 (vaccinia-PSA-TRICOM, $2x10^8$ infectious units subcutaneously) and then fowlpox-vaccine ($1x10^9$ infectious units subcutaneously) given in week 3, week 5 and then every 4 weeks through week 21 [35].

After completing 6 months of vaccine, fowlpox-vaccine ($1x10^9$ infectious units subcutaneously) will be administered every 3 months based on previous clinical trial experience with PSA-TRICOM [17].

Patients will be treated until radiographic progression on scans using Prostate Cancer Working Group Criteria [23] (Response Criteria in this protocol have incorporated the Response Criteria and are discussed in section **5.3**). Re-staging will be done every 3 months similar to the AFFIRM trial [5]. For patients who have stable disease beyond 2 years and a PSA less than 1.0 ng/ml, they can defer restaging scans, based on investigator discretion.

The primary endpoint of this trial will be TTP, with secondary endpoints including overall survival, and exploratory analysis evaluating immunologic responses to vaccine with enzalutamide and to enzalutamide alone.

FOR THE FIRST 18 CYCLES OF TREATMENT:



BEYOND 18 CYCLES OF TREATMENT:

Beyond 18 cycles of treatment, cycle length will increase to 42 days. Patients will return for follow up every 6 weeks instead of every 4 weeks. For those patients beyond 18 cycles of treatment receiving the PROSTVAC vaccine, they will receive a dose every second cycle instead of every third cycle, however, the number of weeks between doses of vaccine will remain unchanged at 12 week intervals. (To allow for the transition between 4 and 6 week cycles, one dose of vaccine will be given at a 10 week interval). Similarly, for patients who have been on treatment more than 18 cycles, restaging scans will be every second cycle instead of every third cycle, however, the number of weeks between restaging scans will remain unchanged at 12 weeks intervals. For patients who have stable disease beyond 2 years and a PSA less than 1.0 ng/ml, they can defer restaging scans, based on investigator discretion.

3.2 ADMINISTRATION

Patients who have been treated for less than 18 cycles will receive enzalutamide 160mg orally, daily on days 1 – 28 in a 28-day cycle, and will continue this dosing throughout the cycle.

Patients who have been treated for more than 18 cycles will receive enzalutamide 160mg orally, daily on days 1 – 42 in a 42-day cycle, and will continue this dosing throughout the cycle. The study drug will be dispensed by the NIH Clinical Center Pharmacy.

Patients randomized to Arm B will receive vaccines at the NIH Clinical Center. The vaccines will be prepared and placed in syringes by the Clinical Center Pharmacy personnel at the NIH. Please see section **3.1** for dose, schedule and route of administration. 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. Patient will be monitored with vital signs prior to subsequent vaccine treatments, and will remain in the Day Hospital for at least 30 minutes following

administration of vaccine for observation of adverse reactions. (Post-vaccine vital signs will not be required except for the first vaccination.)

3.3 DOSE MODIFICATIONS/DELAYS

3.3.1 Vaccine Dose Modification: None

3.3.2 Enzalutamide Dose Modification

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. Treatment may be held if clinically appropriate as determined by the investigator and may be restarted as long as patient has not demonstrated radiographic disease progression as defined in the protocol. If toxicity recurs and or in the judgment of the investigator dose reduction is appropriate, lower dose levels (120 mg /DL-1 or 80 mg/DL-2), may be considered.

3.3.2.1 Enzalutamide Dose Delay

If the planned enzalutamide dose is missed due to scheduling or logistical issues (e.g., vacation, weather) during a cycle or delay in starting a cycle, it may be started within 14 days of the appointed time.

3.3.3 Criteria for individual patient re-treatment

3.3.3.1 Patients with grade 3 non-autoimmune toxicity due to the treatment regimen, may resume treatment provided that the toxicity has decreased to baseline or grade 2 toxicity within 60 days from the last day of treatment. See section **3.6.1** Criteria for removal from protocol therapy.

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

3.3.3.3 Patients who develop any grade 4 toxicity attributable to protocol treatment will be removed from the protocol treatment and followed for resolution of toxicity

3.3.3.4 Patients who develop grade 3 injection site reactions will have their vaccine held until injection site reaction resolves to grade 2 or less.

3.3.3.5 If a schedule vaccine dose is missed due to scheduling or logistical issues, the vaccine may be given within 14 days of the appointed time.

3.4 STUDY CALENDAR

	Screening and Baseline		Treatment 1 cycle = 28 days; C=Cycle; D=Day										
	D -56 to D1	C1 D1	C1 D15	C2 D1	C3 D1	C4 D1	C5 D1	C6 D1	C7 D1	C8 D1	C9 D1	C10+ D1[13]	End of Study
Informed Consent / Registration	X												
NIH Advance Directives Form[11]	X												
Randomization	X												
TREATMENT													
Enzalutamide[1]		X		X	X	X	X	X	X	X	X	X[2]	
rV-PSA-TRICOM[3]		X											
rF-PSA-TRICOM[3]			X	X	X	X	X	X			X[4]		
EVALUATION													
History and PE**,** Height (height only at baseline) and Weight	X		X	X	X	X	X	X	X	X	X	X[5]	X
ECOG Performance Score	X		X	X	X	X	X	X	X	X	X	X[5]	X
Pathologic confirmation of dx	X[6]												
Laboratory Testing													
CBC with differential[12]	X		X	X	X	X	X	X	X	X	X	X[5]	X
Acute Care Panel, Mineral Panel, Hepatic Panel, LDH, CK, Uric Acid, Total Protein[12]	X		X	X	X	X	X	X	X	X	X	X[5]	X
Urinalysis	X												
Serum PSA[12]	X		X	X	X	X	X	X	X	X	X	X[5]	X
Serum CEA[12]	X												

	Screening and Baseline		Treatment 1 cycle = 28 days; C=Cycle; D=Day									
	D -56 to D1	C1 D1	C1 D15	C2 D1	C3 D1	C4 D1	C5 D1	C6 D1	C7 D1	C8 D1	C9 D1	C10+ D1[13]	End of Study
Hep B/HepC, HIV	X[7]											
Lymphocyte Phenotyping TBNK[12]	X			X	X	X	X	X	X	X	X	X[5]	X
HLA testing (results not required prior to enrollment)	X												
ECG	X[8]												
Tc 99 scintigraphy[12]	X[8]					X			X			X[9]	
CT-C/A/P, or MRI[12]	X[8]					X			X			X[9]	
Immunology Assays[10, 12]	X			X		X							
Adverse Events		X →	→	→	→	→	→	→	→	→	→	→	
Concomitant Medications	X	→	→	→	→	→	→	→	→	→	→	→	→

Footnotes:

[1] For patients in Arms A and B

[2] Cycle length will increase from 28 days to 42 days at cycle 19 day 1. Enzalutamide 160mg p.o. daily will be given every 28 days/ cycle until cycle 19 day 1 and then every 42 days/cycle thereafter. For patients who have had stable disease beyond 2 years and no SAEs related to the treatment, patients may be followed up in clinic at intervals of 12 weeks (consistent with clinical practice.) Vaccine dosing may be adjusted to accommodate the new schedule as long as it is not given at less than a 28-day interval.

[3] Only for patients randomized to Arm B (Enzalutamide + Vaccine).

[4] rF-PSA-TRICOM, will be given every 12 weeks after cycle 6 day 1 (To allow for the transition between 4 and 6 week cycles at cycle 19, one dose of vaccine will be given at a 10 week interval).

[5] HPE, ECOG, CBC, Chem, PSA, and Lymphocyte Phenotyping will be obtained every cycle.

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

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

[8] Baseline ECG, Tc99 scintigraphy and CT-CAP or MRI will be obtained within 30 days prior to enrollment.

[9] Restaging will be done every 12 weeks or earlier if clinically indicated. For patients who are on-study beyond 24 months who do not have a rising PSA, CT and bone scan can be deferred based on clinical judgement of the investigator.

[10] After C4D1, samples may be collected prior to treatment at C13, C19, then every 6 months at restaging while on-study.

[11] As indicated in section **11.3**, all subjects will be offered the opportunity to complete an NIH Advance 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.

[12] Blood work and restaging scans may be obtained +/- 7 days of clinic visit/assessment for logistical and scheduling purposes.

[13]Due to the Covid-19 pandemic, subjects with long term stable disease (>3) may have virtual visits and blood work performed with local provider

3.5 COST AND COMPENSATION

3.5.1 Costs

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

3.5.2 Compensation

Participants will not be compensated on this study.

3.5.3 Reimbursement

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

3.6 CRITERIA FOR REMOVAL FROM PROTOCOL THERAPY AND OFF STUDY CRITERIA

3.6.1 Criteria for removal from protocol therapy

- Disease progression as evidenced by radiographic or clinical progression (defined in section **5.3.5**).
- Intercurrent illness or medical circumstances: if at any time the constraints of this protocol are detrimental to the patient's health, the patient may be removed from protocol therapy. In this event, the reasons for withdrawal will be documented.
- Patient's request to be taken off study. In this event, the reasons for withdrawal will be documented.
- If patients are non-compliant with the protocol guidelines, they may be removed from the study at the discretion of the principal investigator.
- Any Grade 4 toxicity that is possibly, probably or definitely related to the protocol treatment will require a patient to be off-treatment.
- Grade ≥3 autoimmune toxicity while on vaccine, will require a patient to be off-treatment.
- Any Grade 3 non-autoimmune toxicity that does not resolve to Grade 1 or baseline within 60 days and is possibly, probably or definitely related to the protocol treatment will require a patient to be off-treatment.
- Any grade of seizure will require discontinuation of enzalutamide but patients may continue vaccine at the discretion of the investigator.

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

3.6.2 Off-Study Criteria

- Patient is off-treatment including an approximately 30 day follow up visit (when logistically feasible)
- Patient's request to be withdrawn from the study

- Death

3.7 POST-STUDY EVALUATION

Patients treated at the NCI will be offered enrollment in the 04-C-0274 "Follow-Up Study of Subjects Previously Enrolled in Immunotherapy Studies Utilizing Gene Transfer" once off study.

3.8 CONCOMITANT TREATMENT

Patients will be on concomitant androgen suppression therapy with a GnRH agonist or GnRH antagonist (unless they have had a prior orchiectomy). Patients may also be on concomitant drugs to prevent bone loss, including bisphosphonates and denosumab.

Other supportive care with blood components, antibiotics, analgesics, general medical therapy, etc., will be delivered as required. Use caution when co-administering medication which may lower the seizure threshold.

Consistent with the PREVAIL trial which demonstrated improved survival in mCRPC and led to FDA approval in the chemotherapy-naïve subgroup, palliative radiation will be allowed for patients on this study.

They will be allowed to continue enzalutamide (with/without vaccine). There is no concern for overlapping toxicity.[36]

3.8.1 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 immunotherapy
- Concurrent anti-cancer radionuclides
- Concurrent systemic corticosteroid use (daily or every other day for continued use > **14 days**)
- Concomitant use of secondary hormonal treatments

3.8.2 Treatment of vaccinia vaccination complication

3.8.2.1 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.

3.8.3 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 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].

4 BIOSPECIMEN COLLECTION

4.1 CORRELATIVE STUDIES FOR RESEARCH

4.1.1 Parameters of Immune Response

(Refer to **4.1.2** Immunologic Assays for detail):

- IFN-gamma ELISPOT assay for PSA-specific T lymphocytes
- CD4 T Cell Proliferation
- Antibodies to PSA and other tumor antigens
- Leukocyte CD3, CD4, CD8 subsets; CD4:CD8 ratio will be drawn at baseline and each cycle while the patient remains on trial.
- Immunologic profiles will be investigated to evaluate whether enhanced thymopoiesis might be present during vaccine administration; these immunologic profiles are to include enumeration of recent thymic emigrants by quantification of T-cell subsets by multiparameter flow cytometry.

- The results of the HIV antibody test need to be available before treatment to determine eligibility.
- HLA class I expression (HLA typing) with A2 subtyping (obtain any time prior to first vaccine).
- Immunologic studies will be repeated more frequently if clinically indicated. Any abnormalities potentially related to treatment will be followed until they have resolved or have been determined not to be treatment-related.

4.1.1.1 All patients who are HLA A02 or A03 (approximately 50% of the US population) will undergo leukapheresis every 12 weeks while on-study when available and with the consent of the patient. Patients may refuse.

- IFN-gamma ELISPOT assay
- T cell proliferation
- Antibody Response
- Flow cytometry analysis of thymic emigrants
- Natural Killer (NK) Cells
- Regulatory T Cells
- 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, chemokines, antibodies, tumor associated antigens, anti-glycan antibodies and/or other markers.

Details on these tests can be found in **4.1.2**.

4.1.1.2 **Collection of blood samples for immune assays:** Blood samples may be obtained at baseline, 1 month, 3 months, 12 months, 18 months, then every 6 months at restaging while on study**.** All patients will have 6 (10ml) green top tubes and 2 (8ml) SST drawn prior to treatment at designated timepoints.

4.1.1.3 The samples will be processed at:

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

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 Caroline Jochems at jochemscm@mail.nih.gov, Jen Bangh at jb478s@nih.gov and Theresa Burks (burkst@mailnih.gov).

4.1.2 Immunological Assays

4.1.2.1 IFN-gamma ELISPOT

We plan to examine the immune response in selected patients (HLA-A2-positive).

HLA class I expression (HLA typing) with A2 subtyping will be done prior to enrollment. Results will not be required prior to enrollment.

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 [37, 38]. 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 [39]. 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 Genitourinary Malignancies Branch, 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.

4.1.2.2 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.

4.1.2.3 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.

4.1.2.4 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.

4.1.2.5 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.

4.1.2.6 Regulatory T Cells

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

4.1.3 Additional Assays

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

All samples will be labeled with the following identifier system.

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

Example: 01-ABC

These labels are used only to send the samples from the NIH Clinical Center to the NCI Frederick Central Repository. The NCI Repository will process all samples, appropriately discard the label on the blood tube, and then store the samples with unique Leidos 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 **4.3** (Sample Storage, Tracking and Disposition).

4.1.4 Circulating Tumor Cells (CTC)

4.1.4.1 Rationale of investigation

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

- Multiplex analysis technologies have been developed to examine the levels and activities of androgen receptor.

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

4.1.4.1 Blood collection

At the time of enrollment and at restaging visits, one 10 mL Streck Cell-Free DNA (brown-black top) tube will be collected. Dr. Figg's lab will pick up the sample and ship to Epic Sciences as noted below. Record the date and exact time of draw on the tube.

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

For sample pickup, page 102-11964.

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

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

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

Epic Sciences
Attn: Joseph Schonhoft
9381 Judicial Drive, Ste. 200
San Diego, CA 92121
858-263-0694

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, and every 3 months thereafter until time of progression. 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 NCIBloodcore@mail.nih.gov at least 24 hours before transporting samples (the Friday before is preferred).

For sample pickup, page 102-11964.

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

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

The samples will be processed, barcoded, and stored in Dr. Figg's lab until requested by the investigator.

4.1.6 Serum analysis

Of the sample collected in Section **4.1.1.2**, 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 ADULT PATIENTS: BLOOD DRAWING LIMITS FOR RESEARCH PURPOSES

The amount of blood that may be drawn from adult patients and volunteers (i.e., those persons 18 years of age or older) for research purposes shall not exceed 10.5 mL/kg or 550 mL, whichever is smaller, over any eight week period.

4.3 SAMPLE STORAGE, TRACKING AND DISPOSITION

Samples will be ordered in CRIS and tracked through a Clinical Trial Data Management System. Should a CRIS screen not be available, the CRIS downtime procedures will be followed. Samples will not be sent outside NIH without IRB notification and an executed MTA.

4.3.1 Samples sent to Clinical Services Program (CSP)

4.3.1.1 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.

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

A subcontractor manages the NCI Frederick Central Repositories under subcontract to Leidos Biomedical Research, Inc. NCI Frederick Central Repositories store, among other things, biological specimens in support of NIH clinical studies. All specimens are stored in secure, limited access facilities with sufficient security, back-up and emergency support capability and monitoring to ensure long-term integrity of the specimens for research.

The subcontractor's role is limited to clinical research databases and repositories containing patient specimens. It does not conduct nor has any vested interest in research on human subjects,

but does provide services and supports the efforts of its customers, many of which are involved in research on human subjects. It is the intent and purpose to accept only de-identified samples and sample information. To the best 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 (BSI) System II. This inventory tracking system is used to manage the storage and retrieval of specimens as well as maintain specimen data. BSI is designed for controlled, concurrent access. It provides a real-time, multi-user environment for tracking millions of specimens. The system controls how and in what order database updates and searches are performed. This control prevents deadlocks and race conditions. For security, BSI has user password access, 3 types of user access levels, and 36 user permissions (levels of access) that can be set to control access to the system functions. BSI provides audit tracking for processes that are done to specimens including shipping, returning to inventory, aliquoting, thawing, additives, and other processes. BSI tracks the ancestry of specimens as they are aliquoted, as well as discrepancies and discrepancy resolution for specimens received by the repository. If a specimen goes out of the inventory, the system maintains data associated with the withdrawal 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 withdrawal 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**. All specimens for analysis will be requested from Leidos Biomedical, Inc. and will be delivered by Leidos Biomedical, Inc. couriers to the Laboratory of Tumor Immunology and Biology.

4.3.2 Samples sent to Blood Processing Core (BPC)

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

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

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

Access to stored clinical samples is restricted. Samples will be stored until requested by a researcher named on the protocol. All requests are monitored and tracked in Labmatrix. All researchers are required to sign a form stating that the samples are only to be used for research purposes associated with this trial (as per the IRB approved protocol) and that any unused samples must be returned to the BPC. It is the responsibility of the NCI Principal Investigator to ensure that the samples requested are being used in a manner consistent with IRB approval.

4.3.3 Protocol Completion/Sample Destruction

All specimens obtained in the protocol are used as defined in the protocol. Any specimens that are remaining at the completion of the protocol will be stored in the conditions described above. The study will remain open so long as sample or data analysis continues. Samples from consenting subjects will be stored until they are no longer of scientific value or if a subject withdraws consent for their continued use, at which time they will be destroyed. Once primary research objectives for the protocol are achieved, intramural researchers can request access to remaining samples providing they have an IRB approved protocol and patient consent.

The PI will report any loss or unanticipated destruction of samples as a deviation. Reporting will be per the requirements of section **6.2**.

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

5 DATA COLLECTION AND EVALUATION

5.1 DATA COLLECTION

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

All adverse events, including clinically significant abnormal findings on laboratory evaluations, regardless of severity, will be followed until return to baseline or stabilization of event.

Document AEs from the first study intervention, Study Day 1, through 30 days after last dose of study treatment. Beyond 30 days, only adverse events that are serious and related to study intervention need to be recorded.

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

- Results in discontinuation from the study
- Is associated with clinical signs or symptoms
- Requires treatment or any other therapeutic intervention
- Is associated with death or another serious adverse event, including hospitalization.
- Is judged by the Investigator to be of significant clinical impact

If any abnormal laboratory result is considered clinically significant, the investigator will provide details about the action taken with respect to the test drug and about the patient's outcome.

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

Loss or destruction of data: Should we become aware that a major breach in our plan to protect subject confidentiality and trial data has occurred, this will be reported expeditiously per requirements in section **6.2.1**.

As the toxicity profile of Enzalutamide, PROSTVAC-V TRICOM and PROSTVAC-F TRICOM is well defined and published, grade 1 clinical adverse events will not be recorded in the database.

Data collection is the responsibility of the clinical trial staff at the site under the supervision of the PI. During the study, the PI must maintain complete and accurate documentation for the study. The PI is responsible to ensure the accuracy, completeness, legibility, and timeliness of the protocol research nurse will collect data according to standardized research nursing procedures. Quality assurance requires maintaining complete records on each patient treated on the protocol, including primary documentation (e.g., laboratory reports, x-ray reports, scan reports, pathology reports, physician notes, etc.) that confirms the following:

- The patient met each eligibility criterion.
- Signed informed consent was obtained prior to treatment. (An on-study confirmation of eligibility form will be filled out before entering the study.)
- Documentation of specific dates and times of all treatments, doses administered, and the reason for any dose modification.
- Toxicity was assessed according to protocol (see section **5.4**).
- Response was assessed according to protocol (x-ray, scan, lab reports, dated notes on measurements, and clinical assessment, as appropriate).
- NCI Drug Accountability Records were kept for each patient.

Clinical data will be entered in a secure electronic database and hard copies will be stored in locked, secured areas. Completed eligibility checklists, patient information/registration forms, and blood sample flow sheets will also be stored. Copies of all records of adverse events will be kept in the research record.

Complete records must be maintained on each patient, including the hospital chart with any supplementary information obtained from outside laboratories, radiology reports, or physician's records. These records will serve as the primary source material that forms the basis for the research record. All relevant data will also be entered in a computer database from which formal analyses will be done.

The primary source documentation will include: on-study information, including patient eligibility data and patient history; flow sheets, records of adverse events, specialty forms for pathology, radiation, or surgery; and off-study summary sheets, including a final assessment by the treating physician.

5.2 DATA SHARING PLANS

5.2.1 Human Data Sharing Plan

What data will be shared?

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

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

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

_X_ Identified or coded, linked data with approved outside collaborators under appropriate agreements

How and where will the data be shared?

Data will be shared through:

_X_ An NIH-funded or approved public repository: www.clinicaltrials.gov

_X_ BTRIS (automatic for activities in the Clinical Center)

_X_ Approved outside collaborators under appropriate individual agreements

_X_ Publication and/or public presentations

When will the data be shared?

_X_ Before publication

_X_ At the time of publication or shortly thereafter

5.3 RESPONSE CRITERIA

5.3.1 Antitumor effect – Solid Tumors

Restaging bone scans and CT scan of chest, abdomen and pelvis will be obtained **every 12 weeks** (refer to Study Calendar in section **3.4**)**.** For patients who have stable disease beyond 2 years and a PSA less than 1.0 ng/ml, they can defer restaging scans based on investigator discretion.

Response and progression will be evaluated in this study using the new international criteria proposed by the revised Response Evaluation Criteria in Solid Tumors (RECIST) guideline (version 1.1) [40]. Changes in the largest diameter (unidimensional measurement) of the tumor lesions and the shortest diameter in the case of malignant lymph nodes are used in the RECIST criteria.

For exploratory purpose, changes in PSA and measurable lesions will be analyzed for efficacy according to the PCWG2 recommendations[23] . The recommended PSA progressions criteria will not be applied to the study as the criteria are arbitrarily proposed and do not necessarily reflect overall disease status. PSA values will be captured at each visit and PSA declines and progression will be followed. PSA is not sufficient in the evaluation of disease progression in this patient population. This is consistent with the recent recommendations by the Prostate Cancer Clinical Trials Working Group 2 [23]. Progression will be determined by radiographic evidence as discussed below or by clinical symptoms (symptomatic clinical progression).

5.3.2 Definitions

Evaluable for toxicity: All patients will be evaluable for toxicity from the time of their first treatment with enzalutamide with or without PSA-TRICOM.

Evaluable for objective response:

All of the patients who met the eligibility criteria (with the possible exception of those who received no study medication) are evaluable for response. Patients that have: progressive disease, early death from malignant disease, early death from toxicity, early death because of other cause, or unknown (not assessable, insufficient data) should be considered to have a treatment failure (disease progression). Thus, an incorrect treatment schedule or drug administration does not result in exclusion from the analysis of the response rate.

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

5.3.3 Disease Parameters

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

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

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

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

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

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

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

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

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

5.3.4 Methods for Disease Evaluation

5.3.4.1 For Measurable Disease

All measurements should be taken and recorded in metric notation using a ruler or calipers. All baseline evaluations should be performed as closely as possible to the beginning of treatment and never more than 4 weeks before the beginning of the treatment.

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

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

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

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

Use of MRI remains a complex issue. MRI has excellent contrast, spatial, and temporal resolution; however, there are many image acquisition variables involved in MRI, which greatly impact image quality, lesion conspicuity, and measurement. Furthermore, the availability of MRI is variable globally. As with CT, if an MRI is performed, the technical specifications of the scanning sequences used should be optimized for the evaluation of the type and site of disease. Furthermore, as with CT, the modality used at follow-up should be the same as was used at baseline and the lesions should be measured/assessed on the same pulse sequence. It is beyond

the scope of the RECIST guidelines to prescribe specific MRI pulse sequence parameters for all scanners, body parts, and diseases. Ideally, the same type of scanner should be used and the image acquisition protocol should be followed as closely as possible to prior scans. Body scans should be performed with breath-hold scanning techniques, if possible.

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

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

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

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

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

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

a. Negative FDG-PET at baseline, with a positive FDG-PET at follow-up is a sign of PD based on a new lesion.
b. No FDG-PET at baseline and a positive FDG-PET at follow-up: If the positive FDG-PET at follow-up corresponds to a new site of disease confirmed by CT, this is PD. If the positive FDG-PET at follow-up is not confirmed as a new site of disease on CT, additional follow-up CT scans are needed to determine if there is truly progression occurring at that site (if so, the date of PD will be the date of the initial abnormal FDG-PET scan). If the positive FDG-PET at follow-up corresponds to a pre-existing site of disease on CT that is not progressing on the basis of the anatomic images, this

is not PD.

c. FDG-PET may be used to upgrade a response to a CR in a manner similar to a biopsy in cases where a residual radiographic abnormality is thought to represent fibrosis or scarring. The use of FDG-PET in this circumstance should be prospectively described in the protocol and supported by disease-specific medical literature for the indication. However, it must be acknowledged that both approaches may lead to false positive CR due to limitations of FDG-PET and biopsy resolution/sensitivity.

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

5.3.4.2 For Metastatic Bone Lesions on Bone Scan

Disease progression is considered if a minimum of two new lesions is observed on bone scan. New lesions seen on first re-staging (at 3 months) may represent disease that was not detected on the baseline scan. In these circumstances, the first re-staging scan then will serve as the baseline comparison for future scans. Patient can be removed at first re-staging at the discretion of the investigator if the clinical scenario is most consistent with disease progression and not "flare" on bone scan. This is consistent with Prostate Cancer Working Group 2 recommendations. [23].

5.3.5 Response Criteria

5.3.5.1 Evaluation of Target Lesions

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

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

Progressive Disease (PD): At least a 20% increase in the sum of the diameters of target lesions, taking as reference the smallest sum on study (this includes the baseline sum if that is the smallest on study). In addition to the relative increase of 20%, the sum must also demonstrate an absolute increase of at least 5 mm. (Note: the appearance of one or more new lesions can also be considered progression if they meet they meet size criteria for target lesions. Smaller, equivocal lesions will be followed to determine their significance and can be used to determine progression if their size continues to increase).

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

5.3.5.2 Evaluation of Non-Target Lesions

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

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

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

Progressive Disease (PD): Appearance of one or more new lesions and/or *unequivocal progression* of existing non-target lesions. *Unequivocal progression* should not normally trump

target lesion status. It must be representative of overall disease status change, not a single lesion increase.

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

5.3.5.3 Clinical Disease Progression

- The need for palliative radiotherapy for cancer related pain
- The need for chemotherapy or other change in therapy based on increased cancer related symptoms
- Worsening ECOG PS to a PS of 3 or 4 based on cancer (not treatment) related symptoms
- Clinical disease progression can also be determined based on the clinical discretion of the investigator

5.3.5.4 Evaluation of Best Overall Response

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

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

Target Lesions	Non-Target Lesions	New Lesions	Overall Response	Best Overall Response when Confirmation is Required*
CR	CR	No	CR	≥4 wks. Confirmation**
CR	Non-CR/Non-PD	No	PR	≥4 wks. Confirmation**
CR	Not evaluated	No	PR	
PR	Non-CR/Non-PD/not evaluated	No	PR	
SD	Non-CR/Non-PD/not evaluated	No	SD	Documented at least once ≥4 wks. from baseline**
PD	Any	Yes or No	PD	no prior SD, PR or CR

Target Lesions	Non-Target Lesions	New Lesions	Overall Response	Best Overall Response when Confirmation is Required*
Any	PD***	Yes or No	PD	
Any	Any	Yes	PD	

* See RECIST 1.1 manuscript for further details on what is evidence of a new lesion.

** Only for non-randomized trials with response as primary endpoint.

*** In exceptional circumstances, unequivocal progression in non-target lesions may be accepted as disease progression.

Note: Patients with a global deterioration of health status requiring discontinuation of treatment without objective evidence of disease progression at that time should be reported as "*symptomatic deterioration."* Every effort should be made to document the objective progression even after discontinuation of treatment.

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

Non-Target Lesions	New Lesions	Overall Response
CR	No	CR
Non-CR/non-PD	No	Non-CR/non-PD*
Not all evaluated	No	not evaluated
Unequivocal PD	Yes or No	PD
Any	Yes	PD

* 'Non-CR/non-PD' is preferred over 'stable disease' for non-target disease since SD is increasingly used as an endpoint for assessment of efficacy in some trials so to assign this category when no lesions can be measured is not advised

5.3.6 Duration of Response

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

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

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

5.3.7 Time to Progression

Time to progression (TTP) is defined as the duration of time from start of treatment to time of disease progression.

5.4 TOXICITY CRITERIA

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

6 NIH REPORTING REQUIREMENTS/DATA AND SAFETY MONITORING PLAN

6.1 DEFINITIONS

Please refer to definitions provided in Policy 801: Reporting Research Events found **here**.

6.2 OHSRP OFFICE OF COMPLIANCE AND TRAINING/ IRB REPORTING

6.2.1 Expedited Reporting

Please refer to the reporting requirements in Policy 801: Reporting Research Events and Policy 802 Non-Compliance Human Subjects Research found **here**. Note: Only IND Safety Reports that meet the definition of an unanticipated problem will need to be reported per these policies.

6.2.2 IRB Requirements for PI Reporting at Continuing Review

Please refer to the reporting requirements in Policy 801: Reporting Research Events found **here**.

6.3 NCI CLINICAL DIRECTOR REPORTING

Problems expeditiously reported to the OHSRP in iRIS will also be reported to the NCI Clinical Director. A separate submission is not necessary as reports in iRIS will be available to the Clinical Director.

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

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

6.4 INSTITUTIONAL BIOSAFETY COMMITTEE (IBC) REPORTING CRITERIA

6.4.1 Serious Adverse Event Reports to IBC

The Principal Investigator (or delegate) will notify IBC of any unexpected fatal or life-threatening experience associated with the use of 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 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.

6.4.2 Annual Reports to IBC

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

6.4.2.1 Clinical Trial Information

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

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

6.4.2.2 Progress Report and Data Analysis

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

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

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

6.5 NIH Required Data and Safety Monitoring Plan

6.5.1 Principal Investigator/Research Team

The Principal Investigator, lead associate investigator and the research nurse will meet at least 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 NIH'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. Events meeting requirements for expedited reporting as described in section **6.2.1** will be submitted within the appropriate timelines.

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.

6.5.2 Safety Monitoring Committee (SMC)

This protocol will be periodically reviewed by an intramural Safety Monitoring Committee (SMC). Initial review will occur as soon as possible after the annual NIH Intramural 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 based on the risks presented in the study. For initial and subsequent reviews, protocols will not be reviewed if there is no accrual within the review period.

The SMC review will focus on unexpected protocol-specific safety issues that are identified during the conduct of the clinical trial.

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.

SMC concluded their oversight of the study on 12/20/2016 when the study closed to accrual.

7 SPONSOR PROTOCOL SAFETY REPORTING

7.1 Definitions

7.1.1 Adverse Event

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

7.1.2 Serious Adverse Event (SAE)

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

- Death,
- A life-threatening adverse event (see section **7.1.3)**
- Inpatient hospitalization or prolongation of existing hospitalization
    - A hospitalization/admission that is pre-planned (i.e., elective or scheduled surgery arranged prior to the start of the study), a planned hospitalization for pre-existing condition, or a procedure required by the protocol, without a serious deterioration in health, is not considered a serious adverse event.
    - A hospitalization/admission that is solely driven by non-medical reasons (e.g., hospitalization for patient convenience) is not considered a serious adverse event.
    - Emergency room visits or stays in observation units that do not result in admission to the hospital would not be considered a serious adverse event. The reason for seeking medical care should be evaluated for meeting one of the other serious criteria.
- Persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- A congenital anomaly/birth defect
- Important medical events that may not result in death, be life-threatening, or require hospitalization may be considered a serious adverse drug experience when, based upon appropriate medical judgment, they may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in this definition.

7.1.3 Life-threatening

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

7.1.4 Severity

The severity of each Adverse Event will be assessed utilizing the CTCAE version 5.0.

7.1.5 Relationship to Study Product

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

- Related – There is a reasonable possibility that the study product caused the adverse event. Reasonable possibility means that there is evidence to suggest a causal relationship between the study product and the adverse event.
- Not Related – There is not a reasonable possibility that the administration of the study product caused the event.

7.2 ASSESSMENT OF SAFETY EVENTS

AE information collected will include event description, date of onset, assessment of severity and relationship to study product and alternate etiology (if not related to study product), date of resolution of the event, seriousness and outcome. The assessment of severity and relationship to the study product will be done only by those with the training and authority to make a diagnosis and listed on the Form FDA 1572 as the site principal investigator or sub-investigator. AEs occurring during the collection and reporting period will be documented appropriately regardless of relationship. AEs will be followed through resolution.

SAEs will be:

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

For timeframe of recording adverse events, please refer to section **5.1**. All serious adverse events recorded from the time of first investigational product administration must be reported to the sponsor with the exception of any listed in section **7.4**.

7.3 REPORTING OF SERIOUS ADVERSE EVENTS

Any AE that meets protocol-defined serious criteria or meets the definition of Adverse Event of Special Interest that require expedited reporting must be submitted immediately (within 24 hours of awareness) to OSRO Safety using the CCR SAE report form. Any exceptions to the expedited reporting requirements are found in section **7.4**.

All SAE reporting must include the elements described in section **7.2**.

SAE reports will be submitted to the Center for Cancer Research (CCR) at: OSROSafety@mail.nih.gov and to the CCR PI and study coordinator. CCR SAE report form and instructions can be found at: https://ccrod.cancer.gov/confluence/display/CCRCRO/Forms+and+Instructions

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

7.4 WAIVER OF EXPEDITED REPORTING TO CCR

As death due to disease progression is part of the study objectives, and captured as an endpoint in this study, it will not be reported in expedited manner to the sponsor. However, if there is evidence suggesting a causal relationship between the study drug and the event, report the event in an expedited manner according to section **7.3**.

7.5 SAFETY REPORTING CRITERIA TO THE PHARMACEUTICAL COLLABORATORS

7.5.1 Reporting to Astellas

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

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

The Investigator should complete and submit an SAE Medwatch 3500 Form, containing all information that is required by the Regulatory Authorities, to 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.

7.5.2 Reporting to Bavarian Nordic, Inc

The investigator should also submit all Medwatch 3500 Forms that are submitted to the FDA to:

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

7.6 REPORTING PREGNANCY

All required pregnancy reports/follow-up to OSRO will be submitted to: OSROSafety@mail.nih.gov and to the CCR PI and study coordinator. Forms and instructions can be found here:
https://ccrod.cancer.gov/confluence/display/CCRCRO/Forms+and+Instructions

7.6.1 Paternal Exposure

Male patients should refrain from fathering a child or donating sperm during the study and for three (3) months after the last dose of enzalutamide.

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 three (3) months after the last dose should, if possible, be followed up and documented.

7.7 REGULATORY REPORTING FOR STUDIES CONDUCTED UNDER CCR SPONSORED IND

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

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

7.8 SPONSOR PROTOCOL DEVIATION REPORTING

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

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

In addition, any deviation to the protocol should be documented in the participant's source records and reported to the reviewing IRB per their guidelines. OSRO required protocol deviation reporting is consistent with E6(R2) GCP: Integrated Addendum to ICH E6(R1): 4.5 Compliance with Protocol; 5.18.3 (a), and 5.20 Noncompliance; and ICH E3 16.2.2 Protocol deviations.

8 CLINICAL MONITORING

Clinical site monitoring is conducted to ensure:

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

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

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

to the protocol, significant or pervasive non-compliance with GCP, or the protocol may trigger CMP updates.

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

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

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

9 STATISTICAL CONSIDERATIONS

The primary objective is to determine if PSA-TRICOM combined with enzalutamide will increase time to progression in chemotherapy-naïve metastatic castration resistant prostate cancer patients compared to enzalutamide alone.

Secondary objectives include determining if PSA-TRICOM combined with enzalutamide will increase overall survival and/ or delay PSA progression compared to enzalutamide alone, and. Tumor growth rates at various time points will also be determined and compared as a secondary objective.

Exploratory objectives include comparing the immune response in patients between the two arms. Immune response evaluation will include CD4 cells, CD 8 cells, PSA-specific T-cells, natural killer cells, Regulatory T-cells, Regulatory T-cell function, cytokines, naïve thymic emigrants. The study will also aim to determine the association between immunologic parameters and clinical outcomes

The study is based on an assumption of a median time to progression of 10 months in chemotherapy-naïve mCRPC patients. Should the PREVAIL trial results be reported prior to the opening of this trial, and if they are substantially different from this estimate, adjustments in this plan will be made accordingly. The primary endpoint will be the comparison of the Kaplan-Meier curves for the two arms using a stratified log-rank test. The trial will be conducted using a phase 2.5 design (one tailed 0.10 alpha level test) [41].

If we assume that the arms will have time to progression medians of 10 vs. 18 cycles (which would have hazards of 0.0693 and 0.0385, respectively, and a hazard ratio of 1.80), and if patients are accrued for 36 months and the last one enrolled is followed for up to 12 more months (48 months total follow-up from time of enrolling first patient), and a total of 52 events are observed, then 36 patients per arm provides 80% power to compare the two arms.

Patients will be stratified based on previous immunotherapy used as cancer treatment.

Survival will be compared between the two arms, as a secondary endpoint, using Kaplan-Meier curves and a stratified log-rank test. Continuous parameters will be compared between the two arms using appropriate non-parametric tests, such as a Wilcoxon rank sum test. Immune outcomes and clinical outcomes will be compared using appropriate measures, such as Spearman correlation, Fisher's exact test, or Wilcoxon rank sum test. All secondary outcomes will be reported without formal adjustment for multiple comparisons but in the context of the number of such tests performed.

In order to allow for a small number of inevaluable patients, up to 76 total patients may be enrolled in order to yield 72 total evaluable patients. If the study is able to accrue approximately 2 patients per month, then accrual can be completed in approximately 3 years.

This projected accrual time frame is based on a previous trial in mCRPC which recently enrolled at the NCI. Trial 11-C-0195 enrolled 52 patients in under 2 years, therefore it would be feasible to accrue the number of patients required in this design.

9.1 EARLY STOPPING RULE

After 50% of the patients (36 total) have been enrolled and followed potentially for 12 months, if the vaccine arm demonstrates the same or worse time to progression than the enzalutamide alone arm, that is, if the arms substantially overlap or if the enzalutamide alone arm appears to have better outcomes than the combination arm, then no further patients will be accrued.

10 COLLABORATIVE AGREEMENTS

10.1 AGREEMENT TYPE

10.1.1 Cooperative Research and Development Agreement (CRADA):

- This study is conducted under a CRADA with Bavarian Nordic (CRADA # 02377)
- This study is conducted under a CRADA with Astellas and Medivation (CRADA # 02859)

10.1.2 Material Transfer Agreement

- A Material Transfer Agreement is in place with Epic Sciences for the studies discussed in section **4.1.4**
- A Material Transfer Agreement is in place with The Cleveland Clinic Foundation for the studies discussed in section **4.1.6**

11 HUMAN SUBJECTS PROTECTIONS

11.1 RATIONALE FOR SUBJECT SELECTION

11.1.1 Selection Based on Ethnicity, and Race

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

are noted, accrual may be expanded or a follow-up study may be written to investigate those differences more fully. Women are not eligible for this protocol as this disease occurs only in men.

11.1.2 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 will be excluded.

11.2 Participation of Children

Because no dosing or adverse event data are currently available on the use of PSA-TRICOM Vaccine or enzalutamide in patients <18 years of age, children are excluded from this study, but may be eligible for future pediatric trials.

11.3 Participation of Subjects unable to Give Consent

Adults unable to give consent are excluded from enrolling in the protocol. However, re-consent may be necessary and there is a possibility, though unlikely, that subjects could become decisionally impaired. For this reason and because there is a prospect of direct benefit from research participation (section **11.4**), all subjects will be offered the opportunity to fill in their wishes for research and care, and assign a substitute decision maker on the "NIH Advance Directive for Health Care and Medical Research Participation" form so that another person can make decisions about their medical care in the event that they become incapacitated or cognitively impaired during the course of the study. Note: The PI or AI will contact the NIH Ability to Consent Assessment Team (ACAT) for evaluation to assess ongoing capacity of the subjects and to identify an LAR, as needed.

Please see section **11.5.1**.

11.4 Evaluation of Benefits/Risks/Discomforts

11.4.1 Alternative Approaches or Treatments

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

11.4.2 Procedure for Protecting Against or Minimizing any Potential Risks

All care will be taken to minimize side effects, but they can be unpredictable in nature and severity. This study may involve risks to patients, which are currently unforeseeable. All patients will have blood tests, examinations and scans as described in the protocol evaluation (Section **3.4** Study Calendar). Patients will also be required to have a local physician to provide long-term care and to monitor for complications. If patients suffer any physical injury as a result of the participation in this study, immediate medical treatment is available at the Clinical Center, National Cancer Institute, Bethesda, Maryland. Although no compensation is available, any injury will be evaluated and treated in keeping with the benefits or care to which patients are entitled under applicable regulations.

11.4.3 Provisions for Monitoring Data Collection to Ensure Safety of Subjects

As information is gathered from this trial, clinical results will be shared with patients as they become available. 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 and/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.

11.5 CONSENT PROCESS AND DOCUMENTATION

The informed consent document will be provided as a physical or electronic document to the participant or consent designee(s) as applicable for review prior to consenting. A designated study investigator will carefully explain the procedures and tests involved in this study, and the associated risks, discomforts, and benefits. 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.

In order to minimize potential coercion, as much time as is needed to review the document will be given, including an opportunity to discuss it with friends, family members, and/or other advisors, and to ask questions of any designated study investigator. A signed informed consent document will be obtained prior to entry onto the study.

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

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

<u>Manual (non-electronic) signature on electronic document:</u>

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

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

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

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

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

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

For participants addressed in section **11.3**, an LAR will be identified consistent with Policy 403 and informed consent obtained from the LAR, as described in Section **11.5.1**.

12 REGULATORY AND OPERATIONAL CONSIDERATIONS

12.1 STUDY DISCONTINUATION AND CLOSURE

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

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

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

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

12.2 QUALITY ASSURANCE AND QUALITY CONTROL

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

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

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

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

12.3 CONFLICT OF INTEREST POLICY

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

12.4 CONFIDENTIALITY AND PRIVACY

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

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

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

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

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

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

the research objectives and promote participation in studies by helping assure confidentiality and privacy to participants.

13 PHARMACEUTICAL INFORMATION

13.1 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.

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.

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.

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

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

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

Special Handling

Fowlpox virus is classified as a Biosafety Level 1 agent. These agents are not known to cause disease in healthy human adults and are of minimal potential hazard to personnel and the environment under ordinary conditions of use. Clinicians can use techniques generally acceptable

for nonpathogenic material. The recombinant vaccine is a preparation of a live virus (infectious for birds) containing DNA sequences derived from the human genome. Handle the recombinant vaccine as a hazardous biological substance and dispose of waste materials as hazardous biological waste, with incineration according to local institutional policy and according to local, state, and federal regulations. Healthcare workers handling the recombinant fowlpox vaccine should avoid direct contact with pet birds for at least 72 hours after working with the agent.

13.1.1 Preparation, Handling and Disposal Recommendations

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

- 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*

13.1.2 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.

13.2 RECOMBINANT VACCINIA-PSA(L155)/TRICOM™

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

13.2.1 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.

13.2.2 How Supplied

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

13.2.3 Preparation

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

13.2.4 Storage

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

13.2.5 Stability

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

13.2.6 Route of Administration

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

13.2.7 Special Handling and Precautions

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

incineration according to local institutional policies and according to local, state, and federal regulations.

13.2.8 Preparation, Handling and Disposal Recommendations

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

gauze soaked in 10% bleach solution, or other appropriate disinfectant, rinsing, then wiping down with sterile gauze soaked in 70% alcohol. Following decontamination, dispose of personal protective apparel in the biohazard safety bag.

17. Place all waste and protective apparel in a leak proof biohazard bag, and place the bag inside a biohazard sharps container for incineration according to institutional policy and according to local, state, and federal regulations.
18. Handle accidental spills similarly to antineoplastic spills and/or according to institutional policy:
    a. Prevent others from entering the area and allow aerosols time to settle (approximately 10 minutes).
    b. Use protective apparel, eyewear, mask, and gloves.
    c. Cover spills with disposable absorbent towels.
    d. Decontaminate the area with 10% bleach solution, or other appropriate disinfectant suitable for decontamination, allowing approximately a 20-minute contact time.
    e. Dispose of all waste and protective apparel as infectious biohazardous waste and incinerate according to institutional policy and according to local, state, and federal regulations.
19. Immediately report spills and accidents resulting in overt exposure to recombinant DNA molecules to the Institutional Biosafety Committee and NIH/OSP (Office of Science Policy). Send reports to the Office of Science Policy, National Institutes of Health, 6705 Rockledge Drive, Suite 750, MSC 7985, Bethesda, MD 20892-7985. Phone (301) 496-9838. 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*

13.2.9 Precautions for Healthcare Workers

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

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

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

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

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

13.2.10.1 Patient Care Implications and Contraindications

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

vaccination, their close household contacts (close household contacts are those who share housing or have close physical contact):

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

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

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

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

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

13.2.11 Potential Complications Associated With Recombinant Vaccinia Vaccination

When vaccinia vaccine is administered by scarification for vaccination against smallpox, expected local reactions in individuals that have not previously been vaccinated with vaccinia include the appearance of a red papule in 3-4 days, followed by vesiculation in 5-6 days, and then the

formation of a pustule on days 8-9. A large area of erythema may surround the vesicle and pustule. A crusted scab usually forms by the second week and sloughs by the third week, leaving a well-formed scar. Maximal viral shedding occurs from days 4-14, but can continue until the scab is shed from the skin. Other normal local reactions can include development of local satellite lesions, regional lymphadenopathy that can persist for weeks following healing of the skin lesion, considerable local edema, and intense inflammation from the vaccination (*i.e.*, viral cellulites), which may be confused with bacterial cellulites. Systemic symptoms typically occur between 8-10 days post-vaccination and include fever, malaise, headache, chills, nausea, myalgia, local lymphadenopathy, soreness and intense erythema surrounding the vaccination site.

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

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

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

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

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

Information has been reported by the US Department of Defense (DoD) during the post-vaccination surveillance assessment of adverse events in military personnel following implementation of a Smallpox Vaccination Program from the period of December 13, 2002 through May 28, 2003. Although not directly comparable to historical numbers, due to differences in multiple population variables, estimated cases (number of cases per million

vaccinations based on vaccination of 450,293 personnel, with a median age of 26 years and 70.5% as primary vaccinees) of these same complications per million vaccinations were:

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

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

1. **Non-specific erythematous or urticarial rashes:** These rashes can appear approximately 10 days after vaccination and may sometimes be confused with generalized vaccinia, but are generally self-limiting. Patients are usually afebrile and these benign rashes usually resolve spontaneously within 2-4 days. Erythema multiforme can present as different types of lesions, including macules, papules, urticaria, and bull's eye lesions (dark papule or vesicle surrounded by a pale zone and an area of erythema). These lesions may be extremely pruritic, lasting up to four weeks. Rarely, more serious bullous erythema multiforme (Stevens-Johnson syndrome) may occur, requiring hospitalization. Vaccinia Immune Globulin (VIG) therapy is not indicated to treat these rashes. Supportive care measures are warranted since these rashes are likely manifestations of an immune response or hypersensitivity reaction to the vaccine and are not likely to contain vaccinia virus.
2. **Bacterial Infection:** Vaccination site infection, most likely due to staphylococcus and streptococcus normal skin flora, is rare. Onset is approximately 5 days post-vaccination and is more common in children. Appropriate antibiotic therapy is required.
3. **Inadvertent Inoculation**: This can occur in the vaccinee (autoinoculation) as well as in close contacts (contact transmission). Accidental infection is the most common complication of vaccinia vaccination, accounting for approximately 50% of all complications associated with vaccination and revaccination. This usually results from autoinoculation of vaccinia virus transferred from the site of the vaccination. Sites typically involved include the face, eyelids, nose, mouth, genitalia, or rectum, but can also involve the arms, legs, and trunk. Contact transmission of vaccinia, with accompanying toxicities, may occur when a recently vaccinated individual has contact with a susceptible individual. In a 1968 ten-state survey, contact transmissions were reported to occur at a rate of 27 infections per million vaccinations. The age group in which contact transmission occurred most commonly was in children ≤ 5 years. Eczema vaccinatum as a result of contact transmission may result in a more severe syndrome than that seen in vaccinees, perhaps because of multiple simultaneous inoculations. About 30% of eczema vaccinatum cases reported in the 1968 ten-state survey were a result of contact transmission. It is possible

that the number of cases of contact transmission would be greater in today's population, due to a largely unvaccinated patient population against smallpox. Contact transmission rarely results in postvaccinial encephalitis or progressive vaccinia. Most cases of inadvertent inoculation usually resolve without specific therapy and resolution of lesions follow the same course as the vaccination site in immunocompetent individuals. VIG can be used for severe cases involving extensive lesions or if comorbid conditions exist. VIG is contraindicated in the presence of isolated keratitis due to the risk of increased corneal scarring. VIG use can be considered in cases of ocular implantation, with keratitis, if vision-threatening or if other life-threatening vaccinia-related complications exist that require VIG therapy.

4. **Generalized vaccinia:** Generalized vaccinia (GV) is characterized by a disseminated maculopapular or vesicular rash of varying extent on any part of the body and typically develops 6-9 days after vaccination. The lesions follow the same course as the vaccination site lesion. The lesions are hematogenously spread and may contain vaccinia virus. In immunocompetent individuals, the rash is generally self-limiting and requires supportive care therapy. VIG treatment can be used in severe cases for patients who are systemically ill and whose condition might be toxic or who have serious underlying immunosuppressive illnesses.

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

5. **Eczema vaccinatum:** Eczema vaccinatum is a serious complication in persons with eczema and other types of chronic or exfoliative skin conditions. It can also occur among eczematous contacts of recently vaccinated persons. Vaccinial lesions (generalized papular, vesicular or pustular lesions) develop on areas of the skin that are, or had at one time been, eczematous. These areas become highly inflamed and lesions may spread to

healthy skin. The rash is often accompanied by fever and individuals are systemically ill. The fatality rate for untreated cases (prior to availability of VIG) has been reported from 30-40%. Following availability of VIG, mortality was reduced to approximately 7%. Early diagnosis and prompt treatment with VIG is necessary to reduce mortality.

6. **Progressive vaccinia:** Progressive vaccinia is the most serious cutaneous complication, occurring when the local vaccination lesion fails to heal and develops progressive necrosis, with destruction of large areas of skin, subcutaneous tissue, and underlying structures. Progressive lesions may spread to other skin surfaces and to bone and viscera. Progressive vaccinia is associated with a high mortality rate. This complication has been seen in patients with a compromised immune system due to a congenital deficiency, lymphoproliferative disease, immunosuppressive treatment, or HIV infection. Management should include aggressive VIG therapy.
7. **Post-Vaccinial Encephalitis/Encephalomyelitis**: Vaccinial complications affecting the CNS are unpredictable. Post-vaccinial encephalitis typically affects children < 2 years of age and is characterized by an onset of symptoms 6-10 days following vaccination, which include seizures, hemiplegia, aphasia, and transient amnesia. Histopathological changes include generalized cerebral edema, mild lymphocytic meningeal infiltration, ganglion degenerative changes and perivascular hemorrhages. Older children and adults can develop encephalitis or encephalomyelitis characterized by an onset of symptoms 11-15 days following vaccination, which include fever, vomiting, headache, malaise, and anorexia, progressing to loss of consciousness, amnesia, confusion, disorientation, restlessness, delirium, drowsiness, seizures and coma. Histopathological changes include demyelination with lymphocytic infiltration, but limited cerebral edema. Mortality rates have ranged from 15-25%, with 25% of patients who recover being left with varying degrees and types of neurological deficits. VIG has not been shown to be effective in treating CNS disease and is not recommended. Post-vaccinial encephalitis/encephalomyelitis are diagnoses of exclusion and are not believed to be a result of replicating vaccinia virus. Although no specific therapy exists, supportive care, anticonvulsants, and intensive care might be required. A review of vaccinia-related deaths (68) during a 9-year period (1959–1966 and 1968) revealed that among first-time vaccines, 36 (52%) patients died as a result of post-vaccinial encephalitis.
8. **Fetal Vaccinia**: Fetal vaccinia is a rare, but serious complication following vaccinia vaccination during pregnancy or shortly before conception (e.g., within four weeks). To date, fewer than 50 cases have been reported and often result in fetal or neonatal death. Efficacy of VIG therapy in a viable infant or used prophylactically in women during pregnancy is unknown. The CDC has established a National Smallpox Vaccine in Pregnancy Registry. This registry will follow women during their pregnancies and their babies, after they are born, to determine the outcome of such pregnancies. The CDC can be contacted at (404) 639-8253.
9. **Myocarditis/Pericarditis:** The CDC has recommended a temporary medical deferral to the voluntary Smallpox Vaccination Program for persons with heart disease or cardiovascular risk factors (March 25, 2003) and issued "interim supplementary information" regarding evidence that smallpox vaccination may cause myocarditis and/or pericarditis (March 31, 2003) in people recently vaccinated with the smallpox vaccine. The cardiac events reported include myocardial infarction, angina, myocarditis, pericarditis, and myopericarditis. Although the CDC believes that there is no evidence to conclude that

the licensed vaccinia vaccine causes angina or heart attacks, it acknowledges a possible causal relationship between the vaccination and heart inflammation. The CDC continues to study the relationship, but in the meantime, recommends that individuals with underlying heart disease be excluded from participation in the current Smallpox Vaccination Program. While it is currently not possible to fully evaluate the risk of cardiac events or the risk of myocarditis, pericarditis, or myopericarditis associated with vaccinia vaccination, it is reasonable to inform patients participating in studies using recombinant vaccinia virus of these reports and provide relevant guidance for evaluating these events. Further investigation from the ongoing vaccine program may provide additional data regarding an association or lack of association with cardiovascular disease. Patients are being immunized with recombinant vaccinia vaccines with therapeutic intent and will be evaluated for cardiovascular risk factors and recent or symptomatic cardiac events per protocol eligibility criteria. Patients will be encouraged to minimize cardiovascular disease risks and encouraged to follow risk reduction according to standard medical practice. At this time the evidence for an association with myocarditis, pericarditis, or myopericarditis seems plausible, but a rare event. If not otherwise excluded, patients with known CHF or clinically significant cardiomyopathy requiring treatment should be excluded from protocol eligibility.

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

13.2.12 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.

13.3 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]. Investigators should obtain cidofovir for second-line therapy through commercial sources if necessary. Investigators should consult the CDC Clinician Information Line at 1-877-554-4625 or the Director's Emergency Operations Center (DEOC) at 770-488-7100 regarding appropriateness of therapy and guidance.

13.4 ENZALUTAMIDE

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

13.4.1 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}z-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 or film-coated tablets 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.

Each tablet contains 40 mg or 80 mg of enzalutamide. The inactive ingredients are hypromellose acetate succinate, microcrystalline cellulose, colloidal silicon dioxide, croscarmellose sodium, and magnesium sterate. The tablet film-coat contains Hypromellose, talc, polyethylene glycol, titanium dioxide, and ferric oxide.

13.4.2 Source

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

13.4.3 Storage

Store enzalutamide capsules and tablets between 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).

13.4.4 Stability

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

13.4.5 Dosage and Administration

Enzalutamide 160 mg administered orally once daily.

Swallow capsules or tablets whole. Do not chew, dissolve, or open the capsules. Do not cut, crush, or chew the tablets. Enzalutamide can be taken with or without food.

13.4.6 Adverse Effects:

13.4.6.1 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.

13.4.6.2 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. Dysgeusia was also reported (i.e., less common – 3.7%). 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. Hypertension was also seen in patients who received enzalutamide relative to placebo (6.6% vs. 3.3%). There have been rare reports of posterior reversible encephalopathy syndrome (PRES), a rare, reversible condition involving the brain, in patients treated with enzalutamide.

13.4.6.3 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.

13.4.6.4 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.

13.4.6.5 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.

13.4.6.6 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.

13.4.7 Drug Interactions

13.4.7.1 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

13.4.7.2 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.

13.4.7.3 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.

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15 APPENDIX A: PERFORMANCE STATUS CRITERIA

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

16 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 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

Physician On Call through NIH page operator (301) 496-1211

17 APPENDIX C: 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
Anti-HBs Antibody Anti-HCV Antibody	1 8 mL SST	CC Department of Transfusion Medicine (DTM)
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)