Bavarian Nordic, Inc.
Study Protocol

Protocol Title	A Randomized, Double-blind, Phase 3 Efficacy Trial of PROSTVAC-V/F ± GM-CSF in Men With Asymptomatic or Minimally Symptomatic Metastatic, Castrate-Resistant Prostate Cancer
Protocol Number	BNIT-PRV-301
IND No. / OBA Protocol Number	BB-IND 13946 / #1104-1101
NCT Number:	NCT01322490
EudraCT No.	2010-021196-85
Investigational Product	PROSTVAC-V/F
Sponsor	Bavarian Nordic, Inc. [redacted]
CRO	[redacted]
Medical Monitor	[redacted]
Drug Safety	[redacted]

[redacted]

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LIST OF ABBREVIATIONS AND DEFINITION OF TERMS

AE(s)	Adverse Event(s)
ALT (SGPT)	Alanine aminotransferase
APC	Antigen presenting cell
AST (SGOT)	Aspartate aminotransferase
AWE	Alive Without Event
BN	Bavarian Nordic A/S (Kvistgaard, Denmark)
BN-B	Bavarian Nordic, Berlin
BNI	Bavarian Nordic, Inc, formerly known as BNIT
BNIT	BN ImmunoTherapeutics, Inc.
BP	Blood pressure
BPI-SF	Brief Pain Inventory – Short Form
BSL	Biological Safety Level
BUN	Blood urea nitrogen
CFR	Code of Federal Regulations
CRF	Case report form
CT	Computerized tomography
CTC	Circulating tumor cell
CTCAE	Common Toxicity Criteria for Adverse Events
CTL	Cytotoxic T-lymphocyte
DLT	Dose-limiting toxicity
DMC	Data Monitoring Committee
ECOG	Eastern Cooperative Oncology Group
ECG	Electrocardiogram
EM	Erythema multiforme
ePIP	electronic Protocol Inquiry Platform
EV	Eczema vaccinatum
FACT-P	Functional Assessment of Cancer Therapy - Prostate
FAS	Full analysis set
FDA	Food and Drug Administration
GCP	Good Clinical Practice
GM-CSF	Granulocyte macrophage-colony stimulating factor
HIV	Human immunodeficiency virus
HLA	Human leukocyte antigen
HR	Hazard ratio
IAP	Interim Analysis Plan

IBC	Institutional Biosafety Committee
ICAM-1	Intercellular adhesion molecule -1
ICH	International Conference on Harmonization
IDT	IDT Biologika, GmbH
IND	Investigational new drug
Inf.U	Infectious units
IRB/EC	Institutional Review Board/ Ethics Committee
IU	International unit
IVRS	Interactive voice response system
IWRS	Interactive web response system
LDH	Lactate dehydrogenase
LFA-3	Leukocyte function associated antigen-3
LLN	Lower limit of normal
LTFU	Long-Term Follow-Up
mCRPC	metastatic Castration-Resistant Prostate Cancer
MHC	Major histocompatibility complex
MRI	Magnetic Resonance Imaging
NCI	National Cancer Institute
NCI-CTCAE	National Cancer Institute-Common Toxicity Criteria for Adverse Events
NYHA	New York Heart Association
PAP	Prostatic acid phosphatase
PBMCs	Peripheral blood mononuclear cells
PCWG2	Prostate Cancer Clinical Trials Working Group 2
PEI	Paul-Ehrlich-Institut
PFS	Progression-free survival
PI	Principal Investigator
[redacted]PVG	[redacted]Pharmacovigilance Group
PROSTVAC	PROSTVAC™, PROSTVAC-V/F, PROSTVAC™-V/F + TRICOM™
PS	Performance status
PSA	Prostate-specific antigen
PV	Progressive vaccinia
PVE	Post-vaccinial encephalitis
QoL	Quality of Life
RAC	Recombinant DNA Advisory Committee
RR	Respiration rate
SADR	Serious Adverse Drug Reaction
SAE	Serious adverse event

sc	Subcutaneous
SUSAR	Suspected Unexpected Serious Adverse Reaction
TAA	Tumor-associated antigen
TRICOM	TRICOM™, Triad of costimulatory molecules
TTP	Time to tumor progression
ULN	Upper limit of normal
VIG	Vaccinia immune globulin
VS	Vital signs

Protocol Synopsis

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NCT Number:	NCT01322490
EudraCT No.	2010-021196-85
Investigational Product	PROSTVAC-V/F
Sponsor	Bavarian Nordic, Inc. [redacted]
Indication	For the treatment of subjects with asymptomatic or minimally symptomatic, metastatic castration-resistant prostate cancer
CRO	[redacted]
Planned Sample Size	1,200 subjects (approximately)
Planned Number of Centers	250 – 300 centers worldwide
Study Type	Pivotal
Study Phase	Phase 3
Medical Monitor	[redacted]
Drug Safety	[redacted]

[redacted]

PROTOCOL SYNOPSIS

Background and Rationale

PROSTVAC™ (PROSTVAC-V and PROSTVAC-F; PROSTVAC-V/F) is a novel candidate prostate cancer immunotherapy for the treatment of prostate cancer. It is a viral vector based product that is administered in seven subcutaneous (sc) vaccinations, over a five month period. In a randomized controlled Phase 2 trial, PROSTVAC therapy was associated with a prolongation of survival in men with metastatic castration-resistant prostate cancer (mCRPC).

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 prostate-specific antigen (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. Both viruses contain four human genes: prostate-specific antigen (PSA) and three genes encoding human immunological costimulatory molecules: B7.1, intercellular adhesion molecule-1 (ICAM-1), and leukocyte function-associated antigen-3 (LFA-3) (designated TRIad of COstimulatory Molecules or TRICOM). The simultaneous expression of PSA and TRICOM enhances the immune response. The PSA transgene is modified at one amino acid (I155L), to enhance binding and immunogenicity of a particular peptide epitope in human leukocyte antigen (HLA-A2)-expressing patients.

PROSTVAC-V/F is the outcome of more than 15 years of poxviral vector development and evaluation by the National Cancer Institute (NCI), the former Therion Biologics Corporation, and Bavarian Nordic, Inc. Over 570 patients have been treated with related PSA-containing poxviral vectors, and over 300 patients have been treated with the current PROSTVAC-V/F product. A large database exists from safety evaluations in animals and humans for PROSTVAC-V/F. (see Investigator's Brochure)

Study Design

BNIT-PRV-301 is a randomized, placebo-controlled, multi-center, multi-country, Phase 3 efficacy trial of PROSTVAC-V/F in men with asymptomatic or minimally symptomatic, metastatic, castration-resistant prostate cancer. It is a 3-arm study and will evaluate overall survival in two separate comparisons, PROSTVAC-V/F plus GM-CSF versus control, and PROSTVAC-V/F without GM-CSF versus control.

Subjects will be randomized with equal probability into one of three double-blind arms. The intended interventions for randomized subjects are:

(Arm V+G) PROSTVAC-V/F plus GM-CSF

(Arm V) PROSTVAC-V/F plus GM-CSF placebo

(Arm P) Double placebo (empty fowlpox vector / plus GM-CSF placebo)

The trial interventions will consist of a single subcutaneous (sc) immunization of PROSTVAC-V or placebo in Week 1, followed by 6 PROSTVAC-F or placebo sc immunizations administered in Weeks 3, 5, 9, 13, 17, and 21.

Each immunization will be accompanied by administration of low dose GM-CSF or placebo on the day of immunization and for the subsequent 3 days (sc injection at same site, within 5 mm).

Study Objectives

Primary Efficacy Objectives:

To ascertain whether the overall survival of subjects randomized to Arm V+G (PROSTVAC-V/F plus GM-CSF) or to Arm V (PROSTVAC-V/F) is superior to that from subjects randomized to Arm P (placebo control).

Secondary Efficacy Objective:

To ascertain whether a greater proportion of subjects randomized to Arm V+G or Arm V remain event-free (radiological progression, pain progression, chemotherapy initiation, or death) at 6 months (or early termination) as compared to the subjects randomized to Arm P.

Safety Objectives:

To further characterize the safety and tolerability of PROSTVAC immunotherapy.

Exploratory Objectives:

To model radiological disease status over the first year of study participation.

To ascertain whether a greater proportion of subjects randomized to either Arm V+G or Arm V remain event-free (radiological progression) 6 months post End-of-Treatment visit (using scans at End-of-Treatment visit as a new baseline) as compared to the subjects randomized to Arm P. This analysis will include all subjects, and the HLA-A2-expressing subgroup.

To assess the role of post-treatment anti-cancer therapies as an alternative explanation for observed survival differences.

To ascertain whether the overall survival of HLA-A2-expressing subjects randomized to Arm V+G, or Arm V is superior to that from HLA-A2-expressing-subjects randomized to Arm P.

To compare arms with respect to immune response to immunizing antigen (PSA), as well as to non-vaccine-contained prostate antigens, and tumor-associated antigens, and assess whether immune responses are prognostic and/or predictive.

	To evaluate for baseline biomarkers which are prognostic and/or predictive of long-term survival outcome. To compare arms with respect to vaccine effects on circulating tumor cell (CTC) levels (US sites).
Study Type	Pivotal
Methodology	• Subcutaneous immunizations o PROSTVAC-V or placebo administered in Week 1 o PROSTVAC-F or placebo administered in Weeks 3, 5, 9, 13, 17, and 21.
Trial Population	Subjects with asymptomatic or minimally symptomatic, metastatic prostate cancer who have progressed despite surgical castration or androgen suppression therapy. Subjects will have evidence of radiological disease progression, or PSA disease progression as described in Prostate Cancer Clinical Trials Working Group 2 (PCWG2) criteria, after being treated with androgen suppression therapy or complete androgen blockade and withdrawal.
Sample Size	It is projected that approximately 1,200 subjects (400 subjects per arm), will be required for evaluation of the primary objective (overall survival). Analysis will require 534 events per each overall efficacy comparison. Arm V+G subjects versus all Arm P subjects, and separately all Arm V subjects versus all Arm P subjects.
Duration of Study	This trial entails the following three phases: Screening, Treatment, and Long-Term Follow-Up. <u>Screening Phase: Week -4 – 0</u> Screening procedures have to be completed no more than 4 weeks prior to first planned dose. <u>Treatment Phase: Weeks 1 – 25</u> Treatment (5 mos), including vaccination at Weeks 1, 3, 5, 9, 13, 17, and 21 followed by End-of-Treatment (Week 25)/Early Termination visit. Due to the delayed effect of vaccine induced immunotherapy, PSA and radiological progression may be expected during the treatment phase of the trial, and is not a cause for stopping vaccination. Subjects who progress clinically (pain) during treatment, or who progress rapidly as measured by PSA-DT <1 month will be counseled with respect to alternative treatment options. If subjects choose to initiate alternative therapy, vaccination will be discontinued. Subjects who stop treatment will continue in the Long-Term Follow-Up phase. <u>Long-Term Follow-Up Phase (LTFU):</u> After completing the End-of-Treatment/Early Termination visit, all subjects will automatically enter the Long-Term Follow-Up phase that extends until 12 months after the required number of 534 events for each between-arm comparison is realized. Subject health status, disease status, and subsequent prostate cancer therapies will be collected at each 6 month visit.
Summary of Eligibility Criteria	. Men ≥ 18 years of age with documented asymptomatic or minimally symptomatic metastatic castration-resistant prostate cancer . Castrate level of testosterone: <50 ng/dl

- PSA (or radiological) progression during androgen-suppression or blockade therapy and withdrawal
- ECOG PS of 0, or 1
- Life expectancy ≥1 year
- Chemotherapy naïve
- Subjects requiring scheduled opioid narcotics for cancer-related pain are excluded
- Subjects with rapidly progressing disease (estimated PSA-DT <1 month), visceral metastases, or with elevated LDH or elevated alkaline phosphatase (≥2xULN) are excluded
- Adequate bone marrow, hepatic, and renal function
- No significant cardiovascular disease
- No contraindication for vaccinia immunization

Concomitant Therapy

- Subjects will be on concomitant androgen suppression therapy with a GnRH agonist or antagonist
- Subjects may be on concomitant drugs to prevent bone loss or skeletal-related events, including bisphosphonates and denosumab
- Subjects may receive palliative radiotherapy during the trial

Excluded Therapy

Chemotherapy: Concomitant use of chemotherapy is not allowed during the Treatment phase (approximately 5 months) of this trial.

Concurrent immunotherapy or immunosuppressive therapy

Anti-cancer radionuclides: Concomitant use of anti-cancer radionuclides is not allowed during the Treatment phase of this trial.

Secondary hormonal therapies: Concomitant use of supplementary hormonal treatments is not allowed during the Treatment phase of this trial.

Corticosteroids: Concomitant chronic use of systemic corticosteroids is not allowed during the Treatment phase of the study. The use of inhaled corticosteroids, nasal sprays, and topical creams for small body areas is allowed.

Dose and Administration

PROSTVAC-V/F is provided at the following doses based on the doses of PROSTVAC-V/F used in the randomized controlled Phase 2 trial of 122 men, TBC-PRO-002.

- 2 x 10^8 Infectious Units (Inf.U)/0.5 ml for vaccinia (PROSTVAC-V).
- 1x 10^9 Inf.U/0.5 ml for fowlpox (PROSTVAC-F).
- 1x 10^9 Inf.U/0.5 ml for placebo (empty fowlpox vector).

PROSTVAC-V/F is administered by sc injection with the following schedule:

- 1 PROSTVAC-V (or placebo) priming vaccination (Week 1)

- 6 PROSTVAC-F (or placebo) booster vaccinations (Weeks 3, 5, and then 9, 13, 17, and 21)

GM-CSF is provided at 100 µg per sc injection (on Days 1 – 4) for each vaccination. GM-CSF placebo is bacteriostatic saline for injection and is delivered on the same schedule.

Statistical Methods

Statistical success with respect to the primary objective is defined as success for either of the following comparisons:

- Superiority of Arm V+G over Arm P for all subjects randomized to these arms.
- Superiority of Arm V over Arm P for all subjects randomized to these arms.

The significance levels to be used for the analyses associated with these two comparisons will protect the overall study type I error probability to be one-sided 0.025 or less. The possibility of delayed vaccine effect is taken into account in computing the required number of deaths, (as modeled from the randomized Phase 2 trial). The projected trial size is 400 subjects per arm (approximately 1,200 subjects) with at least 85% power, and the final analyses will be event-driven and take place when 534 deaths have been realized for the comparisons involving all subjects randomized (Arm V+G versus Arm P, and separately Arm V versus Arm P).

1.0 BACKGROUND AND RATIONALE

1.1 Rationale

Bavarian Nordic, Inc. (BNI) previously known as BN ImmunoTherapeutics, Inc. (BNIT) is developing PROSTVAC-V/F for the treatment of prostate cancer (Madan 2009). The PROSTVAC vaccine product is comprised of two biologic agents, PROSTVAC-V and PROSTVAC-F. These are two different poxvirus vectors, recombinant vaccinia and fowlpox viruses, respectively, encoding the genes for prostate-specific antigen (PSA) with the L155 mutation in combination with a triad of human costimulatory molecules, B7.1, ICAM-1, and LFA-3 (TRICOM™; TRICOM). They are used to introduce tumor-associated antigens (TAAs) and costimulatory molecules into patients with metastatic prostate cancer. The vaccinia and fowlpox viruses encoding PSA and TRICOM are used in a prime-boost vaccination regimen to optimize immune responses against prostate cancer tumor cells.

The proposed treatment is a PSA -based immunization strategy. PROSTVAC-V/F is a viral vector-based product that is administered over seven subcutaneous vaccinations, over a five month period. 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 generate immune responses directed against prostate cancer cells. A double-blind randomized Phase 2 trial found longer survival for men randomized to receive PROSTVAC-V/F, where an overall median survival of 16.6 months was noted for control vector-treated subjects versus 25.1 months for PROSTVAC-V/F-treated subjects, hazard ratio 0.56 (95%CI 0.38-0.88), and p=0.006.

PROSTVAC-V/F is an outcome of more than 15 years of poxviral vector development and evaluation by the NCI, the former Therion Biologics Corporation (Therion), and BNI. Over 570 patients have been treated with related PSA-containing poxviral vectors, and over 300 patients have been treated with the current PROSTVAC-V/F regimen. A large database (see Investigator's Brochure) exists from safety evaluations in animals and in humans for PROSTVAC-V/F. BNIT-PRV-301 will evaluate the efficacy of PROSTVAC-V/F in men with asymptomatic or minimally symptomatic, metastatic castration-resistant prostate cancer (mCRPC).

1.2 Background

1.2.1 PROSTVAC-V/F Development Program

Bavarian Nordic A/S (BN), [redacted], is the parent company of BNI, and is now the sole manufacturer of PROSTVAC-V/F. BN has manufactured poxviral-based products since 1995, ranging from bench-scale through commercial, and has the experience and technical expertise to culture the vaccine vectors, and to produce final frozen liquid formulations. The manufacturing technology and methodology originally developed at Therion have been transferred and further developed [redacted]. Phase 3 clinical trial material will be manufactured [redacted].

The plan for the continued clinical development of PROSTVAC-V/F in men with mCRPC is informed in particular by the Phase 2 study conducted under former BB-IND 10428. This study in metastatic disease demonstrated that (i) recombinant vaccinia and fowlpox viruses are well tolerated vectors for administration to subjects with prostate cancer, and (ii) prolonged survival is achieved in subjects treated with PROSTVAC-V/F versus subjects administered the control.

The planned Phase 3 clinical program to be conducted by BNI will include one well-powered, randomized, placebo-controlled, double-blind, multi-center, multi-country trial designed to evaluate PROSTVAC-V/F with and without GM-CSF for the treatment of mCRPC. This trial will be conducted globally.

1.2.2 Prostate Cancer

Prostate cancer is the second leading cause of cancer deaths in men in the United States. According to the American Cancer Society, approximately 200,000 new diagnoses of, and 28,000 deaths from prostate cancer occur each year in the United States (Jemal 2009). Up to one-third of patients with localized disease will fail local therapy within 10 years (as evidenced by a rising PSA) (Pound 2001, Freedland 2007). Patients with recurrent disease generally undergo hormone suppression therapy, with medical or surgical castration. Unfortunately, in most cases, this is eventually followed (after good response for several years) by progressive disease. Castration-resistant prostate cancer (CRPC) is characterized by disease that is uncontrolled despite low levels of testosterone and/or the presence of androgen receptor antagonist treatment (Rini 2002).

Upon the development of metastasis, overall survival is approximately 1–3 years. Taxane-based chemotherapy (docetaxel) and Provenge™ (Provenge, sipuleucel-T) are the only approved agents for the first-line treatment of patients with metastatic disease that have shown a prolonged survival benefit. Prostate cancer has been shown to be generally poorly responsive to chemotherapy. Taxotere chemotherapy has a beneficial effect on survival of approximately 3 months (Tannock 2004, Petrylak 2004, Berthold 2008). Cabazitaxel-based chemotherapy also offers an apparent 2-3 month improved survival in a second-line setting (after docetaxel chemotherapy) (DeBono 2010). However, as with many other cytolytic agents, the impact on the patient's quality of life is significant. More recently, abiraterone (an androgen hormone synthesis inhibitor) has also been shown to prolong median survival by approximately 4 months, and has been approved for use in second-line setting (De Bono 2011). Provenge has shown a beneficial survival benefit of approximately 4 months (Kantoff 2010, NEJM). Provenge, while having a comparatively favorable safety profile with a slightly better survival benefit, requires extensive processing during the treatment regimen and, therefore, has practical limitations. Consequently, therapies that could significantly improve the therapeutic benefit (survival and safety) as well as practical use of that observed with chemotherapy and Provenge, respectively, are urgently needed.

1.2.3 Prostate Cancer Immunotherapy

Therapeutic cancer vaccines have been investigated in both castration-sensitive and castration-resistant disease (Schlom 2007). Prostate cancer is an ideal candidate for treatments that stimulate the immune system to target cancer cells. Prostate cancer may progress slowly (even in advanced disease), allowing time for the immune system to be stimulated, and to mount an active immune response. It is a poorly chemosensitive disease, so patients are generally not heavily pretreated with cytotoxic agents, and their immune systems are more intact. Prostate cells have a unique and specific marker, prostate-specific antigen (PSA), which is secreted into the blood, and can be detected with even low levels of tumor burden. Several other gene products are unique to prostate cells, and prostate cancer cells over-express tumor-associated antigens (TAA), making for multiple targets suitable for immunotherapy responses (Cunha 2006, Bradford 2006). Many patients with prostate cancer have low levels of cytolytic T-cells capable of recognizing PSA, and this minimal response can be enhanced with immune stimulation by therapeutic cancer vaccines (Chakraborty 2003). Finally, because the prostate is a nonessential organ, targeting PSA- or TAA-specific immune cells to prostatic tissue is unlikely to have a significant negative clinical impact.

The first FDA approved prostate cancer immunotherapy, Provenge (sipuleucel-T), is now available (approved 29 April 2010). However Provenge is available only in the United States. Provenge is a cell-based vaccine consisting of autologous PBMCs that have been activated *ex-vivo* with a recombinant protein representing a fusion of PAP (prostatic acid phosphatase) and GM-CSF. Early clinical work demonstrated prolongation of overall survival (Small 2006), and up-regulations of cellular immunity and reductions in PSA and PAP levels (Patel 2008). The Phase 3 IMPACT study of 512 subjects with metastatic castration-resistant prostate cancer met its primary endpoint of prolongation of overall survival. Provenge was approved for use in metastatic castration-resistant prostate cancer based on an increase in median survival of 4.1 to 4.5 months (Kantoff 2010).

1.2.4 PSA as a Target Antigen

PSA is a promising target antigen for immunotherapy of prostate cancer. PSA is a ~34,000 dalton glycoprotein that is produced in normal, benign, and cancerous prostate epithelia, but not in other normal tissues (Wang 1982, Lilja 2008). Because PSA is expressed essentially only in prostatic epithelial cells (normal and malignant), and the prostate gland is nonessential, this antigen is an enticing choice. The fact that PSA is secreted and not membrane-bound limits its use as a target for humoral immunity, but not its use as a target of specific cellular immune system attack. Cells, including tumor cells, present endogenously expressed proteins on their surface in the form of peptide-major histocompatibility complexes (MHC). Cytotoxic T-lymphocytes (CTLs) recognize and are activated by specific peptides in the context of the appropriate MHC class I molecule on antigen-presenting cells (APC). This activation can in turn lead to killing of tumor targets by the peptide-specific CTLs.

The use of PSA as a target to elicit tumor-specific T-cell-mediated lysis has been validated *in vitro*. Correale *et al*. demonstrated *in vitro* killing of a PSA-peptide-pulsed HLA-A2+ human cell line by a PSA-

specific human CTL cell line, and lysis was blocked by an antibody directed against MHC class I molecules (Correale 1997). It has also been shown that PSA-specific CTLs could be generated that lyse PSA-expressing prostate cancer cells (Correale 1997, 1998). The identification of these peptide epitopes further provides a means of identifying cytolytic T-cells resulting from immunization as an *in vitro* monitoring tool.

1.2.5 PSA-3-epitope modification

Protein antigens are presented to CTLs as small peptides (approximately 9–10 amino acids long) bound to class I molecules of the MHC. One strategy to increase the immunogenicity of a self-antigen such as PSA is to modify selected epitopes within the protein sequence to enhance their binding to MHC class I alleles. The stabilized binding enables more sustained and potent immune cell activation. One such epitope, designated PSA-3 (amino acids 154–163), which is specific for the MHC class I A2 allele, was modified by the introduction of a single amino acid change (I to L) at position 2 in the epitope (designated L155) (Correale 1998, Terasawa 2002). A number of *in vitro* studies suggest that PSA carrying this modification will be more immunogenic in HLA-A2 individuals than the native (unmodified) PSA polypeptide (Correale 1998, Terasawa 2002).

1.2.6 Poxviral Vaccine Approach

Poxviruses are large multi-enveloped DNA viruses which encode about 200 genes. Poxviral vectors are able to accommodate multiple transgenes, and are able to deliver PSA and multiple immunostimulatory genes directly or indirectly to APCs. The activated APCs process and present the PSA antigen, leading to PSA specific T-cell activation (Essajee 2004, Schlom 2008). PSA as a soluble protein is weakly immunogenic, especially in the tumor-bearing host. Vaccine strategies must induce greater activation of T-cells than is being achieved endogenously in the host. The poxviral system achieves this by generating a natural inflammatory context for facilitating activation of immune cells, and inducing PSA-specific immune responses. Extensive pre-clinical studies in experimental murine models have demonstrated the therapeutic utility of using poxviral vaccines to break tolerance (Bernards 1987, Irvine 1993, Kass 1999). Another advantage of poxviral vaccines is that they are well tolerated by the patients. The relative safety of PSA-based poxviral vectors has been well established in previous trials (Madan 2009, Kantoff 2010, Kaufman 2004, Arlen 2007).

1.2.7 Vaccinia Virus

Vaccinia virus has been used for over 200 years as a vaccine for smallpox and has a well-established safety and adverse event (AE) profile (Casey 2005, Poland 2005). The virus actively replicates in human cells, and generates a strong inflammatory response, resulting in the presentation of high levels of antigen to the immune system over a period of one to two weeks, supporting a potent immune stimulation. Subsequently, the immune response specific to vaccinia then eliminates the virus. Host immune responses to vaccinia restrict its replication and thus limit its ability to be re-used for subsequent

vaccinations. Consequently, vaccinia-based vaccines, although potent immunological priming agents, can be used to immunize an individual only once or twice.

1.2.8 Fowlpox Virus

Fowlpox virus is a member of the genus *Avipox*, which is evolutionarily divergent from vaccinia virus and serologically non cross-reactive (Taylor 1988, Beukema 2006). Immune responses to vaccinia are essentially non cross-reactive with fowlpox, and do not block infection and immunization with fowlpox-based vectors. Hence vaccinia-primed immune responses can be boosted with fowlpox vectors. In addition, fowlpox vectors do not replicate in human cells (only in avian cells), and are therefore much less of a safety risk than vaccinia-based vectors. Fowlpox vectors mediate a limited infection in human cells, with early viral and transgene expression, but late gene expression is blocked, and no infectious particles are produced. Thus minimal viral surface antigen is made, and minimal neutralizing antibody immune responses are induced. This enables multiple boosting with the fowlpox-based vectors.

1.2.9 Prime-Boost

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 supported by the results from the completed Phase 1 and Phase 2 trials conducted by the National Cancer Institute (NCI), Eastern Cooperative Oncology Group (ECOG), and Therion.

1.2.10 TRICOM- costimulatory molecules

Destruction of immunological targets requires T-cell lymphocyte recognition, via the T-cell receptor, of antigenic peptides presented in the context of MHC molecules on APCs. In addition to this antigen-specific signal, a second, antigen-independent signal is required for T-cell activation (Boussiotis 1995, Hodge 2006). This second signal is provided by the interaction of specific ligands on the T-cell surface with “costimulatory” molecules expressed on APCs. The most extensively studied pathway of costimulation is that involving the interaction of the costimulatory molecule B7.1 expressed on APCs with CD28 and CTLA4 on the T-cell (Sharpe 2002, Riley 2005). A number of additional costimulatory molecules on APCs have been identified; these include ICAM-1 and LFA-3, whose ligands are LFA-1 and CD2, respectively, on the surface of T-cells (Wingren 1995, Lebedeva 2005, Wang 2008).

Proper engagement of the T-cell receptor and costimulatory receptor requires the expression of both antigen and costimulatory molecules, respectively, in the same cell. Therefore, co-expression of costimulatory molecules using a single recombinant vector presents the potential of cooperation among these proteins to enhance T-cell activation. Recombinant vectors co-expressing three costimulatory molecules, LFA-3, ICAM-1, and B7.1, designated TRICOM™ (TRICOM), have been shown to have synergistic effects on antitumor responses as compared to vectors expressing individual costimulatory molecules (Hodge 1999). Mice immunized with a recombinant vaccinia virus co-expressing

carcinoembryonic antigen (CEA) and murine TRICOM exhibited greater immune responses and anti-tumor responses than mice immunized with a recombinant vaccinia virus co-expressing CEA and murine B7.1. Enhanced anti-tumor immunity was also observed in mice that were transgenic (tolerant) for CEA (Hodge 1999). PROSTVAC-V and PROSTVAC-F have, therefore, been designed to simultaneously express PSA together with B7.1, LFA-3, and ICAM-1.

1.2.11 GM-CSF

Granulocyte macrophage colony-stimulating factor (GM-CSF) has been shown to enhance antigen processing and presentation by dendritic cells (DC). GM-CSF affects many immunologic processes, including increasing expression of class II MHC molecules, augmenting the primary antibody response, and inducing localized inflammation when administered by injection (Warren 2000, Chang 2004). GM-CSF has the ability to promote local DC differentiation, maturation, and subsequent migration to regional lymph nodes. Antigens released by target cells, such as those from tumors, are likely to be taken up and processed by DCs and presented to T-cells, thereby inducing the activation of a cellular immune response. These immunologic properties of GM-CSF have made this cytokine a potentially attractive adjuvant candidate for vaccines. Indeed, use of both recombinant human (rhu) GM-CSF and the recombinant poxviruses expressing GM-CSF has been shown to enhance immunological effects of vaccines in both non-clinical (Kass 2001) and clinical studies (Marshall 2000). GM-CSF was administered as an adjuvant to the PROSTVAC-V/F vaccine in the randomized, placebo controlled Phase 2 trial, and the positive outcome of this trial supports its use in this Phase 3 trial.

More recent data have however, challenged the benefits of GM-CSF. A recent review of approximately 14 different human clinical cancer vaccine studies that employed GM-CSF as an adjuvant (Parmiani, 2007) concluded that the studies reviewed provided limited evidence to justify the use of GM-CSF as an adjuvant and might be even detrimental under certain conditions. There is also growing evidence that systemic use of GM-CSF can compound tumor-mediated immune suppression by acting as a growth factor for myeloid-derived suppressor cells (MDSC) (Talmadge, 2007). Lastly, in a small Phase 2 trial of PROSTVAC-V/F conducted by the NCI, GM-CSF use was not associated with any dramatic clinical benefit. However, only eight subjects per arm were compared (GM-CSF, no GM-CSF, FPV-GM-CSF, FPV-GM-CSF high dose), making firm conclusions difficult. Together these data support going forward with a third arm of the trial comparing PROSTVAC-V/F without GM-CSF to control.

1.3 Placebo Rationale

BNIT-PRV-301 will utilize a placebo control in order to fulfill the double-blind design of the study. In a double-blind trial, neither the investigator nor the subject has knowledge of the treatment being administered. This study design is utilized in order to control variables that could be caused by knowledge of treatment assignment, either on the part of the investigator or the subject. In order to ensure a double-blind design, a study protocol must carefully account for all circumstances in which the Investigator or subject could obtain knowledge of treatment assignment. One of the most obvious requirements to maintain a blind is ensuring that study drug administration is perceived as identical across study arms.

This protocol design minimizes the risk of bias and enhances the impartial analysis of the study drug versus placebo effect. However, it is important to note that in case this information is required for safety purposes it will be provided in an expedited manner.

In minimally symptomatic metastatic prostate cancer there is no standard of care. Most patients and physicians utilize chemotherapy when the disease has become symptomatic, or has taken on accelerated kinetics. These patients are excluded from this trial (BNIT-PRV-301). The side effect profile of chemotherapy and its associated risks have favored later use of chemotherapy, where the potential for pain relief shifts the risk-benefit profile. Immunotherapy is a promising approach in this early stage of metastatic prostate cancer. Although promising, sipuleucel-T is not widely available, and has logistic and cost concerns.

In BNIT-PRV-301 the placebo will be an empty viral vector. Tissue culture adapted fowlpox virus, the same host strain as used in PROSTVAC-F will be used. This vector is non-replicating in humans and has minimal safety risk. In addition, the cutaneous injection site reactions (and side effect profiles) are overlapping (and generally indistinguishable) with subcutaneously administered vaccinia (PROSTVAC-V). Thus this vector forms an ideal placebo. It has no known effects on cancer progression, and is not expected to have any significant effects on its own. It is manufactured by the same process as PROSTVAC-V and PROSTVAC-F, and has the same look and feel of the other investigational vaccine vectors.

1.4 Summary of Findings from Human Clinical Studies

Clinical investigation of the PROSTVAC-V/F vaccine product was initiated in 2002 by Therion in the United States under BB-IND 10428. Therion manufactured all PROSTVAC-V/F product and conducted two clinical trials on PROSTVAC-V/F, including a Phase 1 trial evaluating the safety and immunogenicity of PROSTVAC-V/F in 10 subjects, and a randomized, placebo-controlled Phase 2 trial evaluating the safety and efficacy (as defined by time-to-progression; TTP) of PROSTVAC-V/F in approximately 125 men with castration-resistant metastatic prostate cancer. Both trials were conducted in the US only. Therion also manufactured the PROSTVAC-V/F product for all the NCI and ECOG trials.

1.4.1 Poxviral PSA Vaccine Clinical Trials

The use of poxviral vectors to stimulate an immune response to PSA has been evaluated in several clinical trials. In a Phase 1 clinical trial using an earlier version of a PSA-based vaccine (devoid of any costimulation), Eder *et al*. used three monthly vaccinations of recombinant vaccinia (rV)-PSA (Eder, 2000). Six out of 10 subjects with rising PSA following local therapy who received the highest tested dose, with GM-CSF, had a time of PSA progression of greater than six months. Four of these 6 subjects still showed no progression, with the longest time of follow-up greater than 24 months. This was suggested to be evidence of clinical activity (Eder 2000). Five of 7 HLA-A2+ subjects in this final dose level had at least a 2-fold increase in PSA-specific T-cells in peripheral blood mononuclear cells (PBMCs) as a result of vaccination, as measured by the ELISPOT assay, indicating that tolerance to self-antigens

could be circumvented with these vaccine strategies. Another Phase 1 study of rV-PSA also demonstrated that immune responses could be elicited in subjects with metastatic hormone-refractory prostate cancer and that immune cells taken from these subjects could specifically lyse PSA-expressing cancer cells *in vitro* (Gulley 2002).

ECOG previously conducted and reported a randomized Phase 2 study in which 64 subjects with rising PSA following definitive local therapy with no evidence of disease on imaging studies were randomized to receive four vaccines with rV-PSA (designated V) and/or rF-PSA (designated A for avipox) (Kaufman 2004). The arms were thus VAAA, AAAA and AAAV. PSA progression-free survival (PFS) was about 9 months in the latter arm, and about 18 months in the VAAA arm, lending further support to the use of vaccinia priming and avipox vector boosting.

Vaccinia and fowlpox vectors containing the gene for human PSA with a single amino acid substitution (designated PSA (L155)) along with genes encoding TRICOM have been tested in two fully accrued Phase 1 clinical trials with 25 subjects with metastatic prostate cancer (Arlen 2007, DiPaola 2006). There were no dose-limiting toxicities on these studies attributed to vaccine. One of these studies in subjects with mCRPC has shown that subjects treated with PSA/TRICOM who were evaluable for immune response had an increase in PSA-specific T-cells after treatment; and 9 of 15 subjects had decreases in PSA velocity (Arlen 2007).

The Therion-sponsored Phase 2 trial randomized subjects with mCRPC 2:1 in favor of vaccine vs. an empty fowlpox vector as control. 125 subjects with mCRPC and Gleason scores ≤ 7 were enrolled (122 treated). Subjects randomized to receive vaccine (82 subjects) were given subcutaneous rV-PSA-TRICOM prime with monthly boosts of rF-PSA-TRICOM, while control subjects (40 subjects) were given subcutaneous injections of fowlpox. Although no difference in TTP was seen in the short-term, in an interim analysis at two years of follow-up, median overall survival was 24.4 months in the vaccine arm compared to 16.6 months in the control arm, suggesting that although disease progression occurred at similar times in both groups, there appeared to be a long-term benefit for some subjects treated with PSA/TRICOM (Schlom 2008, Kantoff 2006).

The overall survival results after a final survey (median follow-up 4 years) were recently completed. The formal overall survival update revealed that PROSTVAC-V/F immunotherapy was associated with a significant survival benefit. PROSTVAC-V/F-treated subjects had a longer median survival (25.1 months versus 16.6 months controls), and an increased survival at 3 years post study (25 of 82 subjects, 30%; versus 7 of 40 control subjects, 17%). Statistical analysis of the Kaplan-Meier curves revealed an estimated hazard ratio of 0.56 (95% CI 0.37-0.85), and a stratified log rank of P=0.006 (Kantoff 2010).

In an NCI Phase 2 study (NCI no.5911) of PSA/TRICOM, 32 subjects with metastatic CRPC were treated with an rV-PSA-TRICOM prime and monthly boosts of rF-PSA-TRICOM. In that trial, 47% of subjects had a decrease in PSA velocity, 38% had a PSA decline. Median time to progression was 2.8 months. One subject at 8 months experienced a decrease in his hilar adenopathy associated with declining PSA (> 30%). He remained on study for 12 months. Another subject had a decrease of 29% in his adenopathy

(by RECIST) associated with a decline in his PSA (> 30%). Another subject who enrolled on study with a super-scan on bone scan had a >70% decline in his PSA and remains on trial more than 4 years after enrollment. Overall, 13 of 29 evaluable subjects had a > 2-fold increase in PSA-specific T-cells. In addition, 5 subjects had a > 6-fold increase in PSA-specific T-cells, which was associated with a trend to improved overall survival (p = 0.055) (Madan 2008, Gulley 2009).

1.4.2 Clinical Safety of Recombinant Poxvirus-Based Vaccines

Since 1991, 10 recombinant vaccinia-based vaccines and 8 recombinant fowlpox-based vaccines produced by Therion for the treatment of various cancers have been evaluated in human clinical trials sponsored by CTEP, DCTD, NCI. Over 1,000 cancer subjects, most with metastatic disease, have been treated to date with these poxvirus-based vaccines in 29 CTEP-sponsored or Therion-sponsored clinical trials (Kaufman 2004). These trials represent a large component of the relevant safety database that supports the initiation of the proposed Phase 3 trial of PROSTVAC-V/F. Significant safety experience in humans includes: (a) vaccinia- and/or fowlpox-based vaccines safely administered by a variety of routes including intradermal (by injection or scarification), subcutaneous (sc), intramuscular (im), intravenous (iv), and intra-tumoral at doses up to 2 x 10^9 Inf. U. (vaccinia) or 6 x 10^9 Inf. U. (fowlpox); (b) vaccinia-based and fowlpox-based vaccines containing costimulatory molecules, alone or in combination with CEA or PSA antigens, administered without serious adverse effects, as outlined below.

Poxviral vectors containing PSA, either alone or with B7.1 or TRICOM, have been evaluated in 13 different Phase 1 (5 trials) and 2 (8 trials) comprising over 400 vaccinated subjects with advanced prostate cancer. In addition, there are three other fully enrolled, and two open studies using PROSTVAC-V/F treatment, for an additional 100 treated subjects. There have only been two serious adverse events thought to be possibly due to the PROSTVAC-V/F treatment (both in the same subject): the subject had a myocardial infarction and it was found that he had Grade 4 thrombotic thrombocytopenic purpura, thought to be possibly related to study drug, approximately 3.5 weeks after receiving the fourth dose of his vaccine. This resolved with therapeutic apheresis treatment, however, the subject continued to do poorly (for a more detailed description of this case, see Investigator's Brochure). Thrombotic thrombocytopenic purpura has not been reported as an adverse event in large scale studies of smallpox vaccination with vaccinia.

1.5 Safety of Poxviral Vectors

1.5.1 Vaccinia Virus

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. Vaccinia virus is known to be shed from the wound site in traditional dermal scarification based vaccination.

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. An additional set of data is provided by recent vaccination campaign of military and civilian vaccinations in the US.

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 (Frey 2002, Fulginiti 2003). 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 (Vellozzi 2004). 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) (Fulginiti 2003, Neff 2008).

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 (Bryant-Genevier 2006). The rash appears within a week after vaccination and resolves within a week. Most instances do not require specific therapy (Fulginiti 2003).

Some of the post-vaccinia adverse events, although very rare, are serious and potentially life-threatening. They are described as follows.

Progressive vaccinia (PV) is the most serious complication known. It was almost always fatal prior to the introduction of vaccinia immune globulin (VIG). PV occurs predominantly in persons with T-cell deficiencies or receiving treatments that result in T-cell deficiencies. The primary vaccination site fails to heal leading to a severe local reaction with necrosis, and viremic spread of vaccinia leads to generalized appearance of new lesions without reactive immunoinflammatory response (Bray 2003, Fulginiti 2003). PV is extremely rare; historical incidence is in the order of 1 case per 1,000,000. There were no reports of PV in the military and civilian vaccines in 2002 – 2004 vaccination campaigns (Neff 2008).

Eczema vaccinatum (EV) manifests as rash (papular, vesicular, pustular, erosive) that can be localized or generalized and predominantly occurs in the areas that have been affected by lesions of atopic dermatitis or other eczematous skin condition. Historically it occurred at a rate of ~1 case per 25,000 vaccinations.

EV can occur in a vaccine recipient as well as in susceptible individuals in close contact. Two cases of EV from transmission have been recently reported both in children of recently vaccinated US military personnel (Lederman 2008, Vora 2008). In the military vaccination program in the US there were no reports of EV among 450,239 vaccinees, probably due to careful screening for contraindications (Grabenstein 2003). Review of civilian vaccinations did not detect any cases of EV (Velozzi 2004). EV can be prevented by thorough screening of at-risk individuals and education on importance of avoiding contacts with such persons and proper hygiene.

Postvaccinial encephalitis (PVE) historical case-fatality rate is 25%. The historical (1963 – 1968) reported frequency of PVE in United States was reported at 2.9 cases per 1,000,000 (Aragon 2003). PVE has higher prevalence and mortality rate in children compared to adults. Higher historical rates were reported in Europe compared to United States. Variability is attributed to differences in case definitions, clinical evaluations and differences in vaccine strains used by different countries (Sejvar 2005). Pathogenesis is still under investigation, although several compelling theories focus on autoimmune mechanism. Aside from vaccinia, measles and rabies vaccines have known association with PVE, as well as other viral and bacterial infections (Benneto 2004, Menge 2007). Review of 2002 – 2004 vaccinations in US reported three cases of PVE for the rate of 5 per 1,000,000.

Recent vaccination campaign in the US revealed a higher than historically observed incidence of myo/pericarditis in vaccinees. Predominant symptoms were chest pain, shortness of breath and fatigue, typically mild and transient. Among military contingent, 88% of cases occurred in men with the incidence of 16.11 per 100,000 for primary vaccines and 2.07 per 100,000 in revaccinees (Arness 2004). In civilian population women accounted for 67% of cases and the majority of events (86%) were reported in revaccinees (Casey 2005, Sniadack 2008). Variability between the two sets of data may be explained by differences in demographics of vaccinees, case detection, ascertainment and reporting practices (Morgan 2008). Myo/pericarditis has been long associated with a number of viral infections, although there are very few reports of confirmed viremia. A few cases of myo/pericarditis have been reported following DTP and influenza vaccinations (de Meester 2000, Boccara 2001). It is currently assumed that injury to the heart post viral infection is more of an immune inflammatory than direct nature (Cassimatis 2004, Feldman 2000).

Review of data from 2002 – 2004 vaccinations in US reported ~1 case of autoinoculation per 6,500 vaccinations with 17% of ocular cases, none with corneal involvement (Neff 2008). Vaccinia keratitis is the most serious consequence of autoinoculation, since lesions on the cornea threaten eyesight. Diseased or injured conjunctiva and cornea may increase the risk of this complication. Vaccinia keratitis will respond to treatment with topical antiviral agents and interferon and can be prevented with use of occlusive bandages over the scarification site and by subject education (Fulginiti 2003).

Transmission of vaccinia to close contacts is another known complication. Contact vaccinia may manifest as progressive vaccinia, eczema vaccinatum 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 (Neff, 2002). 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-V/F immunization is subcutaneously administered, which greatly reduces injection site reactions, and skin surface wound formation/viral shedding. PROSTVAC-V/F is also given to vaccinia pre-immune persons. Both of these factors reduce the potential for inadvertent infection versus traditional smallpox vaccine programs.

1.5.2 Fowlpox Virus

Fowlpox virus has been investigated and used in vaccine design for at least two decades. As a pox virus, it offers the advantages of a large genome but provides an additional safety assurance by not being able to replicate in mammalian cells. Fowlpox virus-based vaccines have been tested in both animals and humans for HIV, malaria and cancer. No safety concerns have been raised and the adverse events associated with the use of fowlpox vector have been limited to mild injection site reactions (Beukema 2006, Webster 2006).

1.6 Potential Benefits and Risks to Human Subjects

The intent of vaccination with PROSTVAC-V/F is to induce an immune response to prostate-specific antigen (PSA), and other prostate- and tumor-specific antigens. In the randomized, placebo controlled Phase 2 trial; an over-all survival benefit among patients who received PROSTVAC-V/F was observed. It is unknown whether PROSTVAC-V/F will provide this benefit to subjects participating in this trial, as this product is still currently in development. Benefits of this trial also include the potential boosting (or acquisition) of protective immunity against smallpox.

Based on clinical experience with PROSTVAC-V/F in Phase 2, adverse reactions are expected to be minimal. A local injection site reaction is typical, and comparable to those seen with other modern vaccines. Potential adverse reactions attributable to the administration of PROSTVAC-V/F at ≥ 50% frequency include injection site reactions (pain, swelling, induration, and redness), and at ≥ 10% frequency include headache, fatigue, myalgia, and nausea; the majority of events of Grade 1 and 2 in severity.

The priming vaccination with PROSTVAC-V is with a vaccinia-based replicating virus. Special wound precautions are required. The vaccinia virus has been used in millions of people to eliminate smallpox. In this trial it is given subcutaneously, not by dermal scarification, and local injection site reactions are much less severe, and viral shedding is minimal. However, there are still risks of inadvertent infection of other body sites (through scratching), or transfer of infection to others. Special bandage precautions are required, and subjects need to avoid contact with small children <3 years of age, individuals who are immunocompromised or are receiving immunosuppressive therapies, and pregnant or lactating women

(for 3-4 weeks). With smallpox vaccine programs in vaccinia-naïve subjects immunized by dermal scarification, inadvertent inoculation occurred approximately 1/6,000 vaccinations. Subjects in this trial will be given detailed instructions on proper care of the injection site and rules of hygiene to prevent inadvertent inoculations and transmission of vaccinia virus.

In the recent series of military and civilian vaccinations, vaccinia immunization has been reported to induce myopericarditis. Symptoms of chest pain were typically mild and transient, but occurred in 1/1,000 subjects. The incidence of this complication is lower in re-vaccinees. To prevent potential exacerbation of existing chronic problems, this protocol excludes subjects with known history of heart disease from participating in the trial. In addition, there are rare risks of severe and possibly life-threatening adverse reactions. These include eczema vaccinatum (~1/25,000, usually with history of underlying skin disorder) or progressive vaccinia infections (~1/300,000, usually with underlying immunodeficiency), or post vaccinial encephalitis (~1/500,000, usually infants or small children). These were observed after the administration of conventional smallpox vaccines, and have not been seen in prior studies of PROSTVAC-V/F or related PSA (or other tumor antigen-containing vaccinia virus vaccines) administered to vaccinia pre-immune cancer subjects Under the proposed protocol, age restriction, exclusion of all pre-existing conditions with features of immune suppression and skin disorders minimize the risk of such rare complications.

During study treatments, subjects will be under the continuous care of the investigator and his/her staff and will be assessed for adverse events which will be recorded in the subject's CRF. SAEs must be reported to BNI or its designee within 24 hours of discovery. To minimize risk, potential subjects who have a known allergy to eggs, egg products, aminoglycoside antibiotics or GM-CSF, or other pre-existing medical conditions known to be risk factors for SAEs will be excluded from participation.

In view of the anticipated mild side effects, the potential risks for the subject seem to be limited and to justify the potential benefit of participating in this trial.

1.7 Rationale for Double-Blinding

This study has been designed to be double-blinded despite having an objectively ascertained primary endpoint (survival) for the following reasons:

- There will be no study defections due to randomization disappointment (so subjects randomized to placebo will not quit the study).
- There will be no difference in use of post-treatment anti-cancer therapies or concomitant therapies due to knowing the intervention being delivered.
- There will be no difference in the interpretation of biomarkers or lab tests due to knowing the intervention being delivered.
- The between-arm comparison of adverse events will not be corrupted by knowing the intervention being delivered.

2.0 NAME AND DESCRIPTION OF STUDY AGENTS

2.1 Formulation, Packaging, and Labeling

The PROSTVAC-V/F study vaccines, including placebo, are manufactured at [redacted] in compliance with current good manufacturing practices (cGMPs). The formulations do not contain adjuvants or preservatives. [redacted] the vials are sealed with rubber injection stoppers and plastic/aluminum flip-caps, and a label is affixed to the vial. PROSTVAC-V/F study vaccine vials are then packaged in 6-vial cartons with an outer carton label.

GM-CSF (Leukine®: 250 µg, lyophilized) will be purchased from licensed, US pharmacy wholesalers. Lyophilized GM-CSF is reconstituted with 1.0 mL bacteriostatic water for injection. GM-CSF vials will not be relabeled, and will be packaged in 5-vial cartons with an outer carton label.

GM-CSF placebo is bacteriostatic sodium chloride for injection and will be purchased from licensed, US pharmacy wholesalers. GM-CSF placebo vials will not be relabeled, and will be packaged in 25-vial trays with a label.

Examples of carton and vial labels are included in **Appendix 14.10**.

2.2 Investigational Drug Product (PROSTVAC-V/F)

PROSTVAC-V/F is comprised of two components. PROSTVAC-V is a replication-competent vaccinia virus which has been engineered to encode the sequences for a modified human prostate-specific antigen (PSA) and a triad of co-stimulatory molecules (TRICOM). PROSTVAC-F is a fowlpox virus which does not replicate in human cells and has been engineered to encode the same sequences present in PROSTVAC-V. A full description of the PROSTVAC-V/F product and PROSTVAC-V/F pre-clinical and clinical support are contained in the Investigator's Brochure.

Study vaccine for this trial contains no preservatives or adjuvants and is supplied in single-dose 2 ml clear, borosilicate glass injection vials with rubber injection stoppers covered by a plastic-aluminum flip cap.

Active Ingredient: PROSTVAC-V: PROSTVAC-V-PSA-TRICOM in aqueous solution for injection
PROSTVAC-F: PROSTVAC-F-PSA-TRICOM in aqueous solution for injection

Product Description: PROSTVAC-V/F is comprised of two recombinant pox viruses: a recombinant vaccinia virus (PROSTVAC-V) and a recombinant fowlpox virus (PROSTVAC-F). [redacted]

[redacted]

Dose: 0.5 ml containing at least 2 x 10^8 Infectious Units (Inf.U) PROSTVAC-V

0.5 ml containing at least 1 x 10^9 Inf.U PROSTVAC-F

Route of Administration: Subcutaneous injection

Storage*: −70°C or below

There is no pre-treatment regimen for PROSTVAC-V/F.

* Storage conditions and stability claims for the final PROSTVAC-V/F product are based on long-term manufacturing experience with PROSTVAC-V/F by Therion, the initial manufacturer. The stability data for PROSTVAC-V/F shows virus titer stability for four years when stored at −80°C (± 10°C).

Regimen of Administration: Doses of PROSTVAC-V/F or placebo will be drawn into labeled syringes by an independent, unblinded research pharmacist or designee for use by the clinic staff. PROSTVAC-V will be administered by the study staff once by sc injection on one side (upper arm or upper outer thigh) during Week 1. PROSTVAC-F will be administered by the study staff six times by sc injection during Weeks 3, 5, 9, 13, 17, and 21. Vaccination with PROSTVAC-F will begin on one side (upper arm or upper outer thigh), and subsequent immunization sites should be rotated to the opposite side and/or limb if possible.

2.3 GM-CSF

GM-CSF (Leukine®: 250 µg, lyophilized) will be purchased from licensed, US pharmacy wholesalers and labeled appropriately for the study. Lyophilized GM-CSF will be reconstituted using 1.0 ml of room temperature bacteriostatic water for injection (USP-grade or equivalent). Product reconstituted with bacteriostatic water for injection is stable for 20 days if stored between 2 and 8°C in the original vial. See product label for information related to product description and formulation. Doses of GM-CSF of 100 µg each will be drawn into labeled syringes by an independent, unblinded research pharmacist or designee for use by the clinic staff or by individual subjects for home injection (subjects enrolled in France must return to the clinic to receive GM-CSF injections).

Dose:	0.40 ml (100 µg) GM-CSF
Route of administration:	Subcutaneous injection
Storage:	2 - 8°C (lyophilized and reconstituted); Up to 4 days at 2 - 8°C when in syringes
Regimen of administration:	GM-CSF will be injected sc by the study staff on the day of vaccine or placebo administration and for the subsequent 3 days, within approximately 5 mm of the injection site by the study staff, the subject, or subject's caregiver (subjects enrolled in France must return to the clinic to receive GM-CSF injections).

2.4 PROSTVAC-V/F Placebo: Empty fowlpox vector

The PROSTVAC-V/F placebo will be provided in blinded vials matched to active product. The same placebo will be used for both vaccinia and fowlpox PROSTVAC-V/F components.

Dose:	0.5 ml containing at least 1 x 10^9 Inf.U
Route of administration:	Subcutaneous injection
Storage:	¯70˚C or below
Regimen of administration:	PROSTVAC-V placebo will be administered once by sc injection by the study staff in the upper arm or upper outer thigh during Week 1. PROSTVAC-F placebo will be administered by the study staff six times by sc injection during Weeks 3, 5, 9, 13, 17, and 21. Vaccination with PROSTVAC-F placebo will begin on one side (upper arm or upper outer thigh), and subsequent immunization sites should be rotated to the opposite side and/or limb if possible.

2.5 GM-CSF Placebo

GM-CSF placebo will consist of USP-grade or equivalent bacteriostatic sodium chloride (saline) for injection. Doses of GM-CSF placebo will be drawn into labeled syringes by an independent, unblinded research pharmacist or designee for use by the clinic staff or by individual subjects for home injection (subjects enrolled in France must return to the clinic to receive GM-CSF placebo injections).

Dose:	0.40 ml injection of bacteriostatic sodium chloride (saline) for injection
Route of administration:	Subcutaneous injection
Storage:	Room temperature when in vials; Up to 4 days at 2-8^0 C when in syringes
Regimen of administration:	GM-CSF placebo will be administered sc on the day of PROSTVAC-V/F or PROSTVAC-V/F placebo vaccination by the study staff and for the subsequent 3 days, within approximately 5 mm of the PROSTVAC-V/F or PROSTVAC-V/F placebo vaccination site by the study staff, the subject, or the subject's caregiver (subjects enrolled in France must return to the clinic to receive GM-CSF placebo injections).

3.0 STUDY OBJECTIVES AND ENDPOINTS

3.1 Study Objectives

3.1.1 Primary Efficacy Objective

To ascertain whether the survival of subjects randomized to Arm V+G (PROSTVAC-V/F plus GM-CSF) or to Arm V (PROSTVAC-V/F) is superior to that from subjects randomized to Arm P (placebo control).

3.1.2 Secondary Efficacy Objective

To ascertain whether a greater proportion of subjects randomized to Arm V+G or to Arm V remain event-free at six months (or early termination) as compared to the subjects randomized to Arm P.

3.1.2.1 Definition of Event (To be Used in Secondary Endpoint)

Progression events will be defined as:

- Radiographic progression
    - Two new lesions on bone scan, new metastases on CT scans*, or an increase size of nodal lesions per RECIST 1.1.
- Pain progression
    - Introduction of scheduled opioid narcotics for cancer-related pain control.
- Initiation of chemotherapy
- Death

* For subjects with allergies to contrast agents, MRIs for abdomen and pelvis may be performed. Only in cases where MRI is unavailable may a non-contrast CT be performed. For subjects enrolled in Germany, MRI must be used as the primary imaging modality.

Radiographic progression will be assessed by bone scan at 3 and 6 months, and by CT scan (or MRI for subjects in Germany) at 6 months (and at the time of progression, or any time during the treatment period if clinically indicated). For secondary endpoint analysis, baseline scans and 6 month scans (or early termination scans) will be compared by central radiology review. Bone scan and CT (or MRI for subjects in Germany) data will be archived up to five years past the end of the trial.

In addition, for an exploratory endpoint, we will assess radiological progression at 12 months (6 months post End-of-Treatment visit) by comparison with the 6 month scans (from End-of-Treatment visit or early termination). This will attempt to define effects on secondary progression, and characterize delayed effects of investigational vaccine therapy.

3.1.3 Safety Objective

To further characterize the safety and tolerability of PROSTVAC-V/F immunotherapy, characterize type and number of AEs compared to placebo.

3.1.4 Exploratory Objectives

To model secondary radiological disease progression over the first year of study participation.

To ascertain whether a greater proportion of subjects randomized to either Arm V+G or Arm V remain event-free (radiological progression) six months post End-of-Treatment visit (using scans at End-of-Treatment visit as a new baseline), as compared to the subjects randomized to Arm P. This analysis will include all subjects, and separately the HLA-A2-expressing subgroup.

To assess the role of post-treatment anti-cancer therapies as an alternative explanation for observed survival differences.

To ascertain whether the survival of HLA-A2-expressing subjects randomized to Arm V+G, or Arm V is superior to that from HLA-A2-expressing-subjects randomized to Arm P.

To ascertain whether a greater proportion of HLA-A2 expressing subjects randomized to Arm V+G or to Arm V remain event-free at six months as compared to the HLA-A2 expressing subjects randomized to Arm P.

To compare arms with respect to immune response to immunizing antigen (PSA), as well as to non-vaccine containing prostate antigens, and tumor-associated antigens, and assess whether immune responses are prognostic and/or predictive.

To evaluate for baseline biomarkers which are prognostic and/or predictive of long term survival outcome.

To compare arms with respect to vaccine effects on circulating tumor cell (CTC) levels (US sites only).

3.2 Study Endpoints

3.2.1 Primary Efficacy Endpoint

The primacy efficacy endpoint for the trial is overall survival.

3.2.2 Secondary Efficacy Endpoint

The secondary efficacy endpoint for the study is the proportion of event-free subjects (radiological progression, pain progression, chemotherapy initiation, or death) at six months (or early termination) compared to placebo.

4.0 INVESTIGATIONAL PLAN

4.1 Study Design

BNIT-PRV-301 is a randomized, placebo-controlled, multi-center, multi-country, Phase 3 efficacy trial of PROSTVAC-V/F in men with asymptomatic or minimally symptomatic, metastatic, castration-resistant prostate cancer. Subjects will be randomized with equal probability (1:1:1) to one of three double-blind arms; two investigational arms (Arm V+G; Arm V), and a control arm (Arm P). The study will consist of three periods: a Screening phase, followed by Treatment, and subsequently Long-Term Follow-Up (LTFU) phases.

The intended intervention in Arm V+G is PROSTVAC-V/F plus GM-CSF; Arm V intended intervention is PROSTVAC-V/F plus GM-CSF placebo, whereas the intended intervention in Arm P is vaccine placebo plus GM-CSF placebo. The trial interventions will consist of a single sc immunization of PROSTVAC-V or placebo in Week 1, followed by six PROSTVAC-F or placebo immunizations administered in Weeks 3, 5, 9, 13, 17, and 21. Each immunization will be accompanied by administration of sc GM-CSF or placebo on the day of immunization and for the subsequent three days (sc injection within 5 mm of the original PROSTVAC V/F or placebo injection site). The Treatment phase of this trial lasts for five months and is followed by an End-of-Treatment visit at Week 25. During the Treatment phase, subjects will continue to be treated with vaccination therapy or placebo through PSA and/or radiological progression.

Following the completion of the Treatment phase (after the last dose of PROSTVAC-V/F ± GM-CSF or placebo or early termination of treatment) of the trial, all subjects will automatically enter the LTFU phase with study visits occurring every six months. During LTFU subjects will receive standard-of-care treatment as determined by the Principal Investigator or treating physician. The appropriate timing and the nature of any subsequent treatment will be at the discretion of the Principal Investigator or treating physician. All treatments for mCRPC subsequent to the Treatment phase of the trial will be documented in the CRF. This comprehensive data will be collected until study closure, death, or until a subject withdraws from the study.

The primary endpoint for the study is overall survival. All subjects will be followed for 12 months after the required number of 534 events for each between-arm comparison is realized.

4.2 Sample Size

This study will evaluate approximately 1,200 subjects (400 subjects/arm) with a 1:1:1 randomization of PROSTVAC-V/F plus or minus GM-CSF versus placebo control plus GM-CSF placebo.

4.3 Selection of Study Population

All subjects will be men ≥ 18 years old with asymptomatic or minimally symptomatic, metastatic CRPC. Subjects will have progressive disease following androgen suppression or complete blockade therapy, or surgical castration; and will be chemotherapy naïve for metastatic prostate cancer. Subjects must be

vaccinia experienced (previous smallpox vaccination). Subjects must have a castrate testosterone level of <50 ng/dl at study entry.

For subjects on anti-androgens, a 6-week washout is required (4 weeks if on flutamide).

Prospective subjects may be screened from hospital or clinic records or databases, or may be new patients. At the discretion of the institution, subjects may be recruited by advertisements, on-line, in newsletters or journals, flyers, or on radio provided the content and distribution is approved by BNI and by the IRB/EC prior to use or release. BNI will list the study on the government website, www.clinicaltrials.gov.

4.4 Targeted Enrollment of Minorities

Attempts will be made (in the US) to specifically enroll minority subjects up to the level of representation in the general US population. Cities with high minority populations will be specifically targeted for center recruitment, as will study centers serving primarily minority populations; *e.g.*, Historically Black Colleges and Universities. Attempts will be made to tailor advertising and recruitment tools to minority groups and to enlist the help of subject advocacy groups in high minority population centers.

4.5 Method of Assigning Subjects to Treatment Groups

This study is double-blind and placebo-controlled. Enrollment will be randomized with equal probability to each of three double-blinded arms: two investigational arms (Arm V+G or Arm V) and a control arm (Arm P). The intended interventions associated with the arms are: active treatment with PROSTVAC-V/F + GM-CSF (Arm V+G); PROSTVAC-V/F + GM-CSF placebo (Arm V); or placebo control (Arm P). Block randomization will be used to randomly assign subjects in a 1:1:1 manner. Randomization will be stratified according to the following two factors, PSA (equal and above or below 50 ng/ml) and LDH (equal and above or below 200 U/mL).

4.6 Inclusion Criteria

Eligible subjects must meet all the following criteria (there will be no exemptions for Inclusion/Exclusion criteria):

1. Signed Informed Consent.

2. Men, ≥18 years of age with documented asymptomatic or minimally symptomatic metastatic castration-resistant prostate cancer.

3. Castrate testosterone level < 50 ng/dl.

4. Documented progressive disease post-surgical castration or during androgen suppression therapy, or during complete androgen blockade therapy and withdrawal. Documented by either criterion a (Radiological progression), **OR** criterion b (PSA progression).

a. Radiological progression defined as any new/enlarging bone metastases or new/enlarging lymph node disease, consistent with prostate cancer.

OR

b. PSA progression defined by sequence of rising values separated by > 1 week (2 separate increasing values) over a threshold minimum of 2.0 ng/ml. (PCWG2 PSA eligibility criteria).

5. Chemotherapy naïve. No prior chemotherapy for metastatic prostate cancer. Neo-adjuvant or adjuvant chemotherapy for primary prostate cancer is permissible if >3 years prior.

6. Vaccinia-experienced (previous smallpox vaccination).

7. ECOG Performance Score of 0 or 1.

8. Life expectancy ≥ 1 year.

9. Bone Marrow function:
   - Absolute neutrophil count ≥ 1,500/mm^3
   - Hemoglobin ≥ 10 g/dL
   - Platelet count ≥ 100,000/mm^3

10. Hepatic Function:
    - AST and ALT ≤ 2.5 times upper limit of normal (ULN)
    - Bilirubin ≤ 1.5 times ULN

11. Renal Function:
    - Creatinine ≤ 2.0 times ULN

12. Currently using a GnRH agonist or antagonist (unless surgically castrated).

4.7 Exclusion Criteria

Subjects with any of the following will be excluded from enrollment:

1. Cancer-related pain requiring scheduled opioid narcotics for control (as needed, ≤ 2x per week is allowed).

2. Metastasis to organ systems other than lymph nodes and/or bone.

3. LDH ≥ 2 times ULN.

4. Alkaline phosphatase ≥ 2 times ULN.

5. Estimated PSA doubling time of <1 month as established within 6 months of the anticipated first dose of vaccine or placebo. A minimum of 3 PSA level determinations, at least 2 weeks apart (over a 6 month time-period), are required for assessment.

6. Concurrent or prior Provenge (sipuleucel-T) immunotherapy for prostate cancer.

7. Receipt of an investigational agent within 30 days (or 60 days for an antibody-based therapy) of the first planned dose of PROSTVAC-V/F. There is no exclusion to previous experimental therapy provided dosing/treatment is completed at least 30 days prior to the first planned dose of vaccine unless otherwise noted.

8. History of prior malignancies other than prostate cancer within the past 3 years, excluding successfully resected basal or squamous cell carcinoma of the skin.

9. Congestive heart failure (NYHA Class II, III, or IV), unstable angina, ventricular or hemodynamically significant atrial arrhythmia, or cardiovascular disease such as stroke or myocardial infarction (current or within the past 6 months).

10. Confirmed positive for HIV, hepatitis B, and /or hepatitis C.

11. Prior solid organ or bone marrow transplant.

12. Immunodeficiency or splenectomy.

13. Chronic immunosuppressive therapy within 30 days of screening.

14. Inflammatory eye disease requiring steroid treatment.

15. Chronic administration (defined as daily or every other day for continued use > 14 days) of systemic corticosteroids within 28 days of the first planned dose of PROSTVAC-V/F. Use of inhaled steroids, nasal sprays, and topical creams for small body areas is allowed.

16. 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, Hashimoto's thyroiditis, or Graves disease). Persons with vitiligo are not excluded. Diabetics are not excluded if the condition is well controlled.

17. Known allergy to eggs, egg products, aminoglycoside antibiotics (for example, gentamicin or tobramycin), or GM-CSF. Subjects with a known or suspected allergy to radiological contrast agents are eligible, but this must be noted in the subject's medical history and in the chart notes.

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

19. Previous adverse reactions to smallpox vaccination.

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

21. Significant medical abnormality (defined as a pre-existing condition AE/condition ≥ Grade 3 according to NCI CTCAE v 4.0 and any condition which, in the opinion of the investigator, would prevent full participation in this trial (including the Long-Term Follow-Up), or would interfere with the evaluation of the trial endpoints.

22. Study personnel.

5.0 SAFETY MONITORING

5.1 Data Monitoring Committee (DMC)

An independent Data Monitoring Committee will be established to ensure subject safety, to consider subject risks against the potential for meeting trial objectives, and to provide recommendations to BNI with respect to the conduct and analysis of the trial. The DMC will operate independently of BNI with the exception of the administration of compensation to the DMC members for DMC-related activities and non-voting activities such as clinical trial conduct updates. The voting members of the DMC will be a multi-disciplinary group that may include representative physicians who treat the type of subjects entered onto this trial; one or more individuals with statistical expertise; and if appropriate, one or more biological scientists. The DMC will meet either by phone or in person and will be provided with all available pertinent subject data in a manner specified in the approved Charter. An Executive Summary of Committee recommendations generated at each meeting will be forwarded to all IRBs/ECs as requested or required. Minutes of the closed sessions will be held in confidence by the DMC or its designee until the official close of the trial. The exact constitution and conduct of the DMC will be ultimately governed by the approved Charter. A vital status sweep will be performed in conjunction with each DMC meeting to ensure the most current data is being reviewed.

5.2 Dose-limiting Toxicities and Trial Stopping Rules

5.2.1 Dose-limiting Toxicities

For the purposes of this trial, a dose-limiting toxicity (DLT) is defined as any anaphylactic reaction, a full-thickness ulceration/necrosis, or any AE ≥ Grade 3 definitely related to study drug with the following exceptions:

- Local injection site reactions lasting < 72 hours: pain, redness, swelling, induration, or pruritus
- Systemic injection reactions lasting < 72 hours: fever; myalgia, headache, nausea, fatigue

Study drug should be discontinued for the subject in the case of a definitely related anaphylactic reaction or full-thickness ulceration/necrosis. Re-exposure to study drug after development of other related AE ≥ Grade 3 should only occur if the event resolved or is reduced to grade 1 toxicity.

5.2.2 Trial Stopping Rules

In the event of a death definitely related to study drug within 24 hours of vaccination or ≥ 3 definitely related SAEs (per 20 patients) at any time after receiving study drug during the treatment period, dosing will halt immediately and the DMC will be consulted. All data will be reviewed prior to any further dosing, and dosing will not continue until the DMC agrees that it is safe to continue. At the discretion of the DMC, the FDA or other Health Regulatory Agencies may be consulted. The decision to permanently halt the trial may only be made by BNI Executive Committee.

5.3 Emergency Unblinding Of Treatment Assignment (If Applicable)

All investigational vaccine products for this study have been packaged and labeled in a blinded fashion. GM-CSF will be labeled for investigational use as required by the local and regional Health Authorities, Lyophilized GM-CSF is not packaged and labeled in a blinded fashion. Reconstituted GM-CSF or GM-CSF placebo in single-use syringes is blinded. Unblinding by study site personnel for adverse events should only be performed in emergencies where knowledge of the subject's treatment assignment is essential for further management of the subject's medical care. Unblinding a subject's treatment assignment under any other circumstances will be considered a protocol violation. The Principal Investigator is strongly encouraged to contact the BNI Medical Monitor before unblinding any subject's treatment assignment, but must at least do so within 1 working day after the unblinding. Although the Medical Monitor should be contacted the subject's treatment code should not be communicated to the Medical Monitor. Emergency unblinding for adverse events will be performed through an interactive voice-response system (IVRS). All calls resulting in an unblinding event are recorded and reported by the IVRS. Emergency unblinding must also be documented at the study site. The Medical Monitor will receive notification of unblinding from IVRS but will not be notified of the subject's treatment assignment.

5.4 Removal of Subjects from Therapy or Assessment

Subjects may withdraw consent for participation in the study at any time without prejudice. Additionally, the investigator may withdraw a subject if, in his/her clinical judgment, it is in the best interest of the subject or if the subject cannot or will not comply with the protocol. Wherever possible, the End-of-Study tests and evaluations listed in **Section 7.4** should be carried out at the time the subject withdraws or whenever the Investigator feels that the subject will be unable to make any further visits.

If a subject fails to return for the necessary visits or discontinues prematurely from the study, a genuine effort must be made to determine the reason why. If the subject or his designated contact is unreachable by telephone, the minimum of a registered letter should be sent requesting that contact be made with the Investigator (or designee) to report survival information. Every attempt possible to obtain follow-up blood samples for safety and immune monitoring should be made. Request for an autopsy, autopsy report and death certificate when/if the subject expires while on trial should be pursued.

BNI may discontinue individual subjects, study arms, individual study centers, or the entire study at any time provided sufficient time is given to ensure subject safety.

5.4.1 Clinical or Kinetic Progression: Removal of Subjects from Therapy

Subjects who experience a significant clinical progression during the treatment phase (i.e., before last vaccination is received) will be counseled to seek alternative therapy. Significant clinical progression is defined by the need to institute scheduled narcotic pain relief measures for cancer-related pain, or acceleration of tumor growth kinetics by PSA (PSA doubling time <1 month). Such subjects are allowed to continue vaccine therapy at the discretion of the PI if the decision is made not to seek alternative anti-cancer treatment(s). If subjects choose to initiate alternative anti-cancer treatment, vaccination will be discontinued. All subjects will continue on study and will be followed for survival and safety per protocol.

5.4.2 Biochemical or Radiological Progression: Continuation of Therapy

Vaccination may take several months to induce an immune response. PSA declines and radiological responses are infrequent, and the expectation is for a slowing of ongoing disease progression. Due to the delayed effect of vaccine induced immunotherapy, PSA progression and radiological progression may be expected during the treatment phase of the trial, and are not a cause for stopping vaccination. Continued vaccination to maximize immune response is believed to be beneficial based on earlier clinical trials.

5.5 Replacement Policy

5.5.1 Subjects

No subjects will be replaced in this study. All subjects who receive at least one dose of PROSTVAC-V/F or placebo will be considered evaluable and will be included in all appropriate analyses as described in **Section 9.0**.

5.5.2 Research Centers

Centers will be opened only after all requirements for documentation have been met as defined by Appendix M (M-I-C-1) of the NIH Guidelines for Research Involving Recombinant DNA Molecules (April 2002) or the appropriate regional or national guidelines outside the United States. If an approved institution declines further participation or is dropped for any reason, or if enrollment lags, new centers may be added under the same restrictions.

6.0 STUDY TREATMENTS

6.1 Investigational Study Drug Accountability

Prior to study start, each Investigator will provide the actual location (facility, room number, and freezer/refrigerator number, if any) and identify the pharmacist, study coordinator, sub-investigators, or other personnel who will have access to the drug, and if different from above, any personnel who will have preparation, administration, receipt and disposal responsibilities of the study drug. It should be noted that reconciliation of PROSTVAC-V/F and GM-CSF vials will be performed by an independent unblinded monitor in order to keep the study monitor blinded.

Accurate records of dates, quantities and the lot or vial numbers of all study drug received, to whom dispensed (subject-by-subject accounting), and accounts of any product accidentally wasted or intentionally disposed of must be maintained. The disposal of all used, partially used, or wasted study drug vials and syringes must be performed in accordance with the hospital or institution's drug disposal policy for infectious medical waste. Used vials will not be retained by the pharmacy for drug accountability. The institution's policy for disposal must be kept in the Pharmacy Manual and a copy provided to BNI.

Accountability records must be maintained and readily available for inspection by representatives of BNI or designee and are open to inspection by regulatory authorities at any time.

6.2 Assessment of Study Drug Compliance

Accountability and reconciliation of drug dispensing and administration compliance will be assessed by review of adequate drug dispensing (including return) records performed periodically by the unblinded monitor.

A drug dispensing log must be kept current and will be reviewed periodically throughout the study by the unblinded monitor.

6.3 Disposal of Drug Supplies

Local or institutional regulations require immediate disposal of used study drug. The investigational site staff is to destroy any dispensed study drug vials and syringes in accordance with the institution's policy. Source document verification will be performed on the remaining inventory and reconciled against the documentation of quantity shipped, dispensed, and disposed. Each research center must provide BNI a statement of the institutional policy and procedures for disposing of material with a Biosafety rating of RG2 (**Appendix 14.2**; RAC Guidelines 2002, Appendix B or equivalent outside the United States) and the institution's policy and procedures for disposal of hazardous waste or biologic products. A copy of the policy must be placed in the Pharmacy Manual and provided to BNI.

At the conclusion of the Treatment phase of the study, all unused vaccine, GM-CSF, and placebo supplies (collectively, study medication) will be either disposed of on site or by a licensed facility

contracted by the site following final reconciliation. Subjects who received home-injections will be instructed to return all used and unused GM-CSF syringes to the site for disposal (not applicable to subjects enrolled in France as they must return to the clinic to receive GM-CSF injections). BNI or its designee will provide logs to each site pharmacy for recording IP dispensing (subject ID, date, disposal, etc.). Sites may use their own logs provided the required data is adequately captured.

6.4 PROSTVAC-V/F

PROSTVAC-V is considered a BSL-2 agent; PROSTVAC-F is considered BSL-1 (but may be considered BSL-2 outside the United States, depending on country specific requirements). All vaccines in this trial should be handled using standard infection control procedures and study staff should wear personal protection consisting of at least a lab coat, gloves, and protective eyewear when handling PROSTVAC-V/F. More complete instructions and guidelines are included in the Study Instruction Manual and Pharmacy Manual.

PROSTVAC-V/F is prepared in a phosphate-buffered saline (PBS) with 10% glycerol and stored at -70˚C or colder until the day of use. Once used and accounted for, vials should be disposed of according to institutional standards, local and occupational safety guidelines.

There is no pre-treatment regimen for PROSTVAC-V/F.

PROSTVAC-V/F should not be mixed with any other medicinal product. Refer to the pharmacy manual for instructions regarding administration.

Subjects should remain in the clinic for at least 30 minutes following administration of PROSTVAC-V/F or placebo for observation for signs of adverse reactions.

6.5 GM-CSF

GM-CSF (sargramostim; Leukine®) is a glycosylated, recombinant human GM-CSF manufactured by Genzyme Corporation. GM-CSF is an altered form of the native molecule; the position 23 arginine has been replaced with a leucine to facilitate expression. Leukine is preserved with benzyl alcohol and provided in vials containing 250 µg of lyophilized product. GM-CSF is reconstituted using USP bacteriostatic water for injection (1.0 ml per vial). Refer to the Pharmacy Manual for instructions regarding administration).

If the subject or their caregiver is proficient at self injection (as determined by the study staff), (s)he may be provided with the pre-filled syringes of GM-CSF (or placebo) for self injection for each of 3 consecutive days following the day of vaccination. If the subject or their caregiver is unable or does not wish to self inject, the subject must return to the clinic for three consecutive days following the vaccination to receive the GM-CSF or placebo (subjects enrolled in France must return to the clinic for three consecutive days following the vaccination to receive GM-CSF or placebo).

Site personnel are instructed to query subjects self-injecting GM-CSF for compliance and record the outcome in the subject's chart.

6.6 Subject Instructions and Supplies

Subjects, and if possible, subject caregivers, should be educated as to the care of the injection site, including proper bandage changing, bathing, possible side effects, and minimization of contact with at-risk individuals. Subjects will be provided with injection site care 'kits' containing, for example; instruction sheets, disposable gloves, absorbent toweling, alcohol swabs, non-adherent Telfa-type bandages, band-aids, zip-lock biohazard bags for disposal of used bandages and gloves, pre-filled syringes for home injection of GM-CSF or placebo, a cold pack, a sharps container, and contact information for the study personnel (not applicable to subjects enrolled in [redacted] as they must return to the clinic to receive GM-CSF injections). Subjects should be instructed to return the zip-lock biohazard bag containing all used supplies at their next clinic visit.

6.7 Dose Reductions and Contraindications for PROSTVAC-V/F

There are no dose reductions for this study. Subjects who experience intolerable reactions will be discontinued from treatment but will remain on study and be followed for safety, immune status, and survival per protocol. PROSTVAC-V/F will be discontinued as defined in **Section 5.4**. Subjects discontinued from further vaccination should remain on study and continue to be followed for safety, immune status, and survival per protocol.

For acute illnesses (fever, infectious disease, respiratory insufficiency, chest pain, angina, etc.) present at the time of a scheduled vaccination, the vaccination can be delayed until symptoms subside, the underlying process is treated or resolves, or the subject may be withdrawn at the discretion of the Investigator. If vaccinations are delayed due to acute illness, timing for scheduled assessments and remaining vaccinations should be reestablished according to the original Schedule of Events (**Appendix 14.1**) as soon as possible.

6.8 Procedures for Potential Serious Vaccinia Reaction

The Investigator must notify an Infectious Disease Specialist in the [redacted] Pharmacovigilance Group (PVG) if there is a suspected serious vaccinia reaction as soon as possible, but no later than 24 hours after he/she becomes aware. The Investigator should contact the BNI study Medical Monitor if there are specific questions in regard to the potential vaccinia reaction. A swab of the suspected lesion may be obtained from the study subject for confirmatory testing prior to administration of VIG. 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. For further information or for a supply of VIG in the United States, contact the Centers for Disease Control at (877) 554-4625. For centers outside the United States, VIG suppliers (where known) are listed in the Study Instruction Manual. The Investigator should refer to the Management Plan for Potential Serious Vaccinia Reaction. 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.

6.9 Concomitant and Excluded Medications

6.9.1 Concomitant Medications:

Subjects will be on concomitant androgen suppression therapy with a GnRH agonist or antagonist, unless surgically castrated. Subjects may also be on concomitant drugs to prevent bone loss or skeletal-related events, including bisphosphonates and denosumab. Subjects may receive palliative radiotherapy during the trial.

6.9.2 Excluded Medications:

During the Treatment phase of the study (approximately 5 months) all medications required for the health of the subject are allowed with the following exceptions:

- Concurrent chemotherapy
- Concurrent immunotherapy (e.g. sipuleucel-T, etc.) or immunosuppressive therapy (e.g. etanercept, natalizumab, etc.)
- Concurrent anti-cancer radionuclides
- Concurrent systemic corticosteroid use (daily or every other day for continued use > 14 days)
- Concomitant use of secondary anti-cancer hormonal treatments (e.g. abiraterone)

6.10 Overdose

Because PROSTVAC-V and PROSTVAC-F are provided in single-dose vials there is no potential for an overdose. The highest single dose of PROSTVAC-V ($2x10^8$ Inf. U.) or PROSTVAC-F ($1x10^9$ Inf. U.) used in human studies did not show any dose-limiting toxicity.

Because the GM-CSF dose on this study is approximately five times lower than the approved therapeutic dose, there is no potential for an overdose. Possible side effects with the use of standard dosage are described in the commercial product insert (see the Pharmacy Manual).

7.0 STUDY PROCEDURES BY STUDY PERIOD

7.1 Screening Assessments (Days –28 –1)

Screening activities must be completed within 28 days prior to the first dose of any study medication unless noted. Assessments will be done over one or more visits and are to be completed prior to dosing. Subjects requiring anti-androgen wash-out specifically for this protocol should be consented prior to beginning withdrawal of therapy. No other screening procedures should be performed until it is clear that the subject is eligible as defined in **Sections 4.6** and **4.7**. Subjects requiring wash-out and re-testing will be asked to re-sign an informed consent form prior to any additional screening procedures. Screening assessments performed greater than 28 days prior to first dose will need to be repeated except as specified below. Subjects must be entered into IVRS after signing Informed Consent and prior to any screening procedures being initiated.

For the purposes of this trial, one month = 4 weeks = 28 ± 3 days.

After signing the Informed Consent, Screening assessments will include:

1. Complete medical history, including smallpox vaccination history
2. Prostate cancer history (primary diagnosis, progression history, treatments received; procedures undergone)
3. Concomitant medications (all drugs currently being taken, all drugs prescribed during the past month, and all over-the-counter drugs or supplements (excluding vitamins) taken for ≥5 consecutive days during the past month)
4. Complete physical examination (PE) including vital signs (VS; sitting blood pressure [BP], heart rate, respiration rate [RR]), temperature, height and weight
5. ECOG performance status
6. 12-lead ECG
7. Blood samples will be obtained for:
    a. Hematology (CBC and differential, RBC with indices, platelet count).
    b. Serum chemistry (BUN, creatinine, ALT, AST, alkaline phosphatase, total bilirubin, LDH, sodium, potassium, chloride, bicarbonate, calcium and glucose).
    c. Testosterone level.
    d. C-reactive protein.
    e. Cardiac markers (CK, CK-MB, Troponin T and I).
    f. Coagulation panel (PT (INR)/PTT).
    g. Serum PSA and PAP (prostatic acid phosphatase) levels.

h. Virology (HIV, HepBsAg, HepC) unless known negative within previous 2 months.

8. Urinalysis (dipstick and microscopy).
9. Bone scan, if not performed within 3 months of the start of screening.
10. CT scan with contrast* (abdomen and pelvis) if not performed within 3 months of the start of screening.

*For subjects with allergies to contrast agents, MRIs for abdomen and pelvis may be performed. Only in cases where MRI is unavailable may a non-contrast CT be performed. For subjects enrolled in Germany, MRI must be used as the primary imaging modality.

7.2 Day 1 (Day of first dose of PROSTVAC-V/F or placebo), Week 1

Subjects must be randomized and the first dosing date entered and confirmed in IVRS prior to any Week 1 procedures being performed. Treatment assignment must be obtained through IVRS prior to dosing.

During Week 1 of the Treatment phase of this study, the following procedures will be performed and dosing will occur as follows

To be performed **PRIOR** to administration of study drug:

1. Adverse events (AEs) should be sought by non-directive questioning of the subject, self-reported, and/or directly observed at each clinic visit during the treatment phase of the study.
2. Concomitant medications will be reviewed and any new medications recorded
3. QoL (FACT-P, BPI-SF and EQ-5D-3L) where translations are available
4. Abbreviated physical exam, consisting of VS, temperature, and weight. Only significant changes in PE from Screening will be recorded.
5. ECOG performance status
6. Immune monitoring assessments are to be collected prior to first dose only after a subject is deemed eligible for the study by all other criteria. Blood sample collection will include:
    a. Serum for immune and biomarker status
    b. Peripheral blood RNA//biomarker analysis
    c. Immune functional assay ([redacted])
    d. CTC quantification ([redacted])
7. Blood samples will be obtained for:
    a. Hematology (CBC with differential, RBC with indices, platelet count)

b. Serum Chemistry (BUN, creatinine, ALT, AST, alkaline phosphatase, total bilirubin, LDH, sodium, potassium, chloride, bicarbonate, calcium and glucose).

c. Serum PSA and PAP levels

d. HLA-A2 typing

Dosing is to occur, as follows:

a. Day 1 for PROSTVAC-V or placebo (subcutaneous)*

b. Days 1, 2, 3, and 4 for GM-CSF or placebo (subcutaneous)

* Subjects should remain in the clinic for at least 30 minutes following administration of PROSTVAC-V or placebo for observation of adverse reactions.

7.3 Week 3 Through Week 21 (booster vaccinations)

During the 21-week Treatment phase of this study, the study visits will occur on Weeks 3, 5, 9, 13, 17 and 21 (± 3 days for each individual visit). Medication assignment must be obtained through IVRS prior to dosing for each week of booster vaccinations. The following procedures will be performed and dosing will occur as follows:

To be performed **PRIOR** to administration of study drug:

1. Adverse events (AEs) should be sought by non-directive questioning of the subject, self-reported, and/or directly observed at each clinic visit during the treatment phase of the study.
2. Concomitant medications will be reviewed and recorded at all visits.
3. Abbreviated physical exam (consisting of VS, temperature, and weight). Only significant changes in PE from Screening will be recorded
4. ECOG performance status
5. Blood samples**, except for Week 3**, will be obtained for:
    a. Hematology (CBC with differential, RBC with indices, platelet count)
    b. Serum Chemistry (BUN, creatinine, ALT, AST, alkaline phosphatase, total bilirubin, LDH, sodium, potassium, chloride, bicarbonate, calcium and glucose).
    c. Serum PSA and PAP levels (Weeks 5, 9, 13, 17, and 21)
6. Bone scan
    - Week 13 only
7. CT scan with contrast* (abdomen and pelvis):
    - Week 13, only if clinically indicated

Dosing is to occur, as follows:

a. Day 1 of each week: PROSTVAC-F or placebo**

b. Days 1, 2, 3 and 4 of each week: GM-CSF or placebo

* For subjects with allergies to contrast agents, MRIs for abdomen and pelvis may be performed. Only in cases where MRI is unavailable may a non-contrast CT be performed. For subjects enrolled in Germany, MRI must be used as the primary imaging modality.

**Subjects should remain in the clinic for at least 30 minutes following administration of PROSTVAC-F or placebo for observation of adverse reactions.

7.4 End-of-Treatment Visit – Week 25 or When A Subject Discontinues Treatment

The End-of-Treatment visit is approximately 4 weeks ± 3 days after the last injection of vaccine or placebo. The tests and evaluations listed for the End-of-Treatment/Early Termination Visit should be carried out at the time of any premature subject withdrawal (subject- or investigator-directed) or whenever the investigator feels that the subject will be or is unable or unwilling to make any further visits. If a subject discontinues treatment early for any reason, every attempt should be made to document the reason for the discontinuation.

Any subject who requests to stop study treatment or has been withdrawn from study treatment before completion of the protocol-specified treatment duration will be strongly encouraged to complete the evaluations below, and encouraged to participate in the Long-Term Follow-Up Phase of the study provided the subject has not withdrawn full consent.

1. Adverse events – will be collected by non-directive questioning, self-reported or directly observed occurring since last visit, and discuss and/or resolve any previously reported or ongoing events.
2. Concomitant medications – record all medications being taken currently and record all previously taken, but discontinued drugs.
3. QoL (FACT-P, BPI-SF and EQ-5D-3L) where translations are available
4. Complete PE including VS, sitting BP, heart rate, RR, temperature, and weight
5. ECOG performance status
6. Blood samples will be obtained for:
    a. Hematology (CBC and differential, RBC with indices, platelet count)
    b. Serum chemistry (BUN, creatinine, ALT, AST, alkaline phosphatase, total bilirubin, LDH, sodium, potassium, chloride, bicarbonate, calcium and glucose)
    c. Coagulation panel (PT/(INR) / PTT)
    d. C-reactive protein

e. Serum PSA and PAP

f. Serum for immune and biomarker status

g. Peripheral blood RNA/biomarker analysis

h. CTC quantification (US sites only)

i. Immune functional assays (US sites only)

7. Urinalysis
8. 12-lead ECG
9. Bone scan
10. CT scan with contrast* (abdomen and pelvis)

* For subjects with allergies to contrast agents, MRIs for abdomen and pelvis may be performed. Only in cases where MRI is unavailable may a non-contrast CT be performed. For subjects enrolled in Germany, MRI must be used as the primary imaging modality.

7.5 Assessments During Long-Term Follow-Up

The Long-Term Follow-Up (LTFU) phase begins immediately after the End-of-Treatment visit regardless of the total number of vaccinations actually received by a subject. The first visit is 6 calendar months ± 14 days after the End-of-Treatment visit with consecutive visits every 6 months ± 14 days thereafter. Subjects who progress prior to or during the LTFU phase will continue to be followed for survival and as described below until study closure or death.

First LTFU visit at 6 Months ± 14 Days from the End-of-Treatment visit

1. Concomitant medications (limited to the treatment of prostate cancer therapy). All previously recorded medication entries should be reviewed for stop dates.
2. Prostate cancer treatment update for procedures, including interim unscheduled laboratory tests and/or radiological assessments.
3. Update for newly diagnosed autoimmune disease.
4. Physical exam (limited to assessment of subject's prostate cancer status including prostate cancer progression), vital signs, and weight.
5. QoL (FACT-P, BPI-SF and EQ-5D-3L)
6. Blood samples obtained for:
    i. Serum for immune and biomarker status.
    ii. Peripheral blood RNA/biomarker analysis
    iii. CTC quantification (US sites only)
    iv. PSA and PAP

v. Immune functional assays (US sites only)

7. CT* and bone scan

* For subjects with allergies to contrast agents, MRIs for abdomen and pelvis may be performed. Only in cases where MRI is unavailable may a non-contrast CT be performed. For subjects enrolled in [redacted], MRI must be used as the primary imaging modality.

Subsequent LTFU visits thereafter (every 6 Months ± 14 Days, phone call is acceptable)

1. Survival status
2. Any anti-prostate cancer therapy
3. Update for newly diagnosed autoimmune disease

7.6 Safety Assessments

Subjects will be followed during the Treatment phase of the study for any signs or symptoms of treatment-emergent toxicity by means of an abbreviated physical exam, hematology, serum chemistry panels, ECG, and recording of AEs and concomitant medications.

7.7 Biological and Immune Response Assessments

To evaluate biological responses to vaccinations, serum as well as whole blood samples will be collected from all enrolled subjects at Screening, End-of-Treatment visit, and the first LTFU visit. Serum samples may be analyzed for immune and biomarker status, such as the development of antibodies to the vaccinia and fowlpox virus vectors, the PSA insert, other prostate specific antigens, tumor associated antigens, changes in cytokine and chemokine expression and circulating tumor markers. In addition, whole blood will be drawn into tubes containing an RNA preservative, which will allow for possible future analysis of selected tumor and immune cell gene expression profiles by quantitative PCR as well as other methods.

Circulating tumor cell (CTC) characterization and immune functional analysis will only be performed for the subjects in the United States. Whole blood will be collected for the enumeration of CTC levels and for the isolation of peripheral blood mononuclear cells (PBMCs) for immune functional assays. PBMCs will be evaluated for PSA antigen-specific responses by ELISPOT and flow cytometric-based methods. Determinant spreading to other prostate antigens and tumor-associated antigens will also be examined. All evaluations will be performed at laboratories using standardized procedures. Subject samples may be kept for up to one year past the end of the trial for assay development, implementation, and data review. These described analyses may generate a large dataset that could potentially enable the identification of new potential biomarkers of disease prognosis and/or vaccine response.

7.8 Laboratory Tests and Radiological Assessments

Samples of blood and urine will be obtained for the tests listed in the Schedule of Events. On the day of vaccination all samples will be collected **prior** to vaccine/placebo administration. Follow-up samples will be obtained as clinically indicated. All lab report print-outs will be collected and kept with the appropriate

source documents. Total blood volumes collected for the study will remain within Red Cross guidelines, which specify, “450 ± 50 mL of blood may be collected every 8 weeks.”

8.0 ADVERSE EVENTS

For the purposes of this trial, “study vaccine” is defined as PROSTVAC-V/F. Study medication is defined as study vaccine, GM-CSF, or placebo.

8.1 Investigator’s Responsibilities

Investigators are responsible for monitoring the safety of subjects who have entered this study and for assuring appropriate medical care is provided. In addition, investigators are responsible for alerting BNI or its designee to any event that seems unusual, even if the event may be considered an unanticipated benefit to the subject, and for reporting the event on the appropriate CRF or safety report form.

Investigators are responsible for providing subjects who experience adverse events, especially SAEs that cause subjects to discontinue participation in the study, with appropriate medical care. Frequency of follow-up of any particular adverse event is left to the discretion of the investigator. Duration of follow-up and requirement for immediate SAE reporting (within 24 hours of becoming aware of the event) are described below.

8.2 Definition of Adverse Events (AE)

An AE is any untoward (unfavorable, harmful, or pathologic) medical occurrence in a subject administered a pharmaceutical (investigational) product even if the event does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product whether or not related to the medicinal (investigational) product (ICH E2A II/A/1, 21 CFR 312.32).

For the purpose of this clinical study, adverse events include only treatment-emergent events which are either new or represent detectable exacerbations of pre-existing conditions. Adverse events may include, but are not limited to:

1. Subjective or objective symptoms spontaneously offered by the subject and/or observed by the Investigator or study staff including laboratory abnormalities of clinical significance. Any adverse events experienced by the subject through the completion of final study procedures.
2. AEs not previously observed in the subject that emerge during the protocol-specific AE reporting period, including signs or symptoms associated with metastatic castration-resistant prostate cancer that were not present before the AE reporting period
3. Complications that occur as a result of protocol-mandated interventions

For the purposes of this study, the following exceptions to the definitions above will **NOT** be considered an Adverse Event:

Pre-existing condition: A pre-existing condition (documented on the medical history CRF) is not considered and AE unless the severity, frequency, or character of the event worsens during the study period.

Preplanned hospitalization: A hospitalization planned before signing the informed consent form is not considered an SAE, but rather a therapeutic intervention. However, if during the pre-planned hospitalization an event occurs, which prolongs the hospitalization or meets any other SAE criteria, the event will be considered an SAE. Surgeries or interventions that were under consideration, but not performed before enrollment in the study will not be considered serious if they are performed after enrollment in the study for a condition that has not changed from its baseline level. Hospitalizations for social reasons or due to long travel distances are also not SAEs. Diagnostic testing and procedures: Testing and procedures should not be reported as adverse events or serious adverse events, but rather the cause for the test or procedure should be reported.

8.3 Definition of Expected and Unexpected Events

8.3.1 Expected Events

Expected events are those that have been previously identified as resulting from administration of the investigational product. For the purpose of this study, an adverse event is considered expected when it appears in the current adverse event list, the Investigator's Brochure, the package insert or is included in the informed consent document as a potential risk. An event will be considered expected if the event is thought to be related to a study procedure, and the event occurs with reasonable frequency and similar intensity in the day-to-day care of a patient with prostate cancer treated with GnRH agonists or bisphosphonates. Examples of adverse events that are expected during the course of treatment for prostate cancer with GnRH agonists include, but are not limited to malaise/fatigue, hot flashes/sweating, gynecomastia, testicular atrophy or pain, impotence, and clinically significant and non-significant alterations in laboratory parameters including transient increases in testosterone levels. For subjects taking bisphosphonates (*e.g.*, Zometa), expected events would include flu-like symptoms (fever, chills, myalgia, fatigue), injection site redness, swelling, and pain (for injection or infusion-delivered bisphosphonates); joint and/or muscle pain; osteonecrosis of the jaw, and renal insufficiency. Expected events associated with GM-CSF (at therapeutic doses) include mild to moderate headache, fever, arthralgia, chills, and myalgia. Injection site reactions such as pain, redness, swelling, induration, and pruritus, or systemic injection reactions such as fever ≥ 104°F; myalgia, headache, nausea, fatigue lasting less than 72 hours are expected events for PROSTVAC-V/F.

If, however, the investigator considers an expected event to be unexpectedly severe or more frequent than usual for a patient with prostate cancer treated with GnRH agonists/antagonists, GM-CSF, or bisphosphonates, the event will be captured on the AE CRF (see below). Similarly, if the event is

associated with PROSTVAC-V/F, and is unexpectedly severe, such as a dose-limiting toxicity, it will also be recorded on the AE CRF.

8.3.2 Unexpected Events

Unexpected events are those considered unexpected when it varies in nature, intensity or frequency from the information provided in the current adverse event list, the Investigator's Brochure, the package insert or when it is not included the informed consent document as a potential risk. An event will be considered to be *unexpected* for GnRH agonist/antagonist, GM-CSF, or bisphosphonate therapy or a study procedure if it is not identified in the package insert, or is unexpectedly more severe or more frequent than events described in the package insert, or is related to PROSTVAC-V/F (with the exceptions for injection site and systemic reactions as noted above).

8.4 Collecting and Recording Adverse Events

The Investigator will monitor the occurrence of adverse event for each subject during the course of the study. All AEs (as defined above) reported by the subject, observed by the Investigator, or documented in medical records will be listed on the AE CRF, whether believed by the Investigator to be related or unrelated to the study vaccine.

All AEs must be reviewed, graded and causality determined by an Investigator listed on the form FDA 1572 at the research center reporting the event(s). Collection of adverse events begins at the time the subject signs informed consent and continues for 28 days following administration of the last dose of study medication.

Adverse event terms should be recorded concisely, using acceptable medical terms. When possible, a diagnosis (*i.e.*, disease or syndrome) rather than the component signs and symptoms should be recorded on the CRF (*e.g.*, congestive heart failure rather than dyspnea, rales, and cyanosis). However, signs and symptoms considered unrelated to syndromes or diseases are to be recorded as individual adverse events on the CRF (*e.g.*, if congestive heart failure and severe headache are observed at the same time, each event is to be recorded as an individual adverse event). Only abnormal laboratory values that result in clinical sequelae or require medication for treatment should be recorded as an adverse event. The adverse event should not be recorded as a procedure or clinical measurement (*i.e.*, a laboratory or vital sign). The underlying reason for the procedure or the abnormal clinical measurements, and, whenever possible, a diagnosis, should be recorded. The diagnosis should be recorded; not the individual lab test name.

Death is considered to be an outcome of an adverse event. The cause of death should be recorded on the AE CRF (*e.g.*, congestive heart failure with an outcome of death rather than the term "death").

8.4.1 Progression of Underlying Malignancy

It is anticipated that a proportion of subjects will experience disease progression. When clinical disease progression is identified, the clinical event which marks or identifies the disease progression should be reported as the adverse event term for standard AE reporting, including SAE reporting, per ICH definitions of AE and SAE as reviewed in **Section 8.2** and **Section 8.8** of this protocol, respectively.

For the purpose of this study, progression of underlying malignancy is not considered an (S)AE. Hospitalization, prolonged hospitalization, or death due solely to the progression of underlying malignancy will be captured on the AE CRF, but may NOT be reported as an SAE. Clinical symptoms of progression may be reported as AEs if they cannot be determined to be exclusively due to the progression, or if they do not fit the expected pattern of progression for prostate cancer.

Symptomatic deterioration may occur in the absence of radiographic evidence of tumor progression in some subjects. Alternatively, the disease progression may be so evident that the investigator may elect not to perform further assessments. In such cases, the determination of clinical progression is based on symptomatic deterioration. In these cases, efforts should be made to quantitatively document the progression of the underlying malignancy to the extent possible.

If there is any uncertainty about an adverse event being due to the progression of prostate cancer, it should be reported as an (S)AE.

8.4.2 Pregnancy of a Female Partner

For the purposes of this study, pregnancy of a female partner will be captured using a modified SAE report form, however; the event is not considered an SAE and will be reported to the appropriate regulatory authorities in the annual safety update. Partner pregnancies will be followed through the term of the pregnancy or pregnancy termination and the birth of the child after the female partner has signed the Pregnant Partner Informed Consent Form. Subjects and their partners will be counseled as to any possible known risks to either the partner or the child.

8.4.3 Accidental Transmission of Vaccinia Virus

Accidental transmission of vaccinia virus to a clinic staff member or a member of the subject's family or personal contacts must be reported to the Investigator as soon as possible, but no later than 24hours after he/she becomes aware. The Investigator must notify an Infectious Disease Specialist in the [redacted] Pharmacovigilance Group (PVG) if there is a suspected serious vaccinia reaction as soon as possible, but no later than 24 hours after he/she becomes aware.. The Investigator should contact the BNI study Medical Monitor if there are specific questions in regard to the potential vaccinia reaction. A swab of the suspected lesion may be obtained from the study subject for confirmatory testing prior to administration of VIG. 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. For further information or for a

supply of VIG in the United States, contact the Centers for Disease Control at (877) 554-4625. For centers outside the United States, VIG suppliers (where known) are listed in the Study Instruction Manual. The Investigator should refer to the Management Plan for Potential Serious Vaccinia Reaction. 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.

8.5 Assessment of Relationship to Study Drug (Causality)

Investigators are required to assess the causal relationship (i.e., whether there is reasonable possibility that the study drug caused the event) using the following definitions:

Unrelated: Another cause of the adverse event is more plausible; a temporal sequence cannot be established with the onset of the adverse event and administration of the investigational product; or, a causal relationship is considered biologically implausible.
Possibly Related: There is clinically plausible time sequence between onset of the adverse event and administration of the investigational product, but the adverse event could also be attributed to concurrent or underlying disease, or the use of other drugs or procedures. Possibly related should be used when the investigational product is one of several biologically plausible adverse event causes.
Definitely Related: The adverse event is clearly related to use of the investigational product.

8.6 Severity of Adverse Events (Grading)

Adverse events will be categorized and graded by the Investigator using standard terminology for grading the severity (intensity) of the AE. This study will use the NCI Common Terminology Criteria for Adverse Events (NCI CTCAE v 4.0) five-point scale, Grades 1 to 5 with unique clinical descriptions of severity for each referenced AE.A copy of the CTCAE version 4.0 can be downloaded from the CTEP website at: http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm. A hard copy will be provided by BNI upon request.

Should a subject experience any AE not listed in the CTCAE v4.0, the following grading system should be used to assess severity:

Table 9-1 Adverse Event Grading Criteria

CTCAE Grade	*Equivalent To:*	*Definition*
Grade 1	Mild AE	Experiences which are usually transient, requiring no special treatment, and no disruption of normal daily activity
Grade 2	Moderate AE	Experiences which introduce some level of inconvenience or concern to the subject, and which may interfere with daily activities, but are usually ameliorated by simple therapeutic measures

Grade 3	Severe AE	Experiences which are unacceptable or intolerable, significantly interrupt the subject's usually daily activity, and require systemic drug therapy or other treatment
Grade 4	Life threatening / disabling AE	Experience which causes the subject to be in imminent danger of death
Grade 5	Death related to AE	Experiences resulting in subject death

8.7 Dose Modifications for Toxicity

There are no dose reductions or modifications for dose toxicity for PROSTVAC-V/F. If subjects experience intolerable toxicities due to PROSTVAC-V/F, they will be removed from treatment and will be immediately rolled over onto the Long-Term Follow-Up (LTFU) phase of the study and followed for safety and vital status per protocol.

8.8 Definition of Serious Adverse Events

Note: The terms "severe" and "serious" are not synonymous. Severity (or intensity) refers to the grade of an AE (see below). "Serious" is a regulatory definition. A serious adverse event (experience) or reaction is an untoward medical occurrence that at any dose:

- Results in death (i.e., the AE actually causes or leads to death)
- Is life-threatening (with regards to determining if an AE is serious, "life-threatening" is defined as an AE in which the subject was at risk of death at the time of the event. It does not refer to an event which hypothetically might have caused death if it were more severe. If either the Investigator or the Sponsor believes that an AE meets the definition of life-threatening, it will be considered life-threatening).
- Requires in-patient hospitalization or prolongation of existing hospitalization
- Results in persistent or significant disability/incapacity (i.e., the AE results in substantial disruption of the subject's ability to conduct normal life functions)
- Results in a congenital anomaly/birth defect
- Is medically significant

Medical and scientific judgment should be exercised in deciding whether other situations, such as important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the subject or may require intervention to prevent one of the other outcomes listed in the definition above, should also be considered serious. Examples of such events are intensive treatment in an emergency room or at home for allergic bronchospasm; blood dyscrasias or convulsions that do not result in hospitalization; or development of drug dependency or drug abuse). Given the Investigator's

perspective may be informed by having actually observed the event, the Sponsor is likely to have broader knowledge of the drug and its effects to inform its evaluation of the significance of the event, if either the Sponsor or the Investigator believes that the event is serious, the event will be considered serious.

For the purposes of this trial, death is an expected outcome of disease progression and/or sequelae and will not be considered an SAE. Death due to causes other than underlying disease progression should be reported on the AE and SAE page and should also be reported on the CRF death page.

For the purposes of this trial, the following will NOT be considered SAEs:

- Hospitalizations < 24 hours, or for planned surgeries or procedures.
- Progression of underlying malignancy as a stand-alone event term; clinical events associated with disease progression should be reported as AEs or SAEs as applicable.
- Death due to progression of underlying malignancy

8.9 Definition of Unexpected Serious Adverse Events

An unexpected SAE is one in which the specificity or severity is not consistent with the current applicable product information (Investigators' Brochure or drug prescribing label), or one that has not been observed previously (rather than from the perspective of such experience not being anticipated from the pharmacological properties of the pharmaceutical product).

8.10 Definition of Suspected Adverse Reaction

The FDA has published guidance on the reporting of SAEs (Safety Reporting requirements for INDs and BA/BE studies, December 2012). This document directs Sponsors to consider more carefully the adverse events that are reported in an expedited (urgent) fashion to the FDA. Key elements of this guidance are outlined below:

The guidance defines any adverse event for which there is a "reasonable possibility" that the drug caused the adverse event as a Suspected Adverse Reaction.

"Reasonable Possibility", for the purposes of safety reporting, means there is evidence to suggest a causal relationship between the drug and the adverse event. Examples of evidence that would suggest a causal relationship between the drug and the adverse event are:

- A single occurrence of an event that is uncommon and known to be strongly associated with drug exposure (e.g., angioedema, blood dyscrasias, rhabdomyolysis, hepatic injury, anaphylaxis, and Stevens-Johnson Syndrome).
- One or more occurrences of an event that is not commonly associated with drug exposure, but is otherwise uncommon in the population exposed to the drug (e.g., include tendon rupture or heart valve lesions in young adults, or intussusception in healthy infants). If the event occurs in association with other factors strongly suggesting causation (e.g., strong temporal association, event recurs on rechallenge), a single case may be sufficiently

persuasive; but often, more than one occurrence (from one or multiple studies) would be needed before the Sponsor could make a determination of whether the drug caused the event.

- An aggregate analysis of specific events that can be anticipated to occur in the study population independent of drug exposure. Such events include known consequences of the underlying disease or condition under investigation (e.g., symptoms or disease progression), or events unlikely to be related to the underlying disease or condition under investigation, but commonly occur in the study population independent of drug therapy (e.g., cardiovascular events in an elderly population). An aggregate analysis (across studies) will identify those events that occur more frequently in the drug treatment group than in a concurrent or historical control group.

This definition of "suspected adverse reaction" and the application of the "reasonable possibility" causality standard is considered to be consistent with the concepts and discussion about causality in the International Conference on Harmonization (ICH) E2A guidance.

8.11 Documenting and Reporting of Adverse Events and Serious Adverse Events

The Investigator is responsible for ensuring that all AEs and SAEs that are observed or reported during the study, as outlined in the prior sections, are recorded on the CRF.

An event may qualify for expedited reporting to worldwide regulatory authorities if it is a Serious Adverse Event, Serious Adverse Drug Reaction or Suspected Unexpected Serious Adverse Reaction (SUSAR) in line with relevant legislation, including the European Commission Clinical Trials Directive (2001/20/EC). Expectedness of SAEs will be determined by BNI using the reference safety information specified in the Investigator's Brochure. All investigators will receive a safety letter notifying them of relevant SUSAR reports.

In accordance with the European Commission Directive 2001/20/EC, BNIT or designee will notify the relevant Ethics Committees in concerned Member States of applicable SUSARs as individual notifications or through a periodic line listing.

The Sponsor makes the determination of expedited reporting of an SAE to Regulatory Authorities. Regulatory Authorities, Institutional Review Boards/Research Ethics Boards/Independent Ethics Committees (IRBs/REBs/IECs), and Principal Investigators will be notified of SAEs in accordance with applicable requirements (e.g., GCPs, ICH guidelines, national regulations, and local requirements). The Investigator is responsible for complying with the responsible IRB/IEC's procedures of adverse event reporting. It is the responsibility of the Investigator to promptly inform the relevant IRB/IEC of these new adverse events/risks to subjects, in accordance with the US FDA Code of Federal Regulations (CFR) 21 CFR 312.66.

BNI has an internal medical safety review committee that will review and evaluate accumulating safety data from the entire clinical trial database for PROSTVAC-V/F at appropriate intervals (e.g., quarterly) to identify new safety signals or increased frequency of events.

8.12 Adverse Event Reporting Period

The AE reporting period for this study begins with signing of the informed consent and ends 28 days following administration of the last dose of study medication. If an SAE is present at the EOT Visit, the SAE (and associated AEs and concomitant medications) should be followed to resolution or until the Investigator assesses the subject as stable, or the subject is lost to follow-up or withdraws consent. Resolution/stable means the subject has returned to baseline state of health or the Investigator does not expect any further improvement or worsening of the event.

New SAEs will be collected until 28 days following administration of the last dose of study medication . If an SAE comes to the attention of the investigator after the end of the study, information regarding the SAE should be collected and reported only if assessed as possibly or definitely related by the investigator.

8.13 Assessment of Adverse Events

Investigators will assess the occurrence of AEs and SAEs at all subject evaluation time points during the study. All AEs and SAEs whether volunteered by the subject, discovered by study personnel during questioning, or detected through physical examination, clinically significant laboratory test, or other means will be recorded in the subject's medical record and on the AE CRF and, when applicable, on the Serious Adverse Event (SAE) report form.

Each recorded AE or SAE will be described by its duration (i.e., start and end dates), severity, regulatory seriousness criteria (if applicable), suspected relationship to the investigational product, and any actions taken.

Any SAE that occurs after the subject signs informed consent and until 28 days following administration of the last dose of study medication, irrespective of the treatment received by the subject, must be reported to the [redacted]PVG within 24 hours of the investigator learning of the event.

8.14 Serious Adverse Events

BNI is required to expedite to worldwide regulatory authorities reports of Serious Adverse Events, Serious Adverse Drug Reactions or Suspected Unexpected Serious Adverse Reactions (SUSARs) in line with relevant legislation, including the European Commission Clinical Trials Directive (2001/20/EC); therefore, BNI (or the CRO on the behalf of BNI) must be notified immediately regarding the occurrence of any SAE or SADR that occurs after the subject consents to participate in the study, including SAEs/SADRs resulting from protocol-associated procedures as defined in relevant legislation including 2001/20/EC, performed from screening onwards.

All SAEs (initial and follow-up information) will be reported electronically through [redacted] Remote Data Capture (RDC) platform within 24 hours of the discovery of the event or information. Manual SAE report forms are available in the Study Instructions Manual, however manual SAE report forms may be faxed to [redacted]PVG only in the event RDC is not functioning, and must be entered into RDC when it becomes available. The Sponsor may request follow-up and other additional information from the Investigator (e.g., hospital admission/discharge notes and laboratory results).

[redacted]

All deaths should be reported with the primary cause of death as the AE term, as death is typically the outcome of the event, not the event itself. The primary cause of death on the autopsy report should be the term reported. Autopsy and postmortem reports must be forwarded to the Sponsor's Medical Monitor, or designee, as outlined above.

If study drug is discontinued because of an SAE, this information must be included in the SAE report.

Investigators must report SAEs to their governing IRBs/ECs in writing as soon as possible and in accordance with national and local laws and regulations.

8.15 Treatment and Follow-up of Adverse Events or Serious Adverse Events

From the time the subject signs informed consent until 28 days after the last vaccination, all untoward medical events (as defined in **Section 8.2** and exceptions as noted in **Section 8.4**) will be documented as (S)AEs through the Treatment phase of the study or through the last visit of the Treatment phase of the study for subjects who withdraw early.

All (S)AEs assessed as not related to study vaccine will be followed as clinically indicated until resolved, stabilized, or if non-resolving, until 28 days after the last vaccination, whichever comes first. If a clear explanation is established, it should be recorded on the CRF.

If an SAE is assessed as possibly or definitely related to study vaccine, it must be followed until either the event has resolved, stabilized, or is deemed to be non-resolvable by the investigator.

9.0 STATISTICAL CONSIDERATIONS

The full details of the trial planning computations and planned analyses are contained in a separate Statistical Analysis Plan. Key features are summarized below. This study will be monitored by an independent Data Monitoring Committee and the charter for this committee is based on FDA guidance.

9.1 Study Design

This 1,200 subject (approximately) study is a randomized, double-blind, placebo-controlled, three-arm clinical trial. The primary endpoint is survival time or time to the most recent knowledge of "known to be alive" (censored time). Zero time is defined as the date of randomization.

Subjects will be randomly partitioned with equal probability into one of three double-blinded arms, an investigational arm where the intended intervention is vaccine (PROSTVAC-V/F) plus GM-CSF (Arm V+G), an investigational arm where the intended intervention is vaccine (PROSTVAC-V/F) with placebo GM-CSF (Arm V), and a control arm where the intended intervention is an empty fowlpox vector and placebo GM-CSF (Arm P).

(Arm V+G)	PROSTVAC-V/F plus GM-CSF
(Arm V)	PROSTVAC-V/F plus GM-CSF placebo
(Arm P)	Double placebo (empty fowlpox vector / plus GM-CSF placebo).

The association of intervention to arm will be hidden from clinical personnel and subjects by using a coded identifier for each intervention preparation to be administered. Specifically, the arm designators shown above (V+G, V, and P) are for statistical purposes.

9.2 Randomization

Block randomization will be used to randomly assign subjects in a 1:1:1 manner. Randomization will be stratified by PSA (equal and above, or below 50 ng/mL), and LDH (equal and above, or below 200 u/L). Subjects will be randomized through IVRS/IWRS.

9.3 Analysis Sets

Three analysis sets are defined for this trial. The Intent-to-Treat set (ITT) includes all subjects who are randomized. The full analysis set (FAS) includes all subjects initiating study intervention. The safety analysis set includes all subjects initiating study intervention. The FAS and safety analysis set are the same.

All efficacy analyses will be performed on the ITT set and the FAS. Subjects will be analyzed and summarized based on the arm into which they were randomized. Efficacy analysis performed on the ITT analysis set will be considered to be the primary indicator of efficacy. Analysis performed on the FAS will be considered to be supportive.

All safety analyses will be performed on the Safety set.

9.4 Primary Efficacy Analysis

Two main overall comparisons of survival time are planned: A comparison between Arm V+G and Arm P, and a comparison between Arm V and Arm P. Thus two separate tests will be assessed, and success for the trial is defined as meeting statistical criterion for either of the following comparisons.

- Superiority of Arm V+G over Arm P for all subjects randomized to these arms.
- Superiority of Arm V over Arm P for all subjects randomized to these arms.

The overall type one error probability (alpha error) for the whole study is designed as a one sided P=0.025. There are two main overall comparisons, which therefore necessitate a type one error probability to 0.0125 for each comparison based on using a Bonferroni correction.

The primary analysis methodology will be the stratified log rank test.

9.5 Trial Size

The Arm P reference data for planning this trial is specified as a median survival of 22 months based on the placebo arm of Dendreon D9902B. Based on exponential assumptions the corresponding hazard rate is 0.3781 and the corresponding three-year survival is 32.2%.

The hazard ratio to be used in the specific alternative hypotheses used to compute trial size is specified to be 0.68 for the main comparisons, that is, a 32% smaller hazard rate in Arm V+G or Arm V as compared to Arm P. This hazard ratio is based on hazard ratio estimates from the previously completed randomized Phase 2 trial with an allowance that the Phase 2 trial likely provides an over-optimistic estimate of the true hazard ratio. The hazard ratio estimate from the Phase 2 trial was 0.56, and was approximately 0.5 when delayed effect is taken into account (depending on the characterization of the delayed effect).

Simulations project that 534 events for each between-arm comparison are required for a minimum of 85% power to detect a 32% lower hazard rate. These calculations have taken in to consideration a delayed treatment effect (as found in the randomized phase 2 study). If there is less delayed effect the power will be greater. The projection for the time to realize the required number of deaths for final analysis is 4.6 years following the start of accrual.

9.6 Secondary Efficacy Endpoint

The secondary efficacy endpoint is a binary assessment for Alive without Event (AWE) at six months (or early termination). The event is any of the following on or before six months: radiological progression, pain progression, chemotherapy initiation, or death. Subjects without an event prior to six months will have an evaluation for event at six months. Subjects without event who are not evaluated at six months (or early termination) will be assumed to have had an event. Thus, each subject will be defined as AWE =

1 if the subject is alive and free of event at six months (or early termination) and AWE = 0 otherwise. AWE will have a realization for every subject and therefore is amendable to an intent-to-treat analysis.

The same two comparisons as done for survival will be done for AWE, using the same significance levels. For each comparison the proportions of subjects in each arm for which AWE = 1 will be compared. The probability is 90% or greater that this statistical test will be significant if the true proportion difference is 0.1375 or greater. The critical difference is 0.092 if the control arm proportion is 0.5.

9.7 Safety Analyses

The primary safety analysis set will be all subjects initiating study intervention. The primary focus will be a between-arm comparison of the frequency and severity of safety events without attribution. Supporting analyses will be comparisons of subjects according to intervention received and attribution.

Adverse events will be summarized and tabulated by severity and by relationship to study intervention (attribution). The original terms used by investigators to identify AEs in the CRFs will be translated into preferred terms using current MedDRA Thesaurus. The AEs will be grouped by MedDRA preferred terms into frequency tables according to body system. The number and percentage of subjects experiencing adverse events will be tabulated by body system/preferred term and treatment. When an AE occurs more than once, the maximum severity and causality will be listed only once. Additionally, SAEs and AEs that are possibly or probably related to study intervention will be summarized separately. Data listings of all AEs will be provided by individual subject.

9.8 Interim Analyses

An interim analysis plan (IAP) is included in the SAP for this study. Interim analyses are executed by [redacted] a Contract Research Organization that acts as the independent statistician and assessed by the DMC as described in the DMC Charter. At each interim analysis, both unblinded (closed) and blinded (open) interim analysis reports will be provided to the DMC, while the sponsor will only receive an open (blinded) report unless one of the criteria is met. Even in this case, the DMC will use its discretion regarding the communications of results to the sponsor, with patient safety balanced against benefit being the primary concern.

The IAP was designed to detect early evidence of both efficacy and futility. Interim analyses for the two comparisons of overall survival described in Section 9.4 are to be executed at 40%, 60%, and 80% of information times, corresponding to 214, 321, and 427 deaths per comparison. The final analysis will be completed when 534 deaths per comparison have occurred. To maintain an overall one-sided type I error probability of 0.0125 per comparison, the one-sided significance criteria using one-sided O'Brien-Flemming boundaries for early detection of efficacy analyses are 0.000135, 0.00147, 0.00501, respectively, with the adjusted final analysis to use 0.01063. The significance level to be used for detection of futility at each interim analysis is 0.00001. This futility significance level was chosen in order

to provide a balance between the sought for estimated probability of detecting futility and protecting against falsely detecting futility.

10.0 STUDY ADMINISTRATION AND INVESTIGATOR OBLIGATIONS

10.1 Regulatory and Ethical Compliance

This clinical study was designed and will be implemented in accordance with the protocol, the ICH Harmonized Tripartite Guidelines for Good Clinical Practices, with applicable local regulations (including US Code of Federal Regulations Title 21 and European Directive 2001/20/EC), and with the ethical principles laid down in the Declaration of Helsinki.

10.2 Institutional Review Board (IRB), Research Ethics Board (REB), and Independent Ethics Committee (IEC) Approval

The Investigator will submit this protocol, the informed consent, Investigator Brochure, and any other relevant supporting information (e.g., all advertising materials or materials given to the subject during the study) to the appropriate IRB/REB/IEC for review and approval before study initiation. Amendments to the protocol and informed consent form must also be approved by the IRB/REB/IEC before the implementation of changes in this study.

The Investigator is responsible for providing the IRB/REB/IEC with any required information before or during the study, such as SAE expedited reports or study progress reports.

The IRB/REB/IEC must comply with current United States (US) regulations (§21 CFR 56) (unless a waiver to the IRB requirements of the 1572 has been obtained) as well as country-specific national regulations and/or local laws.

The following documents must be provided to BNI or its authorized representative before entering subjects in this study: (1) a copy of the IRB/REB/IEC letter that grants formal approval; and (2) a copy of the IRB/REB/IEC-approved ICF.

10.3 Institutional Biosafety Committee (IBC) (where applicable)

This protocol and any accompanying material provided to the subject (such as subject information sheets, Informed Consent Form, or descriptions of the study used to obtain informed consent) will be submitted by BNI and/or the site to a legally constituted and chartered Institutional Biosafety Committee (IBC). Additional materials, such as the Investigator's Brochure, will be submitted to the IBC according to the specific Committee and federal (United States' National Institutes of Health or foreign equivalent) requirements. Each site will be approved by the IBC in accordance with local procedures and country-specific regulatory requirements. Documentation of IBC approval must be in place prior to drug shipment to the site.

At the discretion of the specific IBC and within federal requirements, IBC oversight of individual sites may be terminated provided (1) all subjects at that site have completed dosing by at least 28 days, and (2) all investigational materials have been fully accounted for and either returned to BNI, destroyed on site, or

shipped to a duly licensed destruction facility and a shipping and inventory reconciliation records have been filed in the Pharmacy Manual.

10.4 Informed Consent

The informed consent form (ICF) and process must comply with the United States regulations (§ 21 CFR Part 50) as well as country specific national regulations and/or local laws. The ICF will document the study-specific information the Investigator or his/her designee provides to the subject and the subject's agreement to participate.

The Investigator, or designee (designee must be listed on the Delegation of Authority log), **must** explain in terms understandable to the subject the purpose and nature of the study, study procedures, anticipated benefits, potential risks, possible adverse effects, and any discomfort participation in the study may entail. This process must be documented in the subject's source record. Each subject must provide a signed and dated informed consent before any study-related (nonstandard of care) activities are performed (such as screening). The original and any amended signed and dated consent forms must remain in each subject's study file at the study site and be available for verification by study monitors at any time. A copy of each signed consent form must be given to the subject at the time that it is signed by the subject.

11.0 CONTROL AND QUALITY ASSURANCE

11.1 Protected Subject Health Information Authorization

Information on maintaining subject confidentiality in accordance to individual local and national subject privacy regulations must be provided to each subject as part of the informed consent process (refer to **Section 10.4**) either as part of the informed consent form or as a separate signed document (for example, in the US, a site-specific Health Insurance Portability and Accountability Act [HIPAA] consent may be used). The Investigator or designee **must** explain to each subject that for the evaluation of study results, the subject's protected health information obtained during the study may be shared with BNI and its designees, regulatory agencies, and IRBs/REBs/IECs. As the study Sponsor, BNI will not use the subject's protected health information or disclose it to a third party without applicable subject authorization. It is the Investigator's or designee's responsibility to obtain written permission to use protected health information from each subject. If a subject withdraws permission to use protected health information, it is the Investigator's responsibility to obtain the withdrawal request in writing from the subject **and** to ensure that no further data will be collected from the subject. Any data collected on the subject before withdrawal will be used in the analysis of study results.

During the review of source documents by the monitors or auditors, the confidentiality of the subject will be respected with strict adherence to professional standards and regulations.

11.2 Study Files and Record Retention

The Investigator **must** keep a record that lists **all** subjects considered for enrollment (including those who did not undergo screening) in the study. For those subjects subsequently excluded from enrollment, the reason(s) for exclusion is to be recorded.

The Investigator/study staff must maintain adequate and accurate records to enable the conduct of the study to be fully documented and the study data to be subsequently verified. Essential documentation includes, but is not limited to, the Investigator's Brochure, signed protocols and amendments, IRB/REB/IEC approval letters (dated), signed FDA Form 1572 and Financial Disclosures, signed ICFs (including subject confidentiality information), drug dispensing and accountability records, shipping records of investigational product and study-related materials, signed (electronically), dated and completed CRFs, and documentation of CRF corrections, SAE forms transmitted to BNI or designee and notification of SAEs and related reports, source documentation, normal laboratory values, decoding procedures for blinded studies, curricula vitae for study staff, and all relevant correspondence and other documents pertaining to the conduct of the study.

All essential documentation will be retained by the Investigator for at least 2 years after the date the last marketing application is approved for the drug for the indication for which it is being investigated and until there are no pending or contemplated marketing applications; or, if no application is to be filed or if the application is not approved for such indication, until 2 years after formal discontinuation of clinical development of the drug.

The Investigator must notify BNI and obtain written approval from BNI before destroying any clinical study documents or images (e.g., scan, radiograph, ECG tracing) at any time. Should an Investigator wish to assign the study records to another party or move them to another location, advance written notice will be given to BNI. BNI will inform the Investigator of the date that study records may be destroyed or returned to BNI.

BNI must be notified in advance of, and BNI must provide express written approval of, any change in the maintenance of the foregoing documents if the Investigator wishes to move study records to another location or assign responsibility for record retention to another party. If the Investigator cannot guarantee the archiving requirements set forth herein at his or her study site for all such documents, special arrangements must be made between the Investigator and BNI to store such documents in sealed containers away from the study site so that they can be returned sealed to the Investigator for audit purposes.

11.3 Case Report Forms and Record Maintenance

Electronic case report forms (eCRFs) will be used to collect the clinical study data and must be completed for each enrolled subject with all required study data accurately recorded such that the information matches the data contained in medical records (e.g., physicians' notes, nurses' notes, clinic charts and other study-specific source documents). Authorized study site personnel (i.e., listed on the Delegation of Authority form) will complete eCRFs designed for this study according to the completion guidelines that will be provided. The Investigator will ensure that the eCRFs are accurate, complete, legible, and completed within 5 days of each subject's visit. At all times, the Investigator has final responsibility for the accuracy and authenticity of all clinical data.

The eCRFs exists within an electronic data capture (EDC) system with controlled access managed by BNI or its authorized representative for this study. Study staff will be appropriately trained in the use of eCRFs and application of electronic signatures before the start of the study and before being given access to the EDC system. Original data and any changes of data will be recorded using the EDC system, with all changes tracked by the system and recorded in an electronic audit trail. The Investigator attests that the information contained in the eCRFs is true by providing electronic signature within the EDC system. After database lock, the Investigator will receive a copy of the subject data (e.g., paper, CD-ROM or other appropriate media) for archiving at the study site.

11.4 Study Monitoring/ Audit Requirements

Representatives of BNI or its designee will monitor this study until completion. Monitoring will be conducted through personal visits with the Investigator and site staff, remote monitoring, as well as any appropriate communications by mail, fax, email, or telephone. The purpose of monitoring is to ensure that the study is conducted in compliance with the protocol, standard operating procedures (SOPs), and other written instructions and regulatory guidelines, and to ensure the quality and integrity of the data. This study is also subject to reviews or audits.

To assure the accuracy of data collected in the CRFs, it is mandatory that the monitor/auditor have access to all original source documents, including all electronic medical records (EMR) at reasonable times and upon reasonable notice. During the review of source documents, every effort will be made to maintain the anonymity and confidentiality of all subjects during this clinical study. However, because of the experimental nature of this treatment, the Investigator agrees to allow the IRB/REB/IEC, representatives of BNI, its designated agents and authorized employees of the appropriate Regulatory Authority to inspect the facilities used in this study and, for purposes of verification, allow direct access to the hospital or clinic records of all subjects enrolled into this study. A statement to this effect will be included in the informed consent and permission form authorizing the use of protected health information.

BNI or its authorized representative may perform an audit at any time during or after completion of this study. All study-related documentation must be made available to the designated auditor. In addition, a representative of the FDA or other Regulatory Agencies may choose to inspect a study site at any time before, during, or after completion of the clinical study. In the event of such an inspection, BNI will be available to assist in the preparation. All pertinent study data should be made available as requested to the Regulatory Authority for verification, audit, or inspection purposes.

11.5 Investigator Responsibilities

A complete list of Investigator responsibilities are outlined in the clinical trial research agreement and the Statement of Investigator FDA Form 1572, both of which are signed by the Investigator before commencement of the study. In summary, the Investigator will conduct the study according to the current protocol; will read and understand the Investigator's Brochure; will obtain IRB/REB/IEC approval to conduct the study; will obtain informed consent from each study participant; will maintain and supply to the Sponsor or designee, auditors and regulatory agencies adequate and accurate records of study activity and drug accountability for study-related monitoring, audits, IRB/REB/IEC reviews and regulatory inspections; will report SAEs to the Sponsor or designee and IRB/ REB/IEC according to the specifics outlined in this protocol; will personally conduct or supervise the study; and will ensure that colleagues participating in the study are informed about their obligations in meeting the above commitments.

11.6 Sponsor Responsibilities

A complete list of the Sponsor responsibilities is outlined in the clinical trial research agreement and in the laws and regulation of the country in which the research is conducted. In summary, the Sponsor will select qualified Investigators, provide them with the information they need to properly conduct the study, ensure adequate monitoring of the study, conduct the study in accordance with the general investigational plan and protocols and promptly inform Investigators, health and regulatory agencies/authorities as appropriate of significant new adverse effects or risks with respect to the drug.

11.7 Financial Disclosure

A separate financial agreement will be made between each Principal Investigator and BNI or its authorized representative before the study drug is delivered.

For this study, each Investigator and Sub-investigator (as designated on the FDA 1572 form) will provide a signed Financial Disclosure Form in accordance with § 21 CFR 54. Each Investigator will notify BNI or its authorized representative of any relevant changes during the conduct of the study and for 1 year after the study has been completed.

11.8 Liability and Clinical Trial Insurance

In the event of a side effect or injury, appropriate medical care as determined by the Investigator/designee will be provided.

If a bodily injury is sustained, resulting directly from the use of the study drug, BNI will reimburse for reasonable physician fees and medical expenses necessary for treatment of only the bodily injury which is not covered by the subject's medical or hospital insurance, provided that the injury is not due to a negligent or wrongful act or omission by the Investigator/study staff. The Informed Consent Form will include a description of this reimbursement policy, incorporating country-specific national regulations and/or local laws. Financial compensation for lost wages, disability or discomfort due to the study is not available.

Clinical trial insurance has been undertaken according to the laws of the countries where the study will be conducted. An insurance certificate will be made available to the participating sites at the time of study initiation upon request.

11.9 Protocol Amendments

BNI will initiate any change to the protocol in a protocol amendment document. The amendment will be submitted to the IRB/REB/IEC together with, if applicable, a revised model informed consent form (ICF). Written documentation of IRB/REB/IEC and required site approval must be received by BNI before the amendment may take effect at each site. Additionally under this circumstance, information on the increased risk and/or change in scope must be provided to subjects already actively participating in the study, and they must read, understand and sign any revised ICF confirming willingness to remain in the trial.

No other significant or consistent change in the study procedures, except to eliminate an immediate hazard, shall be effected without the mutual agreement of the Investigator and BNI.

12.0 PUBLICATION OF STUDY RESULTS

BNI may use the results of this clinical study in registration documents for Regulatory Authorities in the United States or abroad. The results may also be used for papers, abstracts, posters or other material presented at scientific meetings or published in professional journals or as part of an academic thesis by an Investigator. In all cases, to avoid disclosures that could jeopardize proprietary rights and to ensure accuracy of the data, BNI reserves the right to preview all manuscripts and abstracts related to this study, allowing BNI sufficient time to make appropriate comments before submission for publication.

In most cases, the Principal Investigators at the sites with the highest accruals of eligible subjects shall be listed as lead authors on manuscripts and reports of study results. This custom can be adjusted upon mutual agreement of the authors and BNI.

12.1 Study Discontinuation

The Sponsor reserves the right to terminate the study at any time. Should this be necessary, both the Sponsor and the Investigator will arrange discontinuation procedures. In terminating the study, the Sponsor and the Investigator will assure that adequate consideration is given to the protection of the subjects' interests.

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Relevant Guidelines

- United States Pharmacopoeia
- European Pharmacopoeia
- United States Requirements for Drugs and Biologics (21CFR Parts 200 (Drugs) & 21CFR Parts 600 (Biologics), 2008).
- Guidance on the Development of Vaccinia Virus Based Vaccines Against Smallpox (EMEA/CPMP/1100/02), 2002.
- Recommendations for the Production and Quality Control of Smallpox Vaccine, Revised 2003 (WHO Technical Report Series. No 926), 2004.
- Safety Reporting Requirements for INDs and BA/BE Studies, December 2012

14.0 APPENDICES
14.1 Schedule of Events

All Treatment Visits have a ± 3 day window LTFU Visits have a ±14 day window	Screening	Treatment								Long-Term Follow-Up	
	Week	**1**	**3**	**5**	**9**	**13**	**17**	**21**	**25**	1st visit (6 mos after EoT)	Every subsequent 6 mos[16]*
	Day −28 – −1								EoT		
Dosing: PROSTVAC-V or placebo[1]		X									
Dosing: PROSTVAC-F or placebo[1]			X	X	X	X	X	X			
GM-CSF or placebo[2]		X	X	X	X	X	X	X			
Immune Monitoring**											
Immune/Biomarker Status		X							X	X	
Peripheral Blood RNA		X							X	X	
Tumor CTC (US sites only)		X							X	X	
Immune function (US sites only)		X							X	X	
Procedures											
Informed Consent	X										
Medical History	X										
Prostate Cancer History	X										
Prostate Cancer Treatment Update										X	X
Complete Physical Exam[3]	X								X		
Abbreviated Physical Exam[4]		X	X	X	X	X	X	X		X[13]	X[13]
Vital Signs[5]	X	X	X	X	X	X	X	X	X	X	
Autoimmune Disease Status										X[14]	X[14]
ECOG	X	X	X	X	X	X	X	X	X		
Adverse Events	X	X	X	X	X	X	X	X	X		
Concomitant Medications	X	X	X	X	X	X	X	X	X	X[15]	
ECG	X								X		
Quality of Life Questionnaire***		X							X	X	
Laboratory Assessments											
HLA-A2 typing		X									
PSA and PAP	X	X[8]		X	X	X	X	X	X	X	
Testosterone	X										
Hematology[6]	X	X[8]		X	X	X	X	X	X		
Serum Chemistry[7]	X	X[8]		X	X	X	X	X	X		
Cardiac Markers[9]	X										
HIV, HBsAg, HepC[10]	X										
PT(INR)/PTT	X								X		
C-Reactive Protein	X								X		
Urinalysis	X								X		

14.1 Schedule of Events (Continued)

All Treatment Visits have a ± 3 day window LTFU Visits have a ±14 day window	Screening	Treatment								Long-Term Follow-Up	
	Week	**1**	**3**	**5**	**9**	**13**	**17**	**21**	**25**	1st visit (6 mos after EoT)	Every subsequent 6 mos[16*]
Radiology Assessments											
Bone Scan	X[11]					X			X	X	
CT Scan with contrast (pelvis and abdomen)****	X[11]					X[12]			X	X	

* Until 12 months after the projected number of events is realized or death, whichever is first.

** Immune monitoring samples should be collected only after a patient is eligible by all other criteria and prior to the first dose of study drug (US Only).

*** QOLs include: FACT-P, BPI-SF, and EQ-5D-3L to be performed at Week 1 and Week 25 during the Treatment Phase and at Month 6 during the LTFU Phase.

**** For subjects enrolled in Germany, MRI must be used as the primary imaging modality

1. Subject should remain in the clinic for at least 30 minutes following administration of PROSTVAC-V/F/ placebo for observation of adverse reaction.
2. GM-CSF or placebo will be administered the day of vaccination and following 3 consecutive days.
3. A Complete Physical Examination will include, at a minimum, the general appearance of the subject, height (screening only) and weight, and examination of the skin, eyes, ears, nose, throat, lung, heart, abdomen, extremities, musculoskeletal system, genitourinary system, nervous system, and lymphatic system
4. Abbreviated physical examinations will be driven by problems or issues identified during the Treatment Phase and during the LTFU Phase that have changed since Screening
5. Vital signs will include including sitting BP, heart rate, RR, temperature, and weight
6. Hematology: CBC with differential, RBC with indices, platelets
7. Serum chemistry: BUN, creatinine, ALT, AST, alkaline phosphatase, total bilirubin, LDH, sodium potassium, chloride, bicarbonate, calcium and glucose
8. Blood samples do not need to be repeated at Day 1, if screening samples were run within 7 days of the visit
9. Cardiac Markers (CK, CK-MB, Troponins T and I)
10. Virology testing required unless known negative within the previous 2 months
11. Baseline Bone Scan and CT Scan of the abdomen and pelvis (if not performed within 3 months of the start of Screening); for subjects with allergies to contrast agents, MRIs for abdomen and pelvis may be performed. Only in cases where MRI is unavailable may a non-contrast CT be performed.
12. If clinically indicated
13. Survival status update per **Section 7.5**
14. Update only newly diagnosed autoimmune disease.
15. Only treatments for prostate cancer should be captured during the LTFU Phase.
16. After the initial LTFU visit, all subsequent follow-up LTFU visits can be conducted by phone if the subject cannot visit the clinic.

14.2 NIH RAC Appendix B (excerpt)

Appendix B - Table 1. Basis for the Classification of Biohazardous Agents by Risk Group (RG)

Risk Group 1 (RG1)	Agents that are not associated with disease in healthy adult humans
Risk Group 2 (RG2)	Agents that are associated with human disease which is rarely serious and for which preventive or therapeutic interventions are *often* available
Risk Group 3 (RG3)	Agents that are associated with serious or lethal human disease for which preventive or therapeutic interventions *may* be available (high individual risk but low community risk)
Risk Group 4 (RG4)	Agents that are likely to cause serious or lethal human disease for which preventive or therapeutic interventions are *not usually* available (high individual risk and high community risk)

Appendix B-I. Risk Group 1 (RG1) Agents

RG1 agents are not associated with disease in healthy adult humans.

Appendix B-II. Risk Group 2 (RG2) Agents

RG2 agents are associated with human disease which is rarely serious and for which preventive or therapeutic interventions are *often* available.

Those agents not listed in Risk Groups (RGs) 3 and 4 are not automatically or implicitly classified in RG1 or 2; a risk assessment must be conducted based on the known and potential properties of the agents and their relationship to agents that are listed.

14.3 Criteria of Progression for Trial Eligibility by PCWG2 (2007)

Variable	PCWG2 (2007)
PSA	Obtain sequence of rising values at a minimum of 1-week intervals
	2.0 ng/mL minimum starting value
	Estimate pretherapy PSA-DT if 3 or more values available 4 or more weeks apart

Scher HI, Halabi S, Tannock I, et al. Design and end points of clinical trials for patient with progressive prostate cancer and castrate levels of testosterone: recommendations of the Prostate Cancer Clinical Trials Working Group. J clin Oncol 2008;26:1148-59.

14.4 ECOG Performance Status Criteria

These scales and criteria are used by doctors and researchers to assess how a patient's disease is progressing, assess how the disease affects the daily living abilities of the patient, and determine appropriate treatment and prognosis. They are included here for health care professionals to access.

ECOG PERFORMANCE STATUS*	
Grade	**ECOG**
0	Fully active, able to carry on all pre-disease performance without restriction
1	Restricted in physically strenuous activity but ambulatory and able to carry out work of a light or sedentary nature, e.g., light house work, office work
2	Ambulatory and capable of all selfcare but unable to carry out any work activities. Up and about more than 50% of waking hours
3	Capable of only limited selfcare, confined to bed or chair more than 50% of waking hours
4	Completely disabled. Cannot carry on any selfcare. Totally confined to bed or chair
5	Dead

* As published in Am. J. Clin. Oncol.:
Oken, M.M., Creech, R.H., Tormey, D.C., Horton, J., Davis, T.E., McFadden, E.T., Carbone, P.P.: Toxicity And Response Criteria Of The Eastern Cooperative Oncology Group. Am J Clin Oncol 5:649-655, 1982.

The ECOG Performance Status is in the public domain therefore available for public use. To duplicate the scale, please cite the reference above and credit the Eastern Cooperative Oncology Group, Robert Comis M.D., Group Chair.

14.5 NYHA Classification – The Stages of Heart Failure

In order to determine the best course of therapy, physicians often assess the stage of heart failure according to the New York Heart Association (NYHA) functional classification system. This system relates symptoms to everyday activities and the patient's quality of life.

Class	**Patient Symptoms**
Class I (Mild)	No limitation of physical activity. Ordinary physical activity does not cause undue fatigue, palpitation, or dyspnea (shortness of breath).
Class II (Mild)	Slight limitation of physical activity. Comfortable at rest, but ordinary physical activity results in fatigue, palpitation, or dyspnea.
Class III (Moderate)	Marked limitation of physical activity. Comfortable at rest, but less than ordinary activity causes fatigue, palpitation, or dyspnea.
Class IV (Severe)	Unable to carry out any physical activity without discomfort. Symptoms of cardiac insufficiency at rest. If any physical activity

14.6 NCI Common Terminology Criteria for Adverse Events (NCI CTCAE v 4.0)

A copy of the CTCAE version 4.0 can be downloaded from the CTEP website at: http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm.

A hard copy of the CTCAE version 4.0 can be found in the Study Instruction Manual.

14.7 FACT-P Quality of Life Questionnaire

FACT-P (Version 4)

Below is a list of statements that other people with your illness have said are important. **Please circle or mark one number per line to indicate your response as it applies to the past 7 days.**

	PHYSICAL WELL-BEING	**Not at all**	**A little bit**	**Some-what**	**Quite a bit**	**Very much**
GP1	I have a lack of energy	0	1	2	3	4
GP2	I have nausea	0	1	2	3	4
GP3	Because of my physical condition, I have trouble meeting the needs of my family	0	1	2	3	4
GP4	I have pain	0	1	2	3	4
GP5	I am bothered by side effects of treatment	0	1	2	3	4
GP6	I feel ill	0	1	2	3	4
GP7	I am forced to spend time in bed	0	1	2	3	4

	SOCIAL/FAMILY WELL-BEING	**Not at all**	**A little bit**	**Some-what**	**Quite a bit**	**Very much**
GS1	I feel close to my friends	0	1	2	3	4
GS2	I get emotional support from my family	0	1	2	3	4
GS3	I get support from my friends	0	1	2	3	4
GS4	My family has accepted my illness	0	1	2	3	4
GS5	I am satisfied with family communication about my illness	0	1	2	3	4
GS6	I feel close to my partner (or the person who is my main support)	0	1	2	3	4
Q1	*Regardless of your current level of sexual activity, please answer the following question. If you prefer not to answer it, please mark this box* ☐ *and go to the next section.*					
GS7	I am satisfied with my sex life	0	1	2	3	4

English (Universal)

19 November 2007
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FACT-P (Version 4)

Please circle or mark one number per line to indicate your response as it applies to the past 7 days.

	EMOTIONAL WELL-BEING	Not at all	A little bit	Some-what	Quite a bit	Very much
GE1	I feel sad	0	1	2	3	4
GE2	I am satisfied with how I am coping with my illness	0	1	2	3	4
GE3	I am losing hope in the fight against my illness	0	1	2	3	4
GE4	I feel nervous	0	1	2	3	4
GE5	I worry about dying	0	1	2	3	4
GE6	I worry that my condition will get worse	0	1	2	3	4

	FUNCTIONAL WELL-BEING	Not at all	A little bit	Some-what	Quite a bit	Very much
GF1	I am able to work (include work at home)	0	1	2	3	4
GF2	My work (include work at home) is fulfilling	0	1	2	3	4
GF3	I am able to enjoy life	0	1	2	3	4
GF4	I have accepted my illness	0	1	2	3	4
GF5	I am sleeping well	0	1	2	3	4
GF6	I am enjoying the things I usually do for fun	0	1	2	3	4
GF7	I am content with the quality of my life right now	0	1	2	3	4

FACT-P (Version 4)

Please circle or mark one number per line to indicate your response as it applies to the past 7 days.

	ADDITIONAL CONCERNS	Not at all	A little bit	Some-what	Quite a bit	Very much
C1	I am losing weight	0	1	2	3	4
C6	I have a good appetite	0	1	2	3	4
P1	I have aches and pains that bother me	0	1	2	3	4
P2	I have certain parts of my body where I experience pain	0	1	2	3	4
P3	My pain keeps me from doing things I want to do	0	1	2	3	4
P4	I am satisfied with my present comfort level	0	1	2	3	4
P5	I am able to feel like a man	0	1	2	3	4
P6	I have trouble moving my bowels	0	1	2	3	4
P7	I have difficulty urinating	0	1	2	3	4
BL2	I urinate more frequently than usual	0	1	2	3	4
P8	My problems with urinating limit my activities	0	1	2	3	4
BL5	I am able to have and maintain an erection	0	1	2	3	4

14.8 BPI-SF Quality of Life Questionnaire

STUDY ID# _____ HOSPITAL # _____

DO NOT WRITE ABOVE THIS LINE

Brief Pain Inventory (Short Form)

Date: ____/____/____ Time: ____

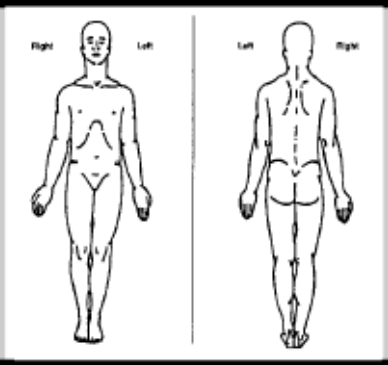
Name: _____ _____ _____

Last First Middle Initial

1. Throughout our lives, most of us have had pain from time to time (such as minor headaches, sprains, and toothaches). Have you had pain other than these every-day kinds of pain today?

1. Yes 2. No

2. On the diagram, shade in the areas where you feel pain. Put an X on the area that hurts the most.



3. Please rate your pain by circling the one number that best describes your pain at its worst in the last 24 hours.

0 1 2 3 4 5 6 7 8 9 10
No Pain — Pain as bad as you can imagine

4. Please rate your pain by circling the one nuimber that best describes your pain at its least in the last 24 hours.

0 1 2 3 4 5 6 7 8 9 10
No Pain — Pain as bad as you can imagine

5. Please rate your pain by circling the one number that best describes your pain on the average.

0 1 2 3 4 5 6 7 8 9 10
No Pain — Pain as bad as you can imagine

6. Please rate your pain by circling the one number that tells how much pain you have right now.

0 1 2 3 4 5 6 7 8 9 10
No Pain — Pain as bad as you can imagine

7. What treatments or medications are you receiving for your pain?

8. In the last 24 hours, how much relief have pain treatments or medications provided? Please circle the one percentage that most shows how much relief you have received.

0%	10%	20%	30%	40%	50%	60%	70%	80%	90%	100%
No Relief										Complete Relief

9. Circle the one number that describes how, during the past 24 hours, pain has interfered with your:

A. General Activity

0	1	2	3	4	5	6	7	8	9	10
Does not Interfere										Completely Interferes

B. Mood

0	1	2	3	4	5	6	7	8	9	10
Does not Interfere										Completely Interferes

C. Walking Ability

0	1	2	3	4	5	6	7	8	9	10
Does not Interfere										Completely Interferes

D. Normal Work (includes both work outside the home and housework)

0	1	2	3	4	5	6	7	8	9	10
Does not Interfere										Completely Interferes

E. Relations with other people

0	1	2	3	4	5	6	7	8	9	10
Does not Interfere										Completely Interferes

F. Sleep

0	1	2	3	4	5	6	7	8	9	10
Does not Interfere										Completely Interferes

G. Enjoyment of life

0	1	2	3	4	5	6	7	8	9	10
Does not Interfere										Completely Interferes

14.9 EQ-5D-3L Quality of Life Questionnaire

By placing a checkmark in one box in each group below, please indicate which statements best describe your own health state today.

Mobility

I have no problems in walking about	❑
I have some problems in walking about	❑
I am confined to bed	❑

Self-Care

I have no problems with self-care	❑
I have some problems washing or dressing myself	❑
I am unable to wash or dress myself	❑

Usual Activities (*e.g. work, study, housework, family or leisure activities*)

I have no problems with performing my usual activities	❑
I have some problems with performing my usual activities	❑
I am unable to perform my usual activities	❑

Pain/Discomfort

I have no pain or discomfort	❑
I have moderate pain or discomfort	❑
I have extreme pain or discomfort	❑

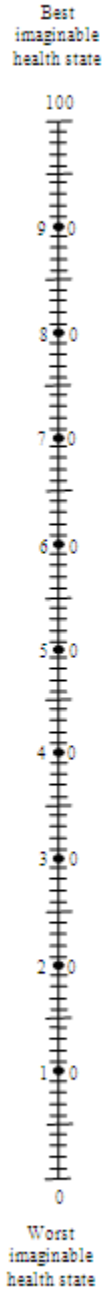
Anxiety/Depression

I am not anxious or depressed	❑
I am moderately anxious or depressed	❑
I am extremely anxious or depressed	❑

To help people say how good or bad a health state is, we have drawn a scale (rather like a thermometer) on which the best state you can imagine is marked 100 and the worst state you can imagine is marked 0.

We would like you to indicate on this scale how good or bad your own health is today, in your opinion. Please do this by drawing a line from the box below to whichever point on the scale indicates how good or bad your health state is today.

Your own health state today



14.10 Vial and Carton Labels

Labels are provided as reference only and are provided as examples. Specific label text and language may vary depending on country.

Labeling for Phase III PROSTVAC-V Outer carton label (Vaccinia)

Labeling for Phase III PROSTVAC-V and placebo vials:

Vial label: (All vial numbers shown are for demonstration purposes only)

Labeling for Phase III PROSTVAC-F Outer carton label (Fowlpox)

Labeling for Phase III PROSTVAC-F and placebo vials:

Vial label: (All vial numbers shown are for demonstration purposes only)

Labeling for Phase III GM-CSF Outer carton label

Bacteriostatic 0.9% Sodium Chloride for Injection, USP,
Secondary Package Label (for tray containing 25 vials, booklet label text)

14.11 List of Research Centers

A list of participating centers will be available on www.clinicaltrials.gov, or upon request to BNI. Actual centers may change over time. Additional sites may be identified and held in reserve in the event that some of the original sites discontinue participation, or if enrollment is slower than expected.

14.12 Rationale and Changes

14.12.1 Rationale

The main reason for this administrative amendment is to address feedback from the [redacted], who requested that the study protocol be updated to contain details regarding the Interim Analyses described in the Statistical Analysis Plan for this study. There is no impact to study design, conduct, or endpoints as a result of the language provided in Section 9.8, Interim Analyses.

In addition, the sponsor address and medical monitor have been updated.

14.12.2 Changes

General changes:

- Update Sponsor Address and Medical Monitor name
- Addition of Section 9.8, Interim Analyses and Section 14.12, Rationale and Changes
- Updated Abbreviation list

New text contained in the new Section 9.8, Interim Analyses is provided below

> An interim analysis plan (IAP) is included in the SAP for this study. Interim analyses are executed by [redacted] a Contract Research Organization that acts as the independent statistician and assessed by the DMC as described in the DMC Charter. At each interim analysis, both unblinded (closed) and blinded (open) interim analysis reports will be provided to the DMC, while the sponsor will only receive an open (blinded) report unless one of the criteria is met. Even in this case, the DMC will use its discretion regarding the communications of results to the sponsor, with patient safety balanced against benefit being the primary concern.
>
> The IAP was designed to detect early evidence of both efficacy and futility. Interim analyses for the two comparisons of overall survival described in Section 9.4 are to be executed at 40%, 60%, and 80% of information times, corresponding to 214, 321, and 427 deaths per comparison. The final analysis will be completed when 534 deaths per comparison have occurred. To maintain an overall one-sided type I error probability of 0.0125 per comparison, the one-sided significance criteria using one-sided O'Brien-

Flemming boundaries for early detection of efficacy analyses are 0.000135, 0.00147, 0.00501, respectively, with the adjusted final analysis to use 0.01063. The significance level to be used for detection of futility at each interim analysis is 0.00001. This futility significance level was chosen in order to provide a balance between the sought for estimated probability of detecting futility and protecting against falsely detecting futility.