UCSF Helen Diller Family Comprehensive Cancer Center

NCT# 02506114

AN OPEN LABEL, RANDOMIZED PHASE 2 TRIAL OF PROSTVAC AND IPILIMUMAB AS MONOTHERAPY OR IN COMBINATION FOR MEN WITH LOCALIZED PROSTATE CANCER UNDERGOING RADICAL PROSTATECTOMY

Protocol Number: CC#14559
Study Drugs: PROSTVAC and IPILIMUMAB

Version Number: 2.0
Version Date: July 10, 2018

IND Number: 16241

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Revision History

Version 2.0	07-10-18
Version 1.9	07-05-17
Version 1.8	02-02-17
Version 1.7	11-05-15
Version 1.6	01-28-15
Version 1.5	12-04-14
Version 1.4	09-22-14
Version 1.3	07-23-14
Version 1.2	07-17-14
Version 1.1	07-01-14
Version 1.0	03-14-14

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Protocol Signature Page

Protocol No.: 14559 **Version Date:** 07/10/2018

1. I agree to follow this protocol version as approved by the UCSF Protocol Review Committee (PRC), Committee on Human Research (CHR), and Data Safety Monitoring Committee (DSMC).
2. I will conduct the study in accordance with applicable CHR requirements, Federal regulations, and state and local laws to maintain the protection of the rights and welfare of study participants.
3. I certify that I, and the study staff, have received the requisite training to conduct this research protocol.
4. I have read and understand the information in the Investigators' Brochure (or Manufacturer's Brochure) regarding the risks and potential benefits. I agree to conduct the protocol in accordance with Good Clinical Practices (ICH-GCP), the applicable ethical principles, the Statement of Investigator (Form FDA 1572), and with local regulatory requirements. In accordance with the FDA Modernization Act, I will ensure the registration of the trial on the www.clinicaltrials.gov website.
5. I agree to maintain adequate and accurate records in accordance with CHR policies, Federal, state and local laws and regulations.

UCSF Principal Investigator / Study Chair

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Printed Name

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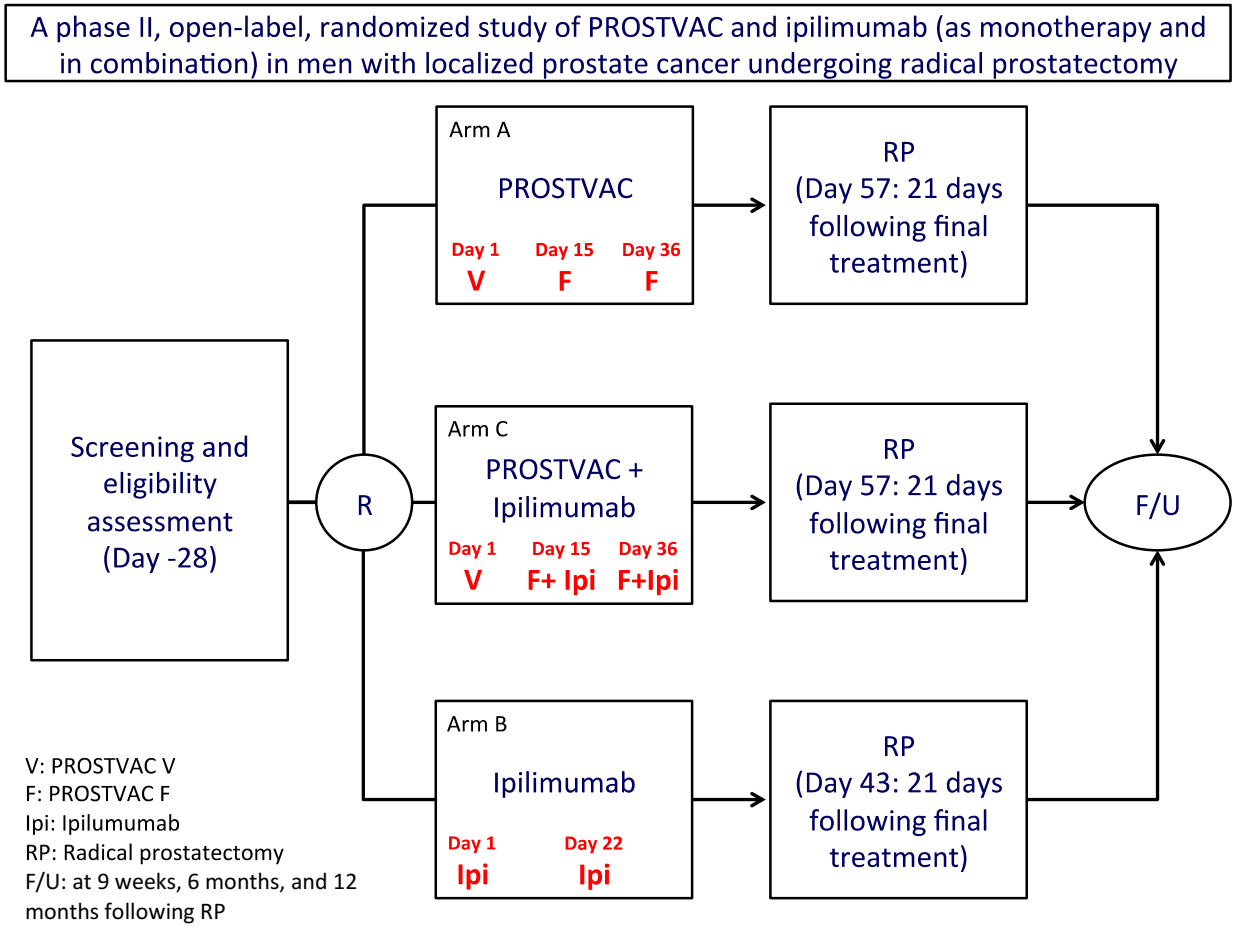
Abstract

Title	An open-label, randomized phase II trial of PROSTVAC and ipilimumab (as monotherapy and in combination) as neoadjuvant treatment for men with localized prostate cancer (PC) undergoing radical prostatectomy (RP).
Patient population	Prostate cancer patients undergoing RP
Rationale for Study	30-40% of patients who undergo RP with curative intent for their localized PC experience relapse of their disease. Therefore, improved therapeutic approaches are needed in this patient population. Enhancing the immune system's response to the tumor may improve long-term outcomes following RP. PROSTVAC is a vaccine therapy consisting of two recombinant viral vectors that may generate systemic immune responses to PSA, and has been shown to improve overall survival in a randomized, placebo-controlled and blinded phase II trial. PROSTVAC is well tolerated, with most adverse events (AEs) related to mild injection site reactions and only a subset of patients showing other mild systemic AEs such as fatigue. CTLA-4 is negative regulatory molecule that is upregulated when the antigen-specific T-cell is activated, which may blunt the immune system's response to malignancy. ipilimumab is a human anti-CTLA-4 monoclonal antibody designed to stimulate immune responses. It has obtained regulatory approval for the treatment of metastatic melanoma, and demonstrated clinical activity in prostate cancer. We hypothesize that PROSTVAC and ipilimumab can each induce specific immune response in the primary tumor as well as in peripheral blood, and that in combination, IPILIMUMAB may augment the T cell-mediated immune response generated by PROSTVAC by blocking the inhibitory signal of the CTLA-4 checkpoint.
Primary Objective	• To assess the immunologic impact induced within the prostate by neoadjuvant administration of PROSTVAC OR ipilimumab OR the combination in men with localized PC undergoing RP, as measured by CD3+ T cell tumor infiltration.
Secondary Objectives	• To characterize T cell subsets and NK cells infiltrating the prostate after neoadjuvant administration of PROSTVAC OR ipilimumab OR the combination. • To measure the treatment-induced effects on circulating T cells following neoadjuvant PROSTVAC OR ipilimumab OR the combination of PROSTVAC and ipilimumab. • To assess the safety of PROSTVAC OR ipilimumab OR the combination in the neoadjuvant setting.

Exploratory Objectives	• To examine the effects of neoadjuvant PROSTVAC OR ipilimumab OR the combination on systemic antigen-specific immune responses. • To assess various immune monitoring parameters of cellular and humoral immune response following neoadjuvant PROSTVAC OR ipilimumab OR the combination in blood and in the tumor. • To determine the impact of neoadjuvant PROSTVAC OR ipilimumab OR the combination on PD-L1 expression in patients with localized PC. • To determine the clinical benefit of neoadjuvant PROSTVAC OR ipilimumab OR the combination in patients with localized prostate cancer measured by: o PSA response o Pathologic response
Study Design	Open label randomized phase II trial of PROSTVAC and Ipilimumab prior to RP. There will be three arms in the study: 1) PROSTVAC alone, 2) Ipilimumab alone, 3) PROSTVAC + ipilimumab. To compare the immune response following treatment, tissue from the prostatectomy specimen will be compared with tissue from the core biopsy specimen obtained prior to treatment, with each subject serving as his own control. The immune response of each arm as well as in aggregate will be compared with a reference control group. **Figure 1** presents a schematic of the study design.
Number of patients	42 (14 patients in each arm)
Duration of Therapy	Patients will be randomized to receive PROSTVAC or ipilimumab alone, or the combination of PROSTVAC and ipilimumab, and will undergo RP 12 weeks after start of therapy.
Duration of Follow up	All subjects will be followed for 1 year following RP.
Duration of study	The study will conclude when all enrolled patients have been followed for 1 year following RP. The estimated duration of study, from start of enrollment to final study report is 2 years.
Study Drugs	PROSTVAC (Bavarian-Nordic) is a therapeutic cancer vaccine. Two component viral vectors (PROSTVAC-V, a recombinant vaccinia vector, and PROSTVAC-F, a recombinant fowlpox vector) are used together in a prime-boost vaccination regimen. Both vectors co-express a human PSA gene and genes encoding three human immunological costimulatory molecules: B7.1, intercellular adhesion molecule-1 (ICAM-1), and leukocyte function-associated antigen-3 (LFA-3). Ipilimumab (Yervoy, Bristol-Myers Squibb) is a human immunoglobulin G-1 kappa monoclonal antibody that targets cytotoxic T lymphocyte-associated antigen-4 (CTLA-4). Ipilimumab was the first in a class of therapies targeting T cell activation and regulation to be licensed in the broad category of agents known as immune checkpoint inhibitors, based on improved overall survival in patients with metastatic melanoma.

Safety Assessments	Safety will be assessed by reviewing adverse events (AEs), laboratory evaluations, and by physical examination. The NCI CTCAE v4.03 will be used. All AEs and serious AEs (SAEs), with the exception of those considered related to RP, will be recorded on case report forms (CRFs) from enrollment through 12 weeks post-surgery
Efficacy Assessments	For the primary endpoint, the change in the number of CD3+ T cell infiltration within prostate tissue between the biopsy and RP specimen will be quantified using immunohistochemistry (IHC), with a positive result if there is ≥2 fold increase in the number of CD3+ T cell infiltration. As secondary endpoints we will look at changes in T cell subsets by IHC in the tissue and circulating T cells by flow cytometry. We will assess the toxicities according to NCI CTCAE v.4.03
Unique Aspects of this Study	This is the first study to: 1) evaluate the safety of systemic PROSTVAC or ipilimumab monotherapy, or the combination of PROSTVAC and ipilimumab, in the neoadjuvant setting in prostate cancer patients; 2) evaluate their direct effect both systemically, and in the tumor microenvironment. The secondary and exploratory studies will enhance the understanding of the immune effects of these therapeutic modalities.

Figure 1 Study Schema



List of Abbreviations

AE	Adverse event
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
ANC	Absolute neutrophil count
AST	Aspartate aminotransferase
BUN	Blood urea nitrogen
CBC	Complete blood cell (count)
CHR	Committee on Human Research (UCSF IRB)
CR	Complete response
CRC	Clinical Research Coordinator
CRADA	Cooperative Research and Development Agreement
CRF	Case report form
CT	Computerized tomography
CTCEA	Common Terminology Criteria for Adverse Events
CTEP	Cancer Therapy Evaluation Program
CTMS	Clinical Trial Management System
DFS	Disease-free survival
DLT	Dose limiting toxicity
DSMC	Data and Safety Monitoring Committee
DSMP	Data and Safety Monitoring Plan
FDA	Food and Drug Administration
GCP	Good Clinical Practice
HBeAg	Hepatitis B “e” antigen
HBV	Hepatitis B virus
HCT	Hematocrit
HCV	Hepatitis C virus
HDFCCC	Helen Diller Family Comprehensive Cancer Center
HGB	Hemoglobin
HIV	Human immunodeficiency virus
ICH	International Conference on Harmonization
IHC	Immunohistochemistry
IND	Investigational new drug application
IP	Investigational product
irAE	Immune related adverse event
IRB	Institutional Review Board
IV	Intravenous

List of Abbreviations

LDH	lactate dehydrogenase
LFT	Liver function test
MedDRA	Medical Dictionary for Regulatory Activities
NCI	National Cancer Institute
ORR	Overall response rate
PC	Prostate cancer
PD	Disease progression
PK	Pharmacokinetics
PO	*Per os* (by mouth, orally)
PR	Partial response
PRC	Protocol Review Committee (UCSF)
QOL	Quality of Life
RBC	Red blood cell (count)
SD	Stable disease
SD	Standard deviation
SGOT	Serum glutamic oxaloacetic transaminase
SGPT	Serum glutamic pyruvic transaminase
TRICOM	TRIad of COstimulatory Molecules
TAA	Tumor associated antigen
ULN	Upper limit of normal
WBC	White blood cell (count)

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1 Introduction

1.1 Background on Indication

Prostate cancer (PC) represents the most common malignancy and the second leading cause of cancer-related death in men in Western countries. In the United States, nearly 240,000 new cases were diagnosed and 30,000 men died in 2013 (1). While surgery and radiotherapy are potential curative treatments in patients with localized PC; treatment failure rates reach 35-40% (2, 3). Neoadjuvant approaches with chemotherapy or androgen deprivation therapy (ADT) in combination with surgery or radiation have been investigated to reduce treatment failure rates. These studies have demonstrated the safety and feasibility of neoadjuvant approaches, and have been shown to decrease the proportion of patients with positive margins at surgery (4, 5). However, this has not translated into significant benefit at meaningful clinical endpoints such as progression-free survival or overall survival (6). Therefore, neoadjuvant therapy remains an investigational approach.

The efficacy of immune therapies in the treatment of human malignancies, and the positive impact on survival or sipuleucel-T (Sip-T) in men with advanced prostate cancer (7) has led to the investigation of the role of immune therapy in the neoadjuvant setting. In a recently completed phase II study(8), patients received 3 infusions of Sip-T at 2 week intervals beginning 6-7 weeks prior to RP. Preoperative sip-T was safe and tolerable, and did not impact surgery. Importantly, significant increases in CD3+ and CD4+ T cells were observed in the prostatectomy specimens, suggesting that a tumor-specific immune response can be induced via this approach (since the effect of systemic Sip-T on localized prostate tumor tissue was unknown prior to this study, a group of 12 patients who underwent RP without any neoadjuvant therapy were used as a negative control to provide confidence that the changes observed were due to treatment effect).

There are multiple strategies for generating therapeutic immune responses, including the use of therapeutic vaccines designed to target specific tumor-associated antigens (TAAs) and immune checkpoint inhibitors that allow for the expansion of an underlying immune response. Antigen-specific active immunotherapy is designed to generate immune responses, particularly T cell-mediated responses, against specific TAAs using vaccines that express one or more of these antigens. The identification and isolation of genes encoding TAAs has allowed the development of recombinant antitumor vaccines designed to elicit immune responses to one or more antigens known to be expressed by a particular tumor type.

1.2 PROSTVAC

PROSTVAC is a PSA (prostate-specific antigen)-based immunization strategy. It is intended to generate immune responses to prostate specific antigens and prostate cancer cells. It uses poxviral vectors to introduce modified PSA to the patient in an immunogenic manner to break self-tolerance, and thereby induce immune responses directed against prostate cancer cells.

PROSTVAC is comprised of two component viral vectors; a recombinant vaccinia (PROSTVAC-V) and a recombinant fowlpox (PROSTVAC-F) virus to be used sequentially in a heterologous prime-boost vaccination regimen. Thus the therapy consists of the following:

- A recombinant vaccinia vector (PROSTVAC-V) as the primary vaccination
- A recombinant fowlpox vector (PROSTVAC-F) as subsequent booster vaccinations

Both vectors contain the transgene to express PSA, as well as transgenes for 3 costimulatory molecules: B7.1, intercellular adhesion molecule-1 (ICAM-1), and leukocyte function-associated antigen-3 (LFA-3), also known as TRICOM (TRIad of COstimulatory Molecules). The PSA gene used in these recombinants has an alteration in a human leukocyte antigen-A2 (HLA-A2) specific epitope (replacement of isoleucine with leucine at amino acid position 155; designated L155 agonist epitope).

Vaccinia virus has been used for over 200 years as a vaccine for smallpox and has a well-established safety profile. Productive fowlpox virus infection is restricted in vivo to avian species; however, fowlpox mediated gene expression does occur in infected non-avian cells (9). In PROSTVAC-V, the recombinant vaccinia virus actively replicates in infected human cells, resulting in the presentation of high levels of antigen to the immune system over a period of one to two weeks, substantially increasing the potential for immune stimulation. The immune system response specific to vaccinia then eliminates the virus. The administration of the recombinant fowlpox vector PROSTVAC-F can then stimulate both humoral and cell-mediated immunity to the expressed transgene.

Recombinant vaccinia and fowlpox vectors are most effective when used in combination in prime-boost regimens. By priming with recombinant vaccinia virus and then boosting repeatedly with the corresponding recombinant fowlpox virus, maximum immune responses to the expressed tumor antigens can be obtained. This phenomenon has been demonstrated in animal models (Hodge 1997, Dale 2006) and has been further supported by results from completed Phase 1 and Phase 2 trials (See Section 1.2.2).

1.2.1 Preclinical studies: PROSTVAC

All components of PROSTVAC, including vaccinia and fowlpox vectors delivering PSA (with the L155 mutated agonist epitope), B7.1, ICAM-1, LFA-3 or murine CEA have been tested in various combinations in mouse, rabbit and non-human primate models as well as in a number of *in vitro* experiments (10). Recombinant vaccinia and fowlpox vaccines targeting PSA as the tumor antigen demonstrated generation predominantly of cell-mediated immune responses to PSA plus antigen spreading to additional tumor antigens which resulted in substantial evidence of anti-tumor activity. Humoral responses to the recombinant vectors and to a much lesser extent to PSA were also observed. No biologically significant changes or signs of untoward toxicological effects (including immunogenicity, biodistribution, neurovirulence and BBB survival) were noted in either rodent or non-human primate safety studies, supporting initiation of human clinical trials (see PROSTVAC Investigator Brochure for more detailed information).

1.2.2 Clinical studies: PROSTVAC

Clinical evaluation of PROSTVAC was preceded by 12 Phase 1 and Phase 2 clinical studies with earlier generation vaccinia and fowlpox constructs showing safety and immunogenicity in different settings.

Sanda et al (11) evaluated the safety and biologic effects of a single dose vaccinia-PSA (rV-PSA) administered in 6 patients with PSA-recurrence after androgen-deprivation interruption. Toxicity was minimal, and DLTs were not observed. Variability in the time required for androgen restoration (after interruption of androgen deprivation therapy) was observed, with one patient

showing 30 weeks of undetectable PSA levels after testosterone restoration. Primary anti-PSA IgG antibody activity was induced after rV-PSA vaccination in one patient.

Eder et al (12) tested three dose levels of rV-PSA (4-week intervals for a total of three doses), in 33 advance PC patients, with GM-CSF as immunostimulatory adjunct for the ten patients at the highest dose level. No virus-related adverse events appeared, and immunological studies demonstrated specific T-cell response to PSA. Certain patients remained without evidence of clinical progression for up to 21 months or longer.

Phase I trial: P1-4 studied PROSTVAC (without GM-CSF) in ten patients establishing its safety and immunogenicity. Only 1 dose of rV (prime, day 1) followed by 1 dose of rF (booster, day 29) was used. There were no SAEs related to the vaccine and anti-vaccinia titers were increased at day 15 (seroconversion) and at the end of eight weeks (13).

Phase I/II trial: (NCI sponsored, Protocol 5911, NCT00062153/NCT00060528) studied patients without previous chemotherapy; visceral disease and narcotic use for cancer pain were allowed. This trial studied different doses of PROSTVAC as well as the effect of GM-CSF and/or rF-GM-CSF on the immunologic response as an addition to these vaccines. The MTD established in the Phase I stage showed no DLT or Grade 3/4 AEs (14). The Phase II portion of the study of 32 patients showed a median OS of 26.6 months, compared to the predicted median OS by the Halabi nomogram of 17.4 months. Overall survival was correlated with the development of this immune response, as patients with greater PSA-specific T-cell responses showed a trend ($p = 0.055$) toward enhanced survival. There were no SAEs related to PROSTVAC or GM-CSF in this study. There was no difference in T-cell responses or survival in cohorts of patients receiving GM-CSF versus no GM-CSF (15). The PSA-TRICOM vaccine appears to have provided marked benefit not apparent during vaccination, but consistent with subsequent development of a beneficial immune response (16).

Phase II trial: TBC-PRO-002 (NCT00078585) was a randomized (2:1), double-blind empty vector-controlled trial that evaluated the safety and efficacy of PROSTVAC in 125 men with castration-resistant metastatic PC patients with no visceral metastases, previous chemotherapy nor narcotic use for cancer-related pain (17). Investigational arm consisted in 1 dose of rV (prime, day 1) followed by 6 doses of rF (boosters on days 14, 28, 56, 84, 112, 140). PFS (primary endpoint) was similar in both arms, but median survival was longer in the investigational arm than for the control group (25.1 and 16.6 months, respectively), with an overall hazard ratio of 0.559 (95% CI 0.367 – 0.852), P=0.0061. The vaccination regimen was well tolerated and safety profile was similar for the two treatment groups.

In collaboration with the NCI under the CRADA agreement and with NCI cooperative groups, clinical experience with PROSTVAC-V and PROSTVAC-F has been evaluated under a variety of study designs. In 2003, the NCI initiated its own BB-IND (10915) on the current PROSTVAC-V and PROSTVAC-F product and to date has conducted multiple studies, which cover a range of study designs evaluating PROSTVAC-V and PROSTVAC-F in early and late-stage disease and in combination with other therapies.

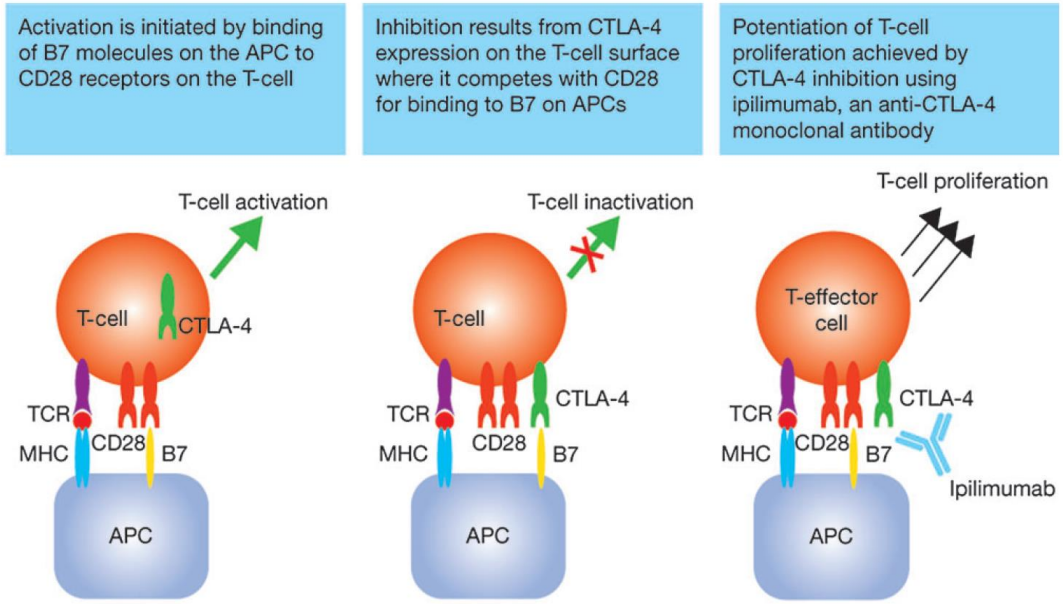
1.3 Ipilimumab

Inhibition of immune checkpoints represents another important major strategy in prostate cancer immunotherapy (Figure 2). Cytotoxic T lymphocyte-associated antigen 4 (CTLA-4) is a molecular “brake” on the immune system. The cell-surface molecule is found on activated T cells and interfaces with the B7 ligands, CD80 and CD86, on antigen presenting cells (APCs). CTLA-4

binds more avidly to the B7 ligands than CD28 receptors, which are responsible for costimulatory signaling, and leads to negative regulation of T-cell activation and proliferation. CTLA-4 also is expressed constitutively on T-reg cells, which contribute to the induction of peripheral self-tolerance.

Ipilimumab is a human immunoglobulin G (IgG1)κ anti-CTLA-4 monoclonal antibody (mAb). *In vitro* studies were performed with ipilimumab to demonstrate that it is specific for CTLA-4, actively inhibits CTLA-4 interactions with B7.1 and B7.2, does not show any cross-reactivity with human B7.1, B7.2 negative cell lines, and stains the appropriate cells without non-specific cross-reactivity in normal human tissues, as demonstrated by immunohistochemistry.

Figure 2 T-cell activation and mechanism of action of Ipilimumab



MHC = major histocompatibility complex; APC = antigen presenting cell; TCR = T-cell receptor; CTLA-4 = cytotoxic T lymphocyte-4

1.3.1 Preclinical studies

Complete information on the pre-clinical toxicology studies can be found in the Ipilimumab Investigator Brochure (IB). Ipilimumab is a human IgG1, an isotype generally capable of mediating CDCC and ADCC, but preclinical studies showed neither of these activities, likely due to a very low expression of CTLA-4 on activated T cells. Therefore, these data suggest that ipilimumab treatment would not result in depletion of activated T cells in-vivo. Indeed, no depletion of T-cells or T-cell subsets were noted in toxicology studies in cynomolgus monkeys, and no mortality or signs of toxicity were observed in three independent 14-day intravenous toxicology studies at multiple doses up to 30 mg/kg/dose. Ipilimumab was well tolerated alone or in combination in all studies. There were no significant changes in clinical signs, body weight values, clinical pathology values or T cell activation markers. In addition, there were no significant histopathology changes in the stomach or colon.

1.3.2 Clinical studies: Non-prostate cancer patients

Ipilimumab has shown to improve overall survival in patients with advanced melanoma (unresectable Stage III or Stage IV) previously treated with chemotherapy (18), and in previously untreated in combination with chemotherapy (19). These results prompted FDA-approval of ipilimumab for the treatment of advanced melanoma in 2011.

In general, the safety profile of ipilimumab administered as single doses of up to 20 mg/kg and multiple doses of up to 10 mg/kg every 3 weeks was characterized by adverse reactions that were mostly immune in nature. Drug-related SAEs were reported in studies of ipilimumab administered as monotherapy, as well as in combination with vaccines, cytokines, chemotherapy, or radiation therapy.

The data on safety of ipilimumab at the dose of 3 mg/kg in phase III trials comes mainly from the metastatic melanoma patients enrolled in the above mentioned study of previously treated patients (18). Immune-related adverse events (irAE) occurred in approximately 60% of patients treated with ipilimumab, of which Grade 3 and 4 were 12.2 and 2.3% respectively, which typically did not occur until 3-4 weeks into therapy. Overall, severe or life-threatening (grade 3 or 4) toxicity was seen in 10-15%. The phase III trial used a dose of 3 mg/kg of ipilimumab every 3 weeks. Grade 4 drug-related toxicities were uncommon (3.8%) and typically immune-related (2.3%).

A higher incidence of side effects was observed with a dose of 10 mg/kg every 3 weeks in the randomized phase II trial that assessed the effects of dose on activity and toxicity. Data from other studies in melanoma and prostate cancer patients show that there is an increase in the frequency of adverse events at increasing dose levels (20, 21):

- Serious adverse events (35, 49, and 53 %, at 0.3, 3.0, and 10 mg/kg, respectively).
- Immune-related adverse events (26, 56, and 70 %, respectively) and serious (grade 3 to 4) immune-related adverse events (0, 7, and 25 %, respectively).
- Adverse events leading to drug discontinuation (13, 10, and 27 %, respectively).

1.3.3 Ipilimumab in prostate cancer patients

The first-in-human study of ipilimumab was performed in patients with prostate cancer at UCSF. In this initial trial, a single dose of ipilimumab at 3 mg/kg given to patients with CRPC resulted in a ≥50% decline in PSA levels in 2 of 14 patients, any of those two having measureable disease. This trial showed that a single 3 mg/kg dose of ipilimumab has acceptable pharmacokinetic and safety profiles, but also indicated the risk of treatment-related immune events as one patient developing grade 3 rash/pruritus requiring systemic corticosteroids (22).

In the setting of prostate cancer, ipilimumab has shown activity across the six other clinical trials that have explored its use alone or in combination with other strategies or immune adjuvants: GM-CSF (23), cancer vaccines like GVAX (24) and PROSTVAC (25), chemotherapy (26), radiation (21) and anti-androgen therapy (27). Although the studies are too small to be definitive, these trials overall have noted declines in PSA and a low frequency of radiographic responses at doses of ipilimumab ≥ 3mg/kg. It is important to note that radiographic responses in metastatic CRPC can be difficult to detect because bone metastasis are difficult to measure. Many of these trials were looking for the optimal dose and schedule of ipilimumab alone or in combination.

Phase I dose-escalation of Ipilimumab with GM-CSF (23). Up to 24 patients were enrolled in this trial where sequential cohorts were treated with increasing doses of ipilimumab, up to 3 mg/kg. At this dose, 22% of patients experienced a PSA response and 50% had a PSA decline of >50%. Subjects experienced irAE in a dose-dependent fashion and also in correlation with PSA response. The three subjects that experienced clinical responses also had irAE: grade 3 pan-hypopituitarism in one, grade 3 colitis in another, and grade 2 rash in the third. All of these irAE were clinically manageable with steroids. The combination enhanced the activation of circulating CD8+ T cells also in a dose-dependent manner.

Phase II of Ipilimumab (3 mg/kg monthly for four doses) with or without a single dose of docetaxel (26) enrolled 43 patients and did not reveal clear difference in safety or PSA response (around 15%) between the two groups, and no radiographic responses where seen. Two patients in the monotherapy group and one in the combination group had PSA responses. 18 patients experienced 52 SAE, 10 of which were attributed to ipilimumab.

Phase II of a single dose of Ipilimumab (3 mg/kg) with ADT vs ADT alone (27). This randomized trial enrolled 108 patients and at 3 months, 55% of ipilimumab patients had undetectable PSA versus 38 % in the ADT-only arm. In the combination arm, grade 3 or 4 irAEs included colitis (4.5%) and diarrhea (4.5%).

Phase I/II testing three different doses (3 mg/kg, 5 mg/kg and 10 mg/kg) with or without radiotherapy (21). PSA decline and radiographic responses were observed in all dose cohorts. Overall PSA response was 20%. Among 45 patients in the 10-mg/kg group, 8 had PSA declines of greater than 50%, 1 had a CR lasting greater than 11.3 months, and 6 had stable disease. Immune-related adverse events were similar to those described in previous studies. No difference in the number of patients who had a decline in PSA ≥ 50% was seen between patients treated with ipilimumab alone (5 out of 16 chemo-naïve patients) versus the ipilimumab and radiotherapy (4 out of 15 chemo-naïve patients), and fewer patients in the post-chemotherapy group had a decline in PSA ≥ 50% when treated with ipilimumab and radiation (1 out of 14).

Various patterns of PSA decline have been reported, occurring at treatment onset, after a short period of stable disease, or within six months after an initial rise in PSA levels. In some cases, response to ipilimumab lasted for 1 year or more. These observed patterns do suggest that an individual´s immune response to prostate cancer may be dynamic and in some patients durable.

Phase III trial of ipilimumab (10 mg/kg) and radiation versus radiation alone in the postdocetaxel setting (NCT00861614, Gerritsen, ESMO 2013) (28). The study's primary endpoint of OS did not reach statistical significance with median OS at 11.2 months with ipilimumab (n=399) and 10 months with the placebo (n= 400; HR=0.85; 95 % confidence interval [CI]=0.72-1.00; p=0.053). Median progression-free survival favored ipilimumab over placebo (HR=0.7; 95 % CI=0.61-0.82) as did prostate-specific antigen (PSA) response rates, as evidenced by declines of ≥50 % in evaluable patients (13.1 % vs. 5.3 %, respectively). Respective 1- and 2-year OS rates for ipilimumab vs placebo were 47% vs 40% and 26% vs 15%. Median PFS also favored ipilimumab over placebo (HR=0.70; 95%CI=0.61–0.82), as did PSA declines of ≥50% in evaluable patients (13.1% vs 5.3%, respectively). Pre-specified subset analyses suggest that ipilimumab may be most active in patients with no visceral disease and favorable laboratory prognostic factors (eg. decreased alkaline phosphatase, elevated hemoglobin). A post hoc analysis in patients who received treatment (n=779; n=387 for ipilimumab vs n=392 for placebo) showed an improvement in OS which favored ipilimumab (HR=0.84; 95%CI=0.71–1.00; p=0.0498). Treatment-related adverse events (AEs) were common and mostly immune-related AEs (irAEs). Grade ≥3 irAEs in the ipi vs pbo arms, respectively, were GI (18% vs 1%), liver (5% vs 1%), endocrine (2% vs 1%) and dermatologic (1% vs 0%); most were reversible using standard ipi management algorithms. Incidences of drug-related death and GI perforation were 1% and 0.6%, respectively.

A second phase III study that compares ipilimumab versus placebo in individuals with metastatic CRPC who have not already been treated with chemotherapy is ongoing (NCT01057810).

Combining cancer vaccines and ipilimumab would presumably improve anti-tumor activity by amplifying immune responses focused to relevant antigens as demonstrated in mice.

Phase I dose-escalation of ipilimumab (monthly, up to 5 mg/kg, with the expansion cohort at 3 mg/kg) with GVAX (24). Five of 28 patients who completed treatment had a ≥ 50% decline in PSA, and 12 had stabilization of metastatic bone disease for extended durations (12 to 21 months) (24). In the escalation cohort (12 patients), 5 patients who received 3 or 5 mg//kg had grade ≥2 irAEs (4 had hypophysitis, 1 had sarcoid alveolitis), and in the expansion cohort (16 patients), 2 patients had grade 2 hypophysitis, 3 had grade 1or 2 colitis and 1 had grade 3 hepatitis.

Phase I dose-escalation of ipilimumab (monthly, at 1, 3, 5 and 10 mg/kg, up to 6 doses) with PROSTVAC and GM-CSF in metastatic CRPC. Twenty-four chemo naïve and 6 postdocetaxel patients were enrolled in this trial. Five of 9 chemo-naïve patients with mCRPC who received 3 or 5 mg/kg of ipilimumab plus PROSTVAC had ≥ 50% declines in PSA (25), and 4 had stable disease ≥ 6 months, and 2 had unconfirmed partial responses. Overall 14 of 24 chemotherapy-naïve subjects at any dose level of ipilimumab (1, 3, 5 and 10 mg/kg) showed decline in PSA. Physical tumor shrinkage however was infrequent. Nine of 15 receiving 10mg/kg of ipilimumab had stable disease ≥ 6 months. No dose-limiting toxicities were recorded and the maximum tolerated dose was not exceeded. Toxicities were primarily local grade 1 and 2 injection-site reactions (three patients with grade 1 events, 26 with grade 2) and immune-related adverse events (table 3). Rash was the most common irAE noted mostly in patients receiving 10 mg/kg ipilimumab. Endocrine irAEs were more common at 5 and 10 mg/kg doses, with grade 2 and 3 diarrhea or colitis noted at all doses above the lowest (1 mg/kg)) ipilimumab dose. Other uncommon irAEs included elevated concentrations of aminotransferases and neutropenia or leucopenia. Overall, 21 patients (70%) had a grade 2 or greater irAE, and 8 patients (27%) had grade 3 or 4 immune-related adverse events. There were no other non-immune-related grade 4 toxic effects, and the remaining grade 3 toxic effects were probably related to the accompanying irAEs in the same patient. Overall, 14 patients discontinued ipilimumab because of disease progression (progressed before the planned initial six doses of ipilimumab) and 13 discontinued ipilimumab because of irAEs (median 2 doses, range 1–3); and three received all six planned initial doses. Of these toxic effects, six patients had irAEs in the first month of treatment. These AEs included rashes in 4 patients (all grade 2) and 2 cases of diarrhea or colitis (grade 2 and 3). With the exception of one subject with rash at the 3 mg/kg dose, all other irAEs in the first month were at the 10 mg/kg dose. The duration of all irAEs varied on the basis of toxic effects and individual patients. For endocrine-related toxic effects, patients had to be placed indefinitely on replacement hormones. Two patients were weaned off these treatments. 10 patients with rash were treated with supportive measures for a median of 27 days (range 6–138). The duration of diarrhea or colitis varied from 2 to 98 days, with a median of 32 days until resolution of symptoms to grade 1 or less. The three episodes of raised aminotransferases lasted for 3, 25, and 28 days, respectively, and the single episode of grade 4 neutropenia lasted 6 days.

1.3.4 Safety: Ipilimumab at 3 mg/kg

The majority of data for drug-related AEs come from the studies with metastatic melanoma patients, where four doses of ipilimumab was the treatment schema. The most common treatment-emergent adverse events (AEs) considered by the investigator to be related to study drug in the metastatic melanoma trial (18) were irAEs, which occurred in approximately 60% of the patients treated with ipilimumab. The frequency of grade 3-4 immune-related adverse events was 10 to 15%. All irAEs occurred during the induction period (within the initial 4 doses). The irAEs most often affected the skin and gastrointestinal tract. The median time to the resolution of irAEs of grade 2, 3, or 4 was 4.9 weeks (95% CI, 3.1 to 6.4). The most common irAE was diarrhea, which occurred at any grade in 27 to 31% of the patients in the ipilimumab treated patients. After the administration of corticosteroids, the median time to the resolution of diarrhea of grade 2 or higher was 2.3 weeks for 14 of 15 patients. In addition to corticosteroids, 4 patients received

infliximab (anti–tumor necrosis factor α antibody) for diarrhea of grade 3 or higher or colitis. Among the 94 persons who survived for 2 years, residual effects of AEs included those related to injection site reactions (16 patients), vitiligo (12), diarrhea or colitis (e.g., proctocolitis with rectal pain) (4), and endocrine irAEs (e.g., inflammation of the pituitary) that required hormone-replacement therapy (8). Ongoing events in the persons who survived for 2 years included rash pruritus, diarrhea, anorexia, and fatigue, generally of grade 1 or 2 (in 5 to 15% of the patients) and grade 3 leukocytosis (in one patient). There were 14 deaths related to the study drugs (2.1%), of which 7 were associated with immune-related adverse events.

In another study of metastatic melanoma patients comparing three different doses of ipilimumab (20), irAEs of any grade arose in 50 of 71, 46 of 71, and 19 of 72 patients at doses of 10 mg/kg, 3 mg/kg, and 0.3 mg/kg, respectively; the most common grade 3-4 adverse events were gastrointestinal irAEs (11 in the 10 mg/kg group, two in the 3 mg/kg group, none in the 0.3 mg/kg group) and diarrhea (ten in the 10 mg/kg group, one in the 3 mg/kg group, none in the 0.3 mg/kg group). Overall, grade 3-4 toxicities were seen in 25.4%, 7 % and 0%, respectively.

Many of the adverse events considered related to ipilimumab may be immune in nature and presumably a consequence of the intrinsic biological activity of ipilimumab. An irAE is defined as any adverse event associated with drug exposure and consistent with an immune-mediated event. Disease progression, infections and other etiologic causes are ruled out or deemed unlikely as contributing to the event. Supportive data, such as autoimmune serology tests or biopsies, are helpful but not necessary to deem an event an irAE. Events of unclear etiology, which were plausibly “immune-mediated” have been conservatively categorized as irAEs even if serologic or histopathology data are absent. These irAEs likely reflect a loss of tolerance to some self-antigens or an unchecked immune response to gut or skin flora. Some breakthrough of immunity may be inseparably linked to the clinical antitumor activity of ipilimumab. Immune-related AEs predominately involve the GI tract, endocrine glands, liver or skin. With few exceptions, irAEs were clinically manageable and reversible with supportive care or corticosteroids. **Management algorithms are included in the IB**.

Corticosteroid treatment did not adversely affect antitumor responses in those subjects who had both an irAE requiring steroid therapy and an objective tumor response. Systemic corticosteroids do not appear adversely associated with ipilimumab-induced clinical response when used to manage irAEs in patients with advanced melanoma. Similar results were observed regardless of whether mWHO or the novel immune-related response criteria (irRC) (29) were used. Steroids can be used promptly to manage severe irAEs and minimize the risk for serious complications(30).

In this study, two doses of ipilimumab will be administered; thus AE data from prior studies may not necessarily be applicable. Here, we report the safety for prostate cancer patients treated with Ipilimumab monotherapy at 3 mg/kg q3 weeks **for up to four doses**:

Table 1 Adverse Events: Ipilimumab 3mg/kg in prostate cancer patients

	Small et al (single dose) N=14 (31)	Slovin et al (up to 4 doses) N=8 (21)
Diarrhea	21%	50%

Colitis (G3-4)	NR	13%
Nausea	14%	38%
Constipation	29%	NR
Vomiting	NR	25%
Abdominal pain	21%	NR
Fatigue	29%	0
Decrease appetite	43%	NR
Pruritus	NR	25%
Rash	14%	13%

1.4 Rationale for the Proposed Study

Results from the PROSTVAC studies Phase I/II trial (Protocol Nº 5911) and the Phase 2 TBC-PRO-002 suggest that it may confer an advantage in overall survival in subjects with mCRPC. Despite limited numbers of patients with noticeable tumor shrinkage, both GVAX and PROSTVAC with Ipilimumab showed significant numbers of patients with stabilization of their disease that can last for several months, with manageable side effects. The presumptive mechanism is that the vaccination activates anti-cancer immune responses, and in particular, induces an intratumoral T cell response (32). Additionally, the median overall survival in the PROSTVAC/ipilimumab combination trial was 31.8 months (25), a notable duration in mCRPC.

The non-overlapping mechanisms of action of these drugs suggest a potential for combination therapy, with the goal of substantially augmenting immune response and ultimately improving clinical outcomes. Although cancer vaccines might induce an antigen-specific T-cell response, once activated, T cells up-regulate CTLA4, a negative regulatory molecule. Preclinical studies in mice have shown that CTLA4 blockade can augment an immune response and increase T cell avidity, leading to enhanced T cell-mediated immune responses to the vaccine. The key role of CTLA4 in regulating immune response is evident in CTLA4 knockout mice, which cannot modulate immune responses (33, 34).

To date, most immunotherapy trials in humans have focused upon assessing immune responses in the blood. While circulating tumor-specific T cells have been detected in a number of studies (35), they have not consistently been demonstrated to be predictive of clinical outcome. Moreover, there is emerging data that antigen-specific T cells can be detected within lymph nodes and tumors of melanoma patients (36).

The underlying hypothesis for this study is that effective immunotherapy induces the recruitment of anti-cancer T cells to the tumor. The study is designed to examine whether immunization with PROSTVAC, or ipilimumab monotherapy, or PROSTVAC and ipilimumab in combination, prior to RP recruits T cells infiltrate PC tissue. Additionally, in a slow-developing disease such as PC it may be that an intervention at an early stage of the disease, when the tumor load is still minimal, may be beneficial for prevention of recurrence after primary treatment. Therefore, we have selected a patient population undergoing curative therapy for their disease, with 2 immune therapies that have shown promising clinical activity in patients with prostate cancer. PROSTVAC, as discussed above, has been shown to be a safe and well-tolerated agent; ipilimumab has been shown to be safe and well-tolerated at a dose of 3mg/kg, and this dose was chosen in order to mitigate the risks associated with treatment.

2 Objectives of the Study

2.1 Primary

- To assess the immunologic impact induced within the prostate by neoadjuvant PROSTVAC or ipilimumab or the combination in men with localized PC undergoing RP, as measured by CD3+ T cell tumor infiltration

2.2 Secondary

- To characterize T cell subsets and NK cells infiltrating the prostate after neoadjuvant PROSTVAC or ipilimumab or the combination.
- To measure the treatment-induced effects on circulating effector and regulatory T cells following neoadjuvant PROSTVAC or ipilimumab or the combination.
- To assess the safety of PROSTVAC or ipilimumab or the combination in the neoadjuvant setting.

2.3 Exploratory Objectives, Other Assessments

- To examine the effects of neoadjuvant PROSTVAC or ipilimumab or the combination on systemic antigen-specific immune responses.
- To assess various immune monitoring parameters of cellular and humoral immune response following neoadjuvant PROSTVAC or ipilimumab or the combination blood and in the tumor.
- To determine the impact of neoadjuvant PROSTVAC or ipilimumab or the combination on PD-L1 expression in patients with localized PC.
- To determine the clinical benefit of neoadjuvant PROSTVAC or ipilimumab or the combination in patients with localized prostate cancer measured by:
    - PSA response
    - Pathologic response

2.4 Endpoints

2.4.1 Primary Endpoint

- The proportion of subjects who demonstrate a positive response following neoadjuvant therapy as measured by change from baseline in CD3+ T cell infiltration within prostate tumor tissue by immunohistochemistry (IHC) assessment following treatment.

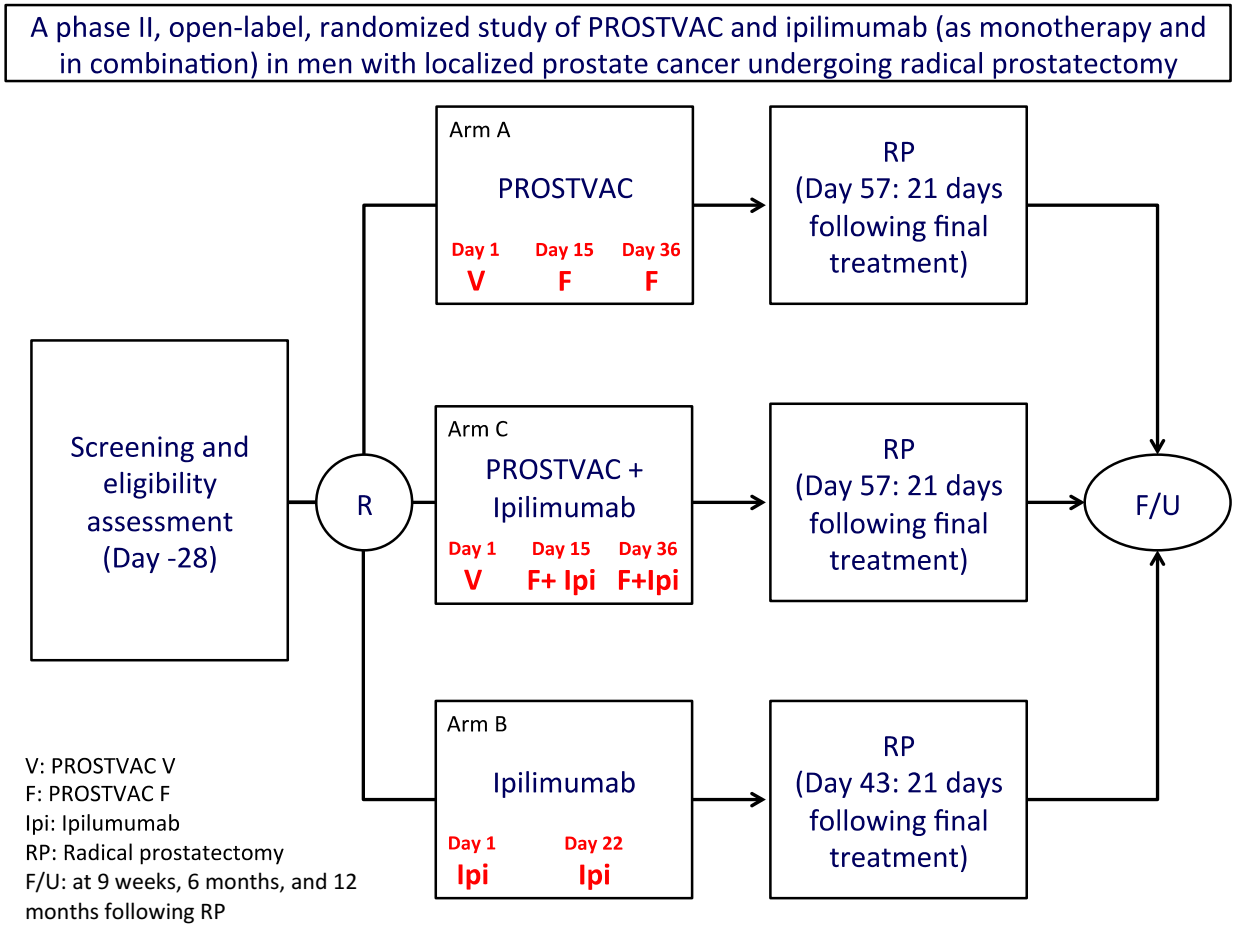
2.4.2 Secondary Endpoints

- The change in immunologic infiltration in tumor tissue quantitated by IHC assessment (e.g. CD4+, CD8+, CD56+, FOXP3, etc.) following neoadjuvant PROSTVAC or ipilimumab or the combination
- Change in circulating effector and regulatory T cells following neoadjuvant PROSTVAC or ipilimumab or the combination by flow cytometry assessment of peripheral blood mononuclear cells
- Treatment related toxicities according to NCI CTCAE v.4.03

2.4.3 Exploratory Endpoints

- Change in antigen-specific T cell responses in peripheral blood from baseline (screening visit) to post-RP (9 weeks post-RP visit) assessed by using a T cell proliferation assay, and interferon gamma (IFN-γ) immunospot (ELISPOT) assays. In a limited number of patients we will also assess for antigen-specific T cells in tumor infiltrating lymphocytes isolated from the RP as fresh tissue is available.
- Development of and changes in humoral immune responses in the periphery following treatment. This will be evaluated by testing serum with methods such as:
    - Measurement of antibodies to PSA,TAA and the viral vector using peptide/protein arrays or standard ELISA assays.
- To characterize the frequency of circulating B and T cells induced by neoadjuvant PROSTVAC or ipilimumab or the combination in patients with localized prostate cancer by flow cytometry. In a limited number of patients we will also assess for antigen-specific T cells in tumor infiltrating lymphocytes isolated from the RP as fresh tissue is available.
- T-cell receptor (TCR) repertoire sequencing following restimulation with prostate specific tumor-associated antigens and viral vector specific antigens to evaluate clonality of T-cell responses in the periphery. TCR repertoire in the tumor may also be evaluated in prostate biopsy samples to allow correlation with responses in the periphery.
- Change in PD-L1 expression by IHC assessment following neoadjuvant PROSTVAC or ipilimumab or the combination
- The clinical benefit of neoadjuvant PROSTVAC or ipilimumab or the combination as measured by:
    - Proportion of patients with a PSA response (≥30% PSA decline) at the time of RP.
    - Proportion of patients with pathologic complete response (pT0) or near complete response at the time of RP.

3 Study Design



3.1 Characteristics

This is a multicentered, open label, randomized phase II trial of PROSTVAC or ipilimumab or the combination of PROSTVAC and ipilimumab as neoadjuvant therapy in patients with localized PC. Eligible patients will be randomized to PROSTVAC monotherapy (Arm A), ipilimumab monotherapy (Arm B), or combination therapy with both PROSTVAC and ipilimumab (Arm C), prior to RP. In arm A, PROSTVAC- V will be administered subcutaneously as the primary vaccine on Day 1, which will be followed 2 weeks later with a series 2 PROSTVAC-F subcutaneous administrations, given 3 weeks apart (Day 15 and 36). In arm B, Ipilimumab will be administered twice, at a dose of 3 mg/kg, 3 weeks apart (Day 1 and 22). In arm C, PROSTVAC will be administered in the same schedule as given in arm A; Ipilimumab will be administered on the same days as PROSTVAC-F. In all three arms, RP will occur 21 days, or 3 weeks, following the final study treatment administration. No further study therapy will be administered following RP.

3.2 Number of Subjects

42 subjects will be enrolled and randomized.

3.3 Eligibility Criteria

Patients must have baseline evaluations performed prior to the first dose of PROSTVAC and/or ipilimumab, and must meet all inclusion and exclusion criteria. In addition, the patient must be

thoroughly informed about all aspects of the study, including the study visit schedule and required evaluations and all regulatory requirements for informed consent. The written informed consent must be obtained from the patient prior to enrollment. The following criteria apply to all patients enrolled onto the study unless otherwise specified.

3.3.1 Inclusion Criteria

For a subject to be eligible for participation in this study, *all* of the following criteria must be satisfied:

1. Patients must have histologically confirmed adenocarcinoma of the prostate without previous therapy for PC.
    a. Treatment-naïve AND
    b. Undergoing RP as initial, locally definitive therapy for PC AND
    c. Eligible for RP in a 3 month timeframe AND
    d. Able to consent for RP
2. Subject's archival prostate biopsy specimen is available, and subject consents to provide tissue for study endpoint analysis. The prostate biopsy slides or blocks must be available prior to starting any study treatment.
3. Age ≥ 18 years
4. Subject has an Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1 (Appendix 1).
5. Subject has adequate organ function, defined as:
    a. White blood cell (WBC) count ≥ 3,000/mcL
    b. Absolute neutrophil count (ANC) ≥ 1,500/mcL
    c. Platelet count ≥ 100,000/mcL
    d. Hemoglobin (Hgb) ≥ 10.0 g/dL
    e. Creatinine ≤ 1.5x institutional ULN
    f. Total bilirubin ≤ 1.5 x institutional ULN
    g. Alanine aminotransferase (ALT) ≤ 1.5 x institutional ULN
    h. Aspartate aminotransferase (AST) ≤ 1.5 x institutional ULN
6. No known history of human immunodeficiency virus (HIV) 1 and 2, human T-cell lymphotropic virus (HTLV)-I/II, and Hepatitis B and C.
7. Ability to understand a written informed consent document, and the willingness to sign it.
8. Because of the unknown potential risk to a gamete and/or developing embryo from these investigational therapies, subjects must agree to use adequate contraception (i.e. barrier method) for the duration of study participation, and for three months after discontinuing therapy.

3.3.2 Exclusion Criteria

A subject will not be eligible for participation in this study if *any* of the following criteria apply.

1. Subject's biopsy specimen reveals neuroendocrine or small cell features.

2. Subject has any evidence of metastatic disease (pre-operative staging will be undertaken per urologic standard of care) as deemed by the Investigator.
3. Subject has prior use of any hormones, including luteinizing hormone-releasing hormone (LHRH) agonists, ketoconazole, antiandrogens (such as bicalutamide, flutamide, or nilutamide), or 5-α-reductase inhibitors.
4. Subject has prior use of any anti-cancer treatment or product, such as PC-SPES (or any other PC-x product: PC-HOPE, PC-CARE, PC-PLUS, etc).
5. Subject has received prior radiation therapy or chemotherapy for prostate cancer.
6. Chronic administration (defined as daily or every other day for continuous use >14 days) of systemic corticosteroids within 28 days of the first planned dose off PROSTVAC. Use of inhaled steroids, nasal sprays, and topical creams for small body areas are allowed.
7. Active atopic dermatitis or skin condition that disrupts the epidermis
8. Inflammatory eye disease requiring steroid treatment
9. History of prior solid organ or bone marrow transplant
10. Previous history of hypersensitivity to eggs or allergy or untoward reaction to prior vaccinia (smallpox) vaccination.
11. Splenectomy
12. Subject, or subject's close household contacts (defined as those who share housing or have close physical contact) have any of the following conditions during the screening and/or treatment periods:
    a. active or a history of atopic dermatitis, eczema or other eczematoid skin disorders that disrupt the epidermis
    b. other acute, chronic or exfoliative skin conditions (*e.g.*, burns, impetigo, varicella zoster, severe acne or other open rashes or wounds) until condition resolves
    c. pregnant or nursing
    d. immunodeficient or immunosuppressed (by disease or therapy), including HIV infection
13. Subject's close household contacts include children less than the age of three
14. History of, or active autoimmune disease (e.g., autoimmune neutropenia, thrombocytopenia, or hemolytic anemia, systemic lupus erythematosis, Sjogren´s syndrome, scleroderma, myasthenia gravis, Goodpasture´s syndrome, Addison´s disease, Hashimotos´s thyroiditis, or Graves disease) as determined by the treating medical oncologist.".
    a. Persons with vitiligo are not excluded.
    b. Persons with a history of type 1 diabetes are not excluded if the condition is well controlled:
        i. Hemoglobin A1C < 7.0, and
        ii. No evidence of end-organ damage due to diabetes, such as diabetic retinopathy, nephropathy, or neuropathy

    Persons with type 2 diabetes are not excluded since this is not an autoimmune disease, and do not need to meet these criteria.

c. Persons with hypothyroidism are not excluded if condition is well controlled, and condition is due to a non-autoimmune etiology.

15. Subject has received treatment with any investigational immunotherapy within 2 years prior to study screening or has received treatment with any other investigational product within 28 days prior to study screening.
16. Subject has participated in any previous study involving PROSTVAC, Sipuleucel-T or ipilimumab, regardless of whether the subject received PROSTVAC, Sipuleucel-T or ipilimumab.
17. Subject has a history of allergic reactions attributed to compounds of similar chemical or biologic composition to PROSTVAC or ipilimumab.
18. Subject has a history of stage III or greater cancer, excluding prostate cancer. Subjects with a history of basal or squamous cell skin cancers are allowed, provided that the subject was adequately treated and is disease-free at the time of study screening. Subjects with a history of stage I or II cancer must have been adequately treated and been disease-free for ≥ 3 years prior to study screening.
19. Subject has any uncontrolled, concurrent illness including, but not limited to the following: ongoing or active infection (bacterial, viral, or fungal), symptomatic congestive heart failure, unstable angina pectoris, cardiac arrhythmia, stroke or myocardial infarction within 6 months, or psychiatric illness that would limit compliance with study requirements.
20. Subject requires any medical intervention(s) or has any other condition(s) that, in the Investigator's opinion, will 1) make the administration of PROSTVAC or ipilimumab hazardous, 2) obscure the interpretation of AEs, 3) compromise adherence with study requirements, or 4) otherwise compromise the study's objectives.
21. Subject has high-risk features (e.g., based on Gleason score, PSA, clinical stage, % positive biopsies), and the treating physician feels the subject should undergo radical prostatectomy sooner than planned within the protocol.

3.4 Duration of Therapy

For patients randomized to receive PROSTVAC (arms A and C), PROSTVAC-V will be administered as the primary vaccine on Day 1, to be followed 2 weeks later by a series of PROSTVAC-F treatments administered on Days 15 and 36; a total of 2 PROSTVAC-F doses will be administered.

For patients randomized to receive ipilimumab alone (arm B), ipilimumab will be administered on Days 1 and 22, at a dose of 3mg/kg; thus a total of 2 doses will be administered.

For those patients randomized to receive the combination (arm C), ipilimumab will be administered on Days 15 and 36 (coinciding with PROSTVAC-F administration) at a dose of 3mg/kg; thus patients will receive 1 dose of PROSTVAC-V, 2 doses of PROSTVAC-F, and 2 doses of ipilimumab.

For all patients, RP will be performed 21 days (+/- 7 days) following the final dose of PROSTVAC and/or ipilimumab. No further therapy will be administered on study following RP.

Subjects should remain on study and continue with protocol-specified tests and evaluations whenever possible. Subjects will remain on study, including randomization, regardless of RP results, initiation of hormonal therapy, chemotherapy, or radiation therapy, or development of biochemical failure or metastatic disease. However, should a subject withdraw early from the study, Bavarian Nordic, Inc and Bristol-Myers Squibb, Inc. should be notified as soon as possible.

The Investigator may withdraw a subject from treatment or assessment at any time if, in his or her clinical judgment, it is in the best interest of the subject.

Subjects may discontinue their participation in the trial at any time without prejudice. If a subject chooses to withdraw early from the study, the Investigator should make every reasonable attempt to ensure that early withdrawal procedures are completed and to determine the reason for withdrawal. The date and reason for withdrawal will be recorded in the source documentation and on the eCRF.

- If the subject fails to respond to requests for follow-up, the clinical site will send a registered letter, at the minimum, to the subject requesting contact with the clinic. All attempts to resume contact (including copies of written correspondence) will be included in the source documentation. Subjects who do not respond to requests for follow-up after all reasonable attempts to establish contact will be considered 'lost to follow-up'.

3.5 Duration of Follow Up and Removal from Assessment.

Following RP, patients will be followed at 9 weeks and at 6 and 12 months. Patients removed from study for unacceptable treatment-related AEs will be followed until resolution or stabilization of all treatment related adverse events to Grade 2 or lower, until the subject is deemed lost to follow-up, or death.

3.6 Randomization Procedures

Once patients have signed the informed consent, patients will be randomized 1:1:1 by stat computing software R, package blockrank, to receive PROSTVAC alone, Ipilimumab alone, or the combination. The randomization is done within blocks to ensure that the balance between treatments stays close to equal throughout the trial. Randomization must be completed within 28 days of day 1 of study.

3.7 Study Timeline

3.7.1 Primary Completion

The study will reach primary completion in 24 months from the time the study opens to accrual.

3.7.2 Study Completion

The study will reach study completion 30 months from the time the study opens to accrual.

4 Study Drugs

4.1 Description, Supply and Storage of Investigational Drugs

4.1.1 PROSTVAC

PROSTVAC is a genetically modified organism (GMO) consisting of a live attenuated viral vector-based vaccine product that is comprised of two component viral vectors (V and F), to be used together in a prime (V) – boost (F) vaccination regimen:

PROSTVAC-V: Recombinant vaccinia virus that contains a modified gene encoding human prostate-specific antigen (PSA) and genes encoding three human immunological costimulatory molecules: B7.1, intracellular adhesion molecule-1 (ICAM-1), and leukocyte function-associated antigen-3 (LFA-3) (or **TRI**ad of **CO**stimulatory **M**olecules, TRICOM™). The PSA gene used in the recombinant has an alteration in an HLA-A2 specific epitope (replacement of isoleucine with leucine at amino acid position 155; designated L155 agonist epitope). All four genes are inserted into the genome of a derivative of the Wyeth strain of vaccinia virus (strain NYCBH).

PROSTVAC-F: Recombinant fowlpox virus that co-expresses the same four human genes as PROSTVAC-V.

Precautions: ***General Vaccinia and Fowlpox Virus Handling and Disposal Precautions:*** Both PROSTVAC-V and PROSTVAC-F are preparations of live viruses and, therefore, materials that may have been exposed to vaccine (empty vials, syringes, swabs, dressings, etc.) should be treated as infectious waste and incinerated according to local institutional protocol.

4.1.1.1 Clinical safety of recombinant poxvirus-based vaccines

Vaccinia virus causes a transient infection, with elimination of viral components over several weeks. Host cells infected with vaccinia virus are short lived (days) and die by a mixed form of apoptosis/necrosis. Vaccinia replicates in the cytoplasm of infected cells, and viral DNA does not integrate into the host cell DNA. The use of vaccinia virus for worldwide eradication of smallpox provides a safety database with number of observations in the millions. Geographical differences in strains of vaccinia virus used as well as differences in reporting practices, diagnostic and follow-up criteria between countries are a cause of some discrepancies in the incidences of adverse events reported, but the overall picture of vaccinia virus safety is very well known.

A number of events post vaccination are expected and considered to be normal: fever, myalgia, headache, fatigue, chills, nausea, soreness and erythema at the vaccination site, local lymphadenopathy. Satellite lesions around the vaccination site have been reported as well as local edema. These symptoms are self-limiting, last for around three weeks after vaccination and rarely are a cause for serious concern (37, 38).

Mild adverse reactions that can occur post vaccination are bacterial superinfection of vaccination site, erythema multiforme (EM) and generalized vaccinia. Superinfection is a rare event with incidence from 0.14 to 55 cases per million according to different reports (39).
EM most often presents as papules, plaques or urticaria which may be symmetrical, may involve palms and soles. EM resolves spontaneously and requires no special care. A development of Stevens-Johnson syndrome with mucosal involvement is extremely rare, with only one case noted in the 2003-2004 vaccination campaign in the US (<1 per 1,000,000) (38).
Generalized vaccinia results from viremic spread of vaccinia virus from the vaccination site. It presents as a generalized rash, which behaves like the vaccination site lesion, progressing through papular, vesicular, pustular and scab-forming stages. The incidence is difficult to assess, since historically there was no strict definition to distinguish generalized vaccinia from other conditions where rash is a dominant symptom (severe chickenpox, smallpox, eczema vaccinatum, EM). Retrospective analysis of 2002 – 2004 vaccinations suggests an incidence of ~50 cases per 1,000,000. The rash appears within a week after vaccination and resolves within a week. Most instances do not require specific therapy (38).

The parent vaccinia vector Therion used to engineer PROSTVAC was further attenuated from the Wyeth strain of vaccinia, with lower neurovirulence than the strains that were used to generate most of the smallpox eradication/prevention safety database. Since 1991, ten recombinant vaccinia-based vaccines and eight recombinant fowlpox-based vaccines produced by Therion for the treatment of various cancers, have been evaluated in human clinical trials sponsored by the NCI. Over 1,000 cancer patients, most with metastatic disease, have been treated to date with these various poxvirus-based vaccines in NCI-sponsored or Therion-sponsored clinical trials (40) and NCI and Therion Annual Reports.

Clinical evaluations of early versions of PSA-containing poxviral vectors involved over 250 patients. The initial constructs tested were vaccinia-PSA, in three Phase I studies totaling 81

patients (11, 12, 41). Later, a prime-boost regimen was devised with boosting with fowlpox-PSA containing vectors, in two studies, one with 64 patients (40) and another unpublished study of approximately 16 patients. The NCI subsequently began a series of trials using additional admixed vectors encoding a single costimulatory molecule (B7.1/CD80). These studies involved 94 patients (42-44). No significant safety issues were identified in these early studies. In addition to the two trials conducted by Therion with PROSTVAC-V and PROSTVAC-F (Phase I trial in ten patients (13); Phase II trial in 125 patients (17)) there are five other fully enrolled studies (14, 15) and one open study using PROSTVAC-V and PROSTVAC-F treatment, for an additional 246 treated patients (NCI-sponsored studies).

No dose-limiting toxicities were observed in early phase trials. The majority of side effects reported across all of the studies involved Grade 1 or 2 injection site reactions; all resolved without sequelae. There have been three SAEs thought to be possibly due to the PROSTVAC-V and PROSTVAC-F treatment; two occurred in the same patient. This patient experienced myocardial infarction and Grade 4 thrombotic thrombocytopenic purpura (TTP), and was discontinued from the study. The third SAE was reported from a subject hospitalized and diagnosed with bilateral pulmonary embolism. Laboratory evaluations likewise did not reveal any untoward effects of treatment.

Additional safety information is described in the Investigator's Brochure, however no vaccinia-related complications were noted in any of the PROSTVAC clinical studies

Overall, based on clinical information available today, recombinant poxviral vaccines present a beneficial safety profile.

4.1.1.2 Investigational Drug #1: PROSTVAC-V [PROSTVAC-V/TRICOM; Recombinant Vaccinia-PSA (L155)/TRICOM; NSC 717170]

Classification: Vaccine; Genus: Orthopox Virus; Species: Vaccinia. PROSTVAC-V is a purified live viral vaccine produced under serum-free conditions in chicken embryo fibroblast (CEF) cells. Bulk Drug Substance (BDS) of PROSTVAC-V is formulated with sterile phosphate-buffered solution (PBS)/10 % glycerol.

Mechanism of Action: Vaccinia virus actively replicates in the cytoplasm of human cells, resulting in the presentation of high levels of antigen to the immune system over a period of one to two weeks, substantially increasing the potential for immune stimulation. A number of studies have shown that immunization of mammalian species by recombinant vaccinia virus can stimulate both humoral and cell-mediated immunity to the expressed transgene.

Contraindications: Immune deficiency of any nature, immunosuppression, history or current eczema or atopic dermatitis, moderate to severe acute illness (deferral of vaccination), myocardial infarction (prior six months), cardiomyopathy, symptomatic CHF, unstable angina

Availability: Each vial of PROSTVAC-V contains a single dose (2×10^8 infectious units (Inf. U.) per 0.50 mL) of PROSTVAC-V in PBS/10% glycerol for subcutaneous injection. The drug product contains no adjuvants or preservatives and is supplied in borosilicate (2R) glass vials, which are sealed with rubber stoppers and aluminium-plastic closures.

Storage and handling: Intact vials must be stored at 80°C±10°C. To date, stability studies indicate that when stored at -80°C±10°C PROSTVAC-V is stable for up to 72 months. Once thawed, potency can be maintained by storing at 2 – 8°C for up to 24 hours. ***Vaccinia Virus Handling and Disposal Precautions:***

- The risk of transmission of recombinant viruses to exposed healthcare workers is very low. There have been no cases of transmission to healthcare personnel in any of the studies with PROSTVAC-V or related vaccinia-based vaccines.
- Vaccinia virus is classified as a Biosafety Level 2 organism.

- Due to the potential for transmission, healthcare personnel who have direct contact with contaminated dressings or other infectious material from participants in clinical studies should adhere to appropriate infection control measures (45, 46).
- Those who are pregnant, have exfoliative skin conditions, or are immunocompromised should avoid exposure to contaminated dressings or to the inoculation site.
- Use of appropriate infection control measures, such as covering the vaccination site (including bandages) and washing hands after contact with the vaccination site, will prevent transmission.
- While there is a theoretical risk of exposure through a needle stick or an accidental spill, the risk can be further minimized by following safety guidelines.

4.1.2 Investigational Drug #2: PROSTVAC-F [PROSTVAC-F/TRICOM; Recombinant Fowlpox-PSA (L155)/TRICOM; NSC 717171]

Classification: Vaccine; PROSTVAC-F: Genus: Avipox Virus; Species: Fowlpox. PROSTVAC-F is a purified live viral vaccine produced under serum-free conditions in CEF cells. Bulk Drug Substance (BDS) is formulated with sterile PBS/10 % glycerol.

Mechanism of Action: Fowlpox virus infects but does not actively replicate in mammalian human cells. Immunization with recombinant PROSTVAC-F results in the presentation of high levels of antigen to the immune system over a period of several days, substantially increasing the potential for stimulation of cell-mediated immune responses. Boosting with PROSTVAC-F has been shown to focus the vaccine-associated cell-mediated immune responses to the PSA antigen.

Contraindications: There are no specific contraindications for PROSTVAC-F, other than those for fowlpox immunization and serious intercurrent illness.

Availability: Each vial of PROSTVAC-F contains a single dose (1×10^9 Inf. U. per 0.50mL) of PROSTVAC-F in PBS/10% glycerol. The drug product contains no adjuvants or preservatives and is supplied in borosilicate (2R) glass vials, which are sealed with rubber stoppers and aluminium-plastic closures. PROSTVAC-F is stored at -70°C or colder.

Storage and handling: Intact vials must be stored at -80°C±10°C. Once thawed, potency can be maintained by storing at 2 – 8°C for up to 24 hours. To date, stability studies indicate that when stored at 80°C±10°C, PROSTVAC-F is stable for up to 72 months. ***Fowlpox Virus Handling and Disposal Precautions****:* Fowlpox virus is classified as a Biosafety Level 1 organism. These agents are not known to cause disease in healthy adult humans and are of minimal potential hazard to personnel and the environment under ordinary conditions of use. They can be handled safely in the laboratory without special apparatus or equipment, using techniques generally acceptable for nonpathogenic material.

Safety of Fowlpox vaccination: Fowlpox vectors do not replicate in human cells (only in avian cells).). No safety concerns have been raised following recombinant fowlpox administration and the adverse events associated with the use of fowlpox vectors have been limited to mild injection site reactions (9).

Complete and updated adverse event information is available in the Investigational Drug Brochure and/or product package insert.

4.2 Viral shedding: PROSTVAC

Vaccinia virus is known to be shed from the wound site in traditional dermal scarification based vaccination. Transmission of vaccinia to close contacts is another known complication. Contact vaccinia may manifest as progressive vaccinia, eczema vaccinatum (EV) or accidental infection of eye, mouth, genital areas. Review of several national and state surveys between 1962 and

1968 gives frequency for EV at 8 - 27 per 1,000,000 and for accidental infections at 3 - 44 per 1,000,000 (47). The rate of contact vaccinia in 2002 – 2004 was <10 cases per 100,000. Education of vaccinees in proper care for the vaccination site, proper hand hygiene and avoidance of contact with at risk individuals seems to be a reasonable and effective prophylactic against contact vaccinia.

PROSTVAC immunization is subcutaneously administered, which greatly reduces injection site reactions, and skin surface wound formation/viral shedding. Both of these factors reduce the potential for inadvertent inoculation and contact transmission versus traditional smallpox vaccine programs.

4.3 Safe handling of recombinant vaccinia virus vaccine

Special consideration must be given to the nature of the initial vaccine: a recombinant replicating vaccinia virus. Subject instruction materials will include detailed descriptions for bandaging, bathing, and reporting any possible side effects. Subjects will also be educated regarding restrictions on contact with certain classes of individuals (children under the age of 3 years pregnant or lactating women, immunocompromised individuals).

PROSTVAC-V is considered a Biosafety Level 2 (BSL2) agent according to the CDC/NIH Biosafety in Microbiological and Biomedical Laboratories Manual. Study staff will be provided specific instructions for storage, use, and destruction of these materials. All subjects will be dosed with the vaccine in the clinic or hospital setting according to local regulations. PROSTVAC-V is aseptically vialed at low volume in a sealed vaccine vial. Loading of syringes may be performed using standard aseptic methods in a clinical pharmacy. Routine, standard universal precautions are required when directly handling the vaccine vials, including the wearing of a lab coat, eye protection, and gloves. There is no need to sequester subjects after dosing. Vials and needles and syringes may be disposed of as for infectious medical waste according to local regulations.

These recommendations are the same as those for administering smallpox vaccine with bifurcated needles.

4.4 Safe handling of recombinant fowlpox vaccine

PROSTVAC-F is classified as BSL1 and because it cannot replicate in mammalian cells, no special precautions beyond standard, universal precautions for infectious materials are required. There are no subject restrictions with respect to activities (such as bathing), personal contact, or bandaging required for vaccination with PROSTVAC-F.

4.4.1 Investigational Drug #3: Ipilimumab (Yervoy®, BMS)

Ipilimumab is available as a sterile solution in the vial is clear and colorless. Ipilimumab is administered via intravenous infusion only.

Patients will receive ipilimumab 3 mg/kg IV administered over 90 minutes every 3 weeks for 2 doses (Arm B: Days 1 and 22; Arm C: Days 15 and 36). Vital signs, including blood pressure, heart rate, respiratory rate, temperature and pulse oximetry, will be monitored before ipilimumab infusion.

Classification

Ipilimumab monoclonal antibody is specific for the CTLA-4 antigen expressed on a subset of activated T-cells. CTLA-4 interaction with the B7 molecule, one of its ligands expressed on antigen presenting cells, can down-regulate T-cell response.

Mechanism of Action

Ipilimumab is thought to act by blocking the interaction of CTLA-4 with the B7 ligand, resulting in a blockade of the inhibitory effect of T-cell activation that is created by the CTLA-4/B7 interaction.

Storage

Ipilimumab must be stored in a secure area according to local regulations. The investigator must ensure that it is stored at a temperature ≥ 2°C and ≤ 8°C.

Handling

As with all injectable drugs, care should be taken when handling and preparing ipilimumab. Whenever possible, ipilimumab should be prepared in a laminar flow hood or safety cabinet using standard precautions for the safe handling of intravenous agents applying aseptic technique. Latex gloves are required. If ipilimumab concentrate or solution comes in contact with skin or mucosa, immediately and thoroughly wash with soap and water. After final drug reconciliation, unused ipilimumab solution should be disposed at the site following procedures for the disposal of anticancer drugs.

4.4.2 Provision of Drugs

PROSTVAC will be obtained directly from Bavarian Nordic pharmaceutical company as study supply.

PROSTVAC is supplied in single use vials. Each vial of PROSTVAC-V contains a single dose (2 x 108 infectious units (Inf. U.) per 0.50 mL) of PROSTVAC-V in PBS/10% glycerol. The drug product contains no adjuvants or preservatives and is supplied in borosilicate (2R) glass vials, which are sealed with rubber stoppers and aluminium- plastic closures. PROSTVAC-V is stored at -80°C±10°C
.

Each vial of PROSTVAC-F contains a single dose (1 x 109 Inf. U. per 0.50mL) of PROSTVAC-F in PBS/10% glycerol. The drug product contains no adjuvants or preservatives and is supplied in borosilicate (2R) glass vials, which are sealed with rubber stoppers and aluminium-plastic closures. PROSTVAC-F is stored at -80°C±10°C
.

Ipilimumab will be obtained from Bavarian Nordic, Inc, as study supply. Ipilimumab is supplied in single-use, glass vials as a sterile, colorless solution for intravenous administration containing 200 mg.
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4.5 Drug Accountability

The Investigational Pharmacist will manage drug accountability records.

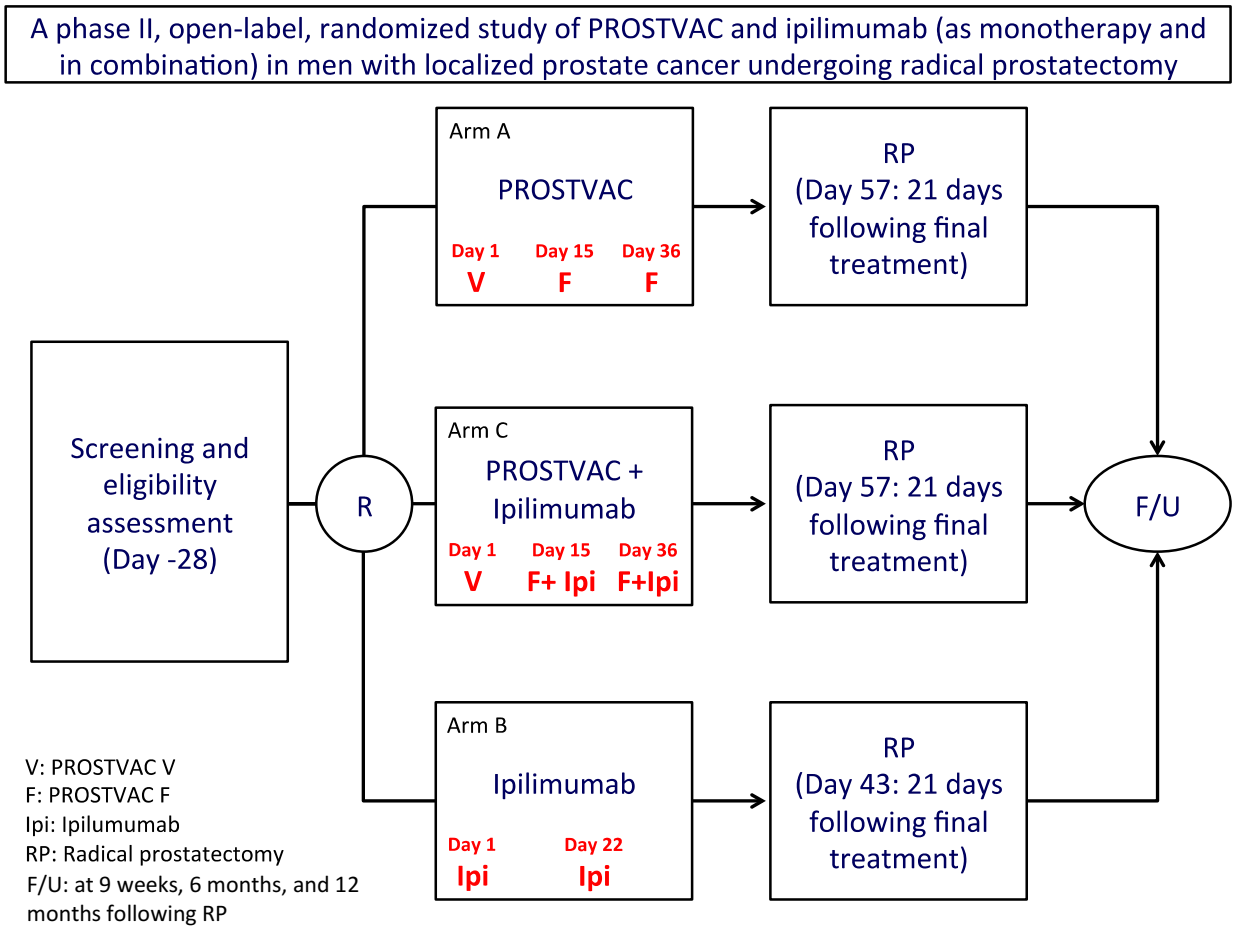
4.6 Drug Ordering

- PROSTVAC and ipilimumab will be directly supplied by Bavarian Nordic, Inc.

4.7 Packaging and Labeling of Study Drugs

Drugs will be packaged and labeled per institutional standards, adhering to applicable local and federal laws.

5 Treatment Plan



5.1 Dosage and Administration

PROSTVAC: Treatment will be administered on an outpatient basis as a subcutaneous injection. No premedication before the administration of the study drug is required. PROSTVAC must only be administered prior to the labeled expiration date. To maintain the correct product temperature, the shipping container should not be opened upon receipt. PROSTVAC must be stored and frozen in its shipping package, at -80°C±10°C until immediately before its use. PROSTVAC is to be thawed at room temperature for approximately 10-15 minutes prior to preparation. Prior to PROSTVAC administration, subjects will be provided written instructions on proper wound care. Subjects will also be provided a wound care kit. Administration time will be recorded, as will vital signs within 30 minutes prior to and after injections. Subjects will be observed for at least 30 minutes after receiving the injections.

Ipilimumab: will be infused over 90 minutes through a low protein-binding in-line filter on an outpatient basis. Flush with NS or D_5W at the end of infusion.

Table 2 Regimen Description

Study Drug	Dose	Route	Schedule
PROSTVAC-V (prime)	2 X 10^8Inf. U	SC	Arms A & C: Day 1
PROSTVAC-F (boost)	1 X 10^9Inf. U.	SC	Arms A & C: Days 15 and 36
Ipilimumab	3 mg/kg	IV	Arm B: Days 1 and 22 Arm C: Days 15 and 36

5.1.1 Criteria for study arm discontinuation

A treatment arm shall be stopped, if Grade ≥3 treatment-associated AEs occur in ≥2 of the first 6 patients, or at any point afterwards if Grade ≥3 adverse events are observed in ≥33% of patients. Exceptions apply to local injection site AEs and transient (no more than 3 days) fevers, chills, myalgias, fatigue, nausea, or dizziness. AEs that are **NOT** clinically relevant, as per Principal Investigator´s assessment, will also be excluded.

5.2 Criteria for stopping treatment

Subjects must discontinue therapy prior to RP if any of the following occurs prior to completion of the treatment regimen:

• The subject develops a concurrent illness that prevents further administration of PROSTVAC;

• The subject elects not to receive all injections of PROSTVAC or withdraws consent;

• At the discretion of the Principal Investigator.

Subjects who receive fewer than planned injections of PROSTVAC or ipilimumab due to any of the reasons noted above will continue to be followed post-RP per protocol as summarized in Section 3.5.

5.2.1 Criteria for holding/skipping ipilimumab

Ipilimumab doses that cannot be given on schedule due to treatment-related AEs will be skipped. No dose adjustment or delay will be allowed for treatment-related AEs (Section 5.3 allows a 3 day window for drug administration for scheduling issues). Decisions to hold ipilimumab must be made on specified safety criteria summarized below. Treatment with ipilimumab will be skipped or discontinued if the subject experiences at least one of the following, considered by the investigator to be “possibly”, probably” or “definitely” related to ipilimumab:

- Any clinically relevant Grade ≥2 non-skin, treatment-related AEs (including irAEs), except for laboratory abnormalities and infusion-related reactions.
- Any Grade ≥3 treatment-related laboratory non-hematologic abnormalities.
- Any AEs, laboratory abnormality or intercurrent illness that, in the judgment of the Principal Investigator, presents a substantial risk to the subject with continued dosing.
- Please see section 5.6.6 for more details, including section 5.6.6.1 for exceptions.

5.3 Radical Prostatectomy

Radical Prostatectomy will be performed at least 14 days but not more than 28 days following the last scheduled subcutaneous injection of PROSTVAC-F or ipilimumab. AEs and concomitant medications will be evaluated and recorded prior to surgery. Adverse events and concomitant medications considered by the Investigator to be related to RP will be recorded in appropriate source documentation; however, such AEs and concomitant medications will not be recorded on eCRFs. Blood samples for PSA and for immune assays will be collected prior to RP.

Standard institutional practices for the care and treatment of surgical patients will be followed before, during, and after the subject's RP. Adverse events and concomitant medications considered by the Investigator to be related to RP will be recorded in appropriate source documentation but will not be recorded on a eCRF.

Patients will be informed in the written consent that radical prostatectomy may be delayed due to adverse events resulting from study treatment, at the discretion of the Investigator and performing surgeon.

5.4 Dose Modifications

There are no dose modifications for this study.

Treatment or visit delays for public holidays or weather conditions do not constitute a protocol violation. Should any delay on receiving the treatment of more than 3 days occur due to these issues, that dose will be skipped; future treatments will continue according to protocol.

5.5 Monitoring and Toxicity Management of PROSTVAC and ipilimumab

Each patient receiving PROSTVAC and/or ipilimumab will be evaluated for safety. The safety parameters include all laboratory tests and hematological abnormalities, physical findings, and spontaneous reports of adverse events reported to the investigator by patients.

Each patient will be assessed periodically for the development of any toxicity as outlined in Section 6 Study Procedures and Observations. Toxicity will be assessed according to the NCI CTCAE v4.03. Criteria for stopping treatment will be made according as outlined in Section 5.1.1.

Acute toxicity will be managed by the treating physician. Further management will depend upon the judgment of the clinician and may include Vaccinia immunoglobulin (VIG) as described below.

5.5.1 Management of PROSTVAC injection site reactions

Subcutaneous injection may induce a local inflammatory response, and pain at the injection site is a commonly reported adverse event. Patients should be carefully assessed for signs of bacterial infection. If there is no evidence of bacterial infection, and the pain is determined to be due to injection site pain:

- Grade 1 injection site pain: should be monitored until resolution.
- Grade 2 injection site pain >72 hours: can be treated with acetaminophen 650mg PO q4-6 hours PRN, and until resolution to grade 1; patient can then be monitored until resolution.
- Grade 3 injection site pain:
    - Initiate treatment with immediate release PO opioid/acetaminophen combination (oxycodone/acetaminophen; hydrocodone/acetaminophen, codeine/acetaminophen)
    - If grade 3 pain is not relieved with treatment as above for >72 hours, patients should then start ibuprofen, 600mg PO q6 hours (or equivalent nonsteroidal anti-

inflammatory drug (NSAID)), as needed for pain. The use of ibuprofen (or other NSAID) must be reported to the Principal Investigator.
- If grade 3 pain is not relieved with NSAID treatment for >72 hours, patient should then be treated with prednisone 20mg po daily in addition to the NSAID. The use of prednisone must be reported to the Principal Investigator.
- If at any point the pain resolves to grade 1/2, subjects can be treated with acetaminophen 650mg q4-6h, and observed until resolution.

5.5.2 Management of PROSTVAC-related systemic adverse events

Systemic adverse events should be treated according to the investigators discretion.

Pyrexia may be treated with acetaminophen 650mg PO q4-6 hours PRN. If necessary, ibuprofen up to 1200 mg daily may be taken until pyrexia resolution.

In the rare, potential event of serious vaccinia virus reaction, treatment of the severe complications of vaccinia caused by dissemination of virus (except for complications involving the CNS and the cornea) is with Vaccinia immunoglobulin (VIG). VIG is an isotonic sterile solution of the immunoglobulin fraction of pooled plasma from individuals inoculated with vaccinia vaccine. It is available through the Center for Disease Control (CDC) by calling the Clinician Information Line [redacted] or the Director's Emergency Operations Center (DEOC) [redacted].

The Investigator must notify an Infectious Disease Specialist [redacted] if there is a suspected serious vaccinia reaction as soon as possible but no later than 24 hours after he/she becomes aware. A swab from the suspected lesion(s) should be obtained and sent to BioReliance for testing (see Appendix 10). The Investigator should refer to the Management Plan for Potential Serious Vaccinia Reaction for recognition, diagnosis and treatment of a rare potential serious vaccinia reaction. This plan also describes when VIG is indicated for use and administration procedures. If VIG treatment is warranted, the person receiving VIG must sign the Consent Form to Administer Vaccinia Immune Globulin (VIG) **PRIOR** to administration of VIG.
Recommended dose: I.V.: 6000 units/kg; may repeat dose based on severity of symptoms and response to treatment (specific data are lacking); 9000 units/kg may be considered if patient does not respond to initial dose. Doses up to 24,000 unit/kg were tolerated in healthy volunteers.

Infusion rate for VIG: Patients ≥50 kg: Infuse at ≤2 mL/minute; Patients <50 kg: Infuse at ≤0.04 mL/kg/minute. Maximum assessed rate of infusion: 4 mL/minute. Decrease rate of infusion in patients who develop minor adverse reactions (eg, flushing) and in patients with risk factors for thrombosis/thromboembolism and/or renal insufficiency.

There is no guarantee that VIG will successfully treat indications, but there are no other known effective antiviral treatments for vaccinia complications besides supportive care.

5.6 Monitoring and Toxicity Management of Ipilimumab

5.6.1 General Guidelines for Managing Toxicity

Clinical monitoring of autoimmunity should be completed at each MD visit (see Appendix 3).

Please see section 5.2.1 regarding guideline for holding ipilimumab for treatment-related AEs. Management algorithms for AEs associated with ipilimumab (including diarrhea, hepatotoxicity,

and endocrinopathies) are provided in Appendices 5-8 and in the Investigator's Brochure. AEs must resolve to grade ≤1 or baseline before resuming ipilimumab

5.6.2 Hypersensitivity Reactions

Since ipilimumab contains only human protein sequences, it is less likely that any allergic reaction will be seen in patients. However, it is possible that infusion of ipilimumab will induce a cytokine release syndrome that could be evidenced by fever, chills, rigors, rash, pruritus, hypotension, hypertension, bronchospasm, or other symptoms. No prophylactic pre-medication will be given unless indicated by previous experience in an individual patient. Reactions should be treated based upon the following recommendations or per institutional protocol.

For mild symptoms (e.g., localized cutaneous reactions such as mild pruritus, flushing, rash):

- Decrease the rate of infusion until recovery from symptoms, remain at bedside and monitor patient.
- Complete the ipilimumab infusion at the initial planned rate.
- Diphenhydramine 50 mg IV may be administered at the discretion of the treating physician and patients may receive additional doses with close monitoring.
- Premedication with diphenhydramine may be given at the discretion of the investigator for subsequent doses of ipilimumab.

For moderate symptoms (any symptom not listed above as mild symptoms; or below as severe symptoms, such as generalized pruritus, flushing, rash, dyspnea, and hypotension with systolic BP >80 mmHg):

- Interrupt ipilimumab.
- Administer diphenhydramine 50 mg IV.
- Monitor patient closely until resolution of symptoms.
- Corticosteroids may abrogate any beneficial immunologic effect, but may be administered at the discretion of the treating physician.
- Resume ipilimumab infusion after recovery of symptoms.
- At the discretion of the treating physician, ipilimumab infusion maybe resumed at *one half the initial infusion rate, then increased incrementally to the initial infusion rate.* If symptoms develop after resumption of the infusion, the infusion should be discontinued and no additional ipilimumab should be administered that day.
- The next dose of ipilimumab will be administered at its next scheduled time and may be given with pre-medication (diphenhydramine and acetaminophen) and careful monitoring, following the same treatment guidelines outlined above. At the discretion of the treating physician additional oral or IV antihistamine may be administered prior to dosing with ipilimumab.

For severe symptoms (e.g., any reaction such as bronchospasm, generalized urticarial, systolic blood pressure < 80 mm Hg, or angioedema):

- Immediately discontinue infusion of ipilimumab, and disconnect infusion tubing from the subject.
- Consider bronchodilators, epinephrine 1 mg IV or subcutaneously, and/or diphenhydramine 50 mg IV, with solumedrol 100 mg IV, as needed.

- Patients should be monitored until the investigator is comfortable that the symptoms will not recur.
- No further ipilimumab will be administered.

In case of late-occurring hypersensitivity symptoms (e.g., appearance within one week after treatment of a localized or generalized pruritus), symptomatic treatment may be given (e.g., oral antihistamine, or corticosteroids).

5.6.3 Management of Hepatotoxicity

Liver function tests (AST, ALT, T. bilirubin) will be evaluated for every subject prior to administration of ipilimumab. Blood samples must be collected and analyzed at local or central labs within 3 days prior to dosing. LFT results must be reviewed by the principal investigator (or designee) to meet dosing criteria specifications: $\leq$ 2.5 x ULN for AST, ALT and $\leq$ 1.5 x ULN for T. bilirubin unless liver metastases are present in which case LFT $\leq$ 5 x ULN for AST, ALT and T. bilirubin $\leq$ 3.0 x ULN) prior to dosing.

If, during the course of treatment abnormal LFT values are detected, the subject must be managed using the hepatotoxicity algorithm section in the appendix 7.

5.6.4 Treatment of Ipilimumab-Related Isolated Drug Fever

In the event of isolated fever, the investigator must use clinical judgment to determine if the fever is related to the ipilimumab or to an infectious etiology. If a patient experiences isolated drug fever, for the next dose, pre-treatment with acetaminophen or non-steroidal anti-inflammatory agent (investigator discretion) should be instituted and a repeated antipyretic dose at 6 and 12 hours after ipilimumab infusion, should be administered. The infusion rate will remain unchanged for future doses. If a patient experiences recurrent isolated drug fever following premedication and post dosing with an appropriate antipyretic, the infusion rate for subsequent dosing should be decreased to 50% of the previous rate. If fever recurs following infusion rate change, the investigator should assess the patient's level of discomfort with the event and use clinical judgment to determine if the patient should receive further ipilimumab.

5.6.5 Immune Related Toxicity

Blocking CTLA-4 function may permit the emergence of auto-reactive T cells and resultant clinical autoimmunity. Rash/vitiligo, diarrhea/colitis, uveitis/episcleritis, hepatitis, and hypopituitarism were drug-related, presumptive autoimmune events, now termed irAEs, noted in previous ipilimumab studies.

For the purposes of this study, an irAE is defined as an AE of unknown etiology associated with drug exposure and consistent with an immune phenomenon. Efforts should be made to rule out neoplastic, infectious, metabolic, toxin or other etiologic causes prior to labeling an AE as an irAE. Serological, immunological, and histological (biopsy) data should be used to support the diagnosis of an immune-mediated toxicity. Suspected irAEs must be documented on an AE or SAE form.

Patients should be informed of and carefully monitored for evidence of clinically significant systemic irAE (e.g., systemic lupus erythematosus-like diseases) or organ-specific irAE (e.g., rash, colitis, uveitis, hepatitis or thyroid disease). If an irAE is noted, appropriate work-up (including biopsy if possible) should be performed, and steroid therapy may be considered if clinically necessary.

It is unknown if systemic corticosteroid therapy has an attenuating effect on ipilimumab activity. However, clinical anti-tumor responses have been maintained in patients treated with corticosteroids and discontinued from ipilimumab. If utilized, corticosteroid therapy should be

individualized for each patient. Prior experience suggests that colitis manifested as ≥ Grade 3 diarrhea requires corticosteroid treatment.

The most common immune-related adverse events are dermatologic, endocrine, gastrointestinal, and hepatic. For any suspected immune-related adverse event, begin with the algorithm in the Appendix 5. For diarrhea, hepatotoxicity, or endocrinopathy, this will refer the investigator to a more specific algorithm (see Appendix 6 – Appendix 8). For rash, see the below.

Grade 3 – skin related AE

Fatal toxic epidermal necrolysis (TEN) has been reported following a grade 3 skin-related AE, which was considered unrelated to ipilimumab on a Bristol-Myers Squibb-sponsored trial. In patients with a drug-related Grade 2 skin immune-mediated toxicity or Grade 3 skin-related adverse event, regardless of causality, additional treatment will be delayed until the event improves to ≤ Grade 1 severity.

A dermatologist should evaluate persistent or severe rash or pruritus. A biopsy should be performed if appropriate and if possible, photos of the rash should also be obtained. Low grade ipilimumab-mediated rash and pruritus were treated with symptomatic therapy (e.g., antihistamines). Topical or parenteral corticosteroids may be required for more severe symptoms. With the appearance of any generalized rash, concomitant medications (e.g antibiotics, anticonvulsants, or proton pump inhibitors) that may be associated with severe skin reactions (e.g., Stevens Johnson Syndrome, TEN) should be discontinued and avoided. Ipilimumab should also be held.

5.6.6 Criteria for stopping ipilimumab

The following treatment-related non-neurological adverse events require permanent discontinuation of ipilimumab:

- Any ≥ Grade 2 eye pain or reduction of visual acuity that does not respond to topical therapy and does not improve to ≤ Grade 1 severity within 2 weeks of starting therapy, OR requires systemic treatment.
- Any ≥ Grade 3 bronchospasm or other hypersensitivity reaction.
- Any other ≥ Grade 3 non-skin related adverse event with the exception of events listed below under “Exceptions to Permanent Discontinuation”.
- Any ≥ Grade 4 laboratory abnormalities, except AST, ALT, or Total Bilirubin
- AST or ALT > 5 x ULN
- Total Bilirubin > 3 x ULN
- Any other Grade 4 adverse event
- Any adverse event, laboratory abnormality or intercurrent illness, which, in the judgment of the investigator, presents a substantial clinical risk to the patient with continued dosing.

The following neurological adverse event requires permanent discontinuation of ipilimumab and defines unacceptable neurotoxicity:

- Any motor neurologic toxicity ≥Grade 3 regardless of causality
- Any ≥ Grade 3 treatment-related sensory neurologic toxicity

5.6.6.1 Exceptions to Permanent Discontinuation

- Potentially reversible inflammation (< Grade 4), attributable to a local anti-tumor reaction and a potential therapeutic response. This includes inflammatory reactions at sites of tumor resections or in draining lymph nodes, or at sites suspicious for, but not diagnostic of metastasis.
- Hospitalization for ≤ Grade 2 adverse events where the primary reason for hospitalization is to expedite the clinical work-up.
- Patients with the following conditions where in the investigator's opinion continuing study drug administration is justified:
  - Ocular toxicity that has responded to topical therapy.

Endocrinopathies where clinical symptoms are controlled with appropriate hormone replacement therapy. **Note:** Ipilimumab may not be restarted while the patient is being treated with systemic corticosteroids except for patients on stable doses of hormone replacement therapy such as hydrocortisone.

6 Study Procedures and Observations

6.1 Schedule of Procedures and Observations

The study schematic is presented in Figure 1. A schedule of study visits and required procedures, tests, and examinations are presented in Table 3(Arms A and C) Table 4(Arm B). All scheduled visits outlined in Table 3 (Arms A and C) Table 4(Arm B) must be conducted at the study site of an Investigator, as indicated on Food and Drug Administration (FDA) Form 1572.

The study-specific assessments are detailed in this section and outlined in Table 3(Arms A and C) Table 4(Arm B). Screening assessments must be performed within 28 days prior to the first dose of investigational product. Any results falling outside of the reference ranges may be repeated at the discretion of the investigator. All on-study visit procedures are allowed a window of 2 days unless otherwise noted. Treatment or visit delays for public holidays or weather conditions do not constitute a protocol violation.

A written, signed, informed consent form (ICF) and a Health Insurance Portability and Accountability Act (HIPAA) authorization must be obtained before any study-specific assessments are initiated. A copy of the signed ICF will be given to the subject and a copy will be filed in the medical record. The original will be kept on file with the study records.

All patients who are consented will be registered in OnCore®, the UCSF Helen Diller Family Comprehensive Cancer Center Clinical Trial Management System (CTMS). The system is password protected and meets HIPAA requirements.

6.1.1 Pretreatment Period

6.1.1.1 Informed consent

Before initiation of any screening procedures, each prospective subject will be provided a verbal, in-depth explanation of the study, requested to read the Institutional Review Board (IRB) approved informed consent form (ICF), and encouraged to ask questions. The Investigator must ensure that each subject understands how the study will be conducted and how they will participate if they so choose. The subject shall be given sufficient time to properly consider the information and to make an informed decision regarding consent. Once all questions have been answered and the Investigator is assured that the subject understands the implications of study participation, the subject will be asked to provide written consent to participate in the study by signing the ICF. The

Investigator will document the informed consent process in the subject's medical chart or progress notes and will provide a copy of the signed ICF to the subject

A written, signed, informed consent form (ICF) and a Health Insurance Portability and Accountability Act (HIPAA) authorization must be obtained before any study-specific assessments are initiated. A copy of the signed ICF will be given to the subject and a copy will be filed in the medical record. The original will be kept on file with the study records.

All patients who are consented will be registered in OnCore®, the UCSF Helen Diller Family Comprehensive Cancer Center Clinical Trial Management System (CTMS). The system is password protected and meets HIPAA requirements.

6.1.1.2 Screening Assessments (ALL ARMS)

Subject screening evaluations must be performed within 28 days prior to enrollment; however, informed consent may be obtained earlier. Written informed consent will be obtained prior to the conduct of any study related procedures. At screening, the subject's name will be entered onto a screening log and a unique subject number will be assigned.

The subject's medical history and demographic information will be reviewed and recorded. The subject's PSA values following prostate cancer diagnosis will be collected and recorded on a eCRF. Each subject will undergo a comprehensive physical examination, including collection of vital signs, performed by the Investigator. The subject's ECOG performance status and American Urological Association (AUA) symptom index will be determined (See Appendix 1 and Appendix 4, respectively).

After eligibility is confirmed, the subject will be enrolled within 1 week prior to the first scheduled administration of study treatment.

The Screening procedures and assessments must be completed within 28 days of first day of infusion (**Week 1 Day 1**).

- Physical examination
- Vital signs including blood pressure, heart rate, respiratory rate, temperature, pulse oximetry, height, weight
- Complete medical history
- Baseline conditions assessment
- Documentation of disease assessment
- ECOG Performance status and International Prostate Symptom Score (IPSS)
- Baseline medications taken within 28 days of Day 1
- Obtain diagnostic biopsy
- Complete blood count (CBC) with differential and platelet count
- Blood chemistry assessment, including: Alkaline phosphatase, aspartate aminotransferase/alanine aminotransferase (ALT/AST), total bilirubin, calcium, phosphorus, blood urea nitrogen (BUN), creatinine, total protein, albumin, fasting glucose, potassium, sodium, chloride, lactate dehydrogenase (LDH), carbon dioxide, Lipase, Amylase
- TSH and T4
- PSA
- Immune monitoring

- HLA typing
- Serum Hepatitis assessment, including Hepatitis B surface antigen (HBsAg), Hepatitis B surface antibody (HBsAb), Hepatitis B core antibody (HBcAb), Hepatitis C virus RNA
- Urinalysis
- ECG

6.1.2 Treatment Period, ARMS A&C ONLY

6.1.2.1 Study Procedures: ARMS A&C Day 1:

- Before administration of study drug:
  - Physical examination
  - Vital signs including blood pressure, heart rate, respiratory rate, temperature, pulse oximetry
  - ECOG PS
  - AE assessment
  - Concomitant medications
  - CBC with differential and platelet count
  - Blood chemistry assessment, including: Alkaline phosphatase, ALT/AST, total bilirubin, BUN, calcium, phosphorus, creatinine, total protein, albumin, fasting glucose, potassium, sodium, chloride, LDH, carbon dioxide, lipase, amylase.
- Arms A&C: Dosing of PROSTVAC-V subcutaneously in the right thigh(both study arms)
  - Vital signs including blood pressure, heart rate, respiratory rate, temperature, pulse oximetry, and weight will be taken within 30 min before dosing of PROSTVAC-V.
- Following administration of study drug:
  - Vital signs including blood pressure, heart rate, respiratory rate, temperature, pulse oximetry, and weight will be taken within 30 min after dosing.
  - Observation of adverse events for 30 min in the clinic.

6.1.2.2 Study Procedures: ARMS A&C, Day 15:

- Before administration of study drug:
  - Physical Exam
  - Vital signs including blood pressure, heart rate, respiratory rate, temperature, pulse oximetry, weight
  - ECOG PS
  - Evaluation of adverse events
  - Concomitant medications
  - CBC with differential and platelet count
  - Blood chemistry assessment, including: Alkaline phosphatase, ALT/AST, total bilirubin, calcium, phosphorus, BUN, creatinine, total protein, albumin, fasting glucose, potassium, sodium, chloride, LDH, carbon dioxide, lipase, amylase
  - TSH and T4
  - Immune monitoring

- Study drug administration
    - Arms A&C: Dosing of PROSTVAC-F subcutaneously in the left thigh
        - Vital signs including blood pressure, heart rate, respiratory rate, temperature, pulse oximetry, and weight will be taken within 30 min before dosing of PROSTVAC-F.
    - Arm C: Dosing of Ipilimumab
- Following administration of both PROSTVAC-F and Ipilimumab:
    - Vital signs including blood pressure, heart rate, respiratory rate, temperature, pulse oximetry, and weight will be taken within 30 min after dosing.
    - Observation for adverse events for 30 minutes in the clinic after each drug administration.

6.1.2.3 Study Procedures: ARMS A&C, Day 36:

- Before administration of study drug:
    - Physical Exam
    - Vital signs including blood pressure, heart rate, respiratory rate, temperature, pulse oximetry, weight
    - ECOG PS
    - Evaluation of adverse events
    - Concomitant medications
    - CBC with differential and platelet count
    - Blood chemistry assessment, including: Alkaline phosphatase, ALT/AST, total bilirubin, calcium, phosphorus, BUN, creatinine, total protein, albumin, fasting glucose, potassium, sodium, chloride, LDH, carbon dioxide, lipase, amylase
    - TSH and T4
    - Immune monitoring
- Study drug administration:
    - ARMS A&C: Dosing of PROSTVAC-F subcutaneously in the left arm/shoulder
        - Vital signs including blood pressure, heart rate, respiratory rate, temperature, pulse oximetry, and weight will be taken within 30 min before dosing of PROSTVAC-F.
    - ARM C: Dosing of Ipilimumab
- Following administration of both PROSTVAC-F and Ipilimumab:
    - Vital signs including blood pressure, heart rate, respiratory rate, temperature, pulse oximetry, and weight will be taken within 30 min after dosing.
    - Observation for adverse events for 30 minutes in the clinic after each drug administration.

6.1.3 Treatment Period, ARM B ONLY

6.1.3.1 Study Procedures: ARM B, Day 1:

- Before administration of study drug:
    - Physical examination

  - Vital signs including blood pressure, heart rate, respiratory rate, temperature, pulse oximetry, weight
  - ECOG PS
  - AE assessment
  - Concomitant medications
  - CBC with differential and platelet count
  - Blood chemistry assessment, including: Alkaline phosphatase, ALT/AST, total bilirubin, BUN, calcium, phosphorus, creatinine, total protein, albumin, fasting glucose, potassium, sodium, chloride, LDH, carbon dioxide, lipase, amylase

- Before Dosing of Ipilimumab
  - Vital signs including blood pressure, heart rate, respiratory rate, temperature, pulse oximetry should be taken within 30 minutes before dosing of Ipilimumab
- Dosing of Ipilimumab

6.1.3.2 Study Procedures: ARM B, Day 22:

- Before administration of study drug:
  - Physical Exam
  - Vital signs including blood pressure, heart rate, respiratory rate, temperature, pulse oximetry, weight
  - ECOG PS
  - Evaluation of adverse events
  - Concomitant medications
  - CBC with differential and platelet count
  - Blood chemistry assessment, including: Alkaline phosphatase, ALT/AST, total bilirubin, calcium, phosphorus, BUN, creatinine, total protein, albumin, fasting glucose, potassium, sodium, chloride, LDH, carbon dioxide, lipase, amylase
  - TSH and T4
  - Immune monitoring

- Before dosing of Ipilimumab
  - Vital signs including blood pressure, heart rate, respiratory rate, temperature, pulse oximetry within 30 min of dosing.
- Dosing of Ipilimumab

6.1.4 Study Procedures: Radical Prostatectomy (ALL ARMS: 21 days following final dose of PROSTVAC and/or Ipi)

- For Arms A&C, Day 57 +/- 7 days
- For Arm B, Day 43 +/- 7 days
- Radical prostatectomy will be performed by the treating urologic oncologist.
- Preoperative Physical exam

- Preoperative Vital signs including blood pressure, heart rate, respiratory rate, temperature, pulse oximetry
- Preoperative Medical history
- Immune monitoring (done preoperatively).
- Preoperative ECOG PS and IPSS.
- Preoperative labs,
    - CBC with differential and platelet count
    - Blood chemistry assessment, including: Alkaline phosphatase, ALT/AST, total bilirubin, calcium, phosphorus, BUN, creatinine, total protein, albumin, fasting glucose, potassium, sodium, chloride, LDH, carbon dioxide, lipase, amylase
    - TSH and T4
    - PSA
- Subjects will have a postoperative telephone follow-up assessment 10-42 days following RP as per the treating urologic and medical oncologists' discretion. At this time the following will be performed:
    - Concomitant medications
    - Evaluation of adverse events
    - Postoperative labs may be requested as per the treating urologic and medical oncologists' discretion
    -

6.1.5 Early Termination Visit (All ARMS)

To be completed within 7 days for subjects who do not proceed to Radical Prostatectomy.

- Physical examination
- Vital signs including blood pressure, heart rate, respiratory rate, temperature, pulse oximetry
- ECOG Performance status
- Evaluation of adverse events
- Concomitant medications
- CBC with differential and platelet count
- Blood chemistry assessment, including: Alkaline phosphatase, ALT/AST, total bilirubin, calcium, phosphorus, BUN, creatinine, total protein, albumin, fasting glucose, potassium, sodium, chloride, carbon dioxide, lipase, amylase
- PSA
- Immune monitoring

6.1.6 Follow-up Study Procedures: (ALL ARMS, 9 weeks following RP (+/- 7 days))

- Physical examination
- Vital signs including blood pressure, heart rate, respiratory rate, temperature, pulse oximetry
- ECOG Performance status

- Evaluation of adverse events
- Concomitant medications
- CBC with differential and platelet count
- Blood chemistry assessment, including: Alkaline phosphatase, ALT/AST, total bilirubin, calcium, phosphorus, BUN, creatinine, total protein, albumin, fasting glucose, potassium, sodium, chloride, carbon dioxide, lipase, amylase
- PSA
- Immune monitoring

6.1.7 Post-treatment/Follow Up Visits (ALL ARMS, 6 and 12 months following RP (+/- 7 days))

Patients will be followed 6 and 12 months following RP, or until disease progression (whichever occurs first). The following procedures will be performed at the Follow Up Visit(s):

- Physical examination
- Vital signs including blood pressure, heart rate, respiratory rate, temperature, pulse oximetry
- Documentation of disease assessment
- ECOG Performance status
- Evaluation of adverse events
- Concomitant medications
- CBC with differential and platelet count
- Blood chemistry assessment, including: Alkaline phosphatase, ALT/AST, total bilirubin, calcium, phosphorus, BUN, creatinine, total protein, albumin, fasting glucose, potassium, sodium, chloride, LDH, carbon dioxide, lipase, amylase
- PSA
- Immune monitoring

6.1.8 Discontinuation of Therapy

The Investigator will withdraw a patient whenever continued participation is no longer in the patient's best interests. Reasons for withdrawing a patient include, but are not limited to, disease progression, the occurrence of an adverse event or a concurrent illness, a patient's request to end participation, a patient's non-compliance or simply significant uncertainty on the part of the Investigator that continued participation is prudent. There may also be administrative reasons to terminate participation, such as concern about a patient's compliance with the prescribed treatment regimen.

Table 3 Schedule of Study Procedures and Assessments (ARMS A&C)

Study Day/Visit Day	Screening Day -28 to 0	Day 1	Day 15 (± 3 days)	Day 36 (±3 days)	Day 57[f] (± 7 days)		Early Termination[e]	9 wks post RP (± 7 days)		6 months post RP (± 7 days)	12 months post-RP (±7 days)
Informed consent	X										
Baseline conditions	X					RP					
AE assessment		X	X	X	X		X	X		X	X
Concomitant medications	X	X	X	X	X		X	X		X	X
Obtain diagnostic biopsy or prostatectomy tissue	X					X					
Treatment/Drug Administration											
PROSTVAC – V **(Arms A&C)**		X				RP					
PROSTVAC – F **(Arms A&C)**			X	X							
IPILIMUMAB **(Arm C only)**			X	X							
Clinical procedures											
Physical exam	X	X	X	X	X	RP	X	X		X	X
Vital signs[d]	X	X	X	X	X		X	X		X	X
Medical history	X										
Disease assessment	X									X	X
Performance status	X	X[a]	X	X	X		X	X		X	X
IPSS	X				X						
ECG	X										

Study Day/Visit Day	Screening Day -28 to 0	Day 1	Day 15 (± 3 days)	Day 36 (±3 days)	Day 57[f] (± 7 days)		Early Termination[e]	9 wks post RP (± 7 days)		6 months post RP (± 7 days)	12 months post-RP (±7 days)
Laboratory procedures											
CBC w/ Diff[b]	X	X[a]	X	X	X	RP	X	X		X	X
Blood chemistry[b]	X	X[a]	X	X	X		X	X		X	X
Thyroid Function Tests	X		X	X	X						
PSA	X				X		X	X		X	X
Hepatitis B, C and HIV	X										
Urinalysis	X										
HLA Typing	X										
Immune Monitoring[c]	X		X	X	X		X	X		X	X

[a] Pre-study clinical (physical exam and performance status) and laboratory parameters are to be measured within 14 days of beginning treatment and do not need to be repeated on Day 1.

[b] CBC with differential and platelet count will be collected prior to study drug(s) dosing. Blood chemistry assessment, including: Alkaline phosphatase, ALT/AST, total bilirubin, calcium, phosphorus, BUN, creatinine, total protein, albumin, fasting glucose, potassium, sodium, chloride, LDH, carbon dioxide, lipase, amylase

[c] Refer to Laboratory Manual for collection procedures.

[d] Vitals that are to be taken at screening and at every provider visit prior to study drug administration are Blood pressure (diastolic & systolic), Heart Rate, Temperature, Respiration, & Pulse Oximetry. Weight is to be measured at screening and prior to Ipilimumab administration. Height is only required at screening. See Section 6.1.2 for Vitals schedule before and after study drug administration.

[e] Early termination visits are to be completed within 7 days for subjects who do not proceed to Radical Prostatectomy.

[f] Subjects will have routine postoperative follow-up assessments 10-42 days following RP as per urologic oncologist's discretion. Concomitant medications, adverse events, and ECOG performance status should be evaluated.

Table 4 Schedule of Study Procedures and Assessments (ARM B)

Study Day/Visit Day	Screening Day -28 to 0	Day 1	Day 22 (± 3 days)	Day 43[f] (± 7 days)		Early Termination[e]	9 wks post RP (± 7 days)		6 months post RP (± 7 days)	12 months post RP (± 7 days)
Informed consent	X									
Baseline conditions	X				RP					
AE assessment		X	X	X		X	X		X	X
Concomitant medications	X	X	X	X		X	X		X	X
Obtain diagnostic biopsy or prostatectomy tissue	X				X					
Treatment/Drug Administration										
IPILIMUMAB		X	X		RP					
Clinical procedures										
Physical exam	X	X	X	X		X	X		X	X
Vital signs[d]	X	X	X	X		X	X		X	X
Medical history	X									
Disease assessment	X				RP				X	X
Performance status	X	X[a]	X	X		X	X		X	X
IPSS	X			X						
ECG	X									
Laboratory procedures										
CBC w/ Diff [b]	X	X[a]	X	X		X	X		X	X
Blood chemistry [b]	X	X[a]	X	X		X	X		X	X
Thyroid Function Tests	X		X	X						
PSA	X			X	RP	X	X		X	X
Hepatitis B, C and HIV	X									
Urinalysis	X									
HLA Typing	X									
Immune Monitoring [c]	X		X	X		X	X		X	X

[a] Pre-study clinical (physical exam and performance status) and laboratory parameters are to be measured within 14 days of beginning treatment and do not need to be repeated on Day 1.

[b] CBC with differential and platelet count will be collected prior to ipilimumab dose. Blood chemistry assessment, including: Alkaline phosphatase, ALT/AST, total bilirubin, calcium, phosphorus, BUN, creatinine, total protein, albumin, fasting glucose, potassium, sodium, chloride, LDH, carbon dioxide, lipase, amylase

[c] Refer to Laboratory Manual for collection procedures.

[d] Vitals that are to be taken at screening and at every provider visit prior to ipilimumab administration are Blood pressure (diastolic & systolic), Heart Rate, Temperature, Respiration, & Pulse Oximetry. Weight is to be measured at screening and prior to Ipilimumab administration. Height is only required at screening. See section 6.1.3 for Vitals schedule before Ipilimumab administration.

[e] Early termination visits are to be completed within 7 days for subjects who do not proceed to Radical Prostatectomy.

[f] Subjects will have routine postoperative follow-up assessments 10-42 days following RP as per urologic oncologist's discretion. Concomitant medications, adverse events, and ECOG performance status should be evaluated.

6.2 Usage of Concurrent/Concomitant Medications

Concomitant medications associated with RP will not be recorded on the concomitant medication eCRF unless the need for the medication is judged to be at least possibly related to treatment with PROSTVAC or ipilimumab or may be immunosuppressive. Concomitant medications that are considered by the Investigator to be related to RP will be documented in the source documentation but will not be recorded on an eCRF
.

6.3 Dietary Restrictions

No dietary restrictions to this protocol.

6.4 Prohibited Medications

The following should not be used at any time during the study:

• Any investigational product other than PROSTVAC or IIpilimumab;

• Corticosteroid administration (inhaled, intra-articular and topical steroids are allowable)

• Vaccine therapy

Additionally, patients in this study may not use vaccines for or prevention of disease (including those for common medical conditions, e.g. influenza) while they are participating in this clinical study, and for 3 months following the final study treatment.

6.5 Restricted Medications

Hormones, including LHRH agonists, antiandrogens (such as bicalutamide, flutamide, or nilutamide), or 5-α-reductase inhibitors may not be administered until after RP. Additionally, radiation therapy may not be initiated until after RP. Following RP, subjects may receive hormone therapy or radiation therapy as deemed appropriate by their medical professional. Systemic anti-cancer therapies not listed above should be discussed with the medical monitor prior to initiating treatment.

7 Reporting and Documentation of Results

7.1 Evaluation of Activity

7.1.1 Primary endpoint: Immunohistological Characterization of $CD3^+$ T Cell Infiltration within Prostate Tissue.

The primary endpoint response measure will be the change in the number of infiltrating CD3+ T cells/μm^2 within the prostatic tumor tissue from the diagnostic core biopsy specimens to the post-treatment prostatectomy tissue specimens, based upon IHC analysis. The infiltrate will be scored by the number of CD3+ cells/μm^2. Thus, the number of inflammatory cells will be normalized to the unit area. Both biopsies and RP specimens will be quantitated with this frequency. For each patient, the change in the number of CD3+ T-cell infiltration from the pre-treatment biopsy to post-treatment prostatectomy tissue specimens will be represented by the ratio of post-treatment prostatectomy tissue specimens vs. pre-treatment biopsy.

7.1.2 Secondary and exploratory endpoints.

7.1.2.1 Immunohistological characterization of T Cell Subsets and other Immune Cells within the Prostate Tissue.

The change in the number of infiltrating T cells/μm^2 of selected subsets (e.g., CD4+, CD8+) within the prostatic tumor tissue from the diagnostic core biopsy specimens to the post treatment prostatectomy tissue specimens will be assessed, based upon IHC analysis.

7.1.2.2 Antigen-specific T Cell Immunity in Peripheral Blood

To assess antigen-specific T cell immunity in the peripheral blood, indicated by the T cell response to PSA-3, peripheral blood samples will be collected from subjects at the screening visit, prior to RP and at 9 weeks post-RP. The frequency of PSA, TAA and vector specific T cells will be enumerated by interferon gamma (IFN-γ) enzyme-linked immunospot (ELISPOT) assays using established methods (41).

T-cell proliferation assay will also be measured at the same timepoints the antigen-specific T cell response by co-culturing previously cryopreserved PBMCs in triplicate with PSA, TAA, viral vector or PHA-L, and assessing with a standardized [^{3}H] thymidine proliferation assay (48),(49).

7.1.3 PSA

The utility of a decline in PSA as a marker of response to neoadjuvant therapies is not well defined. However, the proportion of patients with PSA response, defined as ≥50% PSA decline, will be reported.

7.1.4 Pathologic Response

The proportion of patients with pathologic complete response (pT0) at the time of prostatectomy will be reported.

7.2 Evaluation of Safety

The following will be collected to evaluate safety:

- Adverse events (including SAEs and deaths)
- Physical examinations
- Vital signs
- ECOG performance status
- AUA symptom index
- Laboratory test results

Analyses will be performed for all patients having received at least one dose of study drug. The study will use the CTCAE v4.03 for reporting of non-hematologic adverse events and modified criteria for hematologic adverse events.

For multicenter studies, the Principal Investigator at the UCSF Coordinating Center will hold the role of Study Chair. The Study Chair is responsible for the overall conduct of the study and for monitoring its safety and progress at all participating site.

7.3 Definitions of Adverse Events

7.3.1 Adverse event

An adverse event (also known as an adverse experience) is defined as any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related. More specifically, an adverse event can be any unfavorable and unintended sign (e.g., an abnormal laboratory finding), symptom, or disease temporally associated with the use of a drug, without any judgment about causality. An adverse event can arise from any use of the drug (e.g., off-label use, use in combination with another drug) and from any route of administration, formulation, or dose, including an overdose.

7.3.2 Adverse reaction

An adverse reaction is defined as any adverse event caused by the use of a drug. Adverse reactions are a subset of all suspected adverse reactions for which there is reason to conclude that the drug caused the event.

7.3.2.1 Suspected

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

7.3.2.2 Unexpected

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

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

Adverse events that would be anticipated to occur as part of the disease process are considered *unexpected* for the purposes of reporting because they would not be listed in the investigator brochure. For example, a certain number of non-acute deaths in a cancer trial would be anticipated as an outcome of the underlying disease, but such deaths would generally not be listed as a suspected adverse reaction in the investigator brochure.

Some adverse events are listed in the Investigator Brochure as occurring with the same class of drugs, or as anticipated from the pharmacological properties of the drug, even though they have not been observed with the drug under investigation. Such events would be considered *unexpected* until they have been observed with the drug under investigation. For example, although angioedema is anticipated to occur in some patients exposed to drugs in the ACE inhibitor class and angioedema would be described in the investigator brochure as a class effect, the first case of angioedema observed with the drug under investigation should be considered *unexpected* for reporting purposes.

7.3.2.3 Serious

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

- Death
- Life-threatening adverse event
- Inpatient hospitalization or prolongation of existing hospitalization
- A persistent or significant incapacity or substantial disruption of the ability to conduct normal life function
- Important medical events that may not result in death, are life-threatening, or require hospitalization may be considered serious 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. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

7.3.2.4 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.

7.4 Recording of an Adverse Event

All grade 3 and above adverse events will be entered into OnCore®, whether or not the event is believed to be associated with use of the study drug. Data about these events and their severity will be recorded using the NCI CTCAE v4.0.

The Investigator will assign attribution of the possible association of the event with use of the investigational drug, and this information will be entered into OnCore® using the classification system listed below:

Relationship	Attribution	Description
Unrelated to investigational drug/intervention	Unrelated	The AE *is clearly NOT related* to the intervention
	Unlikely	The AE *is doubtfully related* to the intervention
Related to investigational drug/intervention	Possible	The AE *may be related* to the intervention
	Probable	The AE *is likely related* to the intervention
	Definite	The AE *is clearly related* to the intervention

Signs or symptoms reported as adverse events will be graded and recorded by the Investigator according to the CTCAE. When specific adverse events are not listed in the CTCAE they will be graded by the Investigator as *none*, *mild*, *moderate* or *severe* according to the following grades and definitions:

Grade 0	No AE (or within normal limits)
Grade 1	Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated
Grade 2	Moderate; minimal, local, or noninvasive intervention (e.g., packing, cautery) indicated; limiting age-appropriate instrumental activities of daily living (ADL)
Grade 3:	Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care ADL
Grade 4:	Life-threatening consequences; urgent intervention indicated
Grade 5:	Death related to AE

7.5 Follow-up of Adverse Events

All adverse events will be followed with appropriate medical management until resolved. Patients removed from study for unacceptable adverse events will be followed until resolution or stabilization of the adverse event. For selected adverse events for which administration of the investigational drug was stopped, a re-challenge of the subject with the investigational drug may be conducted if considered both safe and ethical by the Investigator.

7.6 Adverse Events Monitoring

All adverse events, whether or not unexpected, and whether or not considered to be associated with the use of the study drug, will be entered into OnCore®, as noted above.

The Investigator will assess all adverse events and determine report ability requirements to the UCSF Data and Safety Monitoring Committee (DSMC) and UCSF's Institutional Review Board, the Committee on Human Research (CHR); and, when the study is conducted under an Investigational New Drug Application (IND), to the Food and Drug Administration (FDA) if it meets the FDA reporting criteria.

All adverse events entered into OnCore® will be reviewed by the Helen Diller Family Comprehensive Cancer Center Site Committee on a weekly basis. The Site Committee will review and discuss at each weekly meeting the selected toxicity, the toxicity grade, and the attribution of relationship of the adverse event to the administration of the study drug(s).

All grade(s) 3-5 adverse events entered into OnCore® will be reviewed on a monthly basis at the Site Committee meetings. The Site Committee will review and discuss the selected toxicity, the toxicity grade, and the attribution of relationship of the adverse event to the administration of the study drug(s).

In addition, all suspected adverse reactions considered "serious" entered into OnCore®, will be reviewed and monitored by the Data and Safety Monitoring Committee on an ongoing basis and discussed at DSMC meetings, which take place every six weeks.

For a detailed description of the Data and Safety Monitoring Plan please refer to Appendix 2.

7.7 Expedited Reporting

Reporting to the Data and Safety Monitoring Committee

If a death occurs during the treatment phase of the study or within 30 days after the last administration of the study drug(s) and it is determined to be related either to the study drug(s) or to a study procedure, the Investigator or his/her designee must notify the DSMC Chair (or qualified alternate) within 1 business day of knowledge of the event. The contact may be by phone or e-mail.

Reporting to UCSF Committee on Human Research (Institutional Review Board)

The Principal Investigator must report events meeting the UCSF CHR definition of "Unanticipated Problem" (UP) within 5 business days of his/her awareness of the event.

Expedited Reporting to the Food and Drug Administration

If the study is being conducted under an IND, the Sponsor-Investigator is responsible for determining whether or not the suspected adverse reaction meets the criteria for expedited reporting in accordance with Federal Regulations (21 CFR §312.32).

The Investigator must report in an IND safety report any suspected adverse reaction that is both serious and unexpected. The Sponsor-Investigator needs to ensure that the event meets all three definitions:

- Suspected adverse reaction
- Unexpected
- Serious

If the adverse event does not meet all three of the definitions, it should not be submitted as an expedited IND safety report.

The timeline for submitting an IND safety report to FDA is no later than **15 calendar days** after the Investigator determines that the suspected adverse reaction qualifies for reporting (21 CFR 312.32(c)(1)).

Any unexpected fatal or life-threatening suspected adverse reaction will be reported to FDA no later than **7 calendar days** after the Investigator's initial receipt of the information (21 CFR 312.32(c)(2)).

Any relevant additional information that pertains to a previously submitted IND safety report will be submitted to FDA as a Follow-up IND Safety Report without delay, as soon as the information is available (21 CFR 312.32(d)(2)).

Reporting SAEs to Pharmaceutical Companies providing Study Drug

All expedited SAEs (serious, unexpected and related to study drugs) must be reported to Bavarian Nordic. within 24 hours by fax or email to:

Bavarian Nordic
[redacted]

Specify: protocol #, subject #, PI name/site#, SAE/onset dates

In addition, all SAEs – serious, unexpected, and related to ipilimumab – must be reported to Bristol-Myers Squibb, Inc. within 24 hours by fax or email to:

Bristol-Myers Squibb Company Global Pharmacovigilance & Epidemology Department
[redacted]

[redacted]

Reporting Non-Serious AEs to Pharmaceutical Companies providing Study Drug

All non-serious AEs should be transferred to Bavarian Nordic and Bristol-Myers Squibb, Inc. (related to ipilimumab), every six months once the case is closed in the UCSF safety database.

8 Statistical Considerations and Evaluation of Results

8.1 Study Endpoints

See section 2.4 for endpoints.

8.2 Sample size and power estimate

The primary endpoint is the proportion of patients who demonstrate a positive response to neoadjuvant therapy for each arm of the study. A positive response is defined as a ≥2-fold increase in the number of infiltrating CD3+ T cell between the pretreatment biopsy and the post-treatment RP specimen. A negative response is defined as a <2-fold increase. We expect treated patients have more positive responders than the reference cohort with no neoadjuvant treatment (see section 8.6); thus we consider the lower threshold for the probability of positive responders to be 5%. If the true proportion of positive responders is 25%, with 13 patients in each treatment arm, we will have 90% confidence interval of (6.6%, 49.5%). Considering a dropout rate of 7%, a total of 42 patients will be accrued to the study. Additionally, with 13 evaluable patients in each arm, we have 80% power to detect a significantly different response rate between the PROSTVAC and ipilimumab combination arm versus the two monotherapy arms, at a significance level of 0.1, if the monotherapy arms have an average response rate of 20% and the combination arm has a response rate of 66%.

Patients who complete screening but withdraw prior to receiving treatment will be replaced. There are no stratification factors in this analysis.

8.3 Accrual

It is anticipated that 4 subjects will be enrolled on study each month, with accrual to the study completed in approximately 18 months. With 12 months of planned follow-up after RP, the study is expected to be completed in 30 months.

8.4 Interim Analyses and Stopping Rules

There will be no formal interim analysis for efficacy performed in this study. Please refer to section 5.1.1 regarding criteria for stopping treatment for lack of safety.

8.5 Analyses Plans

Safety analyses will be performed on safety-evaluable patients, defined as enrolled patients who received any amount of the study treatment. Efficacy analyses will be performed on efficacy-evaluable patients, defined as safety evaluable patients with measurable disease at baseline.

Demographic and baseline characteristics will be summarized by arm and overall. In general, frequency distribution and percentage will be used to summarize categorical measurements, while mean (with standard deviation) and median (with range) will be used to describe symmetric and skewed continuous measurements, respectively. Univariate analysis among variables will be assessed using the two-sample t-test, Wilcoxon-rank-sum test, Chi-square test, as appropriate.

8.5.1 Primary Analysis (or Analysis of Primary Endpoint)

The primary analysis for immune response within the prostate will include all enrolled subjects who receive at least 1 dose of PROSTVAC or ipilimumab in any of the treatment arms, and undergo RP. Analysis will be done for each arm separately as well as aggregate for all treatment arms.

CD3+ T cell infiltration will be assessed by IHC staining, and quantitated by the number of CD3+ T cells/μm^2. Therefore, the number of T cells will be normalized per unit area. For the analysis of the primary endpoint, T cells will be enumerated from tumor/benign tissue interface (from here on referred to as the “tumor interface”). Subjects will be considered to have a “positive” response if they exhibit a ≥2-fold increase in CD3+ T cell infiltration, and a “negative” response if they exhibit a <2-fold increase. The proportion of patients who have “positive” responses will be reported, with 95% confidence intervals. Furthermore, the positive response rate will be compared between the combination arm vs. the monotherapy arms (pooling the two monotherapy arms together) via two-sample proportion test. Pairwise comparisons among the three arms will also be performed by two-sample proportion test. No multiple testing adjustments will be performed. Additional analyses will be performed for assessment of response at the tumor center and benign glands and will be reported similarly to the primary endpoint.

8.5.2 Secondary Analyses (or Analyses of Secondary Endpoints)

- T cell tumor infiltration (of selected T cell subsets) will be scored by IHC assessment, and scored by the number of T cells/μm^2. Therefore, the number of T cells will be normalized per unit area. The change in T cell infiltration following neoadjuvant therapy will be determined as described above for the primary endpoint. Similar analyses may be performed for other immune cells, such as CD4+, CD8+, CD56+, FOXP3, etc. Results will be reported as described for the primary endpoint, and T cell infiltration will be reported separately for areas of tumor interface, tumor center and benign tissue.
- Flow cytometry will be performed in both pre- and post-treatment peripheral blood mononuclear cells (PBMC) to determine the change in T cell activation (both T^{reg} and $T^{effector}$ cells) after neoadjuvant therapy. Descriptive statistics for continuous measurements will be used to summarize the changes.
- Treatment-related toxicities will be assessed according to NCI CTCAE v4.03, for all patients who receive at least a single dose of investigational therapy. The distribution for the

maximum observed grade for each adverse event will be tabulated and reported with 95% confidence interval.

8.5.3 Exploratory Analyses (or Analyses of Exploratory Endpoints)

- To characterize antibody responses to a broad panel of antigens, sera will be collected at baseline, prior to RP, and 9 weeks following RP, and profiled using spotted antigen array (50). After standard preprocess of the protein array data, Cluster and Tree view software will be used for unsupervised clustering of the data with Pearson correlation and complete linkage. For each array, an antigen is identified as being detected if its value is above the median. To determine the number of up- and down-modulated antibodies, the difference in log2 intensity values of pretreatment and post-treatment samples will be taken for each patient to identify antigens that are detected differentially due to treatment.
- Assessment of change from baseline in antigen-specific T-cell immunity in the blood will be performed using repeated measure analysis of variance (ANOVA) methods with a mixed model approach to allow for a varying number of follow-up measurements. To study the change from baseline (screening visit) to post-baseline visits (up to 9 weeks post-RP), the mixed model will include four visits (up to 9 weeks post-RP) and subject as a random effect. Using polynomial contrast statements, the overall pattern T-cell immunity in the blood (e.g. linear) will be investigated. The specific comparison of change in immunity between baseline and any specific post-baseline time point will be tested also using linear contrast statements. The above analysis will be done for each arm separately as well as aggregately.
- Development of and changes in humoral immune responses in the periphery following treatment. This will be evaluated by testing serum with methods such as:
    - Measurement of antibodies to PSA, TAA and the viral vector may be performed using peptide/protein arrays, standard ELISA assays or flow cytometry to determine neutralizing antibody titers. After standard pre-process of the protein array data, Cluster and Tree view software will be used for unsupervised clustering of the data with Pearson correlation and complete linkage. For each array, an antigen is identified as being detected if its value is above the median. To determine the number of up- and down-modulated antibodies, the difference in log2 intensity values of pre-treatment and post-treatment samples will be taken for each patient to identify antigens that are detected differentially due to treatment.
    - Measurement of cytokines, chemokines or cancer biomarkers by Luminex multiplex analysis will be summarized by descriptive statistics for continuous variables at each timepoint for each arm. Furthermore, repeated measure ANOVA methods with a mixed model approach to allow for a varying number of follow-up measurements will be applied. The specific comparison of change between baseline and any specific post-baseline time point will be tested also using linear contrast statements.
    - PD-L1 expression, by IHC assessment, will be scored on a 0-3 scale (0=0-5% staining, 1=6-33% staining, 2=34-66% staining, 3=67-100% staining). For each subject, the change in PD-L1 expression score following neoadjuvant therapy will be calculated for exploratory characterization of the impact on PD-L1 in localized prostate cancer. Descriptive statistics for categorical measurements will be used to summary the PD-L1 expression change for each location. Furthermore, univariate analysis (proportion test) will be applied to explore the PD-L1 expression score change between locations.

- In order to assess the clinical impact of neoadjuvant treatment with PROSTVAC, ipilimumab, or the combination or PROSTVAC and ipilimumab:

- The proportion of patients who achieve a ≥30% decline in PSA at the time of RP will be reported with 95% confidence intervals.
- The proportion of patients with pathologic complete response (pT0) at the time of RP will be reported with 95% confidence intervals.

- T-cell receptor (TCR) repertoire sequencing following re-stimulation on T cells in vitro with prostate specific, tumor associated antigens and vector specific antigens to evaluate clonality of T-cell responses in the periphery. The responses in the tumor may also be evaluated in prostate biopsy samples to allow correlation with responses in the periphery. For each individual treatment, the change in tumor-infiltrating TCR between pre-treatment and post-prostatectomy after treatment will be assessed by calculating the number of unique clonotypes comprising the top 25th percentile of cumulative reads after sorting by clone abundance. Repertoire change between sequencing experiments will be measured using Morisita's distance.

8.6 Reference population

As discussed in the introduction (section 1.2), in a prior study of neoadjuvant Sip-T, since the effect of systemic Sip-T administration on localized prostate cancer was unknown, a negative control was felt to be important to be confident that the observed changes in the study population were due to treatment effect. Therefore a group of 12 patients, who had undergone RP at UCSF without any neoadjuvant therapy, selected prior to undergoing any tissue analyses and matched to the study population using the UCSF CAPRA-S score for preoperative risk stratification were used as a negative control; these patients were selected from the surgical population at UCSF that provide separate, written consent to the use of their RP specimens for research purposes.

Similarly, in this study, a cohort of 12 patients not treated with any neoadjuvant therapy will be used as a negative control, since the effect of neoadjuvant PROSTVAC, ipilimumab, or the combination of PROSTVAC and ipilimumab in prostate cancer tissue is unknown. Patients will be selected from the RP population at UCSF who have provided separate, written consent to the use of their RP specimens for research purposes, and matched to the phase II study population using the UCSF CAPRA-S score for preoperative risk stratification. This analysis will provide confidence that the changes observed in the prostates of treated patients are due to neoadjuvant treatment effect. These 12 patients will not otherwise be included in the analyses as delineated in section 8.5.

8.7 Evaluation of Safety

Analyses will be performed for all patients having received at least one dose of study drug. The study will use the NCI CTCAE v4.03. In addition, safety data will be tabulated in aggregate and by treatment group within the safety population. The results will be tabulated for all toxicities observed. No statistical testing is planned for AE summaries.

9 Study Management

9.1 Pre-study Documentation

This study will be conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki as stated in 21 CFR §312.120(c)(4); consistent with GCP and all applicable regulatory requirements.

Before initiating this trial, the Investigator will have written and dated approval from the Institutional Review Board for the protocol, written informed consent form, subject recruitment materials, and

any other written information to be provided to subjects before any protocol related procedures are performed on any subjects.

The clinical investigation will not begin until either FDA has determined that the study under the Investigational Drug Application (IND) is allowed to proceed or the Investigator has received a letter from FDA stating that the study is exempt from IND requirements.

The Investigator must comply with the applicable regulations in Title 21 of the Code of Federal Regulations (21 CFR §50, §54, and §312), GCP/ICH guidelines, and all applicable regulatory requirements. The IRB must comply with the regulations in 21 CFR §56 and applicable regulatory requirements.

9.2 Institutional Review Board Approval

The protocol, the proposed informed consent form, and all forms of participant information related to the study (e.g. advertisements used to recruit participants) will be reviewed and approved by the UCSF CHR (UCSF Institutional Review Board). Prior to obtaining CHR approval, the protocol must be approved by the Helen Diller Family Comprehensive Cancer Center Site Committee and by the Protocol Review Committee (PRC). The initial protocol and all protocol amendments must be approved by the IRB prior to implementation.

9.3 Informed Consent

All participants must be provided a consent form describing the study with sufficient information for each participant to make an informed decision regarding their participation. Participants must sign the CHR-approved informed consent form prior to participation in any study specific procedure. The participant must receive a copy of the signed and dated consent document. The original signed copy of the consent document must be retained in the medical record or research file.

9.4 Changes in the Protocol

Once the protocol has been approved by the UCSF CHR, any changes to the protocol must be documented in the form of an amendment. The amendment must be signed by the Investigator and approved by PRC and the CHR prior to implementation.

If it becomes necessary to alter the protocol to eliminate an immediate hazard to patients, an amendment may be implemented prior to CHR approval. In this circumstance, however, the Investigator must then notify the CHR in writing within five (5) working days after implementation. The Study Chair and the UCSF study team will be responsible for updating any participating sites.

9.5 Handling and Documentation of Clinical Supplies

The UCSF Principal Investigator and each participating site will maintain complete records showing the receipt, dispensation, return, or other disposition of all investigational drugs. The date, quantity and batch or code number of the drug, and the identification of patients to whom study drug has been dispensed by patient number and initials will be included. The sponsor-investigator will maintain written records of any disposition of the study drug.

The Principal Investigator shall not make the investigational drug available to any individuals other than to qualified study patients. Furthermore, the Principal Investigator will not allow the investigational drug to be used in any manner other than that specified in this protocol.

9.6 Case Report Forms (CRFs)

The Principal Investigator and/or his/her designee, will prepare and maintain adequate and accurate participant case histories with observations and data pertinent to the study. Study specific electronic Case Report Forms (eCRFs) will document safety and treatment outcomes for safety monitoring and data analysis. All study data will be entered into OnCore® via standardized eCRFs in accordance with the CTMS study calendar, using single data entry with a secure access account. The Clinical Research Coordinator (CRC) will complete the eCRFs as soon as possible upon completion of the study visit; the Investigator will review and approve the completed eCRFs.

The information collected on eCRFs shall be identical to that appearing in original source documents. Source documents will be found in the patient's medical records maintained by UCSF personnel. All source documentation should be kept in separate research folders for each patient.

In accordance with federal regulations, the Investigator is responsible for the accuracy and authenticity of all clinical and laboratory data entered onto eCRFs. The PI will approve all completed eCRFs to attest that the information contained on the eCRFs is true and accurate.

All source documentation and CTMS data will be available for review/monitoring by the UCSF DSMC and regulatory agencies.

The Principal Investigator will be responsible for ensuring the accurate capture of study data. At study completion, when the eCRFs have been declared to be complete and accurate, the database will be locked. Any changes to the data entered into the eCRFs after that time can only be made by joint written agreement among the Study Chair, the Trial Statistician, and the Protocol Project Manager.

Each participating site will complete study specific eCRFs for safety monitoring and data analysis. Each site will enter the study data into OnCore® via standardized eCRFs in accordance with the CTMS study calendar, using single data entry with a secure access account. The participating site's Clinical Research Coordinator (CRC) will complete the eCRFs; the Investigator will review and approve the completed eCRFs – this process must be completed within 5 business days of the visit. Study data from the participating site will be reported and reviewed in aggregate with data from patients enrolled at the coordinating center, UCSF. All source documentation and CTMS data will be available for review/monitoring as needed.

9.7 Registration/Enrollment

Screening procedures will occur when a subject and investigator signs and dates an informed consent form and the subject provides authorization to use protected health information. The informed consent form will be completed prior to any study-specific procedures. Screening procedures that are standard of care and were performed prior to consent, do not need to be repeated if they were conducted within the eligible screening period, unless otherwise specified.

To initiate enrollment, the investigator will verify eligibility according to all inclusion and exclusion criteria and complete the Enrollment Form. All eligible subjects must be centrally registered through the UCSF GU Clinical Research Office. To complete the registration process, the study site must fax or email the signed completed study-specific eligibility checklist, registration form, all source documents verifying eligibility, any supporting documents, and the signed informed consent. These data must also be entered into the OnCore database. Once the enrollment information is received at UCSF, the Study Chair will review the packet and if eligibility is confirmed, he will sign the registration form and assignment to treatment can occur. UCSF will provide the participating site with the signed registration approval. Subjects failing to

meet all study eligibility requirements will not be registered. See Section 3.6 for randomization details.

9.8 Oversight and Monitoring Plan

The UCSF Helen Diller Family Comprehensive Cancer Center DSMC will be the monitoring entity for this study. The UCSF DSMC will monitor the study in accordance with the NCI-approved Data and Safety Monitoring Plan (DSMP). The DSMC will routinely review all adverse events and suspected adverse reactions considered "serious". The DSMC will audit study-related activities to ensure that the study is conducted in accordance with the protocol, local standard operating procedures, FDA regulations, and Good Clinical Practice (GCP). Significant results of the DSMC audit will be communicated to the IRB and the appropriate regulatory authorities at the time of continuing review, or in an expedited fashion, as applicable. See Appendix 2 Data and Safety Monitoring Plan for a Phase 2 or 3 Institutional Study, for additional information.

10 Protection of Human Subjects

10.1 Protection from Unnecessary Harm

Each clinical site is responsible for protecting all subjects involved in human experimentation. This is accomplished through the CHR mechanism and the process of informed consent. The CHR reviews all proposed studies involving human experimentation and ensures that the subject's rights and welfare are protected and that the potential benefits and/or the importance of the knowledge to be gained outweigh the risks to the individual. The CHR also reviews the informed consent document associated with each study in order to ensure that the consent document accurately and clearly communicates the nature of the research to be done and its associated risks and benefits.

10.2 Protection of Privacy

Patients will be informed of the extent to which their confidential health information generated from this study may be used for research purposes. Following this discussion, they will be asked to sign the HIPAA form and informed consent documents. The original signed document will become part of the patient's medical records, and each patient will receive a copy of the signed document. The use and disclosure of protected health information will be limited to the individuals described in the informed consent document.

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Appendices

Appendix 1 Performance Status Criteria

ECOG Performance Status Scale	
Grade	Descriptions
0	Normal activity Fully active, able to carry on all pre-disease performance without restriction
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)
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
3	In bed > 50% of the time Capable of only limited self-care, confined to bed or chair more than 50% of waking hours
4	100% bedridden Completely disabled Cannot carry on any self-care Totally confined to bed or chair
5	Dead

Appendix 2 Data and Safety Monitoring Plan for a Phase II or Phase III Institutional Study

Data and Safety Monitoring Plan for a Phase II or III Institutional Study

1. Oversight and Monitoring Plan

The UCSF Helen Diller Family Comprehensive Cancer Center (HDFCCC) Data and Safety Monitoring Committee (DSMC) is responsible for monitoring data quality and subject safety for all HDFCCC institutional clinical studies. A summary of DSMC activities for this study includes:

- Review of patient data.
- Review of serious adverse events.
- Auditing every six months (depending on study accrual).
- Minimum of a yearly regulatory audit.

2. Monitoring and Reporting Guidelines

Investigators will conduct continuous review of data and patient safety and discuss each patient's treatment at monthly site committee meetings. These discussions are documented in the site committee meeting minutes. The discussion will include the number of patients, significant toxicities in accordance with the protocol, and observed responses.

All institutional Phase II and III studies are designated with a moderate risk assessment (see Appendix H). The data is audited twice per year with twenty percent of the patients monitored (or at least three patients if the calculated value is less than three).

3. Review and Oversight Requirements

3.1 Adverse Event Monitoring

All Grade 3-5 Adverse Events (AEs), whether or not considered to be expected or unexpected and whether or not considered to be associated with the use of the study drug, will be entered into OnCore®, UCSF's Clinical Trial Management System.

Adverse Events are graded according to the Common Terminology Criteria for Adverse Events (CTCAE) (version 4.03) as developed and revised by the Common Therapy Evaluation Program (CTEP) of the National Cancer Institute. Adverse events are further given an assignment of attribution or relationship to treatment or medical procedure. Attribution categories are:

- **Definite** – The adverse event is clearly related to the investigational agent(s) or medical procedure.
- **Probable** – The adverse event is likely related to the investigational agent(s) or medical procedure.
- **Possible** – The adverse event may be related to the investigational agent(s) or medical procedure.

- **Unlikely** – The adverse event is doubtfully related to the investigational agent(s) or medical procedure.

- **Unrelated** – the adverse event is clearly not related to the investigational agent(s) or medical procedure.

All Grade 3-5 adverse events entered into OnCore® will be reviewed on a monthly basis at the Site Committee meetings. The Site Committee will review and discuss the selected toxicity, the toxicity grade, and the attribution of relationship of the adverse event to the administration of the study drug(s).

3.2 Serious Adverse Event Reporting

By definition, an adverse event is defined as a serious adverse event (SAE) according to the following criteria:

- Death.

- Life-threatening (i.e. results in an immediate risk of death).

- Requires inpatient hospitalization or prolongation of existing hospitalization,

- Permanent or significant disability/incapacity

- Gives rise to a congenital anomaly/birth defect, or cancer, or any experience that suggests a significant hazard, contraindication, side effect, or precaution that may require medical or surgical intervention to prevent one of the outcomes listed above.

- Event occurring in a gene therapy study.

- Event that changes the risk/benefit ratio of a study.

- Any other event the Principal Investigator judges to be serious or which would suggest a significant hazard, contraindication, side effect, or precaution.

Serious Adverse Event reporting will be in accordance with the UCSF IRB Regulations and Code of Federal Regulation Title 21 Part 312.32. The SAE will be reported on a Med Watch form.

UCSF IRB website for guidance in reporting serious adverse events:

https://irb.ucsf.edu/adverse-event

FDA website for guidance in reporting serious adverse events:

www.fda.gov/Safety/MedWatch/HowToReport/default.htm

Med Watch forms and information:

www.fda.gov/medwatch/getforms.htm

All serious adverse events are entered into OnCore®, as well as submitted to the IRB (per IRB guidelines) via iMedRIS®. The SAEs are reviewed and monitored by the Data and Safety Monitoring Committee on an ongoing basis and discussed at DSMC meetings, which take place every six weeks. The date the SAE is sent to all required reporting agencies will be documented in OnCore®.

If the SAE involves a subject death, and is determined to be possibly, probably or definitely related to the investigational drug or any research related procedure, the event must be reported to the DSMC Chair or Vice Chair within 1 business day.

The reporting procedure is by communication via phone or in person with written documentation of the one-on-one communication via e-mail, with a copy of the e-mail to the DSMC Manager.

3.3 Review of Adverse Event Rates

If an increase in the frequency of Grade 3 or 4 adverse events (above the rate reported in the Investigator Brochure or package insert) is noted in the study, the Principal Investigator will notify the DSMC at the time the increased rate is identified. The report will indicate if the incidence of adverse events observed in the study is above the range stated in the Investigator Brochure or package insert.

If at any time the Investigator stops enrollment or stops the study due to safety issues, the DSMC Chair and DSMC Manager must be notified within 1 business day via e-mail. and the IRB must be notified within 10 business days via an iRIS Reporting Form.

Data and Safety Monitoring Committee Contacts:

[redacted]
Box 1705
UCSF HDFCCC
San Francisco, CA 94158

DSMC Monitors
Box 0128
UCSF HDFCCC
San Francisco, CA 94143

Appendix 3 Clinical Monitoring for Autoimmunity

(to be completed at each MD visit)

Patient ID_____

UCSF MRN _____

Skin

Symptoms _____

Signs (include pruritis, vitiligo)_____

_____ no rash

_____ rash; description_____

_____ % of body surface

Hepatic

Symptoms _____

Signs _____

Labs: _____ AST/SGOT _____ ALT/SGPT _____ Bilirubin (total) _____ ALP

Pulmonary

Symptoms _____

Signs _____

Gastrointestinal

Symptoms _____

Signs_____

_____ no diarrhea

_____ increase of < 4 stools/day

_____ increase of 4-6 stools/day

_____ increase of ≥ 7 stools/day, or need for parenteral support

_____ diarrhea requiring ICU admission or hemodynamic compromise

_____ nausea / vomiting

Labs

_____ Stool analysis (include. WBC count) for patients with GI symptoms

Rheumatologic

Symptoms_____

Signs (joint pain swelling; #/location of joints involved) _____

Labs

____ ANA (titer, pattern)

____ RF

Other _____

Pancreas

Symptoms _____

Signs _____

Labs: _____ amylase _____ lipase _____glucose

Renal

Symptoms _____

Signs _____

Lab: _____ Cr

Endocrine

Symptoms _____

Signs _____

Labs: ____ TSH; ____T4; ____T3; _____FTI ___ANC

Pituitary_____

Hematologic

Symptoms _____

Signs _____

Labs: ___ WBC; ___ Hgb; ___ Plt;

Neurologic

Symptoms _____

Signs_____

Cardiac

Symptoms _____

Signs _____

Appendix 4 American Urological Association (AUA) symptom

International Prostate Symptom Score (IPSS)

Questions to be answered	Not at all	Less than 1 time in 5	Less than half the time	About half the time	More than half the time	Almost always	Your score
1. Over the past month, how often have you had a sensation of not emptying your bladder completely after you finished urinating?	0	1	2	3	4	5	
2. Over the past month, how often have you had to urinate again less than 2 hours after you finished urinating?	0	1	2	3	4	5	
3. Over the past month, how often have you found you stopped and started again several times when you urinated?	0	1	2	3	4	5	
4. Over the past month, how often have you found it difficult to postpone urination?	0	1	2	3	4	5	
5. Over the past month, how often have you had a weak urinary stream?	0	1	2	3	4	5	
6. Over the past month, how often have you had to push or strain to begin urination?	0	1	2	3	4	5	
7. Over the past month, how many times did you most typically get up to urinate from the time you went to bed at night until the time you got up in the morning?	0 (none)	1 (1 time)	2 (2 times)	3 (3 times)	4 (4 times)	5 (5 or more times)	
						Sum of numbers (AUA symptom score):	

Total score:

0 to 7: Mild symptoms

8 to 19: Moderate symptoms

20 to 35: Severe symptoms

Quality of life due to urinary symptoms	Delighted	Pleased	Mostly satisfied	Mixed - about equally satisfied and unsatisfied	Mostly dissatisfied	Unhappy	Terrible
If you were to spend the rest of your life with your urinary condition the way it is now, how would you feel about that?	0	1	2	3	4	5	6

Modified with permission from: Barry, MJ, Fowler, FJ Jr, O'Leary, MP, et al. The American Urological Association Symptom Index for Benign Prostatic Hyperplasia. J Urol 1992; 148:1549. Copyright © 1992 Lippincott Williams & Wilkins.

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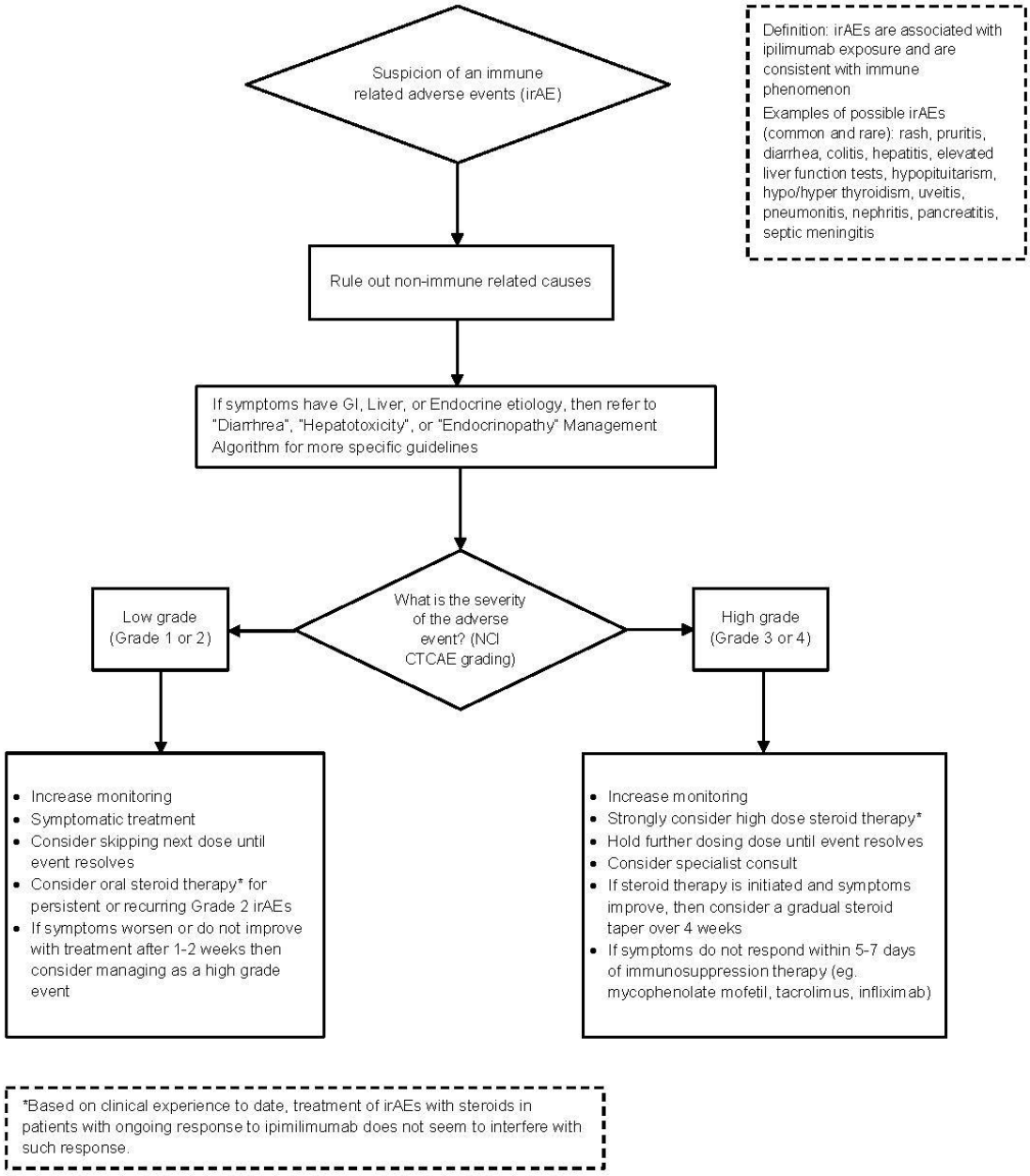
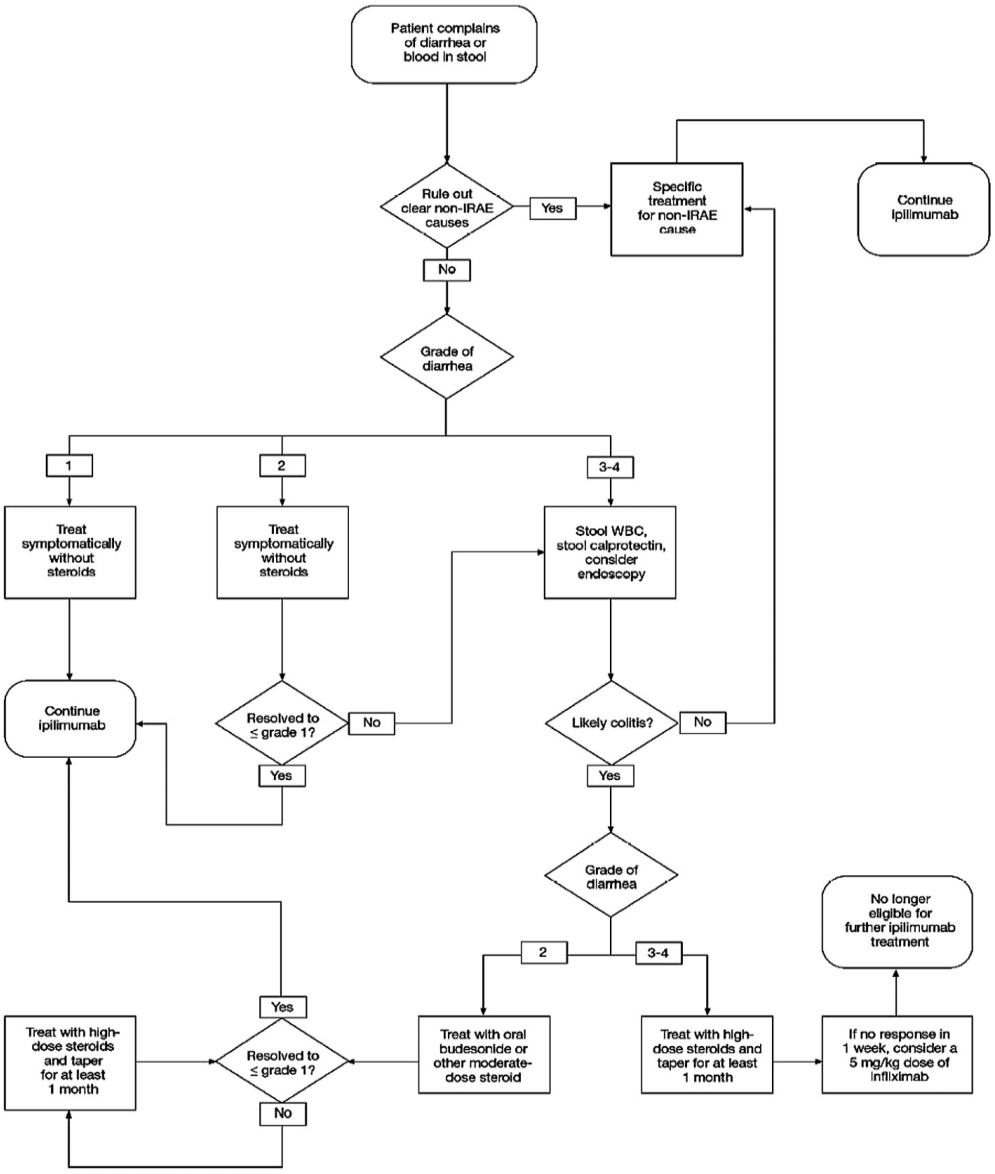
Appendix 5 General recommendations for managements of suspected immune related adverse events



Appendix 6 Diarrhea Management Algorithm



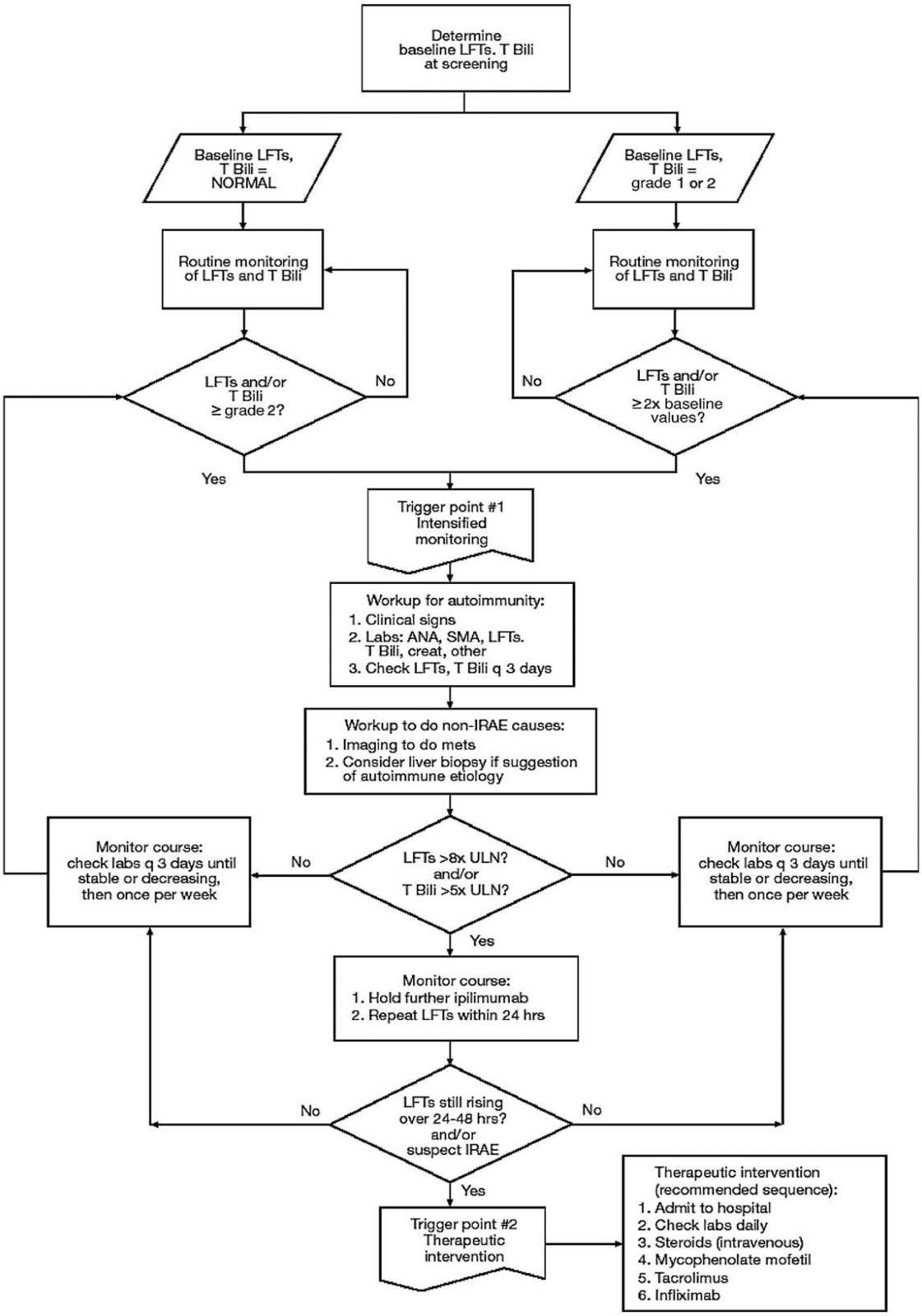
Diarrhea	GRADE 1	GRADE 2	GRADE 3	GRADE 4	GRADE 5
	Increase of < 4 stools per day over baseline; mild increase in ostomy output compared with baseline	Increase of 4-6 stools per day over baseline; IV fluids indicated < 24 hrs; moderate increase in ostomy output compared to baseline; not interfering with ADL	Increase of ≥ 7 stools per day over baseline; incontinence; IV fluids indicated < 24 hrs; hospitalization; severe increase in ostomy output compared to baseline; interfereing with ADL	Life-threatenting consequences (e.g. hemodynamic collapse)	Death

Appendix 7 Hepatotoxicity Management Algorithm

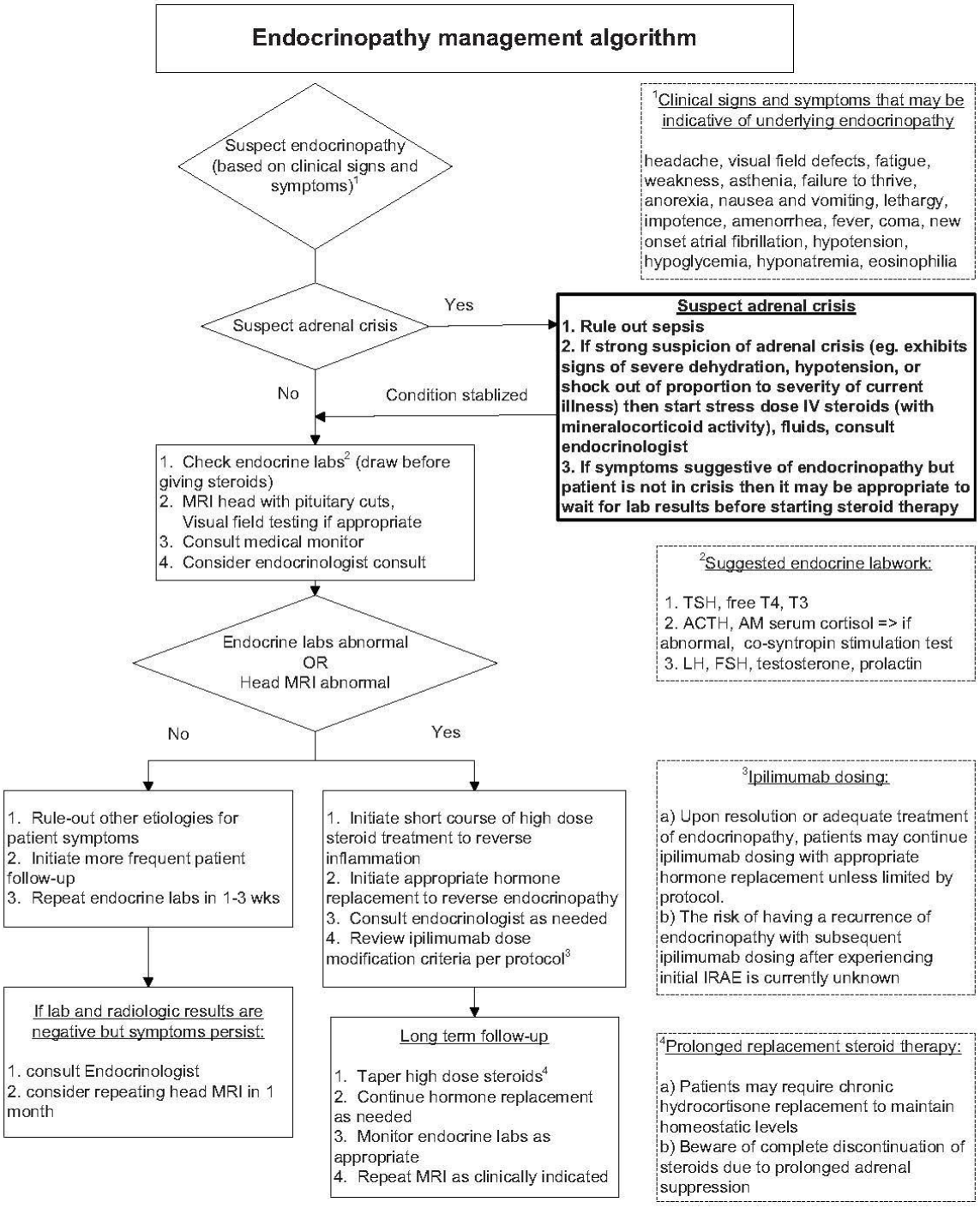
Situation: rising liver function tests (LFTs) > 8X ULN or suspected immune-mediated hepatitis

1) Admit subject to hospital for evaluation and close monitoring
2) Stop further Ipilimumab dosing until hepatotoxicity is resolved. Consider permanent discontinuation of Ipilimumab per protocol (Section 6.2.5 of protocol)
3) Start at least 120 mg methylprednisolone sodium succinate per day, given IV as a single or divided dose
4) Check liver laboratory test values (LFTs, T-bilirubin) daily until stable or showing signs of improvement for at least 3 consecutive days
5) If no decrease in LFTs after 3 days or rebound hepatitis occurs despite treatment with corticosteroids, then add mycophenolate mofetil 1g BID per institutional guidelines for immunosuppression of liver transplants (supportive treatment as required, including prophylaxis for opportunistic infections per institutional guidelines)
6) If no improvement after 5 to 7 days, consider adding 0.10 to 0.15 mg/kg/day of tacrolimus (trough level 5-20 ng/mL)
7) If target trough level is achieved with tacrolimus but no improvement is observed after 5 to 7 days, consider infliximab, 5 mg/kg, once
8) Continue to check LFTs daily for at least 2 weeks to monitor sustained response to treatment

A flow chart of the algorithm is depicted in the following page.


Determine baseline LFTs. T Bili at screening
Baseline LFTs, T Bili = NORMAL
Baseline LFTs, T Bili = grade 1 or 2
Routine monitoring of LFTs and T Bili
Routine monitoring of LFTs and T Bili
LFTs and/or T Bili ≥ grade 2?
No
No
LFTs and/or T Bili ≥2x baseline values?
Yes
Yes
Trigger point #1 Intensified monitoring
Workup for autoimmunity:
1. Clinical signs
2. Labs: ANA, SMA, LFTs. T Bili, creat, other
3. Check LFTs, T Bili q 3 days
Workup to do non-IRAE causes:
1. Imaging to do mets
2. Consider liver biopsy if suggestion of autoimmune etiology
Monitor course: check labs q 3 days until stable or decreasing, then once per week
No
LFTs >8x ULN? and/or T Bili >5x ULN?
No
Monitor course: check labs q 3 days until stable or decreasing, then once per week
Yes
Monitor course:
1. Hold further ipilimumab
2. Repeat LFTs within 24 hrs
No
LFTs still rising over 24-48 hrs? and/or suspect IRAE
No
Yes
Trigger point #2 Therapeutic intervention
Therapeutic intervention (recommended sequence):
1. Admit to hospital
2. Check labs daily
3. Steroids (intravenous)
4. Mycophenolate mofetil
5. Tacrolimus
6. Infliximab

Appendix 8 Endocrinopathy Management Algorithm



Footnote

For numbered footnotes (1,2,3,4), please refer to further explanation and text found in the corresponding dotted line boxes to the right side of the algorithm

Appendix 9 Prohibited Medications

All prostate cancer-directed therapies are prohibited, including, but not limited to:

Leuprolide acetate	Lupron, Eligard
Goserelin	Zoladex
Degarelix	Firmagon
Bicalutamide	Casodex
Flutamide	Eulexin
Nilutamide	Nilandron
Ketoconazole (systemic)	Nizoral
Docetaxel	Taxotere
Cabazitaxel	Jevtana
Abiraterone acetate	Zytiga
Enzalutamide	Xtandi
Sipuleucel-T	Provenge
Radium-223	Xofigo
Diethylstilbestrol	PC-SPES (or any other PC-X product)

Any investigation therapy for the treatment of prostate cancer is prohibited.

Chronic use of prednisone of any corticosteroids (>10mg prednisone daily or its equivalent) is prohibited; the chronic use of any chronic immunosuppressant therapy is prohibited.