A PHASE II TRIAL OF ADJUVANT PROSTVAC-V/F IN SUBJECTS AT HIGH RISK FOR RELAPSE AFTER RADICAL PROSTATECTOMY

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DEFINITION OF TERMS USED

ADT	androgen deprivation therapy
AE	adverse event
AL	allostatic load
ALT	alanine transaminase
APC	antigen-presenting cells
AST	aspartate transaminase
BNI	Bavarian Nordic, Inc, formerly known as BNIT
BUN	blood urea nitrogen
CBCD	complete blood count with differential
CDC	center for disease control
CEA	carcinoembryonic antigen
CFR	Code of Federal Regulations
cGMP	good manufacturing practice
CMP	complete metabolic panel
CRF	case report form
CTCAE	Common Terminology Criteria for Adverse Events
CTSA	Clinical and Translational Science Award
CTEP	Cancer Therapy Evaluation Program
CTL	Cytotoxic T-lymphocytes
DCTD	NCI Division of Cancer Treatment and Diagnosis
DSMC	Data Safety Monitoring Committee
ECOG	Eastern Cooperative Oncology Group
eCRF	electronic case report form
EM	erythema multiforme
EV	eczema vaccinatum
FDA	US Food and Drug Administration
GCP	Good Clinical Practice
GR	glucocorticoid receptor
HCC	Hollings Cancer Center
IB	Investigator's Brochure
IBC	Institutional Biosafety Committee
ICF	informed consent form
ICH	International Conference on Harmonisation
IES	Impact of Events Scale
IGKC	immunoglobulin κ constant
IM	intramuscular
INR	international normalized ratio
IRB	International Review Board
ISEL	Interpersonal Support Evaluation List
IV	intravenous
LLN	lower limit of normal
mCRPC	metastatic castration-resistant prostate cancer
MHC	major histocompatibility complexes
MUSC	Medical University of South Carolina
NCI	National Cancer Institute
PAP	prostatic acid phosphatase
PBL	peripheral blood leukocytes
PBMC	peripheral blood mononuclear cells
Pca	prostate cancer
PFS	progression-free survival
PI	Principal Investigator

PSA	prostate specific antigen
PV	progressive vaccinia
PVE	postvaccinial encephalitis
QOL	quality of life
RBC	red blood cell
RECIST	Response Evaluation Criteria in Solid Tumors
REDCap	Research Electronic Data Capture
RFS	relapse free survival
SAE	serious adverse event
SES	socio-economic status
SIS Unit	Sponsor-Investigator Support Unit
SNP	single nucleotide polymorphism
SNS	sympathetic nervous system
SR	stress response
SUD	subjective units of distress
subQ	subcutaneous injection
SWOG	Southwest Oncology Group
TAA	tumor-associated antigens
TAA	tumor-specific antigens
TRICOM	triad of co-stimulatory molecules
TTP	time-to-progression
TSST	Trier Social Stress Test
ULN	upper limit of normal
VAMC	Ralph H. Johnson Veteran's Administration Medical Center
VIG	Vaccinia Immune Globulin

PROTOCOL SYNOPSIS

Protocol Title:	**A PHASE II TRIAL OF ADJUVANT PROSTVAC-V/F IN SUBJECTS AT HIGH RISK FOR RELAPSE AFTER RADICAL PROSTATECTOMY**
Site Numbers & Names:	1. Hollings Cancer Center, Medical University of South Carolina, Charleston, SC 2. Ralph Johnson VA Medical Center, Charleston, SC
Research Hypothesis:	The hypothesis is that, in patients at high risk for relapse of PCa after radical prostatectomy, treatment with the PROSTVAC-V/F vaccine will improve Relapse Free Survival (RFS) compared with that expected for similar subjects given no adjuvant therapy.
Study Schema:	PROSTVAC-V 2 x 10^8pfu subQ x 1, followed by PROSTVAC-F 1 x 10^9 pfu subQ x 6
Study Objectives:	1. The primary endpoint is to determine the probability of RFS at 2 years after prostatectomy, and to compare it to RFS probability for historical control subjects without adjuvant treatment. 2. Secondary objectives are as follows: a) Compare observed RFSs to the predicted RFS values for the subjects as if they had received no adjuvant therapy. The predicted ("virtual") RFS for each subject will be calculated by standard algorithms. b) Relate serial measurement of immunologic parameters, SNPs to RFS c) Describe the toxicity for the treatment in this patient population
Study Design:	The study will be a Phase II, open-label trial conducted at two centers in the United States.
Accrual Goal:	30 evaluable subjects out of 33 total enrollees
Accrual Rate: **(Number of subjects expected per month)**	1.85 subjects per month x 18 months = 33 subjects. Assuming an in-evaluability rate of about 10%, this will give us 30 evaluable subjects.
Correlative Studies:	1. Lymphocyte, monocytes subsets 2. Immunoglobulin, FcR SNPs 3. Plasma IL6, IL10, VEGF 4. Exon sequencing on tumor from FFPE tissue
Optional Study :	Potential patients will be approached to participate in an optional sub-study looking at sociobiological responses to stress in prostate cancer survivors.

Inclusion Criteria:	1. Age ≥21 2. Completed radical prostatectomy for pathologically-verified adenocarcinoma of the prostate of the prostate no more than 120 days prior to enrollment. The following procedures are acceptable: radical retropubic prostatectomy (RRP), laparoscopic prostatectomy (with or without robotic assistance: RALP), radical perineal prostatectomy (RPP) 3. Post-operative PSA must be undetectable < 0.2 ng/mL by 120 days after prostatectomy. 4. Pts must have one or more of the following: a. pT3b or pT4 primary tumor b. Gleason score 8-10 c. pN1 lymph node disease d. positive surgical margins e. pre-operative PSA of ≥ 10 ng/mL f. presence of any tertiary Gleason 5 component on the prostatectomy pathology report Note: Pts with pT3a disease who lack one of the criteria in 4a-f, and who refuse adjuvant radiation, may also be enrolled. 5. ECOG PS 0-1 6. Adequate hematologic, renal, liver function per parameters described in section 4.3.6 7. Subject of fathering potential must use an adequate method of contraception to avoid conception throughout the study and for at least 4 weeks after the last dose of study drug to minimize the risk of pregnancy. 8. Subjects must have had a negative bone scan, and CT of abdomen and pelvis within 26 weeks prior to registration. Additional forms of imaging (Prostascint scan, MRI) may be substituted for a CT scan of the abdomen and pelvis if clinically indicated.

Exclusion Criteria:	1. Pure small cell carcinoma of the prostate 2. Radiographically-demonstrable metastases prior to, or at the time of enrollment 3. Diagnosis of cancer requiring systemic therapy in the past 5 years 4. Presence of any major medical condition which, in the opinion of the principal investigator, precludes participation in the study 5. Neoadjuvant or adjuvant therapy of any kind 6. Chronic administration (defined as daily or every other day for continued use > 14 days) of systemic glucocorticoids within 28 days of the first planned dose of PROSTVAC-V/F. Use of inhaled steroids, nasal sprays, and all topical preparations (creams, solutions, gels, ointments, etc,) for up to 5% of the body surface area is allowed. 7. Use of systemic immunosuppressant agents including anti-metabolites, glucocorticoids, TNFα antagonists, antibodies to IL6 or IL6R, calcineurin inhibitors, mTOR antagonists 8. Prior history of serious toxicity or systemic reaction to vaccinia immunization such as myopericarditis, progressive vaccinia infection or eczema vaccinatum. 9. Inflammatory or exfoliative skin diseases such as eczema, psoriasis that disrupt epidermis 10. Active infections requiring systemic therapy 11. Serologic evidence of HIV/AIDS. 12. Positive hepatitis C serology or active hepatitis B infection. 13. History of allergy to eggs, egg products, aminoglycoside antibiotics 14. History of myocardial disease, such as myocarditis, cardiomyopathy, congestive heart failure, ischemic cardiomyopathy 15. Prior solid organ or stem cell transplant 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, or Addison's disease). Persons with vitiligo, Hashimoto's thyroiditis, or Graves disease are not excluded. 17. Vaccination with live attenuated vaccine within 28 days prior to day 1 of PROSTVAC-V/F administration or vaccination with a killed vaccine within 14 days prior to day 1 of PROSTVAC-V/F. 18. Inability to avoid close contact or household contact with the following high-risk individuals for three 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. 19. 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.
Criteria for Evaluation:	1. Definition of Relapse: Relapse will be determined if any of the following conditions are met a. increase in PSA to $\geq$ 0.2 ng/mL (confirmed >30 days later) after completion of all PROSTVAC V/F doses. b. development of radiographically-demonstrable metastases c. institution of new prostate cancer therapy d. death Note, RFS at two years will be met if a patient has met none of the above criteria two years post-surgery. RFS as a time to event endpoint will be measured as the time in days from surgery to any of the above conditions being met. 2. Toxicity criteria by the National Cancer Institute Common Toxicity Criteria for Adverse Events (CTCAE) version 4.0

Statistics for Main Study:	**<u>Statistical considerations:</u>** Primary Objective: The primary endpoint will be a binary indicator of relapse status at the 2 year follow-up visit. Enrollment and treatment initiation must take place no more than 120 days after prostatectomy. Two-year RFS will be estimated as a proportion with an exact 95% confidence interval. RFS will also be treated as a time-to-event variable and Kaplan-Meier curves will be created; median and 95% confidence intervals will be reported. The data from this trial will be compared with results from historical control data obtained from a multi-institutional database which includes high-risk, post-prostatectomy subjects without adjuvant therapy (n = 243), and from a published clinical trial (n = 98). The estimated 2-year probability of RFS of high-risk subjects after prostatectomy is 0.6. We define an "interesting" improvement in RFS to be 30% (i.e. increase in the probability of RFS from 0.6 to 0.78). Assuming a one-sided alpha = 0.1, with 30 patients in a group, we have 80% power to detect this difference based on an exact binomial test. Thus if among 30 evaluable subjects, at least 24 are recurrence free at 24 months, we will reject the null hypothesis (2-year RFS=0.60) and conclude that the PROSTVAC intervention improves RFS relative to the RFS of similar patients receiving no adjuvant therapy. Secondary objectives: a) A secondary objective will be a comparison of observed RFS times with the predicted RFS times for the treatment cohort using the Duke and CaPSURE nomograms. The comparison will be performed using the logrank test of the observed and predicted RFSs. b) Biochemical and genomic measurements will be graphically displayed to evaluate their distributions. Transformations will be taken as appropriate. Cox regression will be used to estimate hazard ratios measuring the association between these measures and RFS. 95% confidence will be estimated for the hazard ratios as appropriate. c) Toxicities will be tabulated by CTCAE grade and type.

2 BACKGROUND AND RATIONALE

2.1 RESEARCH HYPOTHESIS

The hypothesis is that the PROSTVAC V/F vaccine will promote the development of an effective immune response leading to long-term disease control in subjects with PCa at high-risk for relapse after prostatectomy.

2.2 BACKGROUND FOR USE OF PROSTVAC-V/F

2.2.1 High-risk PCa

PCa is the most common malignancy among men and the second leading cause of cancer death in men in the United States. The lifetime risk of developing PCa in the United States is 1 in 6, whereas the lifetime risk of death due to PCa is 1 in 34. Treatment options for PCa confined to the gland include watchful waiting, prostatectomy, and radiotherapy. While many cases of early and localized PCa can be cured by radical prostatectomy, one third of patients experience a recurrence of disease. These patients can be identified by clinical parameters through the use of widely-available nomograms (1,2). These high-risk features include 1) T3 or T4 primary PCa, 2) one or more lymph nodes containing metastatic PCa, 3) an initial PSA $\geq$10 ng/mL, 4) involvement of the seminal vesicles, margins, or extraprostatic tissue with cancer. Subjects with these features are at risk for clinically significant recurrence and poor outcomes.

2.2.2 Adjuvant Radiation, Chemotherapy, or Androgen Deprivation Therapy For High Risk PCa

Systemic adjuvant therapy, using cytotoxic agents or hormone manipulation, has been shown to improve overall survival following surgery for subjects with clinical localized cancers of the breast, lung, colon, and other sites. However, adjuvant therapy for subjects treated with radical prostatectomy for PCa has not been widely studied or consistently applied. While traditional local therapies provide adequate control for the vast majority of PCa patients, a substantial minority of patients relapses and is at risk for a fatal outcome. Adjuvant therapy for subjects treated with radical prostectomy for PCa has not been widely studied or consistently applied.

Post-prostatectomy adjuvant therapy for PCa has largely consisted of radiation therapy or ADT. Recent large, prospective, randomized studies demonstrate that external beam radiation to the prostatic fossa can delay biochemical failure, and improve disease-specific survival in subsets of patients with T3a disease, especially those subjects with positive surgical margins (3,4). However, studies are inconsistent as to whether radiation clearly improves outcomes for more advanced cases (pT3b or pN1). In addition radiation can cause significant side effects such as bowel toxicity, increased frequency of strictures, and possibly, secondary malignancies.

Few data exist on the role of adjuvant chemotherapy for patients at high risk of relapse after prostatectomy. The initial studies failed to show clear benefit, however, due to the lack of active agents (5-7). In the past decade the taxanes have been identified as cytotoxic agents with significant anti-tumor activity in PCa (8,9). Attempts have been made to develop chemotherapy regimens with adjuvant activity against PCa. Three large-scale, phase III studies of adjuvant

chemotherapy have been launched, but have been difficult to complete. The SWOG 9921 study compared adjuvant ADT with ADT plus six cycles of mitoxantrone. The study was terminated short of the planned accrual due to an excess of acute leukemia cases in the chemotherapy arm (10). TAX3501 was a multicenter trial comparing ADT with ADT plus six cycles of docetaxel. This study too was terminated after only a small proportion of the total enrollment was accrued. The Veterans Affairs Cooperative Studies Program is sponsoring CSP553, a randomized study comparing prostatectomy versus prostatectomy plus docetaxel for patients with high-risk, localized PCa. Accrual has been completed, but no report has been published. One phase II trial of adjuvant weekly single-agent docetaxel has been presented (11). In this trial 77 post-prostatectomy subjects at high risk for relapse or disease progression were treated. Compared with the predicted PFS for the patients if they had not received adjuvant chemotherapy, there was little or no improvement in PFS from chemotherapy.

The results from studies of adjuvant ADT are also variable. The only randomized, prospective study of adjuvant ADT was the E3886 trial reported by Messing, et al. (12,13). This study demonstrated that ADT given immediately after prostatectomy (rather than waiting for clinical relapse) improves progression-free, disease-specific, and overall survival in PCa patients with positive lymph nodes. Older analyses of adjuvant ADT for other subgroups are ambiguous (14). Recent prospective (15) and retrospective (16) analyses are consistent with a beneficial effect from adjuvant ADT in a wide spectrum of high-risk patients, possibly better than would be expected from observation alone. However, ADT is associated with significant side effects. These include increased body fat, insulin resistance, loss of libido, increased cardiac events, and potentially delay in or prevention of recovery of sexual function after prostatectomy. Furthermore, it is not clear that adjuvant ADT “cures” PCa patients. Progression-free survival curves from the E3886 trial show continuing relapses over time, without a clear plateau or “break” in the downward slope of group survival curves, such as would be seen with a curative treatment.

2.3 STUDY RATIONALE

The rationale is based on the observation that PROSTVAC V/F vaccine can generate an effective immune response against prostate cancer in subsets of patients with advanced disease. Therefore it would be most likely to have the maximum effect when applied at an early stage of cancer, when the tumor bulk is low, when there is not androgen deprivation, and when the patient is in good physical condition. We propose to use PROSTVAC V/F in subjects at high risk of relapse shortly after radical prostatectomy.

2.3.1 Prostate Cancer Immunotherapy

Therapeutic cancer vaccines have been investigated in both castration-sensitive and castration-resistant disease (17). Prostate cancer is an ideal disease 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 not a highly 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, 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 TAA, making for multiple targets suitable for immunotherapy responses (18,19). 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 (20). 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). 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 (21), and up-regulations of cellular immunity and reductions in PSA and PAP levels (22). 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 (23).

2.3.2 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 (24,25). 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-MHC. CTLs recognize and are activated by specific peptides in the context of the appropriate MHC class I molecule on 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 (26). It has also been shown that PSA-specific CTLs could be generated that lyse PSA-expressing prostate cancer cells (26,27). The identification of these peptide epitopes further provides a means of identifying cytolytic T-cells resulting from immunization as an *in vitro* monitoring tool.

2.3.3 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) (27); (28). 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 (27)(28)).

2.3.4 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 (29,30). 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 (31-33). 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 (34-36).

2.3.5 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 profile (37,38). 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.

2.3.6 Fowlpox Virus

Fowlpox virus is a member of the genus *Avipox*, which is evolutionarily divergent from vaccinia virus and serologically non cross-reactive (39,40). 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.

2.3.7 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 (41,42) and has been supported by the results from the completed Phase 1 and Phase 2 trials conducted by the NCI, ECOG, and Therion.

2.3.8 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 (43,44). 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 (45,46). 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 (47-49). 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 (50). Mice immunized with a recombinant vaccinia virus co-expressing carcinoembryonic antigen (CEA) and murine TRICOM exhibited greater immune responses and antitumor 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 (50). PROSTVAC-V and PROSTVAC-F have, therefore, been designed to simultaneously express PSA together with B7.1, LFA-3, and ICAM-1.

2.4 PROSTVAC-V/F FOR PROSTATE CANCER IMMUNOTHERAPY

2.4.1 PROSTVAC-V/F Developmental Program

BNI is developing PROSTVAC-V/F for the treatment of prostate cancer (34). 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, encoding the genes for 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 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.

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, mCRPC.

2.4.2 Summary of Findings from Human Clinical Studies

Clinical investigation of the PROSTVAC-V/F vaccine product in the United States was initiated in 2002 by Therion 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 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.

2.4.3 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 (51). 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 (51). Five of 7 HLA-A2+ subjects in this final dose level had at least a 2-fold increase in PSA-specific T-cells in 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* (52).

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) (53). The arms were thus VAAA, AAAA and AAAV. PSA 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 (36,54). 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 (36).

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-PSATRICOM 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 (30,55). 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 (23).

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) (56); (57).

2.4.4 Clinical Safety of Recombinant Poxvirus-Based Vaccines

2.4.4.1 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 (58,59). Mild adverse reactions that can occur post vaccination are bacterial superinfection of vaccination site, erythema EM and generalized vaccinia. Superinfection is a rare event with incidence from 0.14 to 55 cases per million according to different reports (60). EM most often presents as papules, plaques or 20ocalized20 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) (59,61). 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 20ocaliz, 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 (62). 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.

PV is the most serious complication known. It was almost always fatal prior to the introduction of 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 (59,63). 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 (61).

EV manifests as rash (popular, 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 (64,65). 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 (66). Review of civilian vaccinations did not detect any cases of EV (60). EV can be prevented by thorough screening of at-risk individuals and education on importance of avoiding contacts with such persons and proper hygiene.

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 (67)). 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 (68). 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 (69); (70),). 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 myocarditis or pericarditis among 21ocalize. 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 (71). In civilian population women accounted for 67% of cases and the majority of events (86%) were reported in revaccinees (37,72). Variability between the two sets of data may be explained by differences in demographics of 21 ocalize, case detection, ascertainment and reporting practices (Morgan 2008). Myocarditis and pericarditis has been long associated with a number of viral infections, although there are very few reports of confirmed viremia. A few cases have been reported following DTP and influenza vaccinations (73,74). It is currently assumed that injury to the heart post viral infection is more of an immune inflammatory than direct nature (75,76).

A 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 (61). 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 (77). 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. A 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 (78). The rate of contact vaccinia in 2002 – 2004 was <10 cases per 100,000. Education of 21ocalize 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 preimmune persons. Both of these factors reduce the potential for inadvertent infection versus traditional smallpox vaccine programs.

2.4.4.2 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 (40,79).

2.4.4.3 Safety of Recombinant Poxvirus Vectors in Cancer Immuntherapy

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 (80). These trials represent a large component of the relevant safety database that supported the initiation of the 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), subQ, IM, 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 PROSTVACV/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.

2.5 POTENTIAL BENEFITS AND RISKS TO HUMAN SUBJECTS

The intent of vaccination with PROSTVAC-V/F is to induce an immune response to 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 pre-cautions 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 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 preexisting conditions with features of immune suppression and skin disorders minimize the risk of such rare complications. To minimize risk, potential subjects who have a known allergy to eggs, egg products, aminoglycoside antibiotics, 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.

2.6 BACKGROUND FOR STUDY OF SOCIOBIOLOGICAL RESPONSES TO STRESS IN PROSTATE CANCER

2.6.1 Biological Pathways to Racial Disparities in Prostate Cancer Outcomes

Despite increased access to early detection and the availability of more effective therapeutic strategies, African Americans with prostate cancer continue to experience excess rates of morbidity and mortality from this disease. A vast array of genomic and epigenomic studies of prostate cancer cells, or peri-tumoral stroma, have identified differences among racial groups. An emerging hypothesis about prostate cancer disparities is that social conditions and physiological responses to social stressors influence biological processes, and are important to the aggressive behavior, and poor response to treatment, among African Americans (81). This hypothesis is supported by animal studies showing that chronic stress promotes tumor development and progression in various rodent cancer models (82,83) including prostate cancer models (84). Chronic stress has also been shown to augment the normal age-related senescence of the immune system, enhancing the low of T cell diversity and increasing systemic inflammation (85,86).

The mechanisms through which socio-economic stressors affect several and varied phenotypes in cancer patients are not clear. The postulated mechanisms derive from the HPA axis (1) or the SNS (87) in most studies. In the former case, chronically elevated cortisol has been identified as

a mediator of stress, acting through the polymorphic GR. In the latter case, norepinephrine is thought to regulate VEGF- and PKA-dependent signaling pathways, leading to increased proliferation and migration. Recent work has also implicated stress-induced loss of *TP53* function (88). These pathways are not mutually exclusive, and multiple signaling events may be involved in mediating stress effects.

A variety of social conditions and SES factors may be involved in promoting cancer. Data are most advanced for breast cancer. Social support and perceptions of loneliness are critical to the breast cancer recovery process and morbidity and mortality from disease. Women who have high levels of social support have a decreased risk of breast cancer mortality, even if they are diagnosed with advanced stage disease, whereas social isolation increase the likelihood of breast mortality (89). In addition chronic economic stressors and living in in stressful social environments may contribute to an increased risk of developing advanced breast cancer and triple negative disease. How these stressors function in the setting of prostate cancer is much less clear. Several studies have examined the extent of perceived stress in prostate cancer patients, generally relating these parameters to QOL measurements, or biochemical analyses of stress, or immune system mediators. Hoyt et al (90) found that prostate cancer patients who used avoidant coping strategies had a greater dysregulated cortisol response. Fabre et al (91) has shown that cortisol moderates the effects of adverse life events on testosterone; among those who had high cortisol, the number of stressful life events was positively correlated with total testosterone levels. Similarly, stressful life events were positively correlated with PSA among men with high cortisol levels, but were negatively associated with PSA among those who had low cortisol (92). While stress management methods generally improve QOL, no studies have related stress in prostate cancer patients to an actual anti-cancer effect, or to the development of an anti-cancer immune response.

Even fewer studies have examined the effect of race on stress levels and effects in prostate cancer patients. In a study of multiple socioeconomic stressors, Coker (93) noted a modest association of coping-intensity ("John Henryism") with prostate cancer incidence in African Americans (HR 1.63), but the same effect was noted in Caucasians. Jenkins (94) noted that African Americans were more likely to have concerns about sexual function after primary prostate cancer therapy than were Caucasians. Depression is more likely to be worsened by a prostate cancer diagnosis in African Americans than in Caucasians (95), and African Americans are more likely to have experienced traumatic stress than Caucasians (96). African American prostate cancer survivors generally had poorer mental health QOL than Caucasian, though this difference was erased if there was perceived social support ((97). Dr. Hughes-Halbert and colleagues have recently reported on emotional and physical well-being among African American and Caucasian prostate cancer patients. The former had a significantly better emotional well-being than the latter, a finding at odds with the prior reports of Eton ((98) and Chattre (99). Dr. Hughes-Halbert has also studied the emotional and stress responses of prostate cancer patients shortly after the diagnosis. There were no racial differences in the level of stress or coping mechanisms (100,101).

Despite advances in prostate cancer treatment and increased access to early detection, African American men continue to experience disparities in morbidity and mortality from this disease. Based on data from animal studies, racial disparities in prostate cancer outcomes may involve physiological responses to social stressors that activate biological processes involved in the

initiation and progression of cancer. Until these physiological mechanisms have been examined in human samples, the understanding of the biological basis of disparities in prostate cancer outcomes will be limited. Stress reactivity has not been examined among prostate cancer patients at any stage.

2.6.2 Clinical Model for Study of Stress Effects on Prostate Cancer and Immune Responses

Studying stress response (SR) in conjunction with the immunotherapy clinical trial offers many advantages for identifying SR and other potential immune modulators. An optional sub-study will be made available for patients being screened to the PROSTVAC therapeutic intervention of this study. The goals of the sub-study will be to address critical empirical questions related to underlying physiological processes within the context of allostatic load (AL), by examining the mechanistic underpinnings of immune and stress responses. AL is an indicator of biological dysregulation in response to psychological and social stress and is used as a marker of psychological and social stressor impact on biological functioning (102). The trial is highly feasible and immune responses are likely to be seen in half or more of subjects, providing a wide range on immune response parameters for comparisons.

The sub-study will be the first to use a biobehavioral model to examine biological, psychological, and behavioral pathways that contribute to disease processes and outcomes in this population. Investigators will do this by characterizing stress reactivity among prostate cancer patients based on both biological factors that include individual variation in genetic variants in the GR receptor gene and chronic SES stressors. The sub-study is unique in seeking to relate stress measurements to clinical and immunologic responses. In addition to having a significant impact in terms of understanding the ways in which biological and psychological pathways may contribute to racial disparities in prostate cancer outcomes, the research is highly relevant to precision medicine.

The optional sub-study will involve correlative analyses will demonstrate another distinct feature by studying the pathways within the setting of a clinical trial of prostate cancer immunotherapy. The clinical trial itself was designed and powered to detect a therapeutic effect in prostate cancer patients. It is not a trial designed to measure an irrelevant immune response in normal volunteers or non-cancer patients. Hence, the psychologic insights will be derived from, and applicable to, a “real world” setting. The PROSTVAC vaccine has been in development for several years, and randomized Phase II trials in patients with advanced disease have shown an improvement in overall survival. The PROSPECT trial, designated by the FDA as the registration trial, completed worldwide accrual in December, 2014. Interim analyses will be made in early 2017, with the hope for FDA approval in late 2017 or early 2018. The present trial focuses on a different population, a minimally-treated population after prostatectomy. The physiologic, and likely psychologic, conditions of these subjects are markedly different from those in the PROSPECT trial. MUSC investigators will make comparisons between measurement of SR and other psychologic parameters, and clinical (relapse-free survival), genomic (IgG allotypes), and immunologic (anti-Forssman antibody, antigen-restricted effector T cell populations) measurements. Thus, if the vaccine turns out not to have the desired clinical effect, the investigators will still be able to examine the relationship between stress and immune response development.

Another important aspect of this clinical trial and optional sub-study will be to define the acceptability of stress reactivity measurements in this patient population. The trial will not be powered to detect subtle differences in SR between racial groups. However, an accurate power analysis to establish recruitment goals for a large-scale trial is not possible without initial data on the acceptability of psychologic testing in men with prostate cancer, and the range of SR values to be expected. Project investigators will use the Trier Social Stress Test (TSST) (103) to induce a stress response among the clinical trial participants. The TSST is a standardized psychological stress challenge which has been used extensively in research studies. A meta-analysis supports that it is the gold standard for evoking an HPA axis stress response in a laboratory setting (104). The ACTH and cortisol changes induced by the TSST reflect the sequence of HPA axis activity. That is, ACTH peaks first, at the end of the 15-minute stressor, and returns to baseline level within 40 minutes. Total plasma cortisol peaks 20 minutes after ACTH, and returns to baseline about an hour after initiation of the stressor. Most of the studies of these tools in cancer patients have been in breast cancer and leukemia survivors. Men may be less willing to accurately share their feelings, to participate in role-playing tests, and to learn coping strategies. Indeed, there are no published studies on the use of the Trier Social Stress Test in men with prostate cancer. The data developed during this study will provide a solid foundation for planning large scale studies and will identify core measures of social, psychological, and behavioral factors that are important to measure as part of precision medicine research and health disparities.

3 STUDY OBJECTIVES

3.1 PRIMARY OBJECTIVE

The primary objective is to determine if PROSTVAC-V/F has significant anti-tumor effect when used as a post-surgical adjuvant therapy in subjects at high risk of relapse after prostatectomy. The probability of RFS at 2 years after prostatectomy will be compared with RFS data from similar, published, historical patient series.

3.2 SECONDARY OBJECTIVES

There are four secondary objectives.

- To compare observed RFSs for the study population with the predicted RFSs for the study population. The predicted RFS values will be calculated from each patient's clinical and pathologic data by standard algorithms (Duke and CaPSURE). The observed and predicted RFSs will be presented as Kaplan-Meier plots and comparisons will be perofmed using the logrank test.
- To compare the observed RFS with the RFSs of a historical comparison group of MUSC prostatectomy patients. These patients will be matched to the experimental group for race, Gleason score, and pathological stage. The observed, historical RFSs will be presented as Kaplan-Meier plots and comparisons will be performed using the logrank test.
- To describe associations between the RFS values and various immunologic, genetic, and biochemical parameters on research blood and tissue specimens.
- To describe the toxicity of PROSTVAC-V/F in this patient population.

3.3 SUB-STUDY OBJECTIVES

There are three objectives for the sociobiological response sub-study that is being conducted as part of this trial.

- To evaluate the effects of stress reactivity on the development of an effective anti-tumor immune response in this patient population
- To evaluate the difference in terms of stress reactivity based on SES, perceptions of social stressors, and AL between racial subgroups.
- To evaluate the difference between racial subgroups for the magnitude and distribution of stress responses with biological (AL) and immunologic measure of vaccine effect.

4 INVESTIGATIONAL PLAN

4.1 STUDY DESIGN AND DURATION

This will be a Phase II study of adjuvant PROSTVAC-V/F in subjects at high risk for relapse after radical prostatectomy. The study will be carried out at MUSC and the VAMC. The coordinating center will be at the Sponsor-Investigator Support Unit. Additional sites may be recruited as needed to ensure accrual.

We anticipate a two year period of accrual and an equal period of follow up for each subjects, or approximately four years total, from the point of completions of approvals and contracts.

The study population will be post-prostatectomy patients whose pathology reports documents one or more high risk features, including one or more of the following: pT3b or pT4, pN1, pT3a (without adjuvant radiation), positive margins, pre-operative PSA $\geq$10 ng/mL, Gleason score 8-10. Each site PI will be responsible for reviewing all prostatectomy cases to determine the patient's eligibility and interest in participating in the study.

Forty evaluable subjects (total 44 subjects) will be enrolled. Subjects will be treated with PROSTVAC-V/F alone. Subjects will be followed for relapse for two years after prostatectomy.

Prior to any study specific activities, the patient must be aware of the nature of his/her disease and willingly consent to the study after being informed of study procedures, the experimental therapy, possible alternatives, risks and potential benefits. IRB approval of this protocol and accompanying consent is required.

4.2 PATIENT POPULATION

This study will be conducted at MUSC and the VAMC. There are approximately 80 radical prostatectomies performed yearly at MUSC (30% African American), and 70 at the VAMC (70% African American). Both institutions have extensive experience with prostate cancer clinical trials, and investigators have been particularly successful in recruiting African American subjects. Therapeutic trial accruals in the HCC include approximately 25% African American subjects, reflecting a close match to the 30% prevalence of African Americans in the population of South Carolina. This study will aim to enroll equal numbers of African American and Caucasian subjects.

4.3 INCLUSION CRITERIA

1. Age ≥21

2. Completed radical prostatectomy for pathologically-verified adenocarcinoma of the prostate no more than 120 days prior to start of treatment. The following procedures are acceptable: radical retropubic prostatectomy (RRP), laparoscopic radical prostatectomy (with or without robotic assistance; LAPD), radical perineal prostatectomy (RPP).

3. Post-operative PSA < 0.2 ng/mL by 120 days after prostatectomy

4. Must have one or more of the following:
   - pT3b or pT4 primary tumor
   - Gleason score 8-10
   - pN1 lymph node disease
   - positive surgical margins
   - pre-operative PSA of ≥ 10 ng/mL
   - presence of any tertiary Gleason 5 component on the prostatectomy pathology report.

   Note: Patients with pT3a disease who lack one of the above criteria, and who refuse adjuvant radiation, may also be enrolled.

5. ECOG performance status 0-1

6. Adequate hematologic, renal, liver function per parameters in Table 1

7. Subject of fathering potential must agree to use an adequate method of contraception to avoid conception throughout the study and for at least 4 weeks after the last dose of study drug to minimize the risk of pregnancy.

Table 1. Organ Function Criteria

- Total bilirubin < 1.5x ULN
- Hepatic enzymes (AST, ALT) ≤ 2.5 x ULN
- Serum sodium, potassium, and calcium ≥ LLN
- Serum Creatinine ≤ 1.5 x ULN
- Hemoglobin ≥ 9 gm/dL
- Neutrophil count ≥ 1000/mcL
- Platelets ≥ 100,000/mcL

8. Subjects must have had a negative bone scan, and CT of abdomen and pelvis within 26 weeks prior to registration. Additional forms of imaging (Prostascint scan, MRI) may be substituted for a CT scan of the abdomen and pelvis if clinically indicated.

4.4 EXCLUSION CRITERIA

1. Pure small cell carcinoma of the prostate

2. Radiographically-demonstrable metastases at any time prior to the time of enrollment

3. Diagnosis of cancer requiring systemic therapy in the past 5 years

4. Presence of any major medical condition which, in the opinion of the investigator, precludes participation in the study

5. Neoadjuvant or adjuvant therapy of any kind

6. Chronic administration (defined as daily or every other day for continued use > 14 days) of systemic glucocorticoids within 28 days of the first planned dose of PROSTVAC-V/F. Use of inhaled steroids, nasal sprays, and all topical preparations (creams, solutions, gels, ointments, etc.) for up to 5% of the body surface area is allowed.

7. Use of systemic immunosuppressant agents including anti-metabolites, glucocorticoids, TNFα antagonists, antibodies to IL6 or IL6R, calcineurin inhibitors, mTOR antagonists

8. Prior history of serious toxicity or a systemic reaction to vaccinia immunization such as myopericarditis progressive vaccinia infection, or eczema vaccinatum.

9. Inflammatory or exfoliative skin diseases such as eczema, psoriasis that disrupt epidermis

10. Active infections requiring systemic therapy

11. Serologic evidence of HIV/AIDS.

12. Positive hepatitis C serology or active hepatitis B infection.

13. History of allergy to eggs, egg products, aminoglycoside antibiotics

14. History of myocardial disease, such as myocarditis, cardiomyopathy, congestive heart failure, ischemic cardiomyopathy

15. Prior solid organ or stem cell transplant

16. History of or active autoimmune disease (e.g., autoimmune neutropenia, thrombocytopenia, or hemolytic anemia, systemic lupus, localized lupus, Sjogren's syndrome, scleroderma, myasthenia gravis, Goodpasture's syndrome, or Addison's disease). Persons with vitiligo, Hashimoto's thyroiditis, or Graves disease are not excluded.

17. Vaccination with live attenuated vaccine within 28 days prior to day 1 of PROSTVAC-V/F administration or vaccination with a killed vaccine within 14 days prior to day 1 of PROSTVAC-V/F.

18. Inability to avoid close contact or household contact with the following high-risk individuals for three 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.

19. Any condition which, in the opinion of the investigator, would prevent full participation in this trial (including Follow-Up), or would interfere with the evaluation of the trial endpoints.

4.5 ELIGIBILITY FOR THE OPTIONAL SUB-STUDY

Patients who are being screened for the therapeutic clinical trial will also be approached for participation into the optional sub-study evaluating sociobiological response to stress. Refusal to participate in the sub-study will not impact the patient's eligibility to participate in the therapeutic intervention.

Should a patient consent to the optional sub-study, the patient may proceed with the optional baseline interventions for the optional sub-study as outlined within the protocol's study calendar.

4.6 SUBJECT ENROLLMENT AND REGISTRATION

The SIS Unit will provide patient registration services for the optional sub-study and for the main study.

The SIS Unit will conduct a patient eligibility audit review of all eligibility source documents prior to patient registration. After obtaining signed informed consent(s) and completion of required baseline assessments, eligible subjects will be registered. A unique subject number will be assigned to each patient. The SIS Unit will issue a patient registration confirmation email to the enrolling study team at the time of registration. This confirmation will include the patient's assigned treatment regimen and study ID number. If the patient consented to the optional sub-study, the ID used for that portion of the study will be the subject's study ID. Patient registrations may occur between 8AM and 5PM EST, Monday through Friday.

4.7 DISCONTINUATION OF SUBJECTS FROM STUDY

Subjects must discontinue study treatment and withdraw from the study or optional sub-study for any of the following reasons:

- Withdrawal of informed consent (subject's decision to withdraw for any reason)
- PCa relapse at any time after completion of PROSTVAC administration (see 7.5 for definition)
- Any clinical adverse event (AE), laboratory abnormality, or intercurrent illness which, in the opinion of the investigator, indicates that continued participation in the study is not in the best interest of the subject
- Termination of the study by BNI
- Loss of ability to freely provide consent through imprisonment or involuntarily incarceration for treatment of either a psychiatric or physical (e.g., infectious disease) illness
- Initiation of any form of non-protocol cancer treatment
- Initiation of scheduled narcotics for pain treatment

- Unacceptable toxicity defined as any grade 3-4 possibly or definitely related non-hematologic toxicity that does not improve to grade 1 or resolve within 28 days or any possibly or definitely related grade 3-4 hematologic toxicity that does not improve to grade 1 or resolve with 14 days.
  General or specific changes in the patient's condition render the patient unacceptable for further treatment in the judgment of the investigator.

5 TREATMENTS

5.1 STUDY TREATMENT: PROSTVAC-V/F

Subjects must start study drug within 120 days after surgery.

5.1.1 Identification, Packaging, and Labeling

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 PSA and a 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 IB. 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). Both recombinant pox viruses contain genes for human PSA, B7.1, ICAM-1, and LFA-3. The PSA gene used in these recombinants has an alteration in an HLA-A2 specific epitope (replacement of isoleucine with leucine at amino acid position 155; designated L155). In PROSTVAC-V, all four genes are inserted into the genome of a derivative of the Wyeth strain of vaccinia virus (strain NYCBH). In PROSTVAC-F, all four genes are inserted into the genome of a plaque-purified isolate of the POXVAC-TC vaccine strain of fowlpox virus.

The PROSTVAC-V/F study vaccines are manufactured at IDT's GMP facility in Dessau-Rosslau, Germany in compliance with cGMPs. The formulations do not contain adjuvants or preservatives. The study drug substance is thawed and formulated by dilution in PBS in 10% glycerol. 0.7 ml of the formulated material is dispensed aseptically into sterile 2 mL clear borosilicate glass vials, 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.

5.1.2 Storage, Handling, and Dispensing

Storage*: −70°C or below

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).

PROSTVAC-V/F will be ordered by participating sites directly from the manufacturer.

5.2 SELECTION AND TIMING OF DOSE FOR EACH SUBJECT

5.2.1 PROSTVAC-V/F Treatment

All subjects will receive PROSTVAC V/F in the same dose and schedule.

Drug Administration: Doses of PROSTVAC-V/F will be drawn into labeled syringes by a research pharmacist or designee for use by the clinic staff. PROSTVAC-V will be administered by the designated study staff once by subQ injection on one side (upper arm or upper outer thigh) on Day 1. PROSTVAC-F will be administered by the study staff six times by subQ injection on Days 15, 28, 57, 85, 113 and 141. 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. The doses will contain the following:

- 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

Subjects should be informed on how to care for the wound after each injection of the study agent.

Dose Modification or Interruptions: There will be no modification or delays in dosing of PROSTVAC V/F for toxicity If there are unresolved toxicities related to PROSTVAC V/F at the time the next dose is due, the patient should be taken off study. PROSTVAC V/F treatment should be initiated within 120 days after prostatectomy. If there is a delay due to scheduling, within the +/- 7 days window, it will be at the investigator's discretion whether to have the subject continue.

6 CONCOMITANT TREATMENTS

6.1 PROHIBITED AND/OR RESTRICTED TREATMENTS

- Subjects may not receive any other form of anti-cancer therapy during this study except for PROSTVAC V/F.
- Daily use of finasteride, dutasteride, is not permitted during this study
- Use of supplements reported to delay progression of prostate cancer (vitamin D3 over 1000IU daily, pomegranate juice or extract, lycopene) is not permitted.
- Testosterone, estrogen agonists (estradiol, diethylstilbesterol), progesterone agonists, or DHEA-containing medicines or supplements is not permitted during the study.

6.2 OTHER RESTRICTIONS AND PRECAUTIONS

- Subjects should not start treatment with any immunosuppressive agents during study. This includes daily oral or parenteral glucocorticoids, antimetabolites, calcineurin inhibitors, mTOR inhibitors, cytokine antagonists.
- Subjects should not receive live attenuated vaccine within 28 days prior to PROSTVAC-V/F administration or a killed vaccine within 14 days prior to or after completion of PROSTVAC-V/F.

6.3 TREATMENT COMPLIANCE

Treatment compliance for PROSTVAC V/F will be ensured by administration of the agent in the treating physician's office or infusion center.

7 STUDY ASSESSMENTS AND PROCEDURES

Scheduled treatments, procedures, and assessments are shown in the table contained in Appendix A.

7.1 THERAPEUTIC TRIAL PROCEDURES

A variety of clinical assessments are made during the screening period and while the subject is on study. These include the following. Specific details about the timing of these procedures can be found in the study calendar (Appendix A):

- Patients will be given instructions on how to care for the injection site after each injection of PROSTVAC-V and PROSTVAC-F.
- History and physical examination. The history includes a review of the prostatectomy pathology report to ensure that it includes the following information: date of surgery, subject's age at surgery, pre-op PSA, Gleason score, margin status, extraprostatic extention, seminal vesicle status, lymph node status. A measurement of the PSA at 45 or more days after surgery is also necessary, if the post-prostatectomy PSA nadir is not < 0.2 ng/mL before then.
- CBCD
- CMP (sodium, potassium, chloride, bicarbonate, BUN, creatinine, glucose, albumin, alkaline phosphatase, AST, ALT, calcium);
- Lipid Panel (total cholesterol, HDL cholesterol, triglycerides, LDL cholesterol, non-HDL cholesterol). Lipid panel will only be done at baseline and requires patients to be fasting. If a fasting blood draw cannot be done at screening (prior to registration), it may be done on day 1 prior to agent administration.
- PSA
- Testosterone
- C-reactive protein
- HIV, Hep B and Hep C serology screening, unless known negative within previous 2 years prior to registration.

- Scans: Technetium bone scan (but not ^{18}F-fluoride PET scan); CT scan of abdomen, pelvis (a Prostascint scan or MRI of abdomen, pelvis can be substituted). Scans can be done up to 26 weeks prior to start of treatment.

Correaltive research will include the following:

- FACT-P, AUA SS, and SHIM quality of life questionnaires
- Collection of blood for research (refer to Section 8):
  - 10 mL blood in a sodium heparin tube
  - 7 mL blood in a lithium heparin tube
  - 21 mL blood in a EDTA tube
  - 7 mL blood in a serum (clot) tube
- FFPE tissue, if available: 5 thick (20 micron) sections on plastic slides for genomic analysis, plus 5 additional thin (5 micron) sections
- Social Determinants Survey: Patients will be given the option to complete the survey via telephone administration or in-person during the clinic visit of their first vaccine treatment. Surveys that are completed over the telephone will administered by a trained research assistant from MUSC to obtain information on SES and to measure social factors (e.g., perceived loneliness), perceived stress, behavioral factors (i.e. exercise and diet), shared decision-making with providers, and knowledge and perceptions about precision medicine. Surveys completed during the clinic visit will be self-administered. The survey will take 30 minutes to complete.

7.2 OPTIONAL SUB-STUDY PROCEDURES

For patients who provide consent for the optional sub-study the following additional procedure will be completed after providing consent to the optional sub-study and prior to the first PROSTVAC treatment.

- TSST: 70 minute stress test that includes saliva samples, blood pressure and heart rate monitoring and subjective units of distress (SUD) scale. More information about the TSST procedures is outlined in Section 8.

7.3 OFF-STUDY ASSESSMENTS

Off study assessments will consist of those procedures indicated for day 730 if the patient's participation ends at that time, or at any earlier time. These include:

- clinical examination (history and physical examination);
- assessment of toxicity or adverse events;
- CBCD;
- CMP
- bone scan and CT scan of abdomen, and pelvis (a Prostascint scan or MRI of abdomen, pelvis can be substituted). Scans will not be repeated at the off-study point if they have been performed in the preceding 2 months.
- PSA;

- total testosterone;
- research assessments
    - Blood samples-See Section 8 for details
    - Quality of Life questionnaires

7.4 SAFETY ASSESSMENTS

Safety assessments will be performed continuously throughout the entire study, and will occur at every clinic visit, as indicated in Appendix A. At the minimum assessments will include a complete history and physical examination, and the following laboratory studies: CBCD, CMP, and testosterone.

Toxicities will be defined by the CTCAE v 4.0.

7.5 EFFICACY ASSESSMENTS

Efficacy assessments will primarily be performed by measurement of PSA and by physical assessment. Imaging studies (CT scans of the abdomen and pelvis, and bone scans) will be performed at baseline (up to 16 weeks prior to treatment) and at the end of the study (or within the preceding 2 months before end of study). Otherwise, scans will be performed only if clinically indicated for evaluation of relapse or off study. Additional forms of imaging (Prostascint scan, MRI) may be substituted for a CT scan of the abdomen and pelvis if clinically indicated. However, the imaging method used at baseline will be used for follow up scans. Compliance with therapy may be considered a component of the efficacy assessment. Compliance with PROSTVAC-V/F will be monitored at each visit by interviewing the patient and reviewing the infusion center records for administration.

Both primary and secondary efficacy assessment involve measurement of RFS. Recurrence will be defined as the occurrence of any of the following: :

- Institution of new prostate cancer therapy
- First detection of a PSA level ≥ 0.2 ng/mL (confirmed > 30 days later) after completion of all PROSTVAC V/F vaccine doses
- Development of radiographically-demonstrable metastases
- Death

RFS at two years will be met if a patient has met none of the above criteria two years post-surgery. RFS as a time to event endpoint will be measured as the time in days from surgery to any of the above conditions being met.

7.5.1 Primary Efficacy Assessment

The primary study endpoint is recurrence-free status at 2 years after prostatectomy. RFS will be defined as in Section 10.2. Observed probability of RFS will be compared with the same parameter obtained from similar historical patient series.

7.5.2 Secondary Efficacy Assessments

The secondary efficacy assessment will consist of a comparison of the observed RFS values with predicted RFS values calculated for the enrolled subjects by the Duke and CaPSURE algorithms.

The RFS values will be plotted using Kaplan-Meier methods, and the observed and predicted RFS values will be compared using the logrank test.

8 CORRELATIVE STUDIES

8.1 RESEARCH BLOOD COLLECTIONS

A variety of analyses will be performed on the research blood samples collected for this study. To evaluate biological responses to vaccinations, plasma, serum, as well as whole blood samples will be collected from all enrolled subjects. Plasma will be analyzed for the development of antibodies to the vaccinia and fowlpox virus vectors, the PSA insert, other prostate specific antigens, as well as tumor associated antigens. Serum will also be profiled for changes in cytokine and chemokine expression and circulating tumor markers. Immune function analysis will be performed by isolation of PBMCs for immune function 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 will generate a large dataset that will potentially enable the identification of new biomarkers of disease prognosis and/or vaccine response.

Research assessments will not be made in real time, since they will require analyses that will be performed only in "batch mode" when samples are available. Research samples will be collected as indicated and stored for future assays, in accordance with MUSC and VAMC policies.

All patients will be expected to provide blood for research purposes per the timepoints outlined within the study calendar (Appendix A). Each research blood collection will require 38mL of anticoagulated peripheral whole blood.

Research Blood Collection Timepoints	**At each blood collection, collect the following**	
	Amount of Blood	**Tube type**
Baseline Day 85 Day 225 Day 730 or off study	10 mL	sodium heparin tube
	7 mL	lithium heparin tube
	21 mL *(three 7 mL tubes)*	EDTA tube
	7mL	Blood (clot) tube

8.1.1 Measurement of Antigen-Specific T-Cell Functions

Peripheral blood samples will be collected in EDTA vacutainer tubes prior to vaccination, one week after the third immunization, and one week following final PROSTVAC administration. Red blood cells will be lysed with ACK buffer for isolation of peripheral blood leukocytes (PBL). PBLs will be frozen in 90% FBS/10% DMSO using a controlled-rate freezer, and immediately stored in liquid nitrogen. Phenotypic characterization and detection of prostate specific antigen (PSA)-specific cytokine production from T cells within previously cryopreserved PBL will be performed

simultaneously. Briefly, PBLs will be stimulated at $5x10^6$ cells per test with peptide pools consisting of sequential 15mer peptides overlapping by 11 amino acids spanning the compete human PSA protein at 1µg/ml/peptide (Miltenyi), 1% DMSO negative control or polyclonal activation via 50 ng/mL phorbol myristate acetate (PMA) and 1 µg/ml ionomycin for 6 hours. Brefeldin A (10 mg/mL) will be present for the last 5 hours. Samples will be labeled to exclude dead cells by incubation with amine reactive UV Live/Dead dye (Molecular Probes). Subsequently, T cell memory subsets will be phenotyped by staining for surface antigens using monoclonal antibodies specific for CD3, CD4, CD8, CD45RA, CD45RO, CCR7 and CD64L allowing for detection of naïve, stem cell memory, central memory, effector memory and terminally differentiated effector $CD4^+$ and $CD8^+$ T cells. Cells are then fixed and permeabilized by treatment with BD Cytofix/Cytoperm solution (BD Pharmingen) followed by labeling with monoclonal antibodies specific for IFN-γ, IL-17A, IL-4, and IL-10 for 30 min at 4^0C in the presence of permeabilizing staining buffer to identify predominant T_H1, T_H17, T_H2 and Treg cytokines, respectively. Cells will be collected immediately on a BD Fortessa X-20 flow cytometer capable of detection of up to 18 parameters (Beckman Dickinson). At least 300,000 events will be collected and data analyzed using FlowJo software. Values 3-fold higher than the DMSO background are scored as positive.

The proposed assay provides state-of-the-art immune monitoring of vaccine-induced PSA-specific immunity via multiparametric flow cytometry. Utilizing cutting-edge techniques, project investigators will simultaneously measure T cell memory status and determine the overall cytokine profile of vaccine-induced $CD8^+$ and $CD4^+$ T cell subsets. Detailed characterization of antitumor immunity will allow for the identification of immune correlates of protection from disease progression.

8.1.2 Measurement of Anti-Forssman Antibodies

Antibodies to heterophile antigens such as the glycolipid Forssman antigen commonly develop during certain infections. A recent report documented the appearance of antibodies to Forssman antigen during treatment of prostate cancer patients with PROSTVAC (32). Antibodies appeared in a high proportion of subjects (64%) and in high titer. Moreover, the appearance of anti-Forssman antibodies correlated with overall survival after vaccination, and the antibody titer was directly proportional to length of survival. The appearance of anti-Forssman antibodies during PROSTVAC therapy may represent a generalized activation of the immune system, or possibly antigen spreading. Project investigators will use a competitive immunoassay to detect anti-Forssman antibodies in serum from trial subjects before treatment, after the third injection, and after the final injection. Sheep RBCs will be purchased from commercial sources and used as a source of Forssman antigen. Washed RBCs will be incubated with serial dilutions of patient plasma, and with a FITC-labelled anti-Forssman monoclonal antibody. Binding of the labelled antibody will be measured by flow cytometery. Increasing amounts of anti-Forsmman antibody in patient plasma will compete with the labelled antibody and reduce its binding, leading to decreased signal in the flow histogram. The amount of bound, labelled antibody will be quantified as the difference in maximum fluorescence intensity of the histogram curves, with irrelevant, isotype-matched antibody as a negative control, and labelled anti-Forssman antibody only (no competitor) as the positive controls. The result will be expressed as serum titer that inhibits binding of the labelled antibody probe by 90%.

8.1.3 Measurement of IgG Allotypes GM and KM

Polymorphic hereditary antigenic determinants present on IgG heavy chains are called GM allotypes (33, 34). These determinants are inherited as autosomal codominant genes according to Mendelian laws. They are encoded by three very closely linked genes—immunoglobulin heavy chain G1 (IGHG1), IGHG2, and IGHG3—on chromosome 14q32. GM alleles are expressed on the constant region of ϒ1, ϒ2, and ϒ3 chains. There are currently 18 serologically testable GM specificities—four on ϒ1 (1/a, 2/x, 3/f, 17/z), one on ϒ2 (23/n), and 13 on ϒ3 (5/b1, 6/c3, 10/b5, 11/b0, 13/b3, 14/b4, 15/s, 16/t, 21/g1, 24/c5, 26/u, 27/v, 28/g5). Linkage disequilibrium in the GM gene complex within a racial group is almost absolute and the determinants are transmitted as a group—haplotypes.

Polymorphic hereditary antigenic determinants present on ϒ-type light chains are called KM allotypes. Three alleles—KM 1, KM 1,2, and KM 3—segregate at the immunoglobulin ϒ constant (IGKC) locus on chromosome 2p12. KM 1 allele, without KM 2, is rare; 98% of the individuals positive for KM 1 are also positive for KM 2. Thus, positivity for KM 1 includes both KM 1 and KM 1,2 alleles (33,34).

Immunoglobulin GM genes are one of the most powerful tools for genetic characterization of human populations. For instance, unless there is genetic admixture, GM 3 is not found in African Americans; GM 6 is present only in people of African ancestry; GM 1 is polymorphic only in people of European ancestry. Their racially associated prevalence makes them especially suitable for investigations involving racial disparity in prostate cancer. Furthermore, these markers have been shown to contribute to both cellular and humoral immunity to several tumor-associated antigens in a racially restricted manner ((105-112)(113-115)). Thus, this project could potentially identify racially-specific genetic correlates of PROSTVAC vaccine efficacy. Immunoglobulin GM and KM genotyping may provide an import co-variant to measure along with stress responses. Because of almost absolute linkage disequilibrium between particular GM allotypes within a race, not all markers need to be typed to determine the major haplotypes segregating in a population.

8.1.4 TSC22D3/GILZ Genotyping

Psychological stress negatively influences cancer mortality, but the underlying molecular mechanisms are not known. Stress increases the secretion of glucocorticoids, which upregulates the transcriptional factor TSC22D3, also known as glucocorticoid-induced leucine zipper (GILZ). In mice, TSC22D3 acts as a “checkpoint” for anti-cancer immune responses. In humans, the gene encoding GILZ is located on human chromosome Xq22.3. Several single nucleotide polymorphisms (SNPs) have been identified in this gene. Using TaqMan® genotyping assays from Applied Biosystems Inc. and DNA from the trial participants, we will genotype for the following SNPs with appreciable minor allele frequencies:

rs12858266, rs17254207, rs3747406, rs6523975, rs6523976

These SNPs may influence the qualitative and quantitative expression of the TSC22D3/GILZ protein, resulting in interindividual differences in stress-induced

immunosuppression. We will determine whether these genetic markers are associated with allostatic load, stress reactivity, and the relapse risk in the vaccinees.

Thus, the SNPs to be genotyped in this project could be potential genetic modifiers of psychosocial stress and the outcome of immunotherapy in prostate cancer

8.1.5 GR Genotyping

Trial participants will be genotyped for variants in the BclI polymorphism (rs41423247) by collecting whole blood for genetic analysis. Blood samples will be stored at -80C for batch DNA extraction and genetic testing. Genomic DNA extraction from frozen blood will be done using the Purelink DNA extraction kit (QiaGen). Genotyping will be done using RFLP analysis and verified by sequencing at the MUSC Genomics Center.

8.2 FORMULIN FIXED PARAFFIN EMBEDDED TISSUE

An attempt will be made to acquire FFPE tissue from all subjects, since study enrollment will be in close temporal proximity to the acquisition of the prostatectomy specimen. Should tissue be available for research, the study requires 5 thin (5 micron) sections on glass slides for H&E and IHC staining.

8.3 QUALITY OF LIFE PATIENT QUESTIONNAIRES

All study participants will be administered three questionnaires: FACT-P, AUA SS, and SHIM. The questionnaires will be used to assess the impact of therapy on the patient's perception of their quality of life the intervals as indicated within the study calendar. Questionnaires may be mailed to the subject in pre-metered envelope if they are unable to come into the clinic to complete them (ie: due to COVID19 travel restrictions). The subject will complete the questionnaires at home and then mail the completed questionnaires back to the main site in the provided envelope.

8.4 SUB-STUDY SOCIAL DETERMINANTS SURVEY:

For patients who consent to the main study, SES, social factors, behavioral factors, shared decision-making, knowledge and perceptions of precision medicine, and perceived stress measurements will be obtained by self-report. Patients will have the option to be administered the survey over the telephone, or they can complete the survey during their clinic visit of the first vaccine treatment. This survey will provide a numeric readout. Investigators will measure chronic SES stressors in terms of income, employment status, and financial strain. The Life Events Questionnaire (LEQ; (116)) is a validated measure that captures life events across multiples areas. The Health, Work (e.g., difficulty finding a job); Residence (e.g., difficulty finding housing), and Financial subscales of the LEQ will be used for the purposes of the current study. In addition, the validated Perceived Stress Scale (117) and the Everyday Discrimination Scale ((118)) will be used to capture perceived stress and discriminatory stress, respectively.

Investigators will use the short form of the Loneliness Scale (119) and Interpersonal Support Evaluation List (ISEL; (120)) to measure social isolation and support, respectively. Financial strain will be measured by a validated Likert-style item that asks participants if they have some, just enough, or not enough money left over at the end of the month. Cancer-related stressors will

be measured in terms of perceived stress about being diagnosed with prostate cancer and clinical stressors) and perceived risk and control about prostate cancer recurrence. Perceived risk and control will be measured using items from previous research on cancer recurrence among breast cancer survivors ((121)). Items relating to knowledge and perceptions of precision medicine were adapted from previous research on genetically targeted care (123). Health behavior items measuring moderate exercise and diet behaviors were taken from the validated NCI-HINTS (124). Investigators will also measure clinical stressors in terms of stage and grade of prostate cancer diagnosis and the presence of surgery-related side effects. Clinical stressors will be abstracted from medical records.

8.5 OPTIONAL SUB-STUDY TRIER SOCIAL STRESS TEST

For patients who consent to the optional sub-study, the TSST ((103)) will be used to induce and measure a stress response. The TSST is a standardized psychological stress challenge which has been used extensively in research studies. A meta-analysis supports that it is the gold standard for evoking an HPA axis stress response in a laboratory setting ((104)). The ACTH and cortisol changes induced by the TSST reflect the sequence of HPA axis activity. That is, ACTH peaks first, at the end of the 10-minute stressor, and returns to baseline level within 40 minutes. Total plasma cortisol peaks 20 minutes after ACTH, and returns to baseline about an hour after initiation of the stressor. The TSST will be administered at the South Carolina Clinical and Translational Research Institute (SCTR) at MUSC.

Effort will be made to ensure that the experimental procedures and assessments for the TSST are collected at the same time point for each participant to control for changes in cortisol levels due to time of day (diurnal patterns). However, should a time variation occur, this will be considered a deviation, and patients will have to be rescheduled for their TSST. A time lapse of no more than 15 minutes will be permissible for conducting the TSST. The times of procedures will be collected within the source worksheets and any time delays will be recorded.

Because of the logistics for scheduling and the TSST assessment, the timeframe for conducting the assessment may be prior to the main study registration. The TSST must be administered after the patient provides consent to the optional sub-study and any time before the patient begins PROSTVAC treatment. The TSST experimental stress challenge should begin at 4:00 pm. During the TSST, participants are told at 4:00 pm that he will soon perform to an audience which includes a speech and arithmetic task. The topic of the speech is why he should be hired for a particular job (the individual's "dream job"). The participant will deliver the speech as though speaking to a group of hiring managers. The experimenter then tells the participant that
he has five minutes to prepare the speech, and starts the countdown clock (which is placed in view of the individual). The experimenter leaves the room to allow the participant to prepare. At 4:33 pm, the participant is escorted by the experimenter to an interview room, where three individuals unfamiliar to the participant (the audience) are seated; the individual is instructed by one audience member (the spokesperson) to stand and begin his prepared speech (without notes).

The speech is delivered for five minutes. At the end of the speech task (4:44 pm), the individual is instructed to serially subtract 13 from 1,022 as quickly and accurately as possible. If the

participant makes a mistake, he is told to begin again at 1,022. The mental math recitation continues for five minutes, at the end of which time, the spokesperson instructs the individual to stop. The experimenter enters the room and escorts the participant back to the initial room. The TSST, in total, spans 10 minutes. The first post-stressor assessment occurs immediately following the task (or control condition) at 4:46 p.m. and the last post-stressor assessment occurs at 5:10 p.m.

Investigators will measure stress reactivity using saliva samples that are collected prospectively during the TSST (see Figure 3). The change in cortisol level may be expressed in several ways, such as change from pretest to maximum increase, area-under-the-curve, or an index incorporating multiple values. If needed, heart rate, BP can similarly quantified. Data on cortisol, heart rate, blood pressure will be used to generate allostatic load during an acute stress response. An 11-point scale (0-10) will be used for the Subjective Units of Distress variable (SUD). Investigators will also measure heart rate variability (HRV) and blood pressure using standard medical equipment and procedures during the TSST.

8.6 ALLOSTATIC LOAD

Allostatic load will be determined based on laboratory results and clinical measures that will include the following; blood pressure, heart rate, total cholesterol, HDL cholesterol, body mass

Figure 3. TSST Procedures and Timeline

TIME	EXPERIMENTAL PROCEDURE	STRESS ASSESSMENT
4:00-4:15pm	**Acclimation period (15 minute duration)**	**Patient arrives and given relaxation tools (i.e. spa video, magazine, nature scenes)**
4:16 Baseline (immediately following acclimation period)	**Laboratory Stress measurements**	**Take cortisol, BP, HR, and assess SUD**
4:28pm Immediately before TSST (12 minutes following baseline)	**Laboratory Stress measurements**	**Take cortisol, BP, HR, assess SUD, and explain the task and give participant paper to write notes**
4:33- 4:44pm (5 minutes following 2nd lab measurements)	**TSST**	
5 minutes	Interview task	
5 minutes	Arithmetic task	
Post TSST stress assessments (12 minute increments)		
4:46pm Post TSST (2 minutes following TSST)	**Post-TSST Assessment**	**Cortisol, BP, HR, SUD**
4:58pm 12 mins post TSST	**Post-TSST Assessment**	**Cortisol, BP, HR, SUD**
5:10pm 24 mins post TSST	**Final Post-TSST Assessment**	**Cortisol, BP, HR, SUD**
Note: Patients more than 15 minutes late are unable to participate in the TSST and would need to be rescheduled.		

index (BMI), C-reactive protein (CRP), DHEAS and interlukin-6 (IL-6). Cortisol will also be measured for those participating in the optional sub-study. We will use established methods to determine allostatic load. These methods use established clinical cutoffs to categorize biomarkers as being within normal ranges. For instance, a systolic blood pressure ≥ 140mm/Hg and CRP ≥ 3mg/L are values that are outside of normal ranges. The values for each biomarker will be coded into a dichotomous variable (1=above normal cutoff and 0=below normal cutoff). Scores for allostatic load will be calculated by summing dichotomous values for each biomarker.

9 ADVERSE EVENT REPORTING

For the purposes of this trial, "study vaccine" is defined as PROSTVAC-V/F.

9.1 INVESTIGATOR RESPONSIBILITIES

Investigators are responsible for monitoring the safety of subjects who have entered this study and for assuring appropriate medical care is provided. 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.

9.2 DEFINITION OF ADVERSE EVENTS (AE)

An AE is any untoward (unfavorable, harmful, or pathologic) medical occurrence in a subject administered an 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 investigational product (ICH E2A II/A/1, 21 CFR 312.32).

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

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

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

- **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 planned hospitalization 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 AEs or SAEs, but rather the cause for the test or procedure should be reported.

9.3 COLLECTING AND RECORDING ADVERSE EVENTS

The Investigator will monitor the occurrence of AE 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 AEs begins at the time the subject starts study treatment and continues for 28 days following administration of the last dose of study medication. If an SAE is present at the end of the reporting period, 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.
Adverse event terms should be recorded concisely, using CTCAE v. 4 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). 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.

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”).

9.4 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 SAE report form.

Each recorded AE or SAE will be described by its duration (i.e., start and end dates), severity, suspected relationship to the investigational product, and any actions taken. Any SAE that occurs after the subject signs informed consent, irrespective of the treatment received by the subject, must be reported to the SIS Unit within 24 hours of the investigator learning of the event.

9.5 DEFINITION OF EXPECTED AND UNEXPECTED EVENTS

9.5.1 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 AE is considered expected when it appears in the current adverse event list, the IB, the package insert or is included in the ICF as a potential risk. 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 following prostatectomy the event will be captured on the AE CRF. Similarly, if the event is associated with PROSTVAC-V/F, and is unexpectedly severe, it will also be recorded on the AE CRF.

9.5.2 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 IB, the package insert or when it is not included the ICF as a potential risk.

9.6 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 AE is more plausible; a temporal sequence cannot be established with the onset of the AE 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 AE and administration of the investigational product, but the AE 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 AE causes.

- **Definitely Related:** The AE is clearly related to use of the investigational product.

9.7 SEVERITY OF ADVERSE EVENTS (GRADING)

AEs will be categorized and graded by the Investigator through use of the CTCAE v 4.0 five-point scale, Grades 1 to 5 with unique clinical descriptions of severity for each referenced AE.

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

Table 2. 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 nconvenience or concern to the subject, and which may interfere with daily activities, but are usually ameliorated by simple therapeutic maneuvers
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

9.8 DEFINITION OF SERIOUS ADVERSE EVENTS

An SAE is an untoward medical occurrence that at any dose:

- Results in death (the AE actually causes or leads to death)
- Is life-threatening ("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 (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. If either the Sponsor or the Investigator believes that the event is serious, the event will be considered serious.

9.9 EVENTS OF SPECIAL INTEREST

9.9.1 Appearance of Satellite Lesions

If a patient appears with a lesion that is not at the original injection site, the incident will be reported to the Sponsor-Investigator within 24 hours of occurrence using the SAE CRF in REDCap. The subject will be instructed to return to the clinic and the appropriate safety protocol will be followed. The lesion will be swabbed per instructions from BNI and sent to the designated lab for testing. If the lesion comes back positive, it will be reported to the FDA and NIH as outlined in setion 9.10.

9.9.2 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 but 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.

9.9.3 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 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 CDC at (877) 554-4625. 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.

9.10 EXPEDITED REPORTING REQUIREMENTS FOR SERIOUS ADVERSE EVENTS AND EVENTS OF SPECIAL INTEREST

All events described in section 9.8 and 9.9 must be submitted to the SIS Unit within 24 hours of the investigator learning of the event following the SOPs outlined in the CTO 102377 Operations Manual.

The SIS Unit will be responsible for processing these events for reporting to the applicable regulatory and oversight authorities including the IRB, DSMC and FDA.

All SAEs and events of special interest must be reported to Bavarian Nordic, Inc. within 24 hours by fax or email to:

Bavarian Nordic, Inc.
Attn: Drug Safety and Pharmacovigilance Designee
Fax: 888-465-1219

Email: pharmacovigilance@bavarian-nordic.com

Any suspected, unexpected SAE, skin lesion positive for vaccinia, pregnancy of a female partner, or accidental transmission of vaccinia virus must be reported to the FDA as soon as possible and no later than 7 days (for a death or life-threatening event) or 15 days (for all other SAEs) after the investigator's or institution's initial receipt of the information.

10 STATISTICAL CONSIDERATIONS

We will compare RFS at two years for the patients treated with PROSTVAC to that of untreated historical controls. A secondary efficacy objective will be a comparison of the observed RFSs for the treated subjects with the calculated RFSs for the same subjects as if they had not received adjuvant therapy .

10.1 STATISTICAL CONSIDERATIONS FOR THE PRIMARY OBJECTIVE

Endpoint: The primary endpoint will be a binary indicator of relapse status at the 2 year follow-up visit after prostatectomy. Due to interval-censoring, using a time-to-event RFS outcome may lead us to anti-conservative estimates due to some patients having follow-up weeks to months after the 2 year anniversary of their surgery and recurrences defined at those later timepoints would not be captured in an estimate of 2 year RFS based on a Kaplan-Meier approach.
A time-to-event analysis of RFS will also be performed, but not for making inferences for the primary objective. Patients who do not have data available for a two-year follow-up will be censored at the last visit at which they were known to be recurrence-free. Patients who drop out will be replaced for up to 4 patients. However, those who choose to discontinue treatment prior to a recurrence (or other event defined in Section 4.6) but wish to remain in the study, will be followed through 24 months to evaluate their disease status based on the intent-to-treat principle. These subjects will not be replaced.

Analysis Plan: The primary endpoint will be a binary indicator of relapse status at the 2 year follow-up visit after prostatectomy. Two-year RFS will be estimated as a proportion with an exact 95% confidence interval. RFS will also be treated as a time-to-event variable and Kaplan-Meier curves will be created and median and two year RFS will be reported with 95% confidence intervals.

Sample Size Justification: To estimate the likelihood of RFS at 2 years for subjects at high risk of relapse, we extracted data from two sources. The first source is a database of high risk subjects that we have previously used in published research (122). The database contains clinical synopses of 243 subjects treated with radical prostatectomy (retropubic, laparoscopic, and perineal) for high-risk prostate cancer. These subjects were treated by multiple surgeons at several academic medical centers. None received any adjuvant therapy. High-risk features included one or more of the following criteria: pre-operative PSA $\geq$ 10 ng/mL; Gleason score 8-10; positive surgical margins, seminal vesicles, extraprostatic extension, or lymph nodes; persistently detectable PSA >45 days after surgery. Relapse-free survival was defined as the interval (days) from prostatectomy to the first of the following: a) PSA of ≥ 0.2 ng/mL (confirmed)

>30 days later, b) appearance of lesions consistent with metastases on imaging studies, or c) institution of new prostate cancer therapy. The observed 1yr probability of RFS was 0.751 and the 2yr probability of RFS was 0.645.

An additional source of data for RFS was the ECOG E3886 study (13), in which subjects with node-positive prostate cancer after radical prostatectomy (n = 98) were randomized to immediate ADT, or ADT at the time of clinical relapse. This analysis was performed by Dr. Yu-Hui Chen (ECOG statistical office) at our request, in 2010. Relapse was defined as the first of the following: a) the appearance of local or distant metastases identified by imaging or physical examination, b) the first increase in PSA > 0.1 ng/mL following a post-prostatectomy PSA nadir of < 0.1ng/mL or c) death from any cause. The RFS probability curve is shown in Appendix B. The observed 1yr probability of RFS was 0.73 and the 2yr probability of RFS was 0.56.

While the two groups differ somewhat in the disease status of the subjects, as well as frequency and manner of follow up, the probabilities of RFS agree quite closely. For the purposes of our power analysis and sample size, we will assume that the true probability of RFS at 2 years, for patients similar to our proposed study population, is 0.60.

We define an “interesting” reduction in the risk of relapse to be 30% (i.e., increase in the probability of RFS from 0.6 to 0.78). Assuming a one-sided alpha = 0.1, with 30 evaluable patients, we have 80% power to detect this difference based on an exact binomial test. Thus if among 30 evaluable subjects, at least 24 are recurrence free at 24 months, we will reject the null hypothesis (2 year RFS=0.60) and conclude that the PROSTVAC intervention improves RFS relative to the current standard of care in this patient population (one-sided p-value ≤ 0.025). Anticipating a final sample size of 30 patients with 10% drop out rate, we plan to enroll 33 patietns for this study.

Interim Analysis: An interim analysis for futility will be performed through Simon’s minimax two-stage design (Simon, 1989) when a total number of 19 patients are evaluable. The design is described as follows:

> **Stopping criteria:** Stop the study if there are 11 or fewer recurrence free among the first 19 evaluable patients. Otherwise, additional patients will be accrued resulting in a total evaluable sample size of 30.

Decision rule at N=30 evaluable patients: Reject the null hypothesis if there are 22 or more recurrence free among the 30 evaluable patients. We claim that the PROSTVAC intervention improves RFS relative to the current standard of care in this patient population. This design controls the type I error rate at 0.1 and yields the power of 0.8.

Safety Monitoring: Continuous safety monitoring will be performed with early stopping rules in the case of unacceptable risk to the subjects, defined as evidence in favor of a risk of a grade 3 or greater toxicity deemed to be possibly related to treated of 25% vs. an acceptable rate of 10%. Stopping criteria are shown in the table below. The stopping rules are based on a likelihood ratio. Specifically, the likelihood ratio is calculated based on a hypothesized 10% event rate (safe) vs, a 25% event rate (unsafe) and likelihood ratios in excess of 8 (in favor of an event rate of 25%)

are considered sufficient evidence to stop the trial. For example, 7 events in 33 patients yields an event rate of 0.21, which has a likelihood ratio of 9.21 favoring a rate of 25% vs. 10%.

Stop if:	Likelihood Ratio	Observed Event Rate
3 events in ≤6 patients	≥ 9.04	≥ 0.50
4 events in ≤12 patients	≥ 9.08	≥ 0.33
5 events in ≤18 patients	≥ 9.13	≥ 0.28
6 events in ≤24 patients	≥ 9.17	≥ 0.25
7 events in ≤33 patients	≥ 9.21	≥ 0.23

10.2 SECONDARY OBJECTIVES

10.2.1 Comparison of Observed RFS with Predicted RFS

A secondary endpoint will be a comparison of the observed RFSs with the predicted ("virtual") RFS for the same patients based on clinical and pathologic information collected at baseline. The predicted RFSs will be constructed with published and validated algorithms (Duke and CaPSURE). The observed RFSs of the treated subjects are compared with the predicted RFSs as if the same subjects had not been treated. The comparison is performed using the logrank test of the observed and predicted RFSs. These represent the estimated RFSs for patients with those clinical characteristics if they did not receive any adjuvant therapy.

RFSs will be displayed graphically using Kaplan-Meier curves. We then use the log rank test to compare the virtual or estimated RFSs values of the test patients with the observed values following PROSTVAC-V/F.

The comparative analysis (observed vs predicted RFS) will be performed repeatedly during the course of the study, whenever an additional 5 subjects relapse or reach the end of the 2-year follow up period.

10.2.2 Description of Treatment Toxicities

Toxicities will be tabulated by CTCAE grade and type.

10.2.3 Correlative Laboratory Studies

Biochemical and genomic measurements will be summarized both graphically and numerically using descriptive statistics. Transformations will be performed based on the observed distribution of the endpoints. Cox regression will be used to estimate hazard ratios measuring the association between these measures and RFS. 95% confidence will be estimated for the hazard ratios as appropriate.
For the amount of antibody forcement antigen, graphical displays such as scatterplots of optical density of particular color and dilution will be made for pre- and post-vaccination. To investigate if the distributions of pre- and post-vaccination are different, Kolmogorov-Smirnov test will be performed. The reverse sigmoidal curves will fit to estimate model paprameters such as slope and 50% of maximum effect for the antibody measurement. Depending on the curve results, different percentages of maximum effect (e.g., 65-75%) will be investigated as well. Moreover, those interest of immune parameters will be compared between pre- and post-vaccination. The

difference of the immune parameters will be used for measuring the association between these measures and time to relapse/stress.

10.3 SOCIOBIOLOGICAL RESPONSE TO STRESS OPTIONAL SUB-STUDY OBJECTIVES

The investigators expect around 70-80% of patients will consent for the sub-study, which leads to the sample size around 30-40 patients for the analysis. Through this sub-study, the investigators will explore the relation between stress responses and clinical outcomes for the entire patient population and for racial subgroups. The following statistical plan will be used.

Aim 1: To evaluate the effects of stress reactivity on the development of an effective anti-tumor immune response in this patient population.

We will perform the following analyses to better understand how stress reactivity affects the anti-tumor immune response: 1) correlation analysis between the anti-tumor immune response and stress reactivity measures; 2) logistic regression in which we will regress the anti-tumor immune response of each patient on stress reactivity adjusting for covariates (e.g. age, perceived stress).

Aim 2: To evaluate the difference in terms of stress reactivity based on SES, perceptions of social stressors, and AL between racial subgroups.

Descriptive statistics of stress reactivity measures will be summarized for the entire patient population and for each racial group. Wilcoxon rank sum test or a Fisher's exact test will be used to explore the potential statistical significance among those measures (e.g. SR, clinical history, psychosocial factors (e.g., life stress events, social isolation), and genetic variants in the GR receptor gene) between racial subgroups. In addition, a discriminant analysis (e.g. quadratic discriminant analysis or nearest neighbor approach) for the entire patient population and for each racial group will be used to classify patients as high or low stress responders based on changes in cortisol during the TSST. Cross validation will be used to exam the misclassification rates.

Aim 3: To evaluate the difference between racial subgroups for the magnitude and distribution of stress responses with biological (AL) and immunologic measure of vaccine effect.

Descriptive statistics will be summarized for the AL measurements (e.g. median) at each time point (baseline, day 85, day 225, and day 730 or off study) for the entire patient population and for each racial group. We will compare the mean difference between two consecutive time points between two racial groups using a Wilcoxon rank sum test. A linear mixed model will also be fitted in which we will regress AL measurements on racial group, time, stress reactivity (may consider interaction terms) to explore the AL response profile difference. The outcome of RFS will be evaluated using Kaplan-Meier estimates and the statistical significance of difference in RFS between racial groups will be tested by a logrank test. In addition, a cox regression model will be explored.

10.4 POPULATIONS FOR ANALYSES

Subjects evaluable for toxicity or efficacy will be those who receive at least one dose of PROSTVAC-V.

10.5 ADDITIONAL ANALYSES

Demographic and clinical parameters will be tabulated. Critical parameters to be recorded include age at time of surgery, date of surgery, race, primary Gleason score, secondary Gleason score, pathologic stage, surgical margin status, extraprostatic extension status, seminal vesicle status, lymph node status, pre-operative PSA level, post-operative PSA level (> 6 wks after surgery). Formalin-fixed paraffin-embedded tissue from the prostatectomy specimen will be archived for future "omic" analyses to correlate with RFS.

11 ETHICAL CONSIDERATIONS

11.1 GOOD CLINICAL PRACTICE

This study will be conducted in accordance with GCP, as defined by the International ICH and in accordance with the ethical principles underlying the US CFR, Title 21, Part 50 (21CFR50). The study will be conducted in compliance with the protocol. The protocol, any amendments, and the subject informed consent will receive IRB approval before initiation of the study.

All potential serious breaches must be reported to the institutional IRB and DSMC. A serious breach is a breach of the conditions and principles of GCP in connection with the study or the protocol, which is likely to affect, to a significant degree, the safety or physical or mental integrity of the subjects of the study or the scientific value of the study.

Study personnel involved in conducting this study will be qualified by education, training, and experience to perform their respective tasks. This study will not use the services of study personnel where sanctions have been invoked or where there has been scientific misconduct or fraud (e.g., loss of medical licensure; debarment). Systems with procedures that ensure the quality of every aspect of the study will be implemented.

11.2 INSTITUTIONAL REVIEW BOARD

Before study initiation, the investigator must have written and dated approval/favorable opinion from the IRB for the protocol, consent form, recruitment materials, and any other written information to be provided to subjects. The investigator should also provide the IRB with a copy of the IB or product labeling, information to be provided to subjects, and any updates. The investigator should provide the IRB with reports, updates, and other information (e.g., expedited safety reports, amendments, and administrative letters) according to regulatory requirements or institution procedures.

11.3 INSTITUTIONAL BIOSAFETY COMMITTEE (IBC)

This protocol and any accompanying material provided to the subject (such as subject information sheets, ICF, or descriptions of the study used to obtain informed consent) will be submitted by the investigator to a legally constituted and chartered IBC. Additional materials, such as the IB, will be submitted to the IBC according to the specific Committee and federal (NIH) 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.

11.4 INFORMED CONSENT

The ICF and process must comply with the US regulations (§ 21 CFR Part 50), as well as state and 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. Investigators must ensure that subjects are clearly and fully informed about the purpose, potential risks, and other critical issues regarding clinical studies in which they volunteer to participate. Freely given written informed consent must be obtained from every subject before clinical study participation, including informed consent for any screening procedures conducted to establish subject eligibility for the study. 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. 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.

The rights, safety, and well-being of the study subjects are the most important considerations and should prevail over interests of science and society.

11.5 COMPLIANCE WITH THE PROTOCOL

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 IB; will obtain IRB 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 reviews and regulatory inspections; will report SAEs to the Sponsor or designee and IRB 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 RECORDS RETENTION

The Investigator must keep a record that lists all subjects considered for enrollment (including those who signed consent but were not enrolled) 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 Brochure, signed protocols and amendments, IRB 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.

11.7 DATA COLLECTION

Electronic CRFs 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 7 days of each subject's visit. At all times, the Investigator has final responsibility for the accuracy and authenticity of all clinical data.

All data should be substantiated by clinical source documents organized within a patient research record. ICH Good Clinical Practices are to be followed.

Electronic data for on study and follow-up patient data is submitted via the electronic system called REDCap. REDCap is managed from MUSC as a consortium partner under their CTSA. REDCap is a secure, Web-based application designed to capture and manage research study data.

The system has been reviewed for 21CFR Part 11 compliance and has been deemed "21CFR 11 Capable." Users of the REDCap system are limited to members of the IRB approved research team who are delegated data management responsibilities and those tasked with assuring quality control and quality assurance.

11.8 SUBSTUDY DATA COLLECTION

A data integration core has been established to integrate biological data (i.e., allostatic load) with information collected on social, psychological, behavioral, and clinical variables collected through the social determinants survey, MUSC's EPIC electronic health records (EHR), and MUSC's electronic data warehouse (EDW). This will be accomplished through several data integration strategies that will harmonize diverse types of data on psycho-social factors and biological biomarkers. These strategies include using natural language processing (NLP) techniques to identify social factors and patient stressors. Key data elements from the EHR and EDW will be extracted using NLP to establish discrete fields for patient demographics, encounter details, vitals, diagnostic history, medications, and laboratory results. Textual patient summaries such as clinical notes containing patient's social history will also be extracted from the EHR and EDW and analyzed via NLP. The Data Integration Core will also use data mining strategies to determine the temporal links between allostatic load and disease risk and outcomes in order to validate the associations observed in the study. All longitudinal patient data will be extracted from EDW via dynamic data pull (DDP) and from EHR using medical record numbers (MRNs) and stored in MUSC's REDCap data capture system and then merged with experimental data, and anonymized to create the main data repository. Anonymization will be performed by IRB certified honest brokers during integration of the data into the main data repository. Investigators will only have access to anonymized datasets. On a quarterly basis, data will be updated by honest brokers with any newly acquired clinical data items. Investigators will utilize the datasets to perform correlation analyses in order to examine linkages between biological, social, and clinical factors.

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APPENDIX A: SCHEDULED PROCEDURES AND ASSESSMENTS

		Days on study[f]																
	Procedure[c]	Pre[a]	Active/On Treatment	1[n]	15	29	57	85	113	141	Follow Up[h, p]	225	309	393	477	561	645	730[g]
Therapeutic trial procedures	Informed Consent	X[c]																
	Full Physical Exam	X																
	Focused Phyical Exam			X	X	X	X	X	X	X		X	X	X	X	X	X	X
	Full Medical History	X																
	Interim Medical History[o]			X	X	X	X	X	X	X		X	X	X	X	X	X	X
	AE assessment			X	X	X	X	X	X	X		X						
	CBCD	X		X	X	X	X	X	X	X		X	X	X	X	X	X	X
	CMP	X		X	X	X	X	X	X	X		X	X	X	X	X	X	X
	PSA	X[m]		X[m]			X			X		X	X	X	X	X	X	X
	Testosterone	X																
	CRP	X								X								
	Lipid Panel			X[k]														
	CT (abdomen/pelvis)	X[b]																
	Tc-bone scan	X[b]																
	HIV test	X[l]																
	Hep B and C testing	X[l]																
Treatment	PROSTVAC-V			X														
	PROSTVAC-F				X	X	X	X	X	X								
Correlative Research	FACT-P			X[i,q]				X[q]				X[q]						X[q]
	AUA			X[i,q]				X[q]				X[q]						X[q]
	SHIM			X[i,q]				X[q]				X[q]						X[q]
	Social Determinants Questionnaire			X[i,]														
	Research blood[e]			X[i,]				X				X						X
	Research FFPE tissue	X[l]																
Optional Sub-Study	Optional Sub-study Consent	X[d]																
	Trier Stress Test	X[i]																

a. Screening Assessments ("Pre") may be done up to 30 days prior to registration unless otherwise noted.
b. Radiographic assessments may be done up to 26 weeks prior to registration. PSA at screening is required to document eligibility for post-operative PSA and may be done greater than 30 days prior to registration.
c. The informed consent for the main study can be obtained more than 30 days prior to registration but must be obtained before any research procedures are completed
d. The optional sub-study consent must be obtained prior to conducting the Trier Stress Test. The optional sub-study is voluntary and does not impact the patient's ability to participate in the main therapeutic portion of the trial.
e. See section 8 for blood draw volume and tube type requirements.
f. all specified times are +/- 7 days
g. or off-study day
h. follow up will begin after the last day of study drug administration.
i. Research blood, social determinants questionnaire and quality of life questionnaires may be completed any time after informed consent is signed and prior to Day 1 drug administration.
j. If available: Send 5 thin (5 micron) sections on glass for H+E and IHC staining. These can be sent at any time after consent has been signed.
k. Lipid panel requires patients to be fasting and can be done any time after informed consent is signed and prior to day 1 agent administration.
l. HIV, Hep B and Hep C serology screening, unless known negative within previous 2 years prior to registration.
m. PSA confirming eligibility can be done outside of the 30 day window. However, if PSA confirming eligibility was done greater than 30 days prior to day 1, it should be repeated prior to day 1 agent administration to ensure PSA is < 0.2ng/mL
n. Day 1 assessments completed within 24 hours of screening assessments do not need to be repeated.
o. Interim medical history includes any changes to the patient's medical history and/or any clinical changes since the previous clinic visit.
p. For non-local patients in follow up, all attempts should be made to have subject return to the clinic for all follow up visits, but specifically day 225 and day 703. However, clinical care procedures (PSA, CMP, CBCD, focused physical exam and interim medical history – to include AE assessment and conmed assessment) may be done locally and the records provided. At a minimum, a PSA must be done and results provided to the study team. If a clinic visit is not done during follow up, the study team may conduct a phone interview (using the phone contact guide, Appendix D) in leiu of an clinic visit to assess adverse events, current cancer therapy and PSA level. Quality of life surveys may also be conducted over the phone by the study team if the subject does not return to clinic.
q. Questionnaires may be mailed to the subject in pre-metered envelope if they are unable to come into the clinic to complete them (ie: due to COVID19 travel restrictions). The subject will complete the questionnaires at home and then mail the completed questionnaires back to the main site in the provided envelope.

APPENDIX B: SOURCE DATA FOR RFS

See next page for additional source of data for RFS

E3886 Analysis for Dr. Lilly

September 17, 2010

Background

Dr. Lilly conducted a study of adjuvant chemo-hormonal therapy in patients with high-risk prostate cancer and would like to compare progression-free survival (PFS) of the treated patients to the corresponding values from E3886.

Key Eligibility Criteria

A total of 20 patients were enrolled in Dr. Lilly's study, and all of them had one or more high risk features for early relapse. High risk features included 1) pT3 or pT4 primary prostate cancer, or 2) one or more lymph nodes containing metastatic prostate cancer, or 3) detectable PSA (≥0.2 ng/mL) after prostatectomy, or 4) an 84-month risk of relapse >40% by the 1999 Kattan nomogram.

In E3886, one hundred patients were randomized to either immediate androgen deprivation therapy (ADT) or deferred ADT. Two patients were ineligible due to no prior radical prostatectomy and no lymphadenectomy, resulting in 47 immediate ADT and 51 deferred ADT patients eligible for follow-up. To find a similar cohort from E3886 to evaluate PFS, the 98 eligible patients were evaluated for the high risk features listed above, and Table 1 shows the risk features in these patients. Ten (10%) patients had pT3a prostate cancer and 46 (47%) had pT3b disease. Due to limited information collected on this study, we were not able to determine whether a patient had pT4 disease or not. All patients had at least one positive node. Seventeen patients (17%) had detectable PSA after prostatectomy. The 84-month risk of relapse by the 1999 Kattan nomogram was not assessed for these patients. Based on the information above, all E3886 eligible patients (n=98) had one or more high risk features and were included in the analysis.

Statistical Methods

The method of Kaplan and Meier was used to characterize PFS for both Dr. Lilly's patients and E3886 patients. This report reflects the status of the E3886 database as of 2004. As the disease evaluation methods, assessment schedule, and follow-up duration were different between these two studies, no direct comparison was performed.

Results

PFS is the endpoint of interest in this analysis. In Dr. Lilly's study, PFS is defined as the time from the date of prostatectomy until progression or death. Patients who were alive without documented progression were censored at the date of last disease assessment. Progression is defined as either a rising PSA (PSA greater than 0.1ng/mL in patients with an undetectable PSA after surgery) and/or the appearance of a suspicious new mass on an imaging study, preferably confirmed by an alternative study or biopsy.

In E3886, patients were randomized within 12 weeks after surgery, and PFS is defined as the time from study entry until progression or death. To make the definition of PFS consistent between the two studies, PFS for E3886 was recalculated from the date of radical prostatectomy to progression or death. Patients who were alive with no recorded progression were censored at the date they were last known to be progression-free. In this study, progression is defined as local recurrence, regional recurrence, distant metastases, PSA progression or death, whichever occurs first.

As the median follow-up was 11.9 years and 5.1 years among living patients in E3886 and Dr. Lilly's study, respectively, a 5-year PFS rate along with a 90% confidence interval (CI) was computed for each cohort. The 5-year PFS rate for Dr. Lilly's patients was 83% (90% CI, 62-93%; Figure 1). For E3886, the 5-year PFS rates were 83% (90%

CI, 72-90%; Figure 2) and 27% (90% CI, 18-38%; Figure 3) in the immediate ADT and deferred ADT arms, respectively.

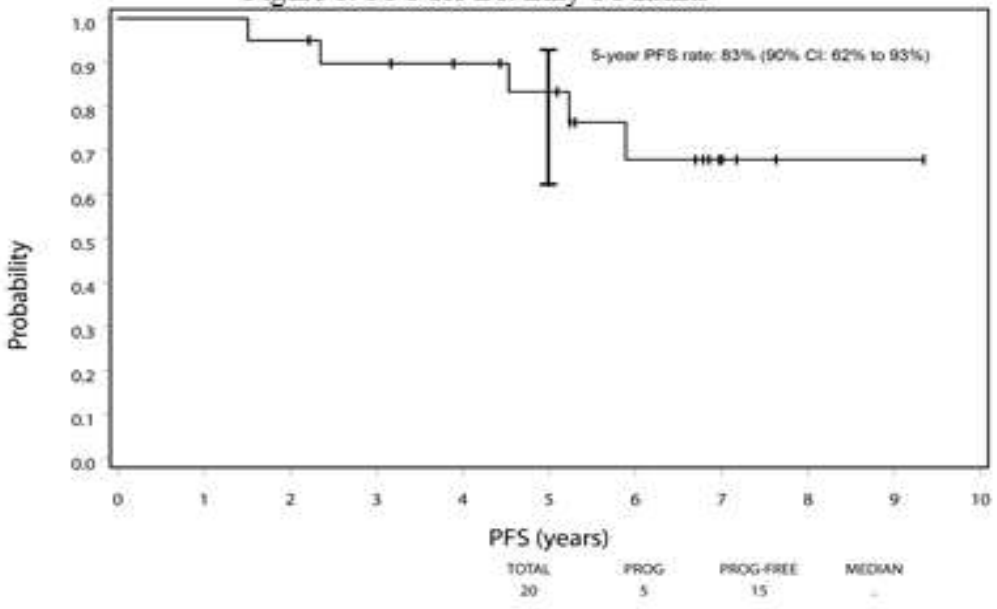
E3886 Analysis for Dr. Lilly

September 17, 2010

Table 1: High Risk Features for E3886 Patients

	Immediate ADT (n=47)	Deferred ADT (n=51)
Pathologic T stage		
pT2	19 (40%)	23 (45%)
pT3a	6 (13%)	4 (8%)
pT3b	22 (47%)	24 (47%)
Nodal status		
Number assessed (median, range)	11(3-36)	14 (2-39)
Number positive (median, range)	2(1-19)	2 (1-20)
Postoperative serum PSA		
Range	<0.2-87.4 µg/L	<0.2-48.7 µg/L
Detectable	8 (17%)	9 (18%)

Figure 1: PFS for Dr. Lilly's Patients



E3886 Analysis for Dr. Lilly

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Figure 2: PFS for E3886 Patients with Immediate ADT

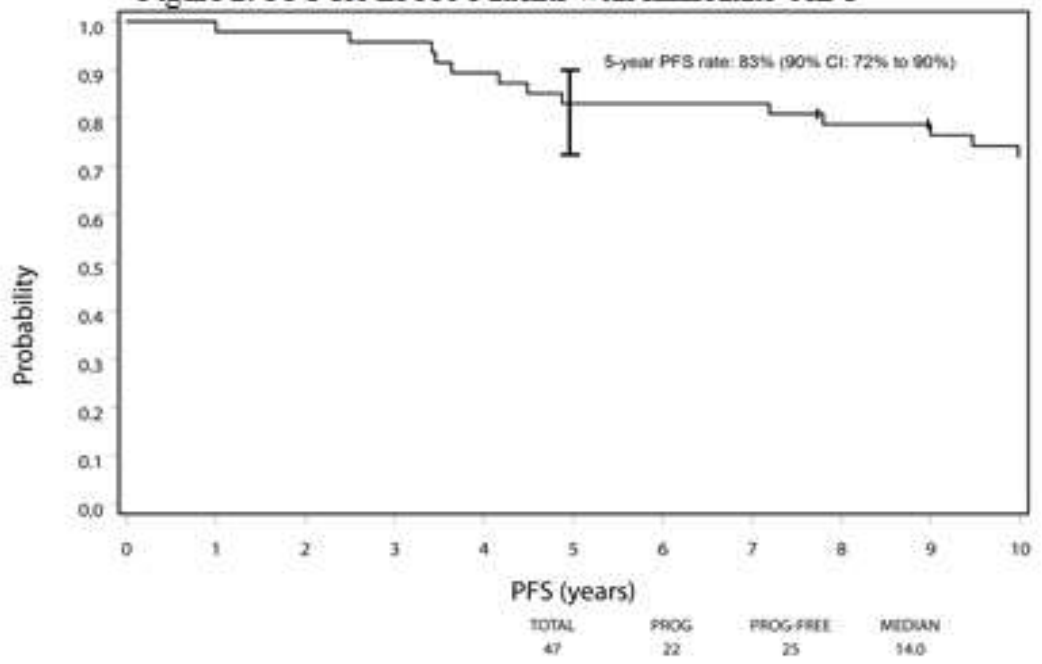
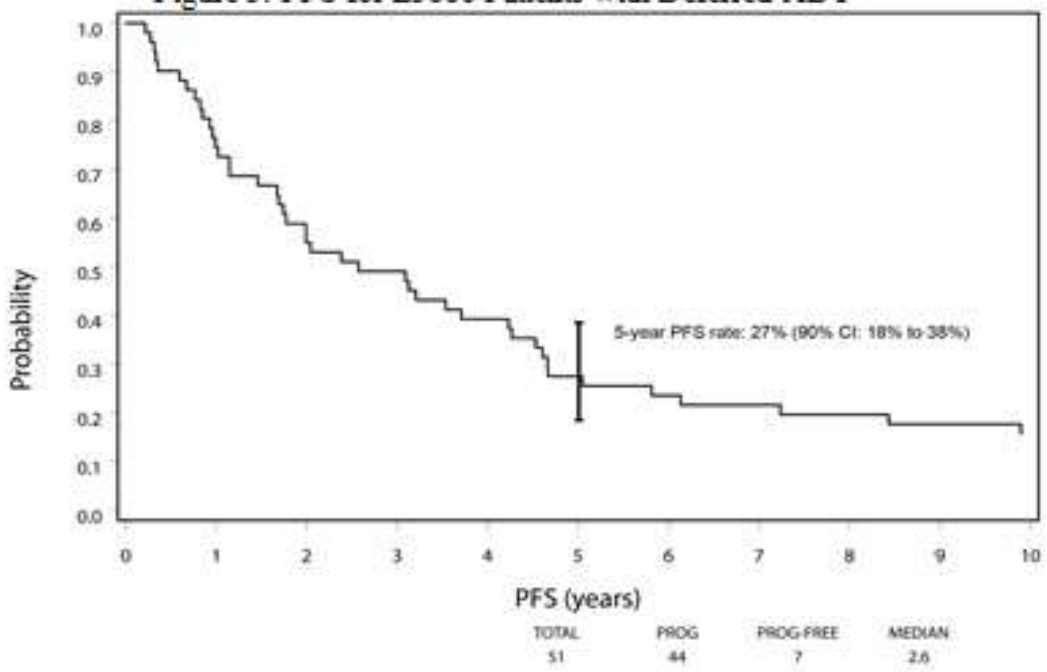


Figure 3: PFS for E3886 Patients with Deferred ADT



APPENDIX C: PROCEDURE FOR OBTAINING VIG AND CIDOFOVIR FOR TREATMENT OF SERIOUS ADVERSE REACTIONS CAUSED BY THE SMALL POX VACCINE.

Information in this document is taken from the Smallpox fact sheet – Information for Clinicians and Public Health Professionals. The fact sheet can be reviewed in full here: https://emergency.cdc.gov/agent/smallpox/vaccination/pdf/mgmt-adverse-reactions-clinic.pdf

Indications for VIG/cidofovir release:

- Vaccinia Immune Globulin (VIG) and cidofovir are indicated for the treatment of certain serious smallpox vaccine adverse events, including progressive vaccinia, eczema vaccinatum, generalized vaccinia (severe form or if underlying illness), and inadvertent inoculation (if judged to be severe due to the number of lesions, toxicity of affected individual, or significant pain). VIG is recommended as the first line of therapy. Cidofovir may be considered as a secondary treatment, and will only be released by CDC after all inventories of VIG have been exhausted, after a patient fails to improve with VIG treatment, or as a last effort for a patient who is otherwise near death.
- VIG and cidofovir are available for civilians through the CDC under Investigational New Drug (IND) protocols for treatment of specific smallpox vaccine reactions. Based on the anticipated number of adverse events resulting from the planned vaccination program for healthcare workers, CDC's supply of VIG should be adequate.

Process for obtaining VIG/cidofovir under Investigational New Drug Protocol (IND):

1. **Contact the South Carolina Department of Health:** Physicians should first contact their State Health Department when seeking consultation for civilian patients experiencing a severe or unexpected adverse event following smallpox vaccination or when requesting VIG or cidofovir.
2. If further consultation is required, or VIG or cidofovir is recommended, the physician will be referred to the **CDC Clinical Information Line (CIL) at 1- 877-554-4625**. The nurses staffing the CIL will take basic information and then expedite the call to the CDC Smallpox Vaccine Adverse Events Clinical Consultation Team. The CDC Clinical Consultation Team will provide in-depth consultation and will facilitate VIG or cidofovir release as appropriate.
3. According to FDA regulations, VIG or cidofovir released from the CDC must be administered according to their investigational new drug protocols (IND). The IND mandates that the treating physician must become a co-investigator. The responsibilities of the co-investigator are primarily to complete follow-up forms describing the clinical status of the patient being treated with VIG and/or cidofovir, including the prompt report of any significant adverse reaction in the recipient. Detailed information on the requirements of the IND will be shipped with the products.

Shipment of VIG/cidofovir

- VIG/cidofovir will be shipped by the National Pharmaceutical Stockpile (NPS). The CDC Smallpox Vaccine Adverse Events Clinical Consultation Team will coordinate the shipment of VIG/cidofovir with NPS. The cost of VIG and cidofovir and the cost of shipping will be covered by the U.S. Government. Arrival of shipments should be expected within 12 hours of the approval for release.

APPENDIX D: PHONE CONTACT GUIDE

This form is to be used if a patient does not come back to HCC for follow up visits after the day 141 PROSTVAC-F administration.

Study ID/Initials:_____ Date:_____Timepoint:_____

☐ Call conducted (complete information below)
☐ Patient could not be contacted after ____ attempts.

1. **Are you experiencing any side effects?**

   [Document any side effects reported by the subject. Severity to be confirmed by a study-approved investigator. Attribution to done by a study-approved investigator.]

Side Effect	Severity	Attribution	Investigator Initials

2. **Are you receiving any form of prostate cancer treatment?**
   ☐Yes ☐No Date treatment was started:_____

3. **What is your most recent PSA value?**_____ng/dL

4. **Day 225 and Day 730 only: May we ask you some quality of life questions?** ☐Yes ☐No

   [If yes – administer the quality of life questionnaires]

Recorder's signature and date: _____

Investigator's signature and date:_____