Abbreviated Title: Prostvac-Nivo in Prostate CA
NIH Protocol #: 17-C-0007
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Title: Phase I/II Study of PROSTVAC in Combination with Nivolumab in Men with Prostate Cancer

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Drug Name	PROSTVAC-V/F	Nivolumab (BMS-936558, MDX-1106, and ONO-4538) (NSC 748726)
IND Number	17104	17104
Sponsor	CCR, NCI	CCR, NCI
Manufacturer	Bavarian Nordic, Inc.	Bristol-Myers Squibb
Supplier	Bavarian Nordic, Inc.	Bristol-Myers Squibb

PRÉCIS

Background:

- Immune checkpoint inhibitors interfere with the immune system's autoregulatory mechanisms, allowing for a potentially expanded and prolonged T-cell response with the possibility of greater antitumor effects.
- Nivolumab is a fully human IgG4 monoclonal antibody that targets the PD-1 protein. Specifically, the antibody binds to the PD-1 receptor and blocks its interaction with PD-L1 and PD-L2, thereby releasing PD-1 pathway-mediated inhibition of the immune response, including anti-tumor immune response.
- PROSTVAC (developed by the National Cancer Institute [NCI] and licensed to Bavarian Nordic Immunotherapeutics, Mountain View, CA) is a therapeutic cancer vaccine for prostate cancer. Early studies have demonstrated immunologic efficacy and suggested clinical benefit. A phase III trial has completed accrual.
- A previous study combining the immune checkpoint inhibitor ipilimumab and PROSTVAC suggested greater efficacy than PROSTVAC alone. Additional studies have demonstrated the potential efficacy of immunologic combination therapy with the immune checkpoint inhibitor nivolumab.
- This study will aim to evaluate the impact of the combination of PROSTVAC and the immune checkpoint inhibitor nivolumab on the tumor microenvironment focusing on immune cell infiltration as the primary endpoint.
- US-MRI imaging technology will be employed to sample the tumor before treatment and after radical prostatectomy.
- The findings from this study could serve as the basis for future studies with this combination in this population of participants and more advanced disease.

Objectives:

- Safety (For castration resistant prostate cancer (CRPC) lead-in cohort)
- Evaluate changes in T-cell infiltration in the tumor after neoadjuvant treatment with PROSTVAC and nivolumab, relative to changes seen in a phase 2 trial with PROSTVAC alone in the neoadjuvant setting- NCT02153918 (For the neoadjuvant cohort).

Eligibility:

- Participants must have histopathological documentation of adenocarcinoma of the prostate prior to starting this study and evaluable biopsy tissue (e.g., unstained slides or blocks) available for analysis.
- For the castration resistant lead in cohort, if histopathological documentation is unavailable, a rising PSA and a clinical course consistent with prostate cancer would be acceptable.
- Participants must have a performance status of 0 to 1 according to the ECOG criteria.
- Hematological eligibility parameters (within 16 days of starting therapy):

- Granulocyte count 1,500/mm^3
- Platelet count 100,000/mm^3
- Hgb ≥ 8 g/dL
- Biochemical eligibility parameters (within 16 days of starting therapy):
- Hepatic function: Bilirubin < 1.5 mg/dl (OR in participants with Gilbert's syndrome, total bilirubin ≤ 3.0 mg/dL), AST and ALT ≤ 2.5 times upper limit of normal.
- Creatinine ≤ 1.5 X ULN

Design:

- The primary focus of this study will be to evaluate PROSTVAC and nivolumab in the neoadjuvant setting.
- Lead-in cohort evaluating the safety and tolerability of this combination in the castration resistant setting (CRPC cohort)
- Following this lead-in cohort in the CRPC setting, we will enroll a cohort in the neoadjuvant setting evaluating the combination of PROSTVAC and nivolumab.
- The lead-in safety cohort will require 10 participants and the neoadjuvant cohort will require 17 evaluable participants. In order to allow for a small number of inevaluable participants, the accrual ceiling will be set to 29 participants.

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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

- Safety (For castration resistant prostate cancer (CRPC) lead-in cohort)
- Evaluate changes in T-cell infiltration in the tumor after neoadjuvant treatment with PROSTVAC and nivolumab, relative to changes seen in a phase 2 trial with PROSTVAC alone in the neoadjuvant setting – NCT02153918 (For neoadjuvant cohort).

1.1.2 Secondary Objectives

- To determine the change in peripheral PSA-specific T cells in participants treated with PROSTVAC and nivolumab.
- To document any intraprostatic T_{reg} cell infiltration with CD4+FOX-P3 staining.
- To document any PSA changes secondary to immune treatment, including rate of biochemical recurrence after prostatectomy.
- To document any MRI changes secondary to treatment (including changes in ADC mapping)
- To evaluate changes in PDL-1 expression in tumor.
- To document pathologic responses (including pathologic CR).
- To evaluate changes in immune cell subsets in the periphery.
- To evaluate changes in soluble immune mediating factors (such as cytokines, etc.) in sera.
- To evaluate changes in circulating tumor cells levels (for CRPC cohort only)
- Safety (for localized prostate cancer cohort)

1.2 BACKGROUND AND RATIONALE

It is expected that 26,120 men will die from prostate cancer in the US in 2016.[1-3] While some men are definitively treated for localized disease, many men will ultimately develop castrate resistant prostate cancer (CRPC) and succumb to the disease; [2] this is due to the lack of an optimal treatment strategy. Treatment options typically involve radical prostatectomy (RP) or radiation therapy (RT) in combination with androgen deprivation therapy (ADT). Following RP upwards of 50% of patients with high-risk disease will experience a biochemical recurrence at 5 years, [4] and approximately 20% will die of their disease in 10-15 years. [5]

1.2.1 Immunotherapy in prostate cancer

Vesalainen *et al* [6] found that the density of tumor-infiltrating lymphocytes in primary prostatic adenocarcinoma was independent of the tumor differentiation (Gleason score), and low numbers of tumor-infiltrating lymphocytes were an independently negative predictor of survival (n=325, P<0.05) suggesting a role the immune system may have in the control of this disease.

Several characteristics of prostate cancer make it an ideal target for immunotherapy. Its relatively indolent disease course allows sufficient time to generate immune responses, which usually take weeks or months to mount. Therapeutic vaccines for prostate cancer offer a therapeutic approach and recent data have renewed interest in this form of treatment for metastatic prostate cancer.[7]

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 [8, 9]. A phase III trial (n = 512) demonstrated an overall survival benefit for the vaccine (25.8 months vs. 21.7 months; P = 0.032)[10]. 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.

1.2.2 PROSTVAC

PROSTVAC, an off-the-shelf therapeutic cancer vaccine, offers an alternative strategy to enhance immune targeting of the tumor antigen, PSA.[11] PROSTVAC 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 cells including 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 cytotoxic T lymphocytes activation.[11] (**Figure 1**) This approach does not require logistically complex, labor-intensive *ex vivo* preparation of patients' peripheral blood. PROSTVAC is thus potentially more feasible and practical over the long-term than sipuleucel-T[12].

Early clinical trials demonstrated that PROSTVAC was well tolerated with common side effects including injection site reactions and flu-like symptoms.[13] PROSTVAC has subsequently been investigated in 2 phase II trials in metastatic CRPC (mCRPC), both of which administered the vaccine at monthly intervals until disease progression. An industry-sponsored, placebo-controlled, multicenter trial in 125 mCRPC patients randomized them 2:1 in favor of PROSTVAC; the placebo was an empty poxviral vector containing no transgenes. As was seen in the sipuleucel-T studies, patients receiving vaccine showed no change in progression free survival, yet had an overall survival benefit (25.1 months with PROSTVAC vs. 16.6 months with placebo; P = 0.0061).[14] (**Figure 2**) A second phase II study of PROSTVAC 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 appeared to have superior outcomes. [15]

The immune impact induced by PROSTVAC has also been described more broadly. Of 104 patients tested for T-cell responses, 57% (59/104) demonstrated a ≥2-fold increase in PSA-specific T cells 4 weeks after vaccine (median 5-fold increase) compared with pre-vaccine. In addition, 68% (19/28) of patients tested mounted post-vaccine immune responses to tumor-associated antigens not present in the vaccine demonstrating the ability of the immune response to expand once generated *in vivo* (antigen spreading).[16] Based on the findings in these early trials, a phase III trial of PROSTVAC in mCRPC is currently underway (NCT01322490).

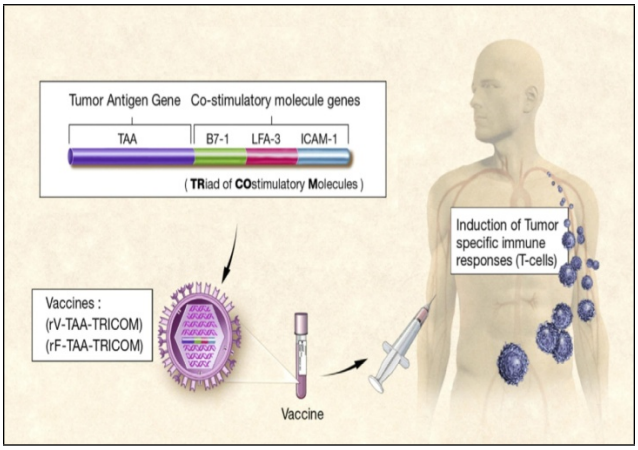

Figure 1. Poxviral vaccine strategy: Modified poxvirus contains transgenes for the tumor-associated antigen PSA and 3 T-cell costimulatory molecules [11]

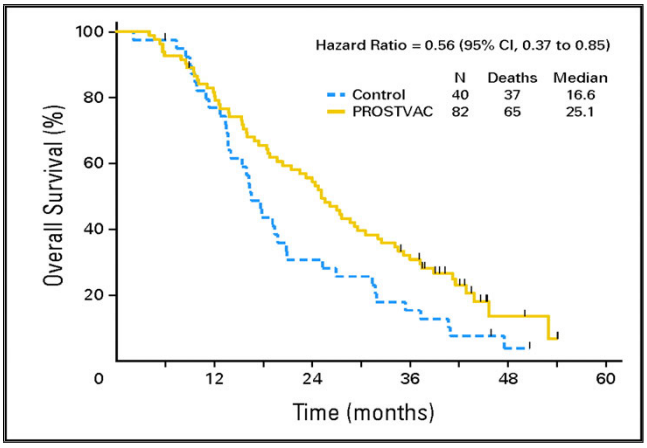

Figure 2. PROSTVAC improved survival in mCRPC patients in a randomized multicenter phase II trial.[14]

1.2.3 Immune Checkpoint Inhibitors

1.2.3.1 Ipilimumab

Immune checkpoint inhibitors interfere with the immune system's autoregulatory mechanisms, allowing for a potentially expanded and prolonged T-cell response with the possibility of greater antitumor effects. [17]

Ipilimumab, a fully human anti-CTLA-4 monoclonal antibody, is a first-in-class immune checkpoint inhibitor. CTLA-4 is expressed on cytotoxic T lymphocytes (CTLs) after activation by APCs. The CTLA-4 receptor on CTLs is a negative regulator of T-cell activation that outcompetes CD28 for binding to B7 on APCs. In contrast to CD28/B7 binding, which acts as a costimulatory signal, the binding of CTLA-4 by ipilimumab removes the physiologic brake, augmenting the immune response by blocking the interaction of CTLA and B7.[17] Preclinical and clinical studies also suggest ipilimumab may also increase T-cell avidity, decrease Tregs within tumor), and expand the T-cell repertoire. [18-20]

Ipilimumab was FDA-approved in 2011 for the treatment of unresectable or metastatic melanoma. The approval was based on a randomized (3:1:1) double-blind clinical trial that demonstrated an OS benefit in patients receiving ipilimumab; but, similar to the earlier trials involving sipuleucel-T and PROSTVAC, there was no short term change in disease progression.[21] Despite the clinical efficacy, because ipilimumab generates a more non-specific immune response, it characteristically has frequent immune related adverse events (irAEs). IrAEs are mechanism-based adverse events and chiefly include skin and mucosal rash, diarrhea/colitis, hepatotoxicity, and hypophysitis.[21] Immunosuppressive agents such as systemic steroids are the standard treatment for many irAEs which are thus reversible. For irAEs that impact endocrine glands, replacement hormones may be required.

Ipilimumab has also been investigated in mCRPC. A recently completed phase III trial compared ipilimumab and limited radiation (serving as an immune enhancing therapy) to limited radiation and placebo. This study was conducted in advanced mCRPC, patients who had progressive disease on docetaxel and thus had only a limited expected survival (approximately 1 year). The study did not meet its primary endpoint of overall survival, but results of this trial showed a median overall survival favoring ipilimumab over placebo (11.2 vs. 10 months; HR 0.85, 95% CI 0.72–1.00; p = 0.053.[22] Interestingly PSA declines of ≥ 50% in evaluable

patients (13.1% vs. 5.3%) favored patients getting ipilimumab. In subset analyses, patients with more indolent disease features had a substantially improved survival if they were treated with ipilimumab compared to placebo (22.7 vs. 15.8 months; n=142; HR 0·62, 95% CI 0·45–0·86; p=0·0038). These results support the concept that immunotherapy given earlier in the course of disease may yield greater benefit. [23] An on-going second phase III trial of ipilimumab vs. placebo in chemotherapy-naïve mCRPC patients has completed accrual and results are anticipated in the next year (NCT01057810). Given that these patients have an earlier stage of disease, the results from this study will suggest whether this is indeed a more appropriate population of patients.

1.2.4 Anti-PD1 and Anti-PDL1

Agents blocking programmed cell death protein-1 (PD-1) and its ligand PD-ligand-1 (PD-L-1) are other examples of immune checkpoint molecules shown to have antitumor activity in solid malignancies. [24, 25] These interventions represent a form of immune checkpoint inhibition disrupting the interaction between PD1 (e.g., on immune cells) and PDL1 (e.g. on tumor cells).[26]

1.2.4.1 Nivolumab

Nivolumab is a fully human IgG4 monoclonal antibody that targets the PD-1 protein. Specifically, the antibody binds to the PD-1 receptor and blocks its interaction with PD-L1 and PD-L2, thereby releasing PD-1 pathway-mediated inhibition of the immune response, including anti-tumor immune response.

Nivolumab was granted accelerated approval by the US FDA in December of 2014 for the treatment of patients with unresectable or metastatic melanoma and disease progression following ipilimumab and, if BRAF V600 mutation positive, a BRAF inhibitor. Approval was based on objective response rate (ORR) and durability of response in the first 120 patients who were treated with nivolumab and had a minimum 6 months follow up from an ongoing, randomized, open-label trial in which 370 patients with unresectable or metastatic melanoma received nivolumab 3 mg/kg intravenously every 2 weeks (n=268) or investigators choice of chemotherapy (n=102). Patients were excluded from the trial if they had an autoimmune disease, a medical condition that required corticosteroids or immunosuppression, or a history of severe ipilimumab-related adverse reactions.

The major efficacy endpoints were confirmed ORR and response duration. The ORR was 32% (95% CI:23,41) with four complete responses and 34 partial responses. At the time of the FDA approval, five responding patients had progressed, while the remaining 33 patients (87%) had ongoing responses (range 2.6+ to 10+months). Thirteen patients had ongoing responses of 6 months or longer. The most common adverse reactions noted were rash in greater or equal to 20%. Clinically significant immune-mediated adverse reactions included pneumonitis, colitis, hepatitis, nephritis, hypo and hyperthyroidism.[27]

As of August 2021, nivolumab is FDA approved for 10 different cancers, some with multiple lines of approval [28]. As with all inhibitors of PD1/PDL1 signaling, nivolumab can cause inflammation in virtually any organ or tissue in the body. Per the Nivolumab Investigator Brochure version 21 the side effects include:

Very Common Side Effects (Occurring in greater than 1 in 10 people)

- Fatigue
- Diarrhea
- Rash, itching

Common Side Effects (Occurring in up to 1 in 10 people)

- Nausea
- Vomiting
- Constipation
- Mouth ulcers and sores
- Dry mouth
- Decreased appetite
- Fever
- Chills (feeling cold)
- Muscle pain, weakness, stiffness, spasms
- Joint pain or stiffness
- Headache
- Dizziness, fainting
- Abdominal pain
- Difficulty breathing
- Cough
- Dry skin
- Skin color changes
- Allergic reactions during/after infusion
- Infusion related reactions
- Anemia, may require blood transfusion
- Thrombocytopenia
- Pain or swelling in your arms, legs, ankles or body
- Peripheral neuropathy
- Inflammation of the colon (colitis)
- Inflammation of the lung (pneumonitis)
- Abnormal blood chemistry, including abnormal liver, kidney, and pancreas function tests
- High blood sugar (hyperglycemia)
- Hypothyroidism
- Hyperthyroidism
- Hyponatremia

Uncommon Side Effects (Occurring in up to 1 in 100 people)

- Alopecia
- Adrenal insufficiency
- Hypertension
- Hypotension
- Fast heart rate
- Irregular heart rhythm

- Blurry vision, dry eyes, eye inflammation
- Orbital myositis
- Inflammation of the liver (hepatitis) – Can be fatal
- Hives
- Erythema multiforme
- Psoriasis
- Pemphigoid
- Can't sleep
- Neutropenia
- Inflammation of the kidney, kidney failure requiring dialysis
- Dehydration
- Upper respiratory infections
- Pancreatitis
- Inflammation of the pituitary gland
- Condition in which the pituitary gland does not produce enough hormones
- Sarcoidosis
- Bronchitis
- Inflammation of the mouth and lining of digestive tract
- Inflammation of the muscles
- Hypoxia
- Diabetes mellitus

Rare Side Effects (Occurring in up to 1 in 1000 people)

- Increased blood creatine phosphokinase levels
- Increased blood acid levels caused by diabetes
- Blood glucose increased for too long causing severe dehydration and confusion caused by diabetes
- Rosacea
- Fluid around lungs
- Inflammation of the brain (encephalitis) – Can be fatal
- Inflammation of the blood vessels which could cut off blood supply to tissues and organs
- Inflammation of the duodenum
- Cranial nerve disorder which may affect smell, taste, vision, sensation in the face, facial expression, hearing, balance, speech, swallowing and muscles of the neck
- Guillain-Barre Syndrome
- Anaphylaxis
- Drug hypersensitivity
- Rhabdomyolysis and polymyositis
- Toxic epidermal necrolysis
- Stevens-Johnson syndrome
- Myasthenia gravis
- Polymyalgia rheumatica
- Inflammation of the spinal cord

- Inflammation of the heart (myocarditis)
- Liver injury
- Double vision
- Demyelination or loss of protective coating around your nerve fibers
- Meningitis or swelling of the brain or spinal cord membranes
- A hole developing in your intestinal tract
- Haemophagocytic lymphohistiocytosis
- Kikuchi- Fujimoto disease

The Nivolumab investigator brochure may be referenced for additional information.

While there doesn't appear to be significant activity of anti-PD1 or anti-PDL1 targeted agents alone in prostate cancer outside of the ~5% of mCRPC that are MSI hi [29], it is likely because this is a T-cell poor tumor with little underlying immune response to unleash. [30] A vaccine could potentially turn this into a T-cell enriched tumor as described below.

1.2.5 Rationale to Combine Immunotherapies

1.2.5.1 Immunogenic intensification

One of the most compelling areas of study in immunotherapy is the effects of combining immunotherapeutic approaches, a process known as immunogenic intensification. Novel approaches such as combining vaccine with immune checkpoint inhibitors highlight the potential of immunogenic intensification, whereby combining immunotherapies could potentially generate a greater immune response and enhance tumor-cell killing. [31] Previous clinical studies have shown evidence of enhanced clinical outcomes with manageable toxicity.

A phase I study treated 30 patients with mCRPC with a fixed dose of PROSTVAC in conjunction with escalating doses of ipilimumab given at monthly intervals. No dose-limiting toxicities were seen and again side effects typical of ipilimumab were observed. Of the 24 patients who were chemotherapy-naïve, 14 (58%) had PSA declines, six of which (25%) were > 50%. The median OS of patients treated with this combination was 34.4 months. This OS compares favorably to other studies with PROSTVAC and sipuleucel-T which employed vaccine alone in the same population with similar prognostic features. [32],[15],[14]

As expected, the range of toxic effects identified exceeded those in single-agent studies involving PROSTVAC, where grade 1 or 2 local injection-site reactions were most common. However, the proportion of patients affected by grade 3-4 toxic effects in this study (eight [27%] of 30 patients) was similar to previous phase I trials involving ipilimumab. [33],[34],[12] These data suggest that the combination of a vaccine that enhances co-stimulation with an immune checkpoint inhibitor does not seems to be associated with increased immune-related adverse events compared with ipilimumab alone. [23]

A second phase I study that combined the whole tumor cell vaccine GVAX with escalating doses of ipilimumab (0.3 – 5 mg/kg) in patients with mCRPC.[35] Seven patients (25%) who received either 3 or 5 mg/kg ipilimumab had PSA declines of ≥ 50% while 2 patients showed a clear regression of bone metastases. irAEs were similar in incidence and character to those that had been previously observed with single agent ipilimumab.

These findings provide a clinical proof of concept for use of an immune checkpoint inhibitor in combination with a therapeutic cancer vaccine in the treatment of mCRPC. Given the limited toxicity profile of anti-PDL1 treatments, there may be stronger rationale for the use PROSTVAC and antiPDL1 in patients with prostate cancer, although randomized trials are required to estimate any potential clinical benefit of such treatments.

1.2.5.2 Immune Response Can Drive PDL1 Expression in the Tumor

Preliminary data also suggests that an activated immune response, particularly those associated with increased INF-gamma in the tumor microenvironment can drive PDL1 expression on cancer cells, making it a relevant target for a PDL1 inhibitor. Based on this rationale, not only would a therapeutic cancer vaccine potentially have a therapeutic anti-tumor impact, associated CD8 T-cell activation and the ensuing production of INF-gamma could drive PDL1 expression on the tumor cells. These data provide further rationale for the combination of a therapeutic cancer vaccine and anti-PDL1 therapy. [36]

Preclinical data with therapeutic vaccines suggests that one can give tumor associated antigen (TAA) based vaccines (where the TAA is a selfantigen and the tumor doesn't express PDL1) and induce a response that leads to infiltration of activated T-cells and subsequent up-regulation of PDL1 expression (NCI preclinical data, not published).

1.2.6 Rationale for Evaluating Immunotherapies in the Neoadjuvant Setting

The neoadjuvant setting of prostate cancer provides an opportunity to assess the immunologic impact of these therapies in the tumor microenvironment. This proof of concept has been provided by a study of sipuleucel-T in untreated prostate cancer. When given in the neoadjuvant setting prior to radical prostatectomy sipuleucel-t was associated with increased immune cells seen at the tumor/prostate interface (periphery of the tumor). [37] A trial of PROSTVAC alone prior to radical prostatectomy (NCT02153918) has completed accrual and evaluation of the immunologic impact on the tumor is in progress. Data from that study will be used for comparison in this study.

The data acquired from the tumor microenvironment in this study could provide the rationale for a larger clinical trial in this same population to determine if combination immunotherapy (checkpoint inhibitors and vaccine) could enhance cure rate or significantly impact time to disease recurrence. In addition, data from this study could be extrapolated to more advanced disease and provide rational for combination immunotherapy in those patients as well. The findings of this study could also contribute to biomarker development in the use of immunotherapy and immunotherapy combinations.

1.2.6.1.1 Evaluation using multiparametric MRI imaging of the prostate

As described below, patients will have multiparametric MRI imaging before and after neoadjuvant immunotherapy. This will provide an opportunity to evaluate qualitative and quantitative changes in the multiparametric MRI. A previous study here at the NCI (NCT01496131) has demonstrated that immunotherapy combined with ADT led to substantial improvements in tumor density (i.e. decreased tumor density as measured by ADC mapping) in patients who received vaccine with ADT compared to ADT alone. This will be prospectively evaluated in this trial.

1.2.7 MR-US Technology to Acquire and Compare Tissue prior to Treatment and after Surgery

The MR-US "fusion" approach pioneered by Drs. Pinto and Choyke can be used to specifically biopsy lesions seen on mpMRI. Such targeted biopsies will be evaluated in the men prior to neoadjuvant combination immunotherapy. All men will have a 3T MR examination and MR/US fusion guided biopsy will utilize custom biopsy probe with the embedded passive electromagnetic (EM) tracker that allows real-time tracking of the US image and needle location. 3D reconstructed US volume will be registered and fused with the pre-biopsy MRI dataset. Continuous correction of rigid motion between the reconstructed reference 3D US volume and real-time 2D US images will enable tracking of the target lesions over the course of the biopsy procedure. The technical aspects and validation of this approach have been described earlier.[38] Biopsy targets identified in the MRI and re-identified in TRUS by means of registration will be sampled under real-time US guidance. The EM tracking and fusion technology we plan to use for this procedure has been developed and thoroughly validated by the NCI and has been applied in more than 195 cases. [39, 40] Data from these biopsies will establish baseline immunologic assessments of the tumor microenvironment.

Custom made patient –specific MR based prostate gland molds: A 3D model of the prostate can be created in a novel technique developed and pioneered in the NIH Clinical Center (**Figure 3**), as described previously.[41] In brief it is a 3D model based upon MR images, which begins with manual segmentation of the MR T2W images. The prostate is outlined and converted via software into a 3D object. This is then imported into SolidWorks (SolidWorks, Dassault Systèmes SolidWorks Corp., Concord, MA, USA). There it is subtracted to create an internal cavity that precisely shapes the patients' gland. Slots for slicing the gland at 6mm intervals are designed and orientation markings (Superior/Inferior, Right/Left etc.) are added and it is then created using a 3D printer

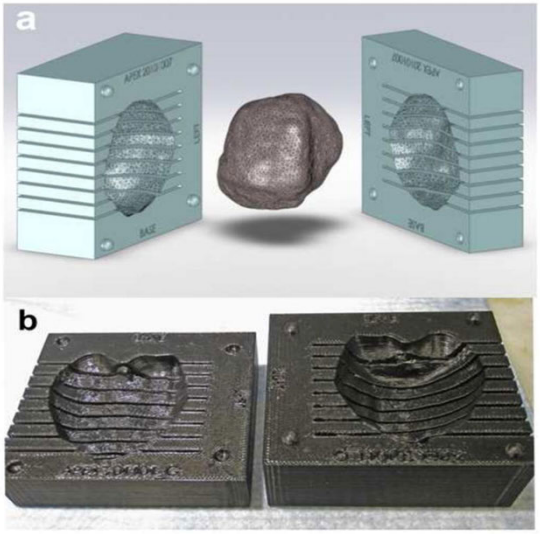

Figure 3: Customized, patient-specific prostate specimen mold. (a) Three-dimensional representation of final prostate mold in green, shown in two halves with a 3D model of the patient's prostate shown in brown. (b) Mold is created using a 3D printer, shown here in two halves fit together using small pegs/slots in the corners.

The mold approach has also been shown to allow for decision system support development [42] that provides a probability map of peripheral zone cancers based upon MRI. This expertise will be critical to the imaging, allowing suspicious lesions to be targeted for biopsy and subsequent

analyses (of immune cells in the tumor microenvironment after neoadjuvant therapy) to be performed on the tissue from the index lesion(s). This unique tool allows for the most accurate alignment of the prostate specimen to MR images that is available today. This resource available here at the NCI will be essential for the success of this proposal allowing for precise validation of in vivo imaging studies by enabling registration for focal lesion core biopsy and whole mount histopathology.

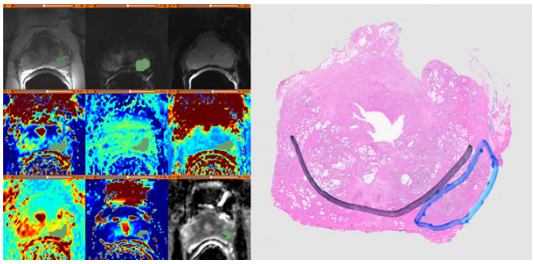

Figure 4: Digitized multiparametric MRI maps (left) and the corresponding digitized whole-mount histopathology (right) slice with tumor outlined by a prostate cancer pathologist, and used as a landmark for lesion confirmation on mp MRI maps determine lesion localization.

1.2.8 Summary

PROSTVAC is a pox viral vaccine in clinical development in prostate cancer. Preliminary data suggest that it can enhance clinical outcomes in advanced prostate cancer and can induce systemic immune responses. A previous study combining the immune checkpoint inhibitor ipilimumab and PROSTVAC suggested greater efficacy than PROSTVAC alone. Additional studies have demonstrated the potential of the checkpoint inhibitor nivolumab with the possibility of less toxicity. This study will aim to evaluate the impact of immunologic combination therapy on the tumor microenvironment focusing on immune cell infiltration as the primary endpoint. US-MRI imaging technology will be employed to sample the tumor before treatment and after radical prostatectomy. The findings from this study could serve as the basis for future studies with combination immunotherapy in this population of patients and more advanced disease.

2 ELIGIBILITY ASSESSMENT AND ENROLLMENT

2.1 ELIGIBILITY CRITERIA

2.1.1 Inclusion Criteria

For the neoadjuvant cohort, participants must have histopathological documentation of adenocarcinoma of the prostate prior to starting this study and evaluable biopsy tissue (e.g., unstained slides or blocks) available for analysis. If evaluable tissue is not available, the participant must agree to undergo a pre-vaccination prostate biopsy on study.

For the CRPC lead in cohort, if histopathological documentation is unavailable, a rising PSA (see Section **2.1.1.9**) and a clinical course consistent with prostate cancer would be acceptable.

2.1.1.1 Age ≥18 years. Because no dosing or adverse event data are currently available on the use of PROSTVAC in combination with nivolumab in participants <18 years of age, children are excluded from this study, but will be eligible for future pediatric trials.

2.1.1.2 ECOG performance status of 0 or 1 (see Appendix A, Section **16.1**).

2.1.1.3 Participants must not have other active invasive malignancies within the past 2 years (with the exception of non-melanoma skin cancers) (for CRPC cohort only).

2.1.1.4 Participants must be willing to travel to the study site for follow-up visits

2.1.1.5 All participants who have received prior vaccination with vaccinia virus (for smallpox immunization) must not have a history of serious adverse reaction to the vaccine.

2.1.1.6 The effects of PROSTVAC in combination with nivolumab on the developing human fetus are unknown. For this reason men must agree to use adequate contraception (abstinence, vasectomy) or female partner must use (intrauterine device (IUD), hormonal [birth control, pills, injections, or implants], tubal ligation] prior to study entry and for up to 7 months after the last dose.

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

2.1.1.8 Participants must have normal organ and marrow function as defined below:

a.	hemoglobin	≥ 8 g/dL
b.	granulocytes	≥1,500/mcL
c.	platelets	≥100,000/mcL
d.	total bilirubin	< 1.5 mg/dL (or ≤ 3.0 mg/dL in participants with Gilbert syndrome)
e.	AST(SGOT)/ALT(SGPT)	≤ 2.5 X institutional upper limit of normal
f.	creatinine	≤ 1.5 X ULN

2.1.1.9 **For the lead in cohort:**

- Castrate testosterone level (<50ng/dl or 1.7nmol /L)
- Progressive disease at study entry defined as one or more of the following criteria occurring in the setting of castrate levels of testosterone:

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

OR

- 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 participants had been on flutamide, PSA progression is documented 4 weeks or more after withdrawal. For participants on bicalutamide or nilutamide disease progression is documented 6 or more weeks after withdrawal.
- Participants must agree to continuation of androgen deprivation therapy (ADT) with a gonadotropin-releasing hormone agonist/antagonist or bilateral orchiectomy

2.1.1.10 **For the neoadjuvant cohort:**

1. Participants must be a surgical candidate for radical prostatectomy based on standard workup of PSA, biopsy results, and if necessary supplemental imaging.
2. Participants must have chosen radical prostatectomy as their definitive treatment of choice for management of their prostate cancer as part of their standard of care treatment.
3. No systemic steroid or steroid eye drop use within 2 weeks prior to initiation of experimental therapy. Limited doses of systemic steroids to prevent IV contrast, allergic reaction or anaphylaxis (in participants who have known contrast allergies) are allowed.

2.1.2 Exclusion Criteria

2.1.2.1 Prior splenectomy.

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

- persons with active or a history of eczema or other eczematoid skin disorders
- those with other acute, chronic or exfoliative skin conditions (e.g., atopic dermatitis, burns, impetigo, varicella zoster, severe acne or other open rashes or wounds) until condition resolves
- pregnant or nursing women; children under 3 years of age

2.1.2.3 Participants should have no evidence, as listed below, of being immunocompromised:

- HIV positivity due to the potential for decreased tolerance and risk for severe side effects.
- Hepatitis B or C positivity.

2.1.2.4 Concurrent use of systemic steroids or steroid eye drops. This is to avoid immunosuppression which may lead to potential complications with vaccinia (priming vaccination). Nasal, topical or inhaled steroid use is permitted.

2.1.2.5 Participants with known allergy to eggs or to compounds with a similar chemical or biologic composition to PROSTVAC or nivolumab.

2.1.2.6 No prior immune checkpoint inhibitors (e.g., anti-CTLA4, anti-PD-1 or anti-PDL1 are allowed.

2.1.2.7 Other serious intercurrent illness.

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

2.1.2.9 Participants with significant autoimmune disease that is active or potentially life threatening if activated.

2.1.2.10 Participants with clinically significant cardiomyopathy requiring treatment.

2.1.2.11 Participants with ongoing toxicities related to prior therapies targeting T cell coregulatory proteins (immune checkpoints) such as anti-PD-1, anti-PD-L1, or anti-CTLA-4 antibody are excluded

2.1.2.12 No transfusion of blood or blood products within 2 weeks and no G-CSF or GM-CSF within 2 weeks prior to initiations of experimental therapy.

2.1.2.13 Contraindication to biopsy or prostatectomy (for neoadjuvant cohort only):

- Bleeding disorders
- Artificial heart valve
- PT/PTT ≥ 1.5 x ULN in participants not taking anticoagulation. Participants on anticoagulation (e.g., enoxaparin, oral anticoagulants) are eligible regardless of PT/PTT. Prior to biopsy, anticoagulation will be held per standard practice.

2.1.2.14 For participants with localized prostate cancer contraindication to MRI:

- Participants weighing >136 kilograms (weight limit for the scanner tables)
- Allergy to MR contrast agent
- Participants with pacemakers, cerebral aneurysm clips, shrapnel injury or implantable electronic devices

2.1.2.15 History of radiation proctitis (for lead-in CRPC cohort only)

2.1.3 Recruitment Strategies

This protocol may be abstracted into a plain language announcement posted on NIH websites and on NIH social media platforms. Participants may also be recruited from the current patient population at NIH.

Screening Evaluation

2.1.4 Screening activities performed after a consent for screening has been signed

The following activities will be performed only after the subject has signed the consent for study # 01-C-0129 (Eligibility Screening and Tissue Procurement for the NIH Intramural Research Program Clinical Protocols) on which screening activities will be performed. Assessments performed at outside facilities or on another NIH protocol within the timeframes below may also be used to determine eligibility once a participant has signed the consent.

2.1.4.1 For Lead-in CRPC Cohort

- Pathological confirmation of diagnosis. May be obtained any time prior to enrollment.

- The following parameters will be obtained within 8 weeks prior to start of enrollment:

1. HIV test
2. Hepatitis B and C
3. Height

- The following parameters will be obtained within 4 weeks prior to start of enrollment:

4. Tc-99 whole-body scintigraphy
5. CT (or MRI may be substituted at investigator's discretion) of chest, abdomen and pelvis

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

6. History and physical examination including vital signs
7. ECOG performance status
8. Complete blood count plus differential and platelet count
9. Hepatic (alkaline phosphatase, AST, ALT, total bilirubin, direct bilirubin), Mineral (albumin, calcium, magnesium, phosphorus) and Acute Care (sodium, potassium, chloride, bicarbonate, creatinine, glucose, BUN, eGFR) Panels
10. Serum lipase
11. Amylase
12. Serum PSA
13. Testosterone level
14. HIV serology (within 60 days prior to enrollment)
15. Serology for hepatitis B and C (within 60 days prior to enrollment).
16. TSH
17. PT/PTT

2.1.4.2 For neoadjuvant cohort

- Pathologic Confirmation of Diagnosis by the Laboratory of Pathology, CC, NIH, or Walter Reed National Military Medical Center (if specimen is available).
- Participants that do not have previously collected biopsy material available will be asked to undergo a prostate biopsy.
- The following parameters will be obtained within 35 days prior to start of enrollment:
  - History and Physical Exam, including height measurement
  - Performance Status Evaluation
  - EKG
  - Laboratory Evaluation
  - CBC with differential and platelet count
  - Acute care panel
  - Hepatic Panel
  - HIV serology (within 60 days prior to enrollment)
  - Serology for hepatitis B and C (within 60 days prior to enrollment).
  - Lipase

- Amylase
- TSH
- Serum PSA
- PT/PTT

2.2 PARTICIPANT REGISTRATION AND STATUS UPDATE 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 at: https://ccrod.cancer.gov/confluence/pages/viewpage.action?pageId=73203825.

2.2.1 Treatment Assignment Procedures

Cohorts

Number	Name	Description
1	Cohort 1	Men with Prostate Cancer

Arms

Number	Name	Description
1	Lead-in CRPC Cohort	PROSTVAC-V on week 0 followed by booster injection called PROSTVAC-F on 2, 4, and 8 weeks. Nivolumab will be administered every 2 weeks, starting at week 2. When administered on the same day, the preferred order of administration is PROSTVAC first followed by nivolumab. Participants will undergo restaging scans on week 12 prior to receiving treatment. If no PD, option to continue treatment with nivolumab every 2 weeks and PROSTVAC-F every 4 weeks until intolerance or progression (evaluated radiographically every 12 weeks). Participants who remain on protocol beyond 1 year will have the option of extending the nivolumab dosing interval to every 4 weeks.
2	Neoadjuvant Cohort	PROSTVAC-V on week 0 followed by booster injection called PROSTVAC-F on 2, 4 and 8 weeks. Nivolumab will be administered on weeks 2, 4, 6, and 8. When administered on the same day, the preferred order of administration is PROSTVAC first followed by nivolumab. Participants will undergo prostatectomy on week 9. (If surgery is scheduled earlier than 9 weeks after initial dosing, the 6 and 8 week dosing may skipped and surgery may be done as early as week 5.)

Arm Assignment

Participants in cohort 1 will be assigned to arm 1 until the expected number of participants enrolled (n=10), then participants will be assigned to arm 2 (n=17).

2.3 BASELINE EVALUATION

2.3.1 For Lead-In CRPC Cohort

To be performed within 16 days prior to initiation of study therapy (these tests will not need to be repeated if they were done at screening within the appropriate timeframe):

- History and physical exam including weight and vital signs
- ECOG performance status
- CBC with differential and platelet count, prothrombin time/INR, activated partial thromboplastin time
- HLA class 1 profile (May be obtained any time prior to enrollment)
- Urinalysis (may be omitted in participants with incontinence who cannot produce a clean sample)
- Serum chemistries (Na+, K+, Cl-, CO2, BUN, creatinine, glucose, AST/ALT, bilirubin, calcium, phosphorous, albumin, magnesium, alkaline phosphatase, LDH, ionized calcium, amylase, lipase, total protein, uric acid)
- Serum Testosterone level
- Serum PSA
- CPK
- Lymphocyte Phenotyping (TBNK)
- Baseline electrocardiogram 12 lead ECG (EKG) on all participants, and appropriate cardiologic evaluation, as clinically indicated, to provide baseline function and identify any participants who should be monitored closely for cardiac risks associated with vaccinia vaccination
- Immunology assays (including immune T-cell response assay, CD3, 4, 8 subsets and CD4:CD8 ratio, Class II immune responses, CTL assay, and sera antibody analyses)
- Biopsy (May be obtained any time after enrollment and prior to treatment initiation; optional)
- Scans (CT CAP and bone scan)

2.3.2 For neoadjuvant cohort

To be performed within 16 days prior to enrollment unless specifically stated otherwise (these tests will not need to be repeated if they were done at screening within the appropriate timeframe):

- Baseline examinations need not be repeated if they have been performed within the appropriate time frame.
- History and Physical Exam
- ECOG Performance Status
- HLA class 1 profile (May be obtained any time prior to enrollment)
- Serum PSA (tumor marker)
- CBC
- Urinalysis (may be omitted in participants with incontinence who cannot produce a clean sample)
- Serum chemistries (Na+, K+, Cl-, CO2, BUN, creatinine, glucose, AST/ALT, bilirubin, calcium, phosphorous, albumin, magnesium, alkaline phosphatase, LDH, ionized calcium, amylase, lipase, total protein, uric acid, C-reactive protein)
- Lymphocyte Phenotyping (TBNK)
- Baseline electrocardiogram 12 lead ECG (EKG) on all participants, and appropriate cardiologic evaluation, as clinically indicated, to provide baseline function and identify any participants who should be monitored closely for cardiac risks associated with vaccinia vaccination
- Research Studies (See Section **5** for details)
- MRI prostate (can be obtained within one year prior to treatment) MRI of the prostate will be performed prior to and after vaccination course if logistically feasible.
- Immunology assays (including immune T-cell response assay, CD3, 4, 8 subsets and CD4:CD8 ratio, Class II immune responses, CTL assay, and sera antibody analyses)
- Prostate biopsy (performed only if previously collected tissue is not available. If biopsy was performed at screening, leftover material may be used for research study.)

3 STUDY IMPLEMENTATION

3.1 STUDY DESIGN

3.1.1 Rationale for Serial Cohort Design

The primary focus of this study will be to evaluate PROSTVAC and nivolumab in the neoadjuvant setting. However, given the concern that treating with these agents prior to radical prostatectomy may delay or compromise definitive radical prostatectomy (due to systemic adverse events or local inflammation) we will have a lead-in cohort evaluating the safety and tolerability of this combination in the castration resistant setting.

Participants in this initial cohort will be enrolled not more than 3 a week.

Following this lead-in cohort in the CRPC setting, we propose a cohort in the neoadjuvant setting evaluating the combination of PROSTVAC and nivolumab.

3.1.2 Study Schema

Lead-in CRPC Cohort for safety:

	Week 0	**Week 2**	**Week 4**	**Week 6**	**Week 8**	**Week 10**	**Week 12**
N = 10	PROSTVAC- V	PROSTVAC-F Nivolumab	PROSTVAC-F Nivolumab	Nivolumab	PROSTVAC-F Nivolumab	Nivolumab	Restaging CT and Bone scan and if no PD option to continue treatment until intolerance or progression (evaluated radiographically every 12 weeks)

PROSTVAC-V $2x10^8$ Infectious Units

PROSTVAC-F $1x10^9$ Infectious Units

Nivolumab 240 mg (Flat dose) every 2 weeks. Alternately, participants who remain on protocol beyond 1 year may transition to nivolumab 480 mg (flat dose) every 4 weeks.

Neoadjuvant Cohort:

	Week 0	**Week 2**	**Week 4**	**Week 6***	**Week 8***	**Week 9***
N=17	PROSTVAC-V	PROSTVAC-F Nivolumab	PROSTVAC-F Nivolumab	Nivolumab	PROSTVAC-F Nivolumab	Prostatectomy

PROSTVAC-V $2x10^8$ Infectious Units

PROSTVAC-F $1x10^9$ Infectious Units

Nivolumab 240 mg (Flat dose)

*If surgery is scheduled earlier than 9 weeks after initial dosing, the 6 and 8 week dosing may be skipped and surgery may be done as early as week 5.

3.2 DRUG ADMINISTRATION

3.2.1 PROSTVAC Vaccine

All vaccines in this trial should be handled according to the guidelines outlined by each department. Study staff administering the vaccine or assessing the vaccine site should wear personal protection consistent with the standard of practice outlined by each department or unit. Participants receiving PROSTVAC-V should be isolated just prior to the administration of

vaccine and can be removed from isolation after the vaccine is administered and bandage is secured over the injection site. Appendix B (Section **16.2**) defines individuals at risk for vaccinia exposure.

PROSTVAC-V (vaccinia) will be administered subcutaneously in an extremity (e.g., thigh) at a dose of $2x10^8$ infectious units.

PROSTVAC-F (fowlpox) will be administered subcutaneously in an extremity (e.g., thigh) at a dose of $1x10^9$ infectious units.

3.2.2 Nivolumab

Nivolumab is to be administered as a flat dose of 240 mg or 480 mg over approximately 30-minutes via IV infusion, using a volumetric pump with a 0.2/1.2 micron in-line filter at the protocol-specified dose. Nivolumab injection can be infused undiluted or diluted so as not to exceed a total infusion volume of 160 mL. Nivolumab may be diluted with 0.9% sodium chloride injection, USP or 5% dextrose injection, USP. It is not to be administered as an IV push or bolus injection. At the end of the infusion, flush the line with a sufficient quantity of normal saline.

3.2.3 PROSTVAC with Nivolumab

Toxicity management for the combined agents follows the same template guidelines and algorithms that are provided in Section **16.3** for single agent nivolumab.

When administered on the same day, the preferred order of administration is PROSTVAC first followed by nivolumab.

3.3 DOSE MODIFICATIONS

Participants must have recovered to ≤ grade 1 attributable toxicity for the parameters used to assess levels of organ function required for eligibility (see Section **2.1**) after each treatment in order to receive a subsequent treatment. Participants who experience irAE may continue PROSTVAC alone if irAE appears to be driven by nivolumab. In such circumstances, investigator will discuss fully the potential risks with the participant and document that conversation appropriately. Any dosing interruption lasting > 6 weeks from the last dose will require participants to be removed from protocol with the following exceptions:

- Dosing interruptions to allow for prolonged steroid tapers to manage drug-related adverse events are allowed. Tumor imaging assessments should continue as per protocol even if dosing is interrupted
- Dosing interruptions > 6 weeks from the last dose that occur for non-drug-related reasons may be allowed
- For subjects with mCRPC who have stopped receiving nivolumab because of grade 3 or higher irAEs, nivolumab could be re-started if the participant had partial response according Prostate Cancer Working Group 3 (PCWG 3) and RECIST 1.1 criteria but subsequently develops PSA progression (by PCWG 3) while on PROSTVAC alone.

3.4 STOPPING RULES

The study may be halted if any of the following safety conditions are met:

- If one or more deaths occur, we will immediately stop accrual and we will promptly discuss this with the NIH Intramural IRB and the FDA. In addition, the study will be halted if any death occurs within 30 days of treatment regimen.
- If two or more participants develop a Grade 4 toxicity at any point in the study attributable to the treatment regimen that does not resolve to Grade 2 within 10 days.

3.5 STUDY CALENDAR

3.5.1 Lead-in CRPC Cohort

	Screening	Week 0 (Baseline)	Week 2 (+/- 3 days)	Week 4 (+/- 3 days)	Week 6 (+/- 3 days)	Week 8 (+/- 3 days)	Week 10 (+/- 3 days)	Follow-up q 2 weeks until off treatment	Safety Visit 4-5 weeks after treatment discontinuation[9]
Treatment									
Vaccinia-PROSTVAC		X							
Fowlpox-PROSTVAC			X	X		X		X[4]	
Nivolumab (Flat dose)			X	X	X	X	X	X[4]	
Assessments									
History and PE, Weight	X[2]	X[3]	X	X	X	X	X	X[4]	X
Height	X[5]								
ECOG Performance Score	X[2]	X[3]	X	X	X	X	X	X[4]	X
ECG		X[3]							
HIV/Hep B and C tests	X[5]								
HLA class I profile		X[3]							
Immunology Assays/TBNK	X	X[3]							

	Screening	Week 0 (Baseline)	Week 2 (+/- 3 days)	Week 4 (+/- 3 days)	Week 6 (+/- 3 days)	Week 8 (+/- 3 days)	Week 10 (+/- 3 days)	Follow-up q 2 weeks until off treatment	Safety Visit 4-5 weeks after treatment discontinuation[9]
Pathologic confirmation of dx[1]	X[1]								
Labs (see Sections **2.1.4** and **2.2.1**)	X[2]	X[3]							
Labs: CBC, TSH, amylase, lipase, PSA, PT, PTT and the following panels: acute care, mineral, hepatic			X	X	X	X	X	X	X
Research labs: 6 green tops (PBMCs) and 2 SSTs (serum).		X	X	X			X	X[7, 8]	X
Prostate biopsy (optional)		X							
Restaging CT and Bone scan[4]								X[8]	X
Tc 99 scintigraphy	X[6]	X							

	Screening	Week 0 (Baseline)	Week 2 (+/- 3 days)	Week 4 (+/- 3 days)	Week 6 (+/- 3 days)	Week 8 (+/- 3 days)	Week 10 (+/- 3 days)	Follow-up q 2 weeks until off treatment	Safety Visit 4-5 weeks after treatment discontinuation[9]
CT-C/A/P or MRI	X[6]	X							

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

[2]Screening assessments obtained within 16 days prior to enrollment

[3]Baseline assessments obtained within 16 days prior to initiation of study therapy (these tests will not need to be repeated if they were done at screening within the appropriate timeframe)

[4]If no PD option to continue treatment until intolerance or progression (evaluated radiographically using irRC every 12 weeks). PROSTVAC-F is administered every 4 weeks (i.e., weeks 12, 16, 20, etc.) Nivolumab is administered at a flat dose of 240mg every 2 weeks (+/- 3 days). For participants who remain on protocol beyond 1 year, nivolumab 480mg (flat dose) every 4 weeks (+/- 3 days) may be administered instead of the 240 mg every 2-week (+/- 3 days) regimen.

[5]To be obtained within 8 weeks prior to start of enrollment

[6]To be obtained within 4 weeks prior to start of enrollment

[7]Restaging visits only

[8]Per PI discretion. For example, participants who have had a complete response to treatment may have the interval between re-staging imaging increased to 1 year. Research samples for these participants will also be collected yearly with staging.

[9] Assessment of SAEs at approximately 100 days (± 1 week) after nivolumab discontinuation. Assessment may be conducted by phone if the participant cannot come to the NIH. Assessments may also be performed by a local provider.

3.5.2 Neoadjuvant Cohort

	Screening	Week 0 (Baseline)	Week 2 (+/- 3 days)	Week 4 (+/- 3 days)	Week 6[6] (+/- 3 days)	Week 8[6] (+/- 3 days)	Post study treatment visit [7]	Safety Visit 6-8 weeks after treatment discontinuation[4]
Treatment								
Vaccinia-PROSTVAC		X						
Fowlpox-PROSTVAC			X	X		X		
Nivolumab 240 mg (Flat dose)			X	X	X	X		
Assessments								
History and PE, and Weight	X[2]	X[3]	X	X	X	X	X	X
Height	X[2]							
ECOG Performance Score	X[2]	X[3]	X	X	X	X		X
ECG	X[2]	X[3]						
HLA class I profile		X[3]						
Pathologic confirmation of dx[1]	X[1]							
Labs (see Sections **2.1.4** and **2.2.1**)	X[2]	X[3]						
Labs: CBC, TSH,			X	X	X	X	X	X

	Screening	Week 0 (Baseline)	Week 2 (+/- 3 days)	Week 4 (+/- 3 days)	Week 6[6] (+/- 3 days)	Week 8[6] (+/- 3 days)	Post study treatment visit [7]	Safety Visit 6-8 weeks after treatment discontinuation[4]
amylase, lipase, PSA, PT, PTT and the following panels: acute care, mineral, hepatic								
Research labs: 6 green tops (PBMCs) and 2 SSTs (serum).		X	X	X			X	X
Gene expression profiling (PaxGene tube)		X					X	
Immunology Assays/TBNK		X	X	X	X	X	X	
Prostate biopsy		X						
Serum PSA	X	X	X	X	X	X	X	
MRI prostate		X					X	
PT/PTT/INR	X						X	

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

[2]Screening assessments obtained within 35 days prior to enrollment

[3]Baseline assessments obtained within 16 days prior to enrollment (these tests will not need to be repeated if they were done at screening within the appropriate timeframe)

[4]Assessment of SAEs at approximately 100 days (± 1 week) after nivolumab discontinuation**.** Assessment may be conducted by phone if the participant cannot come to the NIH. Assessments may also be performed by a local provider.

[5]A standard of care radical prostatectomy will be performed between week 5 and week 12 week to allow for logistical constraints, but ideally as close to week 9 as possible. This may occur at NIH or at an outside institution.

[6]If surgery is scheduled earlier than 9 weeks after initial dosing, the 6 and 8 week dosing and study visits/procedures may skipped and surgery may be done as early as week 5.

[7] This visit should occur one week after the final planned study immunotherapy treatment (-3/+7 days).

3.6 COST AND COMPENSATION

3.6.1 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 are performed outside the NIH Clinical Center, participants may have to pay for these costs . Medicines that are not part of the study treatment will not be provided or paid for by the NIH Clinical Center.

3.6.2 Compensation

Participants will not be compensated on this study.

3.6.3 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.

3.7 CRITERIA FOR REMOVAL FROM PROTOCOL THERAPY AND OFF STUDY CRITERIA

Prior to removal from study, effort must be made to have all subjects complete a safety visit approximately 4-5 weeks for Lead-in mCRPC cohort and 6-8 weeks for neoadjuvant cohort) following the last dose of study therapy and at approximately 100 days after nivolumab discontinuation for both cohorts.

3.7.1 Criteria for removal from protocol therapy

Participants will be removed from treatment for the following:

- Grade 2 or greater toxicity attributed to treatment that does not resolve to grade 1 within 42 days from time of scheduled treatment except as described in Section **3.3**.
- Grade 2 or greater autoimmune disease that threatens vital organs.
- Intercurrent illness or medical circumstances. If at any time the constraints of this protocol are detrimental to the participant's health, the participant may be removed from protocol therapy and reasons for withdrawal will be documented.
- Greater than 3-month delay in the performance of radical prostatectomy from week 12.
- Completion of protocol therapy
- Participant requests to be withdrawn from active therapy
- Investigator discretion
- Clinical or confirmed radiographic progression of disease (for CRPC Cohort only) (irPD)

3.7.2 Off-Study Criteria

1. Patient is off-treatment and has agreed to be followed on a long-term therapy protocol as outlined in Section **3.9** or has completed the study follow up period.

2. The study is stopped.
3. Participant requests to be taken off study. Reasons for withdrawal will be documented.
4. Noncompliance with protocol guidelines (participant removed at discretion of Principal Investigator).
5. Disease progression
6. Death

3.8 FOLLOW-UP EVALUATIONS

After subjects have stopped taking the study medication for any of the reasons listed in Section **3.6**, they will be seen at NIH, if logistically feasible, for a safety visit within 4-5 weeks for Lead-in mCRPC cohort and 6-8 weeks for neoadjuvant cohort of drug discontinuation. The safety assessments may be performed by a local physician and laboratory if participants unable to return to the NIH Clinical Center at this time.

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

- History and Physical Examination
- CBC with differential and platelet count, prothrombin time/INR, activated partial thromboplastin time
- Serum chemistries (Na+, K+, Cl-, CO2, BUN, creatinine, glucose, AST/ALT, bilirubin, calcium, phosphorous, albumin, magnesium, alkaline phosphatase, LDH, ionized calcium, amylase, lipase, total protein, uric acid), TSH
- Serum PSA level
- Adverse event reporting

After the safety visit, if there are no unresolved grade 3 or higher AEs, we will offer participants the opportunity to enroll on our long term follow up study. If there are unresolved grade 3 – 4 AEs, participants will be followed either at the NIH Clinical Center or by their local physician. In the latter case, we will obtain the physician's record of AEs.

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

3.9 POST-STUDY EVALUATION

In 2006, the FDA excluded pox virus based vaccine platforms from required long term follow-up. Following this guidance in 2006 we haven't built in long term follow-up for our pox-viral studies as such vectors have not been shown to have a propensity to integrate or reactivate following latency and, in the absence of evidence to the contrary, present a low risk of gene therapy-related delayed adverse events. Therefore no long term follow up is required with this study, though participants will be offered the opportunity to enroll on our long term follow up study.

4 CONCOMITANT MEDICATIONS/MEASURES

Subjects must inform the investigators of the current or planned use or all other medications during the study (including prescription medications, vitamins, herbal and nutritional supplements, and over-the-counter medications).

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

Other supportive care with blood components, antibiotics, analgesics, general medical therapy, etc., will be delivered as required. Any participants taking antibiotics for an active infection must complete that course of therapy and be free of evidence of further infection before receiving any immunotherapy treatment. Antibiotics for prophylaxis are allowed while on treatment.

Concurrent systemic corticosteroid use (daily or every other day for continued use > 14 days) should be avoided within 14 days before the first planned dose of PROSTVAC. Use of inhaled steroids, nasal sprays, and topical creams for small body areas is allowed. Limited use of steroids is allowed on study (e.g., for immune related adverse events or for premedication for imaging).

Symptomatic anemia should be treated with appropriate red blood cell or erythropoietin support.

Thrombocytopenia should be treated conservatively. In the absence of bleeding or a planned invasive procedure, platelet transfusions should be given for a platelet count below 10,000/mm3. If invasive procedures are planned or the participant develops bleeding, platelet transfusions should be administered in accordance with the standard of practice, usually maintaining a platelet count of > 50,000/mm3.

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

A standard of care radical prostatectomy will be performed between week 5 and week 12 to allow for logistical constraints, but ideally as close to week 9 as possible. This may occur at NIH or at an outside institution. Surgical specimens will be requested for research use (Section **5**).

5 CORRELATIVE STUDIES FOR RESEARCH

5.1 IMMUNE CELL INFILTRATE (TISSUE SPECIMENS)

Slides made from prostate biopsy specimens (collected at baseline) and prostatectomy specimens (collected at surgery performed after last dose of vaccine) will be stained for CD4 and CD8 cells and digitized for analysis of immune cell infiltrate. Other markers such as CD4/FoxP3 may also be stained for further correlative studies. These studies will be performed by the Laboratory of Tumor Immunology and Biology. At a minimum, 5 slides from one biopsy core confirmed to contain cancer will be needed for baseline quantification. Attempts will be made based on anatomic location notation or when available, MRI based location, to correlate biopsy location with pathologic location on prostatectomy specimens. When feasible, quantitation will be performed in triplicate. Quantitation will be reported as number of stained cells per micron squared of surface area. Wilcoxon matched pair signed rank test, Friedman test with Dunn's multiple comparisons, or alternative statistical models will be used as appropriate. Aperio ScanScope digital scanner systems with Aperio ImageScope software algorithms will be used. Manual quantitation will be performed in case of software malfunction. Isotype antibodies

negative controls will be included in all runs. Tonsil or lymph node tissue will be positive control for CD4 and CD8. Slides will be developed with primary and secondary antibodies per manufacturer specifications for visualization of cells.

Peripheral Blood Correlative Studies for Research/Pharmacokinetic Studies

Blood Studies	**Blood Tube/Comments****	**Destination**
CBC with differential	One light lavender tube	CC Department of Laboratory Medicine (DLM)
Hepatic Panel, Acute Care Panel	One 4 mL SST	CC DLM
Anti-HIV-1/2	One 8 mL SST	CC TTV lab
PT/PTT/INR	One 4.5 mL citrate blue top	CC DLM
HBs Antigen Screening Anti-HCV Antibody	One 8 mL SST	CC Department of Transfusion Medicine (DTM)
Prostate Specific Antigen	One 4 mL SST	CC DLM
Lymphocyte Phenotyping, TBNK	One light lavender tube	CC DLM
Immunology Assays	Six 10 mL Na Heparin tubes Two 8 ml SST tubes	NCI-Frederick 1-301-846-5893
Gene expression profiling*	Two 2.5 mL RNA PaxGene tubes	Blood Processing Core (Figg lab)

*RNA PaxGene to be drawn only on the weeks of biopsies or standard of care prostatectomy

** Please note that tubes and media may be substituted based on availability with the permission of the PI or laboratory investigator.

5.1.1 Sera Antibody Analysis

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

5.1.2 PSA-Specific Immune Responses

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

Unlike previous studies involving PROSTVAC, which evaluated immune responses to a single 9 amino acid section of the 244 amino acid PSA protein, the immune analysis in this trial will evaluate overlapping 15 amino acid sequences of the entire PSA protein. This more extensive analysis will provide a more thorough understanding of the immune response initiated by

PROSTVAC and a greater opportunity for clinical correlations. This type of analysis has not been previously prospectively performed in localized prostate cancer participants. Advantages of this approach include the ability to assess both CD4 and CD8 T cells, identification of multifunctional T cells (those producing ≥2 cytokines), and identification of T lymphocytes with lytic potential (CD107a expression). (**Table 1**).

		Immune Responses to PSA											
		CD4				CD8				Immune Responses to PSA			
	PT	CD107a	IFNg	IL2	TNF	CD107a	IFNg	IL2	TNF	Any	Only CD4	Only CD8	Both CD4 & CD8
Cohort 1 – No Vaccine	11									2/8	0/8	2/8	0/8
	13												
	20												
	22					1427			274				
	25								630				
	3												
	5												
	10												
Cohort 2 with Prostvac	2		786		374	5269	453		323	6/10	3/10	1/10	2/10
	8		345			633							
	12												
	18				402								
	21												
	24					1242							
	14	821											
	16												
	26	815											
	27												

Table 1. Preliminary Data from a trial of Prostvac in mCRPC. This table shows how this multi peptide approach can be used in this proposal. In this previous (unpublished) trial, 2 cohorts of patients were evaluated using the proposed methods. (Responses are listed as absolute # of CD4 or CD8 T Lymphocytes Producing Cytokine or Positive for CD107a per $1x10^6$ cells plated.) This readout shows the breadth of this analysis as both CD4 and CD8 tell cells are evaluated using multiple parameters (cytokine production signifying activation – IFN-γ, IL-2 and TNF-α) and lytic potential as measured by CD107a expression. Had only one peptide been used to analyze this immune response it is likely that many of these immune responders would not have been identified. (unpublished).

A positive "response" will be defined as increased CD107a expression or increased intracellular cytokine production for given antigen for CD4 or CD8 cells. For example, Participant #2 in Table 1 will be scored has having 5 responses (CD4: INFg, TNF and CD8: CD107a, INFg and TNF).
In order to give added weight to the spread antigens (MUC1 and Brachyury) they will be weighted 150% higher than PSA antigens. Thus, the scoring will be as follows:

For PSA:
1 point each of the four CD4 parameters (CD107a, IFN-γ, IL-2 and TNF-α) – max total of 4
1 point each of the four CD8 parameters (CD107a, IFN-γ, IL-2 and TNF-α) – max total of 4
Total possible score for PSA response is 8

For MUC1:
1.5 points each of the four CD4 parameters (CD107a, IFN-γ, IL-2 and TNF-α) – max total of 6
1.5 points each of the four CD8 parameters (CD107a, IFN-γ, IL-2 and TNF-α) – max total of 6

Total possible score for PSA response is 12.

For Brachyury:
1.5 points each of the four CD4 parameters (CD107a, IFN-γ, IL-2 and TNF-α) – max total of 6
1.5 points each of the four CD8 parameters (CD107a, IFN-γ, IL-2 and TNF-α) – max total of 6
Total possible score for PSA response is 12.

5.1.3 Flow Cytometry Analysis of Immune subsets (Biomarker Development)

As part of the extensive immune interrogation, peripheral immune cells will be evaluated by 30 markers to assess 127 immune subsets. (**Table 2**) This methodology has been previously described [43]. This added analysis will provide preliminary data to develop a Peripheral Immunoscore based on the frequency of specific pre-determined immune cell subsets in the blood of participants prior to therapy. The score is calculated from the changes in frequency of certain immune cell subsets after vaccine treatment. The resultant panel of markers would reflect immune response to the vaccine.

Table 2. Analysis of Peripheral Immune Cells

A. **CD4**: Helper T lymphocytes (32 subsets)
B. **CD8**: Cytotoxic T lymphocytes (29 subsets)
    a. **Markers of PD-1 pathway and T cell activation (in CD4 and CD8):**
        i. **EOMES:** activation
        ii. **TCR-αβ:** activation
        iii. **Tbet:** activation
        iv. **BATF:** activation/exhaustion
    b. **Maturation status of T lymphocytes (in CD4 and CD8)**:
        i. **Naïve**: $CD45RA^{+}$ $CCR7^{+}$
        ii. **Effector Memory**: $CD45RA^{-}$ $CCR7^{-}$
        iii. **Terminal (EMRA)**: $CD45RA^{+}$ $CCR7^{-}$
    c. **T lymphocyte markers (in CD4 and CD8)**:
        i. **CTLA-4:** inhibition
        ii. **PD-1:** activation/inhibition
        iii. **PD-L1:** activation/cross-inhibition
        iv. **TIM-3:** inhibition
        v. **ICOS**: activation (only on CD4)
C. **Tregs**: Regulatory T lymphocytes ($CD4^{+}$ $CD25^{+}$ $FoxP3^{+}$ $CD127^{-}$) (7 subsets)
    a. **CD45RA**: Tregs highly expandable *in vitro*
    b. **CTLA-4**: Treg suppression
    c. **CD49d:** "contaminating" effector lymphocytes (non-Tregs)
    d. **ICOS**: Treg suppression
    e. **PD-1:** activation/inhibition
    f. **PD-L1:** cross-inhibition
D. **B lymphocytes**: $CD19^{+}$ (5 subsets)
    a. **CTLA-4:** inhibition
    b. **TIM-3:** inhibition
    c. **PD-1:** activation/inhibition
    d. **PD-L1:** cross-inhibition
E. **NK**: Natural killer cells ($CD56^{+}$ $CD3^{-}$) (20 subsets)
    a. **$CD16^{+}$ $CD56^{br}$**: Functional intermediate, lytic and cytokine production
    b. **$CD16^{+}$ $CD56^{dim}$**: Mature NK, cytokine production
    c. **$CD16^{-}$ $CD56^{br}$:** Immature, abundant in human placenta
    d. **$CD16^{-}$ $CD56^{dim}$**: non-lytic, non-cytokine production
    e. **TIM-3:** activation
    f. **PD-1:** activation/inhibition
    g. **PD-L1:** cross-inhibition
F. **NK-T**: $CD56^{+}$ $CD3^{+}$ (4 subsets)
    a. **TIM-3:** activation
    b. **PD-1:** activation/inhibition
    c. **PD-L1:** cross-inhibition
G. **cDCs (Conventional DCs):** $CD3^{-}CD56^{-}$ $CD1c^{+}CD303^{-}$ (5 subsets)
H. **pDCs (plasmacytoid DCs):** $CD3^{-}CD56^{-}CD1c^{-}$ $CD303^{+}$ (5 subsets)
    a. **Markers of DC activation**
        i. **CD83:** activation
        ii. **TIM-3:** inhibition
        iii. **PD-1:** activation/inhibition
        iv. **PD-L1:** cross-inhibition
I. **MDSCs**: Myeloid-derived suppressor cells ($CD11b^{+}$ $HLA\text{-}DR^{low/-}$ $CD33^{+}$) (20 subsets)
    a. **CD14**: Common Myeloid Marker (high in monocytes, dim in granulocytes)
    b. **CD15**: Granulocyte marker
    c. **CD16**: most immature monocytic MDSCs
    d. **PD-1:** activation/inhibition
    e. **PD-L1:** cross-inhibition

5.1.4 MRI study

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

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

5.1.5 Additional Assays

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

All samples will be labeled with the following identifier system.
- Participant's enrollment #
- Trial number
- Participant's initials Example: 01-ABC

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

5.1.6 Analysis of pre and post treatment samples

5.1.6.1 Pre and post treatment samples will be coded and analysis of tissues using multiplexed immunofluorescence or multispectral imaging to identify specific markers, including markers of immune cell subsets, may be performed in collaboration with:

PerkinElmer
68 Elm St.
Hopkinton, MA 01748
[redacted]

and

General Electric (GE) Global Research
[redacted]
Lifesciences and Molecular Diagnostics Org
Building K1-5B27
One Research Circle
Niskayuna, NY 12309

5.1.6.2 Scanned images and/or tissue samples and linked, coded data (clinical parameters, outcomes data, laboratory data, and other relevant data) may be sent to Definiens for analysis to explore the tumor microenvironment and the impact of treatment. The Definiens tissue phenomics platform allows for quantitative histopathology and spatial pattern analysis of scanned images of tissue samples, and correlation of results with outcomes and clinical parameters.

Definiens
[redacted]
1808 Aston Avenue, Suite 190
Carlsbad, CA 92008, U.S.
[redacted]

5.1.6.3 DNA and RNA-based analysis and quantitative proteomics may be performed in collaboration with:

5.1.6.3.1 HTG Molecular Diagnostics, Inc.

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

HTG Molecular Diagnostics, Inc. has a platform to obtain RNA from formalin-fixed and paraffin-embedded (FFPE) tissues and from PBMC cells. NCI would send HTG Molecular paired coded samples of pretreatment and post treatment PBMC and FFPE slides (H&E and serial unstained slides) for sequencing analysis.

Correlative analysis may be planned at a later point in conjunction with Larry Fong at UCSF who is doing a study in a similar participant population with a different design and different agents (PROSTVAC vs combination) to allow for a better ability to compare across studies.

5.1.6.3.2 NantOmics

NantOmics
9600 Medical Center Drive, Suite 300
Rockville, MD 20850
[redacted]

NantOmics performs comprehensive molecular profiling of samples through whole genome sequencing, whole transcriptome sequencing, and quantitative proteomics. Coded tissue and blood samples may be sent for analysis.

5.1.6.3.3 Certificate of Confidentiality

As part of study efforts to provide confidentiality of subject information, this study will obtain a Certificate of Confidentiality which helps to protect personally identifiable research information. The Certificate of Confidentiality allows investigators on this trial to refuse to disclose identifying information related to the research participants, should such disclosure have adverse consequences for subjects or damage their financial standing, employability, insurability or reputation. The informed consent includes the appropriate coverage and restrictions of the Certificate of Confidentiality.

5.1.6.3.4 Management of Results

Subjects will be contacted if a clinically actionable gene variant is discovered. Clinically actionable findings for the purpose of this study are defined as disorders appearing in the American College of Medical Genetics and Genomics recommendations for the return of incidental findings that is current at the time of primary analysis. (A list of current guidelines is maintained on the CCR intranet: https://ccrod.cancer.gov/confluence/display/CCRCRO/Incidental+Findings+Lists). Subjects will be contacted at this time with a request to provide a blood sample to be sent to a CLIA certified laboratory. If the research findings are verified in the CLIA certified lab, the subject will be offered the opportunity to come to NIH (at our expense) to have genetic education and counseling to explain this result. If the subject does not want to come to NIH, a referral to a local genetic healthcare provider will be provided (at their expense).

This is the only time during the course of the study that incidental findings will be returned. No interrogations regarding clinically actionable findings will be made after the primary analysis.

5.1.7 Circulating Tumor Cells (CTC)

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, to include multiplex analysis.

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

Blood collection:

At baseline and at one later timepoint (as close as possible to the time of planned prostatectomy or week 9 for initial safety cohort), one 10 mL Streck Cell-Free DNA (brown-black top) tube will be collected. Dr. Figg's lab will be paged at 102-11964 for tube pick up and shipping 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°C for immunology assays may also be shipped to Epic Sciences for CTC analysis. Samples will be shipped to the following address:

Epic Sciences
[redacted]
9381 Judicial Drive, Ste. 200
San Diego, CA 92121
[redacted]

5.2 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 appropriate approvals and/or agreements, if required.

5.2.1 Samples Managed by Dr. Figg's Blood Processing Core (BPC)

All samples processed by the Blood Processing Core (BPC) will be barcoded, with data entered and stored in the 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 participants without Labmatrix access. The data recorded for each sample includes the participant ID, name, trial name/protocol number, time drawn, cycle time point, dose, material type, as well as box and freezer location. Participant demographics associated with the clinical center participant 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.

5.2.2 Samples Sent to Clinical Services Program (CSP)

All data associated with participant samples is protected by using a secure database. All Clinical Support Laboratory Staff receive annual training in maintaining records' confidentiality. All samples drawn at the NIH Clinical Center will be transported to the Clinical Support Laboratory at the Frederick National Laboratory for Cancer Research by couriers.

Samples will be tracked and managed by the Central Repository database, where there is no link to personal identifiable information. All samples will be stored in either a -80˚C freezer or vapor phase liquid nitrogen. These freezers are located at NCI Frederick Central Repository in Frederick, Maryland.

NCI-Frederick Central Repositories (managed under a subcontract) 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.

Specimens are stored in accordance will applicable HHS and FDA Protection of Human Subjects Regulations in accordance with the subcontractor's Federal-wide Assurance. The subcontractor's role is limited to clinical research databases and repositories containing participant specimens. The subcontractor does not conduct nor has any vested interest in research on human subjects, but does provide services and support the efforts of its customers, many of which are involved in research on human subjects. The subcontractor's IRB reviews policies and procedures for labeling, data collection and storage, access, and security. The IRB will review protection of privacy issues prior to acceptance of any new work and in the event of change impacting privacy issues in existing work.

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

Sample data is stored in the BioSpecimen Inventory System II (BSI). This inventory tracking system is used to manage the storage and retrieval of specimens as well as maintain specimen data. BSI is designed for controlled, concurrent access. It provides a real-time, multi-user environment for tracking millions of specimens. The system controls how and in what order database updates and searches are performed. This control prevents deadlocks and race conditions. For security, BSI has user password access, three types of user access levels, and 36 user permissions (levels of access) that can be set to control access to the system functions. BSI

provides audit tracking for processes that are done to specimens including shipping, returning to inventory, aliquoting, thawing, additives, and other processes. BSI tracks the ancestry of specimens as they are aliquoted, as well as discrepancies and discrepancy resolution for specimens received by the repository. If a specimen goes out of the inventory, the system maintains data associated with the withdraw request. Vials are labeled with a unique BSI ID which is printed in both eye readable and barcoded format. No participant specific information is encoded in this ID.

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

5.2.3 Protocol Completion/Sample Destruction

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

Samples and associated data will be stored permanently unless the participant withdraws consent. If the participant withdraws consent the participant's data will be excluded from future distributions, but data that have already been distributed for approved research use will not be able to be retrieved. The PI will record any loss or unanticipated destruction of samples as a deviation. Reporting will be per the requirements of section **7.2.1**.

6 DATA COLLECTION AND EVALUATION

6.1 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 the subject received the last study drug administration. Beyond 30 days after the last study drug and through approximately 100 days, only adverse events which are serious and related to the study intervention need to be recorded.

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

- Results in discontinuation from the study
- Is associated with clinical signs or symptoms
- Requires treatment or any other therapeutic intervention

- Is associated with death or another serious adverse event, including hospitalization.
- Is judged by the Investigator to be of significant clinical impact

If any abnormal laboratory result is considered clinically significant, the investigator will provide details about the action taken with respect to the test drug and about the participant'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 **7.2.1**.

- Eligible participants must be confirmed and checklist completed. Consent form must be signed prior to registration with Central Registration Information Services.
- Data will be secured in study database. Data will be collected using protocol-specific case report forms and verified for accuracy and completeness. Hard copies of data will be stored in locked secured areas and data will be entered onto a secured electronic data base. The following protocol-specific study forms will be complete and stored: eligibility checklist (developed by Central Registration Office, CRO). A copy of all serious AE forms will be kept in the research record.
- Treatment is given according to protocol (dated notes about doses given, complications, and clinical outcomes).
- Toxicity is assessed according to protocol (laboratory report slips, etc.)
- Response is assessed according to protocol (X-ray, scan, lab reports, and date noted on clinical assessment, as appropriate).
- Drug Accountability Records are kept for each participant.

6.2 DATA SHARING PLANS

6.2.1 Human Data Sharing Plan

What data will be shared?

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

- Coded, linked data in an NIH-funded or approved public repository.
- Coded, linked data in BTRIS (automatic for activities in the Clinical Center)
- 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:

- An NIH-funded or approved public repository: clinicaltrials.gov, dbGaP

- BTRIS (automatic for activities in the Clinical Center)
- Approved outside collaborators under appropriate individual agreements.
- Publication and/or public presentations.

When will the data be shared?

- Before publication.
- At the time of publication or shortly thereafter.

6.2.2 Genomic Data Sharing Plan

Unlinked genomic data will be deposited in public genomic databases such as dbGaP in compliance with the NIH Genomic Data Sharing Policy.

6.3 RESPONSE CRITERIA (APPLICABLE TO CRPC COHORT)

Recent clinical studies evaluating checkpoint inhibitors in solid tumors have shown that in a fraction of cases even if some new lesions appear at the beginning of a treatment or if the total tumor burden does not increase substantially, tumor regressions or stabilizations might still occur later. For the CRPC cohort of this trial, a modified immune related response criteria (irRC) based on RECIST 1.1 [44] will be used (See Appendix D, Section **16.4**). Changes in the largest diameter (unidimensional measurement) of the tumor lesions and the shortest diameter in the case of malignant lymph nodes will be used as per RECIST 1.1 criteria.

This modified irRC will be based upon RECIST 1.1 criteria (described below) except for 2 major changes:
1) require confirmation of progression by imaging at 4 weeks after initial imaging (evidence of progression of prostate cancer within the first 3 months on bone scan only should be interpreted with extreme caution due to risk of tumor flare) and
2) do not necessarily score the appearance of new lesions as progressive disease if the sum of lesion diameters of target lesions (minimum of 10 mm per lesion, maximum of 5 target lesions, maximum of 2 per organ) and measurable new lesions does not increase by ≥ 20%.

6.3.1 Definitions

Evaluable for toxicity: All participants will be evaluable for toxicity from the time of their first treatment

Evaluable for objective response: Only those participants who have measurable disease present at baseline, have received at least one cycle of therapy, and have had their disease re-evaluated will be considered evaluable for response. These participants will have their response classified according to the definitions stated below. (Note: Participants who exhibit objective disease progression prior to the end of cycle 1 will also be considered evaluable.)

Evaluable Non-Target Disease Response: Participants 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.

6.3.2 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:

.1 Scan slice thickness 5 mm or under: as ≥10 mm;

.2 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 participant, 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.

6.3.3 Methods for Evaluation of 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 8 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.

Tumor markers: Tumor markers alone cannot be used to assess response. If markers are initially above the upper normal limit, they must normalize for a participant to be considered in complete clinical response. Specific guidelines for both CA-125 response (in recurrent ovarian cancer) and PSA response (in recurrent prostate cancer) have been published. [45-47] In addition, the Gynecologic Cancer Intergroup has developed CA-125 progression criteria which are to be integrated with objective tumor assessment for use in first-line trials in ovarian cancer.[48]

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:

- Negative FDG-PET at baseline, with a positive FDG-PET at follow-up is a sign of PD based on a new lesion.
- 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.
- 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.

6.3.4 Response Criteria

6.3.4.1 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 of 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 is also considered progressions).

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

6.3.4.2 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 participant 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).

6.3.4.3 Metastatic Bone Lesions

Disease progression is considered if a minimum of two new lesions is observed on bone scan.

6.3.4.4 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 participant's best response assignment will depend on the achievement of both measurement and confirmation criteria.

For Participants 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
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: Participants 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 Participants 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		

6.3.5 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.

6.3.6 Progression-Free Survival

6.3.6.1 Serologic Response

PSA will be assessed at each visit during treatment and every 12 weeks thereafter. Participants will be assigned response based on the following criteria.

Complete Serological Response: PSA level less than 0.2 ng/ml measured for 2 consecutive measurements at least 4 weeks apart.

Serological Partial Response: decline of PSA at least 50% measured for 2 consecutive measurements at least 4 weeks apart.

Serological Progression:

- Serological progression will only be measured once PSA has risen above 4 ng/ml and this value must be 50% above the PSA level before commencing ADT and;
- Increase in PSA more than 50% of nadir (lowest PSA on ADT).
- Values must be measured for 2 consecutive measurements at least 2 weeks apart.
- The date of the first increase will be recorded as progression.

Time to Serologic Progression will be defined as interval between initiation of ADT and date of PSA progression or death from any cause.

6.4 TOXICITY CRITERIA

The following adverse event management guidelines are intended to ensure the safety of each participant 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).

7 NIH REPORTING REQUIREMENTS/DATA AND SAFETY MONITORING PLAN

7.1 DEFINITIONS

Please refer to definitions provided in Policy 801: Reporting Research Events found at: https://irbo.nih.gov/confluence/pages/viewpage.action?pageId=36241835#Policies&Guidance-800Series-ComplianceandResearchEventReportingRequirements.

7.2 OHSRP OFFICE OF COMPLIANCE AND TRAINING/ IRB REPORTING

7.2.1 Expedited Reporting

Please refer to the reporting requirements in Policy 801: Reporting Research Events and Policy 802 Non-Compliance Human Subjects Research found at: https://irbo.nih.gov/confluence/pages/viewpage.action?pageId=36241835#Policies&Guidance-800Series-ComplianceandResearchEventReportingRequirements. Note: Only IND Safety Reports that meet the definition of an unanticipated problem will need to be reported per these policies.

7.2.2 IRB Requirements for PI Reporting at Continuing Review

Please refer to the reporting requirements in Policy 801: Reporting Research Events found at: https://irbo.nih.gov/confluence/pages/viewpage.action?pageId=36241835#Policies&Guidance-800Series-ComplianceandResearchEventReportingRequirements.

7.3 NCI CLINICAL DIRECTOR REPORTING

Problems expeditiously reviewed by the OHSRP in the NIH eIRB system will also be reported to the NCI Clinical Director/designee; therefore, a separate submission for these reports is not necessary.

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

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

7.4 INSTITUTIONAL BIOSAFETY COMMITTEE (IBC) REPORTING CRITERIA

7.4.1 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 PROSTVAC as soon as possible but in no event later than 7 calendar days of initial receipt of the information. Serious adverse events that are unexpected and associated with the use of the PROSTVAC, but are not fatal or life-threatening, must be reported to the NIH IBC as soon as possible, but not later than 15 calendar days after the investigator's initial receipt of the information. Adverse events may be reported by using the FDA Form 3500a.

7.4.2 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.

7.4.3 Clinical Trial Information

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

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

7.4.4 Progress Report and Data Analysis

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

  - a narrative or tabular summary showing the most frequent and most serious adverse experiences by body system

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

7.5 NIH Required Data and Safety Monitoring Plan

7.5.1 Principal Investigator/Research Team

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

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 **Error! Reference source not found.** will be submitted within the appropriate timelines.

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

8 SPONSOR PROTOCOL/SAFETY REPORTING

8.1 Definitions

8.1.1 Adverse Event

Any untoward medical occurrence in a participant 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))

8.1.2 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 **8.1.3**)
- 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.

8.1.3 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)

8.1.4 Severity

The severity of each Adverse Event will be assessed utilizing the CTCAE version 4.0.

8.1.5 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.

8.2 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 **6.1**. All serious adverse events recorded from the time of first investigational product administration must be reported to the sponsor.

8.3 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. All SAE reporting must include the elements described in section **8.2**.

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.

8.4 SAFETY REPORTING CRITERIA TO THE PHARMACEUTICAL COLLABORATORS

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

The CCR Office of Sponsor and Regulatory Oversight (OSRO) will send all reports to the manufacturer as described below.

8.4.1 Bavarian-Nordic

To be sent by Office of Sponsor and Regulatory Oversight (OSRO), CCR, NCI

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

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

Bavarian-Nordic, Inc.
Email: drug.safety@bavarian-nordic.com
[redacted]

The following minimum information is required:

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

Follow-up information for the event should be sent within 7 days as necessary.
SAEs that are deemed unrelated or unlikely to be due to the study vaccines are submitted regularly to BNIT at the following address:

Bavarian-Nordic, Inc.
Email: drug.safety@bavarian-nordic.com
[redacted]

8.4.2 Bristol-Myers-Squibb

To be sent by OSRO, CCR, NCI, NIH:

Following the subject's written consent to participate in the study, all SAEs, whether related or not related to study drug, must be collected, including those thought to be associated with protocol-specified procedures. All SAEs must be collected that occur within 100 days of discontinuation of dosing.

All SAEs must be collected that occur during the screening period. If applicable, SAEs must be collected that relate to any protocol-specified procedure (e.g., a follow-up skin biopsy). The investigator should report any SAE that occurs after these time periods that is believed to be related to study drug or protocol-specified procedure.

SAEs, whether related or not related to study drug, and pregnancies must be reported to BMS within 24 hours. SAEs must be recorded on BMS or an approved form; pregnancies on a Pregnancy Surveillance Form.

SAE Email Address: Worldwide.Safety@BMS.com

SAE Facsimile Number: 609-818-3804

If only limited information is initially available, follow-up reports are required. (Note: Follow-up SAE reports should include the same investigator term(s) initially reported.)

If an ongoing SAE changes in its intensity or relationship to study drug or if new information becomes available, a follow-up SAE report should be sent within 24 hours to the BMS (or designee) using the same procedure used for transmitting the initial SAE report.

All SAEs should be followed to resolution or stabilization.

The Investigator will ensure that all SAEs in the clinical database are reported to BMS and any applicable health authority during the conduct of the study. This reconciliation will occur at least quarterly and be initiated by the investigator. Investigator will request a reconciliation report from: aepbusinessprocess@bms.com. During reconciliation, any events found to not be reported previously to BMS must be sent to Worldwide.Safety@BMS.com.

BMS will be provided with a simultaneous copy of all adverse events filed with the FDA.

SAEs should be reported on MedWatch Form 3500A, which can be accessed at: http://www.accessdata.fda.gov/scripts/medwatch/ or CCR SAE report form.

All SAEs should simultaneously be faxed or e-mailed to BMS at:

Global Pharmacovigilance & Epidemiology

Bristol-Myers Squibb Company

Fax Number: 609-818-3804

Email: Worldwide.safety@bms.com

8.5 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

8.5.1 Paternal exposure

Male participants should refrain from fathering a child or donating sperm during the study and for 7 months after the last dose of study agent.

Pregnancy of the participant's partner is not considered to be an AE. However, the outcome of all pregnancies occurring from the date of the first dose until 7 months after the last dose should, if possible, be followed up and documented. Pregnant partners may be offered the opportunity to participate in an institutional pregnancy registry protocol (e.g., the NIH IRP pregnancy registry study) to provide data about the outcome of the pregnancy for safety reporting purposes.

8.6 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.

8.7 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.

9 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) Sponsor 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.

10 STATISTICAL CONSIDERATIONS

For the initial lead-in cohort, the primary objective is safety, especially detection of clinically important inflammation defined as a grade 3 diarrhea or colitis requiring steroids or anti cytokine therapy or not resolving to grade 1 or less within 28 days. If there are 10 participants evaluated in the lead-in cohort and if 0/10 have clinically important inflammation, then this will be considered acceptable for proceeding with the neoadjuvant cohort. On the other hand, if 1/10 or more have significant inflammation, then we will discuss with the IRB, FDA and sponsor amending the trial, since the upper one-sided 90% confidence interval on 1/10 is 33.7% which would be consistent with an excessive rate of inflammation.

After the lead-in cohort, participants on this study will be assigned to PROSTVAC and Nivolumab (neoadjuvant cohort) for 9 weeks. Utilizing computer automated staining analysis, density of cell infiltrate will be calculated and the pre- and post-treatment values will be compared to determine response to treatment. All measurement will be determined in a blinded fashion with respect to the treatment groups. The primary evaluation will be of a comparison of differences between the neoadjuvant cohort and participants treated on the 14-C-0112 protocol (NCT02153918), of the changes of the CD3 + T cells, measured from baseline to the radical prostatectomy at 9 weeks on the present trial, and from baseline to the radical prostatectomy at 12 weeks in participants treated on the 14-C-0112 trial.

With 17 total evaluable participants having both CD3+/CD4+ and CD3+/CD8+ T cell measurements from baseline to 9 weeks on the present trial, and comparable data from 26 participants on the 14-C-0112 trial, there would be adequate participants to have 80% power to detect a difference between the cohort from this trial and that from the prior trial of a change in each type of CD3+ T cells from baseline to 9 weeks (or baseline to 12 weeks in the 14-C-0112 trial) equal to 1.0 SD of the change in the CD3 + T cells (1.0 SD effect size) using a 0.025 significance level two-tailed two group t-test to allow adequate participants for a Bonferroni adjustment holding the overall significance level to 0.05 for both tests. In practice, a Hochberg adjustment may be used instead of the overly stringent Bonferroni adjustment. Also, if the distributions of the CD3 + T cells are not normally distributed ($p<0.05$ by a Shapiro-Wilks test), then a Wilcoxon rank sum test may be used instead of a two-sample t-test. The results from the

comparison of data from the two studies will be reported with appropriate caveats to reflect differences in study characteristics, and to reflect the lack of randomized participant assignment between the two trials.

Secondary objectives are to determine the change in peripheral PSA-specific T cells in participants treated with these vaccines; to document any intraprostatic Treg cell infiltration with CD4+FOX-P3 staining; to document any PSA changes secondary to vaccination, including rate of biochemical recurrence after prostatectomy; to document any MRI changes secondary to treatment; to evaluate changes in PDL-1 expression; and to document pathologic responses (including pathologic CR). When analysis is required, each of these endpoints will be analyzed using appropriate nonparametric methods when applicable and the results from these analyses will be presented without formal adjustment for multiple comparisons, but in the context of the number of such evaluations undertaken.

Slides made from prostate biopsy specimens (collected at baseline) and prostatectomy specimens (collected at surgery performed after last dose of vaccine) will be stained for CD4 and CD8 cells and digitized for analysis of immune cell infiltrate. Other markers such as CD4/FoxP3 may also be stained for further correlative studies. These studies will be performed by the Laboratory of Tumor Immunology and Biology. At a minimum, 5 slides from one biopsy core confirmed to contain cancer will be needed for baseline quantification. Attempts will be made based on anatomic location notation or when available, MRI based location, to correlate biopsy location with pathologic location on prostatectomy specimens. When feasible, quantitation will be performed in triplicate. Quantitation will be reported as number of stained cells per micron squared of surface area. Wilcoxon matched pair signed rank test, Friedman test with Dunn's multiple comparisons, or alternative statistical models will be used as appropriate. Aperio ScanScope digital scanner systems with Aperio ImageScope software algorithms will be used. Manual quantitation will be performed in case of software malfunction. Isotype antibodies negative controls will be included in all runs. Tonsil or lymph node tissue will be positive control for CD4 and CD8. Slides will be developed with primary and secondary antibodies per manufacturer specifications for visualization of cells.

The lead-in safety cohort will require 10 participants and the neoadjuvant cohort will require 17 evaluable participants. In order to allow for a small number of inevaluable participants, the accrual ceiling will be set to 29 participants. If 3-4 participants per month enroll onto this study, accrual is expected to be completed in one year.

11 COLLABORATIVE AGREEMENTS

11.1 COLLABORATIVE RESEARCH AND DEVELOPMENT AGREEMENT (CRADA)

The PROSTVAC vaccines will be provided under a Collaborative Research and Development Agreement (CRADA) with Bavarian Nordic, Inc., CRADA# 02377.

11.2 CLINICAL TRIALS AGREEMENT (CTA)

Bristol-Myers Squibb will be providing the study agent, nivolumab through a Clinical Trials Agreement (CTA), CTA# 1034-17.

11.3 MATERIALS TRANSFER AGREEMENT (MTA)

Samples will be sent to the following collaborators through a Materials Transfer Agreement (MTA): General Electric (section 5.1.6.1); PerkinElmer (section 5.1.6.1); Definiens (section 5.1.6.2); HTG Molecular Diagnostics (section 5.1.6.3.1); NantOmics (section 5.1.6.3.2); and Epic Sciences (section 5.1.7).

12 HUMAN SUBJECTS PROTECTIONS

12.1 RATIONALE FOR SUBJECT SELECTION

Subjects treated on this study, will be men with castration resistant cancer (lead-in cohort) and localized adenocarcinoma of the prostate (neoadjuvant cohort).

Eligibility assessment will be based solely on the participant's medical status. Recruitment of participants onto this study will be through standard CCR mechanisms. No special recruitment efforts will be conducted.

12.2 INCLUSION OF WOMEN AND MINORITIES

Men of all races and ethnic groups are eligible for this trial. Women are excluded as prostate cancer does not exist in this population.

12.3 PARTICIPATION OF CHILDREN

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

12.4 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 enrolled in the protocol may permanently lose capacity to consent for themselves during the course of this study. For this reason and because there is a prospect of direct benefit from research participation (section **12.6**), all subjects ≥ age 18 will be offered the opportunity to fill in their wishes for research and care, and assign a substitute decision maker on the "NIH Advance Directive for Health Care and Medical Research Participation" form so that another person can make decisions about their medical care in the event that they become incapacitated or cognitively impaired during the course of the study. Note: The PI or AI will contact the NIH Ability to Consent Assessment Team (ACAT) for evaluation to assess ongoing capacity of the subjects and to identify an LAR as needed. Please see section **12.7.1** for consent procedure.

12.5 EVALUATION OF BENEFITS AND RISKS/DISCOMFORTS

Potential risks of vaccine in this participant population include the range of side effects outlined in Section **14**. PROSTVAC has been well tolerated in previous large trials.

12.5.1 Alternative Approaches or Treatments

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

12.5.2 Procedures to Eliminate or Minimize Potential Risks

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

12.5.3 Provisions for Monitoring Data Collection to Ensure Subject Safety

As information is gathered from this trial, clinical results will be shared with participants. 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 participant's willingness to participate further, will be explained.

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

12.5.4 Risks due to biopsy

All care will be taken to minimize risks that may be incurred by tumor sampling. However, there are procedure-related risks (such as bleeding, infection and visceral injury) that will be explained fully during informed consent. If participants suffer any physical injury as a result of the biopsies, immediate medical treatment is available at the NCI's Clinical Center in Bethesda, Maryland. Although no compensation is available, any injury will be fully evaluated and treated in keeping with the benefits or care to which participants are entitled under applicable regulations.

12.5.5 Research Blood Collection Risks

Risks of blood draws include pain and bruising in the area where the needle is placed, lightheadedness, and rarely, fainting. When large amounts of blood are collected, low red blood cell count (anemia) can develop.

12.5.6 Risks due to Radiation

The study will involve radiation from the following sources for CRPC cohort:

- Up to 5 CT scans (C/A/P) per year for disease assessment not including one performed on separate study at screening
- Up to 5 Tc99 scans per year for disease assessment not including one performed on separate study at screening
- 1 CT-guided biopsy

Subjects in this study may be exposed up to 8.25 rem maximum annually.

The CT and bone scans that participant get in this study will expose them to the roughly the same amount of radiation up to 27.5 years of background radiation depending upon how much they weigh. The risk of getting cancer from the radiation exposure in this study is 0.8 out of 100 (0.8%) and of getting a fatal cancer is 0.4 out of 100 (0.4 %).

The study will have no radiation exposure for Neoadjuvant cohort:

12.5.7 Risks due to contrast dye (for CT and/or MRI)

If contrast dye is used, there is a risk for allergic reaction to the dye. Participants who are allergic to or sensitive to medications, contrast dye, iodine, or shellfish should notify their physician. Participants with kidney failure or other kidney problems should notify their physician. Participants may receive a contrast agent such as Gadolinium as part of your CT scan or MRI. Risks due to gadolinium are explained in consent document.

12.5.8 Risks due to MRI

Risks due to MRI are explained in the consent document.

12.6 RISKS/BENEFITS ANALYSIS

This study involves clinical research with an experimental vaccine designed to generate an immune response against antigens found in prostate cancer. Participants will undergo multiple vaccinations. Side effects of the vaccine are outlined elsewhere. Whether the vaccine will have any clinical effect is unknown; therefore, benefit cannot be promised from vaccine in addition to standard of care, nor the chance of benefit accurately predicted.

The aim of this study is to see if PROSTVAC vaccine in combination with nivolumab is safe for castration resistant prostate cancer (CRPC) participants and to evaluate changes in the immune cells in the tumor after treatment in all other cancer participants. The study drugs may help to control the disease. There is a possibility of benefit for participants in this study, though it can't be certain.

Participation in this study is voluntary, and refusal will not result in penalty or loss of benefit to which the participant is otherwise entitled.

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

12.7 CONSENT PROCESS AND DOCUMENTATION

The informed consent document will be provided as a physical or electronic document to the participant or consent designee(s) (legally authorized representative [LAR] if participant is an adult unable to consent) 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. 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 local 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.

Manual (non-electronic) signature on electronic document:

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 subject 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 at:
https://ccrod.cancer.gov/confluence/pages/viewpage.action?pageId=73203825.

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

For participants addressed in section **12.4**, an LAR will be identified consistent with Policy 403 and informed consent obtained from the LAR, as described in Section **12.7**.

13 REGULATORY AND OPERATIONAL CONSIDERATIONS

13.1 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, 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).

13.2 QUALITY ASSURANCE AND QUALITY CONTROL

Each 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.

13.3 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.

13.4 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 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 NCI CCR.

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.

14 PHARMACEUTICAL INFORMATION

14.1 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 by Bavarian Nordic, Inc. 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.

Preparation, Handling and Disposal Recommendations

- Strictly adhere to standard microbiological practices and techniques.
- Limit/restrict access to preparation areas while dose preparation is in progress.
- Use appropriate infection control measures (e.g., thorough hand washing) after handling any materials.
- Institute and follow policies for safe handling of sharps.
- Perform all dose preparations in a certified Class II biological safety cabinet, generally using procedures, guidelines and personal protective apparel used during preparation of antineoplastic agents [*e.g.*, minimizing creation of aerosols; no eating, drinking, handling contact lenses or applying cosmetics in the work area; using appropriate personal protective apparel - gowns, sterile latex gloves (double-gloving is recommended), respirator masks, protective eye wear, hair cover].
- 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.
- 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.
- 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.
- Transport the agent from the freezer to the work area in leak proof bag.
- 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.
- 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.
- 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.
- Incinerate waste according to institutional policy and according to local, state, and federal regulations.
- 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.
  For more information about biohazard risk group classification and biohazard safety levels see:

A. *Biosafety in Microbiological and Biomedical Laboratories; U. S. Department of Health and Human Services Centers for Disease Control and Prevention and National Institutes of Health. See current edition at:*
   http://www.cdc.gov/biosafety/publications/index.htm

Patient Care Implications and Contraindications

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

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

14.2 RECOMBINANT VACCINIA-PSA(L155)/TRICOM™

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

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

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

humoral and cellular immune response. Vaccinia virus is replication competent in mammalian cells, making systemic infections possible.

How Supplied: Recombinant Vaccinia-PSA(L155)/TRICOM™ is supplied by Bavarian Nordic, Inc. 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.

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.

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

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

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

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

Preparation, Handling and Disposal Recommendations

- Prepare a biosafety manual which advises personnel of special hazards and specific instructions on practices and procedures.
- 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.
- 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.
- Strictly adhere to standard microbiological practices and techniques.
- Limit/restrict access to preparation areas while dose preparation is in progress.

- Use appropriate infection control measures (*e.g.*, thorough hand washing) after handling any materials.
- 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.
- 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].
- Perform all procedures carefully to minimize aerosol creation.
- 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.
- 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.
- Place an empty biohazard sharps container in the biosafety cabinet to dispose of all waste generated.
- Transport the agent from the freezer to the work area in leak proof bag.
- 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.
- 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.
- 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.
- 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.
- 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 and protective apparel as infectious biohazardous waste and incinerate according to institutional policy and according to local, state, and federal regulations.

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

B. Biosafety in Microbiological and Biomedical Laboratories; U. S. Department of Health and Human Services Centers for Disease Control and Prevention and National Institutes of Health. See current edition at: http://www.cdc.gov/biosafety/publications/index.htm

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):

1. have active eczema or a history of eczema or atopic dermatitis, or Darier's disease.
2. 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.
3. are pregnant or intend to become pregnant within 4 weeks of vaccination or are breast-feeding.
4. are immunodeficient or immunocompromised (by disease or therapy), including HIV infection.

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

- has a moderate or severe acute illness, until the illness resolves.
- is less than 18 years of age, unless specifically indicated.
- is undergoing topical steroid therapy for inflammatory eye diseases or undergoing therapy with systemic steroids; potential immune suppression increases risk for vaccinia-related complications.
- has a history of allergy or serious reaction to prior vaccinia vaccination or any of the vaccine's components.

- As a precaution, the CDC recommends that individuals with known cardiac disease (e.g., previous MI, angina, CHF, cardiomyopathy, stroke or TIA) or who have ≥ 3 known risk factors for cardiac disease (e.g., hypertension, hypercholesterolemia, diabetes, first degree relative with onset of cardiac complications prior to age 50, smoker), not receive routine, non-emergency, prophylactic vaccination with the licensed vaccinia vaccine while a possible causal relationship between vaccination and cardiac events is being evaluated.

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

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

Recombinant Vaccinia Vaccine Patient Care Implications, Contraindications and Potential Complications

Patient Care Implications and Contraindications

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

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

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

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

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

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

Potential Complications Associated With Recombinant Vaccinia Vaccination

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

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

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

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

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

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

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

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

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

- **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.
- **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.
- **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.

- **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.
- **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.
- **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.

- **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.
- **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.
- **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.

14.3 NIVOLUMAB

Amino Acid Sequence: 4 polypeptide chains, which include 2 identical heavy chains with 440 amino acids and 2 identical light chains.

Other Names: BMS-936558, MDX1106

Classification: Anti-PD-1MAb

M.W.: 146,221 daltons

Mode of Action: Nivolumab targets the programmed death–1 (PD-1, cluster of differentiation 279 [CD279]) cell surface membrane receptor. PD-1 is a negative regulatory receptor expressed by activated T and B lymphocytes. Binding of PD-1 to its ligands, programmed death–ligand 1 (PD-L1) and 2 (PD-L2), results in the downregulation of lymphocyte activation. Nivolumab inhibits the binding of PD-1 to PD-L1 and PD-L2. Inhibition of the interaction between PD-1 and its ligands promotes immune responses and antigen-specific T-cell responses to both foreign antigens as well as self-antigens.

Description: Nivolumab Injection is a clear to opalescent, colorless to pale yellow liquid; light (few) particulates may be present. The drug product is a sterile, nonpyrogenic, single-use, isotonic aqueous solution formulated in sodium citrate, sodium chloride, mannitol, diethylenetriamine pentacetic acid (pentetic acid) and polysorbate 80 (Tween® 80), pH 6.0.

How Supplied: Nivolumab will be supplied by Bristol-Myers Squibb as 100 mg vials (10 mg/mL) with a 0.7mL overfill. It is supplied in 10 mL type I flint glass vials, with butyl rubber stoppers and aluminum seals.

Preparation: Nivolumab injection can be infused undiluted (10 mg/mL) or diluted with 0.9% Sodium Chloride Injection, USP or 5% Dextrose, USP to concentrations no less than 0.35 mg/mL. When the dose is fixed (e.g., 240 mg or 480 mg flat dose), nivolumab injection can be infused undiluted or diluted so as not to exceed a total infusion volume of 160 mL.

Storage: Vials of Nivolumab injection must be stored at 2°-8°C (36°-46°F) and protected from light, freezing and shaking.

Stability: Shelf-life surveillance of the intact vials is ongoing.

The administration of undiluted and diluted solutions of Nivolumab must be completed within 24 hours of preparation. If not used immediately, the infusion solution may be stored up to 24 hours in a refrigerator at 2°-8°C (36°-46°F) and a maximum of 8 hours of the total 24 hours can be at room temperature (20°-25°C, 68°-77°F) and room light. The maximum 8-hour period under room temperature and room light conditions includes the product administration period.

Toxicity: The following related adverse events have been reported with nivolumab monotherapy at an incidence of > 5%: fatigue, rash, pruritis, diarrhea, nausea, hypothyroidism, decreased appetite, and musculoskeletal pain. Immune-related adverse events have also been reported and may be severe or life-threating.

Route of Administration: Intravenous infusion. Do not administer as an IV push or bolus injection.

Method of Administration: Administer through a 0.2 micron to 1.2 micron pore size, low-protein binding polyethersulfone membrane in-line filter.

Potential Drug Interactions: No incompatibilities between Nivolumab injection and polyvinyl chloride (PVC), non-PVC/non-DEHP (di(2-ethylhexyl)phthalate) IV components, or glass bottles have been observed.

Destruction of nivolumab provided by BMS:
On-site destruction of unused vials of investigational nivolumab should be conducted according to institutional standards. A copy of the drug destruction certificate must be maintained for provision to BMS at the end of the study.

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16 APPENDICES

16.1 APPENDIX A: PERFORMANCE STATUS CRITERIA

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

16.2 APPENDIX B: VACCINIA-PROSTVAC 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 you 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 the 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 non-bandaged site because live virus could be washed onto other areas of 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 a minimum of 3 weeks 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 the research nurse, call the research nurse's office during regular business hours; if your question is not urgent, you may leave a message for the research nurse on the answering machine. If your question is urgent or if you have a question outside of the regular business hour, please contact the 3NW inpatient unit and speak with the charge nurse.

In an emergency, call 911 or report to the local emergency room/urgent care center. If you have to go to an emergency room, please ask the doctors to call NIH for more information.

PHONE NUMBERS

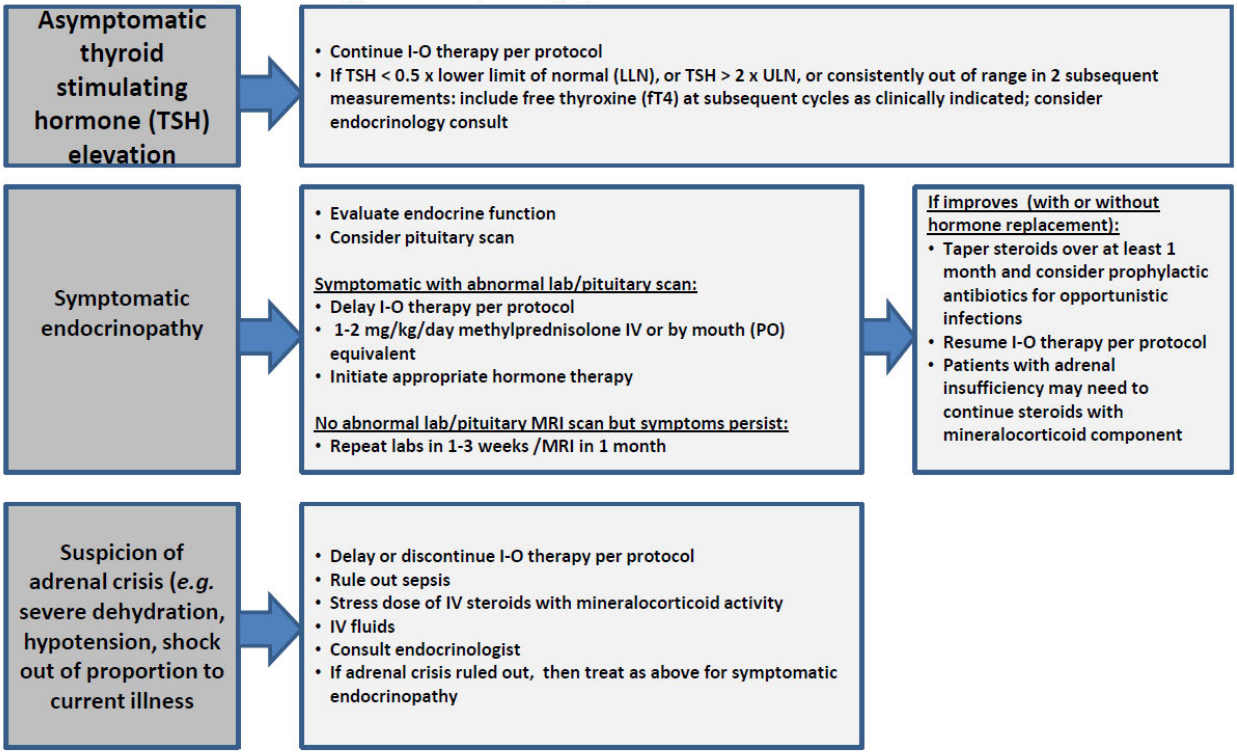
3NW Inpatient unit	(301) 451-0789
12th floor Oncology Clinic	(301) 496-4026
[redacted]	[redacted]
[redacted]	[redacted]

*after regular business hours, please contact 3NW inpatient unit.

16.3 APPENDIX C: MANAGEMENT ALGORITHMS FOR ENDOCRINOPATHY, GASTROINTESTINAL, HEPATIC, NEUROLOGICAL, PULMONARY, RENAL, AND SKIN ADVERSE EVENTS

16.3.1 Endocrinopathy Management Algorithm

Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue immuno-oncology (I-O) therapy. Consider visual field testing, endocrinology consultation, and imaging.



Patients on IV steroids may be switched to an equivalent dose of oral corticosteroids (e.g. prednisone) at start of tapering or earlier, once sustained clinical improvement is observed. Lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of oral corticosteroids.

16.3.2 GI Adverse Event Management Algorithm

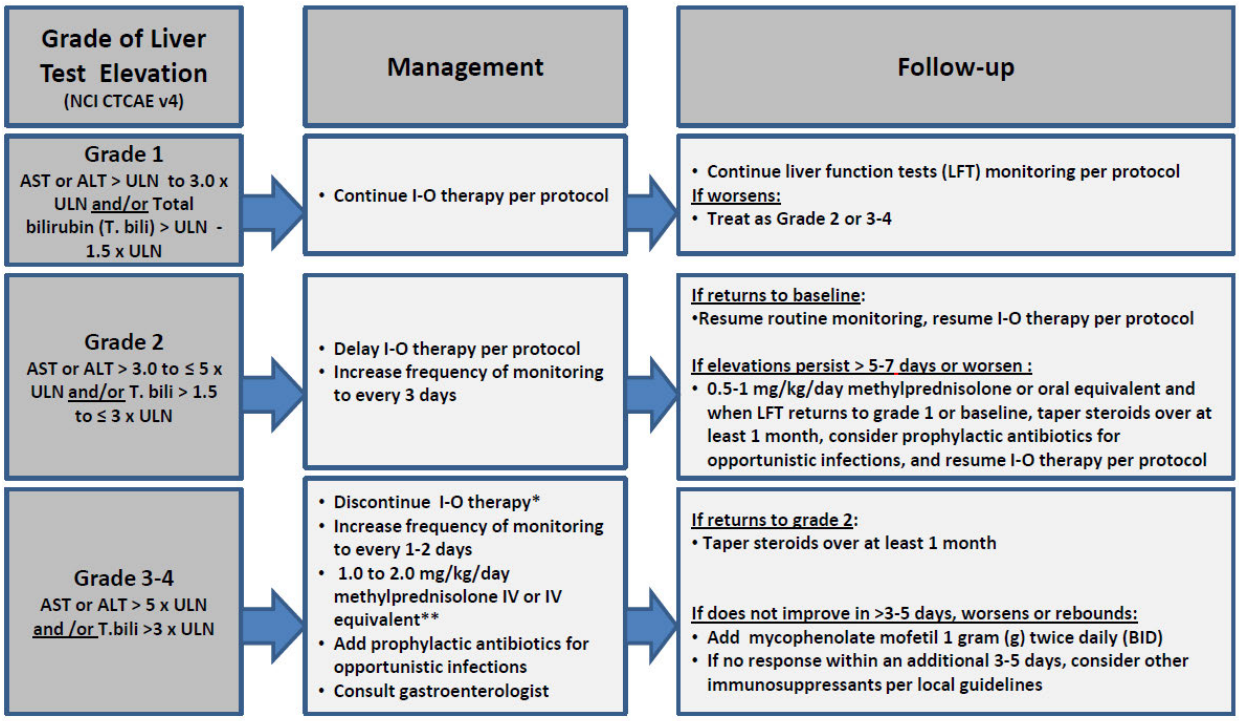
Rule out non-inflammatory causes. If non-inflammatory cause is identified, treat accordingly and continue I-O therapy. Opiates/narcotics may mask symptoms of perforation. Infliximab should not be used in cases of perforation or sepsis.

Grade of Diarrhea/ Colitis (NCI CTCAE v4)	Management	Follow-up
Grade 1 Diarrhea:< 4 stools/day over baseline; Colitis: asymptomatic	• Continue I-O therapy per protocol • Symptomatic treatment	• Close monitoring for worsening symptoms. • Educate patient to report worsening immediately If worsens: • Treat as Grade (G) 2 or 3/4
Grade 2 Diarrhea: 4-6 stools per day over baseline; IV fluids indicated <24 hours (hrs); not interfering with ADL Colitis: abdominal pain; blood in stool	• Delay I-O therapy per protocol • Symptomatic treatment	If improves to grade 1: • Resume I-O therapy per protocol If persists > 5-7 days or recur: • 0.5-1.0 mg/kg/day methylprednisolone or oral equivalent • When symptoms improve to grade 1, taper steroids over at least 1 month, consider prophylactic antibiotics for opportunistic infections, and resume I-O therapy per protocol. If worsens or persists > 3-5 days with oral steroids: • Treat as grade 3/4
Grade 3-4 Diarrhea (G3): ≥7 stools per day over baseline; incontinence; IV fluids ≥24 hrs; interfering with activities of daily living (ADL) Colitis (G3): severe abdominal pain, medical intervention indicated, peritoneal signs G4: life-threatening, perforation	• Discontinue I-O therapy per protocol • 1.0 to 2.0 mg/kg/day methylprednisolone IV or IV equivalent • Add prophylactic antibiotics for opportunistic infections • Consider lower endoscopy	If improves: • Continue steroids until grade 1, then taper over at least 1 month If persists > 3-5 days, or recurs after improvement: • Add infliximab 5 mg/kg (if no contraindication). Note: Infliximab should not be used in cases of perforation or sepsis

Patients on IV steroids may be switched to an equivalent dose of oral corticosteroids (e.g. prednisone) at start of tapering or earlier, once sustained clinical improvement is observed. Lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of oral corticosteroids.

16.3.3 Hepatic Adverse Event Management Algorithm

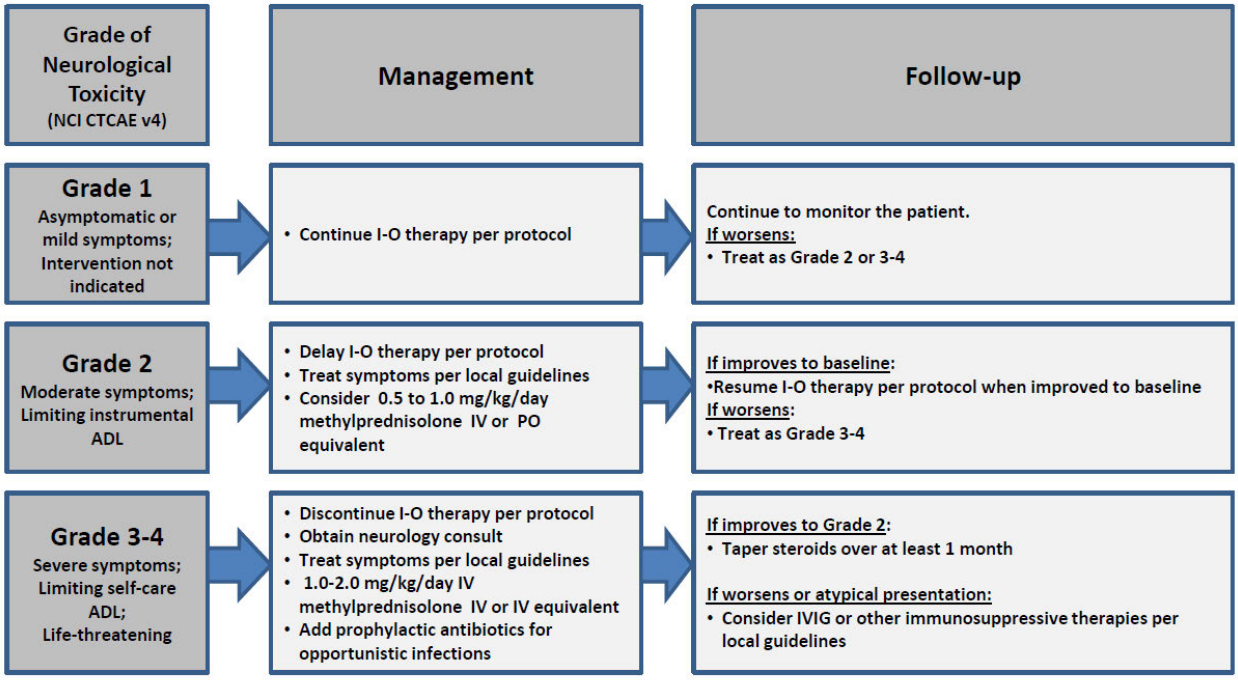
Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue I-O therapy. Consider imaging for obstruction.



Patients on IV steroids may be switched to an equivalent dose of oral corticosteroids (e.g. prednisone) at start of tapering or earlier, once sustained clinical improvement is observed. Lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of oral corticosteroids.
***I-O therapy may be delayed rather than discontinued if AST/ALT ≤ 8 x ULN and T.bili ≤ 5 x ULN.**
**The recommended starting dose for grade 4 hepatitis is 2 mg/kg/day methylprednisolone IV.

16.3.4 Neurologic Adverse Event Management Algorithm

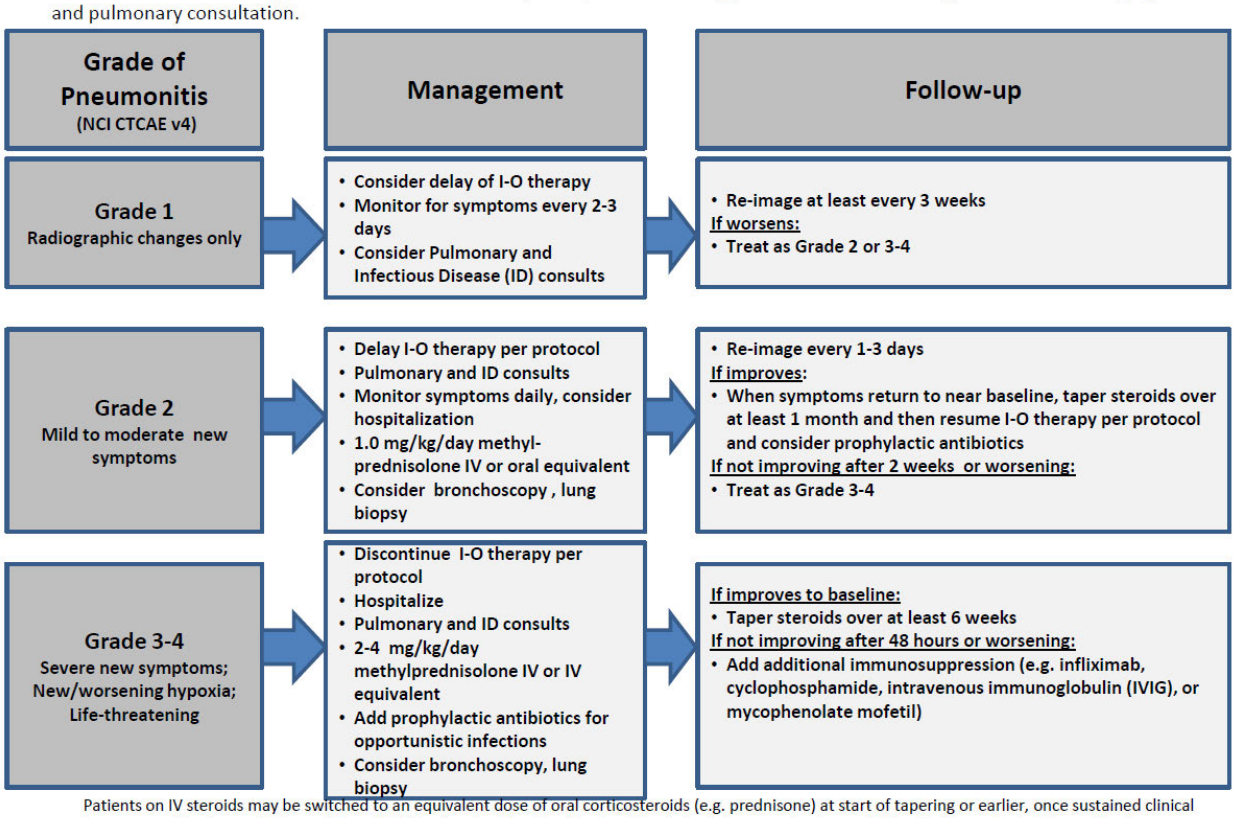
Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue I-O therapy.



Patients on IV steroids may be switched to an equivalent dose of oral corticosteroids (e.g. prednisone) at start of tapering or earlier, once sustained clinical improvement is observed. Lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of oral corticosteroids.

16.3.5 Pulmonary Adverse Event Management Algorithm

Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue I-O therapy. Evaluate with imaging and pulmonary consultation.



Patients on IV steroids may be switched to an equivalent dose of oral corticosteroids (e.g. prednisone) at start of tapering or earlier, once sustained clinical improvement is observed. Lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of oral corticosteroids.

16.3.6 Renal Adverse Event Management Algorithm

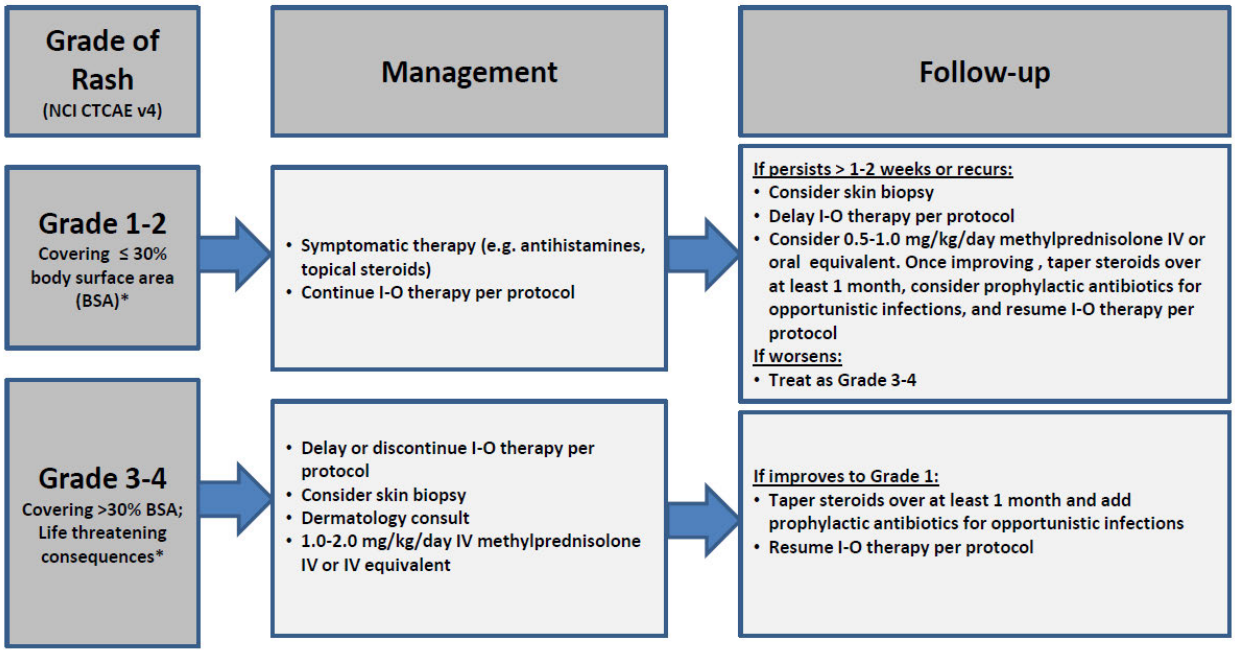
Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue I-O therapy

Grade of Creatinine Elevation (NCI CTCAE v4)	Management	Follow-up
Grade 1 Creatinine > upper limit of normal (ULN) and > than baseline but ≤ 1.5x baseline	• Continue I-O therapy per protocol • Monitor creatinine weekly	<u>If returns to baseline</u>: •Resume routine creatinine monitoring per protocol <u>If worsens:</u> •Treat as Grade 2 or 3/4
Grade 2-3 Creatinine > 1.5x baseline to ≤ 6x ULN	• Delay I-O therapy per protocol • Monitor creatinine every 2-3 days • 0.5 to 1.0 mg/kg/day methylprednisolone IV or oral equivalent • Consider renal biopsy	<u>If returns to Grade 1:</u> •Taper steroids over at least 1 month, consider prophylactic antibiotics for opportunistic infections, and resume I-O therapy and routine creatinine monitoring per protocol <u>If elevations persist > 7 days or worsen:</u> •Treat as Grade 4
Grade 4 Creatinine > 6x ULN	• Discontinue I-O therapy per protocol • Monitor creatinine daily • 1.0-2.0 mg/kg/day methylprednisolone IV or IV equivalent • Consult nephrologist • Consider renal biopsy	<u>If returns to Grade 1</u> : Taper steroids over at least 1 month and add prophylactic antibiotics for opportunistic infections

Patients on IV steroids may be switched to an equivalent dose of oral corticosteroids (e.g. prednisone) at start of tapering or earlier, once sustained clinical improvement is observed. Lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of oral corticosteroids.

16.3.7 Skin Adverse Event Management Algorithm

Rule out non-inflammatory causes. If non-inflammatory cause, treat accordingly and continue I-O therapy.



Patients on IV steroids may be switched to an equivalent dose of oral corticosteroids (e.g. prednisone) at start of tapering or earlier, once sustained clinical improvement is observed. Lower bioavailability of oral corticosteroids should be taken into account when switching to the equivalent dose of oral corticosteroids.
*Refer to NCI CTCAE v4 for term-specific grading criteria.

16.4 APPENDIX D: IMMUNE RELATED RESPONSE CRITERIA

Because of direct clinical observations of immune cell influx into tumor causing enlargement in some patients prior to sustained response, recently it has been suggested that clinical trials involving the use of immunotherapy use alternative guidelines, called immune related response criteria (irRC) to determine radiographic response or progression after therapy[49]. These recommendations have been used in recent clinical trials. One study of 227 subjects with metastatic melanoma showed that the approximately 10% of patients who had PD by modified WHO criteria but either CR, PR or SD by irRC had a similar overall survival as those patients who had SD, PR or CR by both criteria. The irRC was created using bidimensional measurements (as previously widely used in the WHO criteria). We have taken the concepts of the irRC and combined them with the recently revised RECIST 1.1[44] to come up with the modified irRC used in this protocol. Consistent with the irRC, the main changes from RECIST 1.1 are (a) a requirement for confirmation of both progression and response by imaging at least 4 weeks after initial imaging and (b) not automatically calling the appearance of new lesions progressive disease if the total measurable tumor burden has not met criteria for progressive disease.

For immune-related response criteria (irRC), only index and measurable new lesions are taken into account. At baseline tumor assessment on this trial, target lesions will be measured along the longest axis and the measurements will be summed, called sum of largest diameter (SLD). These lesions must be a minimum of 10mm per lesion, maximum of 5 target lesions, maximum of 2 per organ system. At each subsequent tumor assessment, the unidimensional measurement of target lesions and of new measureable lesions are added together to provide the total tumor burden: As per the modified definitions below, all responses and progression except stable disease (SD) require confirmation on a consecutive scan at least 4 weeks from the initial observation).

Definitions of irRC:

Response	**irRC**
New measurable lesions	**Incorporated into tumor burden**
New non-measurable lesions	**Do not define progression (but precludes irCR)**
Non-index lesions	Contributes to defining irCR (complete disappearance required)
Overall irCR	100% disappearance of all lesions, whether measurable or not, and no new lesions, in two consecutive observations not less than 4 wks from the date first documented. All measurable lymph nodes also must have reduction in short axis to <10mm.
Overall irPR	≥ 30% decrease in SLD compared with baseline confirmed by a consecutive assessment at least 4 wk after first documentation
Overall irSD	Not meeting criteria for irCR or irPR, in absence of irPD: 30% decrease in SLD compared with baseline cannot be established nor 20% increase compared with nadir.
Overall irPD	At least 20% increase in SLD compared with nadir (minimum recorded tumor

	burden) and an increase of at least 5mm over the nadir, confirmed by a repeat, consecutive observations at least 4 wk from the date first documented.

Overall responses derived from changes in index, non-index and new lesions as demonstrated in the following table:

Measurable response	**Non-measurable response**		**Overall response using irRC**
Index and new, measurable lesions (tumor burden)* %	**Non-index lesions**	**New, non-measurable lesions**	
Decrease 100	Absent	Absent	irCR[&]
Decrease 100	Stable	Any	irPR[&]
Decrease 100	Unequivocal progression	Any	irPR[&]
Decrease ≥ 30%	Absent / Stable	Any	irPR[&]
Decrease ≥ 30%	Unequivocal progression	Any	irPR[&]
Decrease < 30 to increase < 20	Absent / Stable	Any	irSD
Decrease < 30 to increase < 20	Unequivocal progression	Any	irSD
Increase ≥ 20	Any	Any	irPD

* Decreases assessed relative to baseline

[&] Assuming response (irCR) and progression (irPD) are confirmed by a second, consecutive assessment at least 4 weeks apart.