PART A1: INFORMATION REQUIRED UNDER ARTICLE 11 (SCHEDULE 2) OF THE 2002 REGULATIONS

PART I
GENERAL INFORMATION

1. The name and address of the applicant and the name, qualifications and experience of the scientist and of every other person who will be responsible for planning and carrying out the release of the organisms and for the supervision, monitoring and safety of the release.

Name and address of the applicant:

BN ImmunoTherapeutics, Inc.
2425 Garcia Avenue
Mountain View,
CA 94043,
USA

2. The title of the project:

Clinical Study BNIT-PRV-301, “A Randomized, Double-blind, Phase 3 Efficacy Trial of PROSTVAC ± GM-CSF in Men With Asymptomatic or Minimally Symptomatic Metastatic, Castrate-Resistant Prostate Cancer”

PART II
INFORMATION RELATING TO THE ORGANISMS

Characteristics Of The Donor, Parental And Recipient Organisms

3. Scientific Name and Taxonomy

PROSTVAC-V/F is a live attenuated viral vector-based investigational vaccine product that is comprised of two component viral vectors, to be used together in a prime-boost vaccination regimen:

- **PROSTVAC-V**: Recombinant vaccinia virus that co-expresses a human prostate-specific antigen (PSA) gene and genes encoding three human immunological costimulatory molecules: B7.1, intercellular adhesion molecule-1 (ICAM-1), and leukocyte function-associated antigen-3 (LFA-3) (designated TRIad of COstimulatory Molecules, or TRICOM™). The PSA gene used in these recombinants has an alteration in a human leukocyte antigen-A2 (HLA-A2) specific epitope (replacement of isoleucine with leucine at amino acid position 155; designated L155).
- **PROSTVAC-F:** Recombinant fowlpox virus that co-expresses the same four genes as PROSTVAC-V.

Both PROSTVAC-V and PROSTVAC-F are large, double-stranded DNA viruses that belong to the subfamily *Chordopoxvirinae* of the *Poxviridae* Family. The subfamily is divided into genera according to host range (*Moss, 1996*).

PROSTVAC-V

Family: Poxviridae
Subfamily: *Chordopoxvirinae* (vertebrate poxviruses)
Genus: Orthopox Virus
Species: Vaccinia

PROSTVAC-F

Family: Poxviridae
Subfamily: *Chordopoxvirinae* (vertebrate poxviruses)
Genus: Avipox Virus
Species: Fowlpox

4. Usual strain, cultivar or other strain

PROSTVAC-V: New York City Board of Health Vaccine (NYCBH) strain of vaccinia virus

PROSTVAC-F: POXVAX-TC, a tissue culture-adapted vaccine strain of fowlpox virus

5. Phenotype and Genetic Markers

PROSTVAC-V

The parental vaccinia virus used for the generation of PROSTVAC-V was derived from a plaque isolate (designated TBC-Wy) from the seed stock of virus used by Wyeth Pharmaceuticals to produce the licensed Dryvax® Smallpox Vaccine. This vaccine is the New York City Board of Health (NYCBH) strain, which has been associated with the lowest incidence of clinical complications following immunization (*Fenner, 1988*).

Notably, TBC-Wy and PROSTVAC-V are significantly more attenuated than the mixture of vaccinia viruses that comprise the Dryvax Smallpox Vaccine, as measured in an intracranial LD_{50} test in weanling mice. This neurovirulence test has traditionally been the most sensitive method for measuring virulence of vaccinia virus *in vivo*.

PROSTVAC-V can be distinguished genetically from TBC-Wy by the presence of the inserted human genes encoding PSA and TRICOM and by the unique recombination junctions between the inserted genes and the parental vaccinia sequences.

In primary chicken embryo fibroblast cells, PROSTVAC-V grows approximately as well *in vitro* as its non-recombinant parental vacciniaf virus.

PROSTVAC-F

The parental fowlpox virus used for the generation of PROSTVAC-F is a plaque isolate (designated TBC-FPV) from the tissue culture-adapted vaccine strain POXVAC-TC®. POXVAC-TC is a USDA-licensed poultry vaccine manufactured by Schering-Plough Corporation.

PROSTVAC-F can be distinguished genetically from TBC-FPV by the presence of the inserted human genes encoding PSA and TRICOM and by the unique recombination junctions between the inserted genes and the parental fowlpox sequences.

In primary chicken embryo fibroblast cells, PROSTVAC-F grows approximately as well *in vitro* as its non-recombinant parental fowlpox virus. In the rodent neurovirulence assay, both TBC-FPV and PROSTVAC-F are significantly more attenuated than the Dryvax smallpox vaccine.

6. The degree of relatededness between the donor and recipient or between parental organisms

PROSTVAC-V

In PROSTVAC-V, the PSA and TRICOM genes are all inserted at a single site in the Hind F region (*Goebel, 1990*) of the parental virus TBC-Wy. T hese four genes are inserted into an intergenic region between open reading frames F12L and F13L, which are transcribed to the right. No vaccinia genes are interrupted by this insertion.

PROSTVAC-F

In PROSTVAC-F, the PSA and TRICOM genes are all inserted at a single site in the parental virus genome. One fowlpox gene in TBC-FPV was disrupted by the insertion of the human genes in PROSTVAC-F. The absence of this gene, FPV246, which has homology to the ankyrin repeat gene family (*Afonso, 2000*), is not predicted to have an effect on the properties of fowlpox virus.

For both PROSTVAC-V and PROSTVAC-F, comparison of the respective parental and recombinant viruses indicates that the added human transgenes in these recombinant viruses have not fundamentally altered the biologic properties of the viruses with respect to virulence, replicative ability, or stability in the environment.

7. The description of identification and detection techniques.

Detection and identification of PROSTVAC-V and PROSTVAC-F are accomplished using the following assays:

- Quantitative FACS assay to measure titers of infectious virus
- Polymerase Chain Reaction (PCR) of inserted genes and recombination junctions for virus identity
- Transgene expression by Western Blot

8. The sensitivity, reliability (in quantitative terms) and specificity of detection and identification techniques.

Quantitative FACS Assay

The FACS assay is a quantitative *in vitro* assay that confirms the expression of viral products, TRICOM® (B7.1, ICAM-1 and LFA-3) and PSA. In this assay, cells are incubated *in vitro* with serial dilution of virus then evaluated for the expression of both viral proteins as well as transgene products simultaneously. Transgene products and viral antigens in individual cells are detected with specific antibodies conjugated to fluorophores that are detected by FACS. Binding of antibodies to their specific targets is performed after fixation and permeabilization of infected cells. FACS analysis reveals the percentage of cells that are infected with virus, from which an

infectious unit (Inf. U.) titer is calculated. It also reveals the percentage of cells that express the trangenes, from which a transducing unit (Txg. U.) titer is calculated. This assay is a fully-validated potency assay used for the release of clinical material.

Polymerase Chain Reaction (PCR) of inserted genes and recombination junctions

The identity of PROSTVAC-V and PROSTVAC-F is demonstrated using a qualitative PCR test. Briefly, for each test, DNA is extracted from the test sample and subjected to PCR amplification using specific primers homologous to sequences flanking the inserted PSA-TRICOM genes. The resulting PCR amplicons are visualised by agarose gel electrophoresis and ethidium bromide staining and compared with DNA markers of known fragment sizes. Analysis of the recombinant viruses is conducted in parallel with analysis of PROSTVAC-V/F reference standards.

The PCR identity tests for PROSTVAC-V and PROSTVAC-F were validated with respect to Lower Detection Limit, Specificity, Repeatability (intra-assay precision), and Intermediate Precision.

Western Blot Analysis

Western blot analysis using antibodies specific for PSA, B7.7, ICAM-1 and LFA-3 is used to examine the molecular weight and identity of these polypeptides expressed by a mammalian cell line following uptake of recombinant virus. Analysis of the recombinant viruses is conducted in parallel with analysis of PROSTVAC-V and PROSTVAC-F reference standards. This assay is a fully-validated identity assay used for the release of clinical material.

9. The description of the geographic distribution and of the natural habitat of the organisms including information on natural predators, prey, parasites and competitors, symbionts and hosts.

Vaccinia virus has been used for over 200 years as a vaccine for smallpox and has a well established safety and adverse event profile (*Casey, 2005*; *Poland, 2005*). However, the origins of vaccinia virus in nature and as a vaccine remain a mystery. During the smallpox eradication campaign, the dogma held that vaccine strains could not survive in nature and that wild-type vaccinia virus was extinct. However, although vaccinia virus has no known natural animal reservoirs, buffalopox virus, first isolated in India and still associated with sporadic outbreaks in Pakistan, India, Bangladesh, Russia, Indonesia, Egypt, and Italy, has been proposed to be a subspecies of vaccinia virus. Additionally, several strains of vaccinia virus have been isolated throughout Brazil. Recent studies have suggested an independent origin for South American VACV isolates, distinct from the vaccine strains used on this continent during the smallpox eradication campaign (*Trindade, 2007*).

Avipoxviruses (APV) are distributed worldwide. APV host range is restricted *in vivo* to certain avian species. The virus species fowlpox virus infects and causes disease in poultry. The parental fowlpox used for the generation of PROSTVAC-F is a non-virulent, vaccine strain.

10. The potential of the organisms for genetic transfer and exchange with other organisms.

The potential for gene transfer to other species under the proposed release of the GMO is extremely low.

Recombination between the DNA genome of PROSTVAC-V or PROSTVAC-F with the DNA genome of a host cell is unlikely. The poxvirus life cycle is carried out in the cytoplasm (*Moss, 1996*); poxviruses do not integrate into the genome of the infected cell. Therefore, the risk of poxvirus persistence by integration into the host chromosome is very low. Although the human genes expressed in PROSTVAC-V and PROSTVAC-F share homology with their counterparts in the human genome, the physical segregation between host and viral genomes renders recombination between PROSTVAC-V or PROSTVAC-F and the human genome an unlikely event. The frequency, already unlikely, of any such recombination events in humans or non-avian species after administration of PROSTVAC-F would be further reduced by the lack of replicative capacity of fowlpox virus in these species.

Recombination between the two PROSTVAC-V/F components, PROSTVAC-V and wild type fowlpox, or PROSTVAC-F and wild type vaccinia, is unlikely because the vaccinia and fowlpox do not share homology. No interviral recombination between vaccinia and fowlpox DNA has been observed (*Scheiflinger, 1992*). In addition, vaccinia is not normally found in nature.

Recombination between PROSTVAC-V or PROSTVAC-F and a wild-type vaccinia or fowlpox virus in an infected host organism is theoretically possible. However, such recombination would not be expected to alter the virulence, growth properties, or environmental persistence of the virus. In addition, the likelihood of recombination between PROSTVAC-V and wild type vaccinia *in vivo* is extremely low because vaccinia is not normally found in nature. Recombination of vaccinia virus with other poxviruses, such as orf, molluscum contagiosum, or shope fibroma virus, has not been observed *in vitro*. Recombination between PROSTVAC-F and a wild or vaccine strain of fowlpox would require release of PROSTVAC-F into an environment containing poultry. Such release is highly unlikely under the proposed conditions of release.

Recombination with other viral genomes is also unlikely due to the lack of homology between different families of viruses as well as to the physical segregation between the genomes of the poxviruses and those of other viruses that replicate in the nucleus. Additionally, in non-avian species susceptible to infection by PROSTVAC-F, few opportunities for genetic recombination with other could occur, since the level of replication that the vector DNA undergoes *in vivo* is low, and limited to cells infected by the inoculum (no generation of infectious particles).

11. Verification of the genetic stability of the organisms and factors affecting stability.

The entire genome of the Working Seed Virus (WSV) and the entire genome of one production lot of PROSTVAC-V and PROSTVAC-F are sequenced. In addition, for each production lot, identity is demonstrated by PCR, Western blot, and restriction site analysis. In aggregate this testing provides verification of genetic stability.

In addition, a comparability assessment of the full genomes Phase 2 and Phase 3 IMP material was completed. These data further support the genetic stability of PROSTVAC-V and PROSTVAC-F.

12. The following pathological, ecological and physiological traits:

a. The classification of hazard according to existing Community rules concerning the protection of human health and the environment

In terms of classification of hazard, the human vaccinia virus is classified as a group 2 biological agent according to the European Economic Community (EEC) classification for the protection of workers with biological agents {Directive 2000/54/EC}.

Fowlpox virus is classified as a Biosafety Level 1 organism for practices involving biological materials and containment facilities (as defined by the United States Center for Disease Control and Prevention, Laboratory Biosafety Level Criteria).

b. The generation time in natural ecosystems, and sexual and asexual reproductive cycles.

Vaccinia Virus

Vaccinia virus has no known natural animal reservoirs, although buffalopox virus has been proposed to be a subspecies of vaccinia virus, and several strains of vaccinia virus have been isolated throughout Brazil. Vaccinia virus causes a transient infection in susceptible hosts, 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. Latent infection of humans with vaccinia virus has not been observed (*Fenner, 1988*).

Fowlpox Virus

The vaccine strain of fowlpox virus used for the generation of PROSTVAC-F is widely used for the prevention of disease in chickens by wild type fowlpox virus. It is not virulent and does not cause disease.

Wild-type fowlpox virus causes a slow-spreading viral infection of chickens and turkeys. The course of the disease in the individual bird takes three to five weeks. The virus replicates in the cytoplasm of infected avian cells, which results in a characteristic cytopathic effect (CPE) 4 to 6 days post infection. Viral DNA does not integrate into the host cell DNA.

c. Survivability, including seasonability and the ability to form survival structures, including seeds, spores, and sclerotia

PROSTVAC-V and PROSTVAC-F are live viruses and do not form structures. Survivability is dependent upon the ability to replicate within a host cell.

Poxviruses have the capacity to survive for considerable periods in dried material such as detached vaccination scabs. They are also relatively stable when stored frozen or lyophilized under carefully controlled conditions. However stability decreases significantly as temperature is increased. Under normal environmental conditions, PROSTVAC-V and PROSTVAC-F are expected to lose viability within days or weeks.

d. Pathogenicity, including infectivity, toxigenicity, virulence, allergenicity, carrier (vector) of pathogen, possible vectors, host range including non-target organisms and possible activation of latent viruses (proviruses) and ability to colonise other organisms

PROSTVAC-V

Vaccinia virus has been used for over 200 years as a vaccine for smallpox and has a well-established safety profile. US experience, published in 1968, delineate 8 deaths that occurred in a program of over 14 million primary and secondary smallpox vaccinations (*Lane, 1969*). Normal reactions to smallpox vaccination are mild and self-limited, and include fever, myalgia, headache, fatigue, chills, nausea, soreness and erythema at the vaccination site, local lymphadenopathy. Mild adverse reactions that can occur post vaccination are bacterial superinfection of vaccination site, erythema multiforme and generalized vaccinia. Very rare, but serious and potentially life-threatening adverse events, include progressive vaccinia (PV), eczema vaccinatum (EV) and postvaccinial encephalitis (PVE).

Replication *in vivo* of vaccinia virus is restricted to certain warm-blooded vertebrate hosts, including humans and animal species such as cattle, cats, rodents, rabbits and pigs. However, the virus does not appear to occur naturally in humans and has no known animal reservoir.

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. Thus, vaccinia is incapable of colonizing the host organisms that it infects.

The parental virus used for the generation of PROSTVAC-V was derived from TBC-Wy, a plaque isolate from the seed stock of virus used by Wyeth Pharmaceuticals to produce the licensed Dryvax® Smallpox Vaccine. This vaccine is the New York City Board of Health strain, which has been associated with the lowest incidence of clinical complications following immunization (*Fenner, 1988*). Both TBC-Wy and PROSTVAC-V are considerably more attenuated than Dryvax as measured in a rodent neurovirulence assay (see item 5); these results indicate that the insertion of foreign sequences into TBC-Wy does not increase the virulence of the resulting recombinant virus.

With respect to toxigenicity and allergenicity, no biologically significant changes or signs of untoward toxicological effects were noted in either rodent or non-human primate safety studies. In addition, PROSTVAC-V has previously been administered to over 300 subjects in eight phase 1 and 2 clinical studies (see Item 32) and no toxic or allergenic effects were reported. The most common AEs related to PROSTVAC-V observed to date have been injection site reactions, all of which were ≤ Grade 2 severity.

PROSTVAC-F

Productive fowlpox virus infection is restricted *in vivo* to certain avian species, including chickens, turkeys, and pigeons, and *in vitro* to cells derived from avian species. Although fowlpox-mediated gene expression does occur in infected non-avian cells, infection of mammalian species does not cause disease. Further, because fowlpox virus is incapable of replication in mammalian species, it is incapable of colonizing these species.

The parental virus used for the generation of PROSTVAC-F, designated TBC-FPV, was a plaque isolate from a tissue culture-adapted vaccine strain of FPV (POXVAC-TC), which is a USDA-licensed poultry vaccine manufactured by Schering-Plough Corporation. No untoward effects on the environment, other bird species, or animal handlers have been reported from the use of POXVAC-TC.

In the rodent neurovirulence assay, both TBC-FPV and PROSTVAC-F are significantly more attenuated than the Dryvax smallpox vaccine. With respect to toxigenicity and allergenicity, no biologically significant changes or signs of untoward toxicological effects were noted in either rodent or non-human primate safety studies. In addition, PROSTVAC-F has previously been administered to over 300 subjects in eight phase 1 and 2 clinical studies (see Item 32) and no toxic or allergenic effects were reported.

e. Antibiotic resistance, and potential use of these antibiotics in humans and domestic organisms for prophylaxsis and therapy

Not applicable. PROSTVAC-V and PROSTVAC-F are viruses and therefore do not confer antibiotic resistance properties.

f. Involvement in environmental processes including primary production, nutrient turnover, decomposition of organic matter and respiration.

There is no evidence of poxviruses having any involvement in environmental processes.

13. The sequence, frequency of mobilisation and specificity of indigenous vectors and the presence in those vectors of genes which confer resistance to environmental stresses.

Not applicable. Infection of a cell with PROSTVAC-V or PROSTVAC-F results in cell death.

14. The history of genetic modification.

The gene encoding PSA was isolated at the National Cancer Institute by polymerase chain reaction amplification of cDNA derived from RNA from the human LNCaP cell line (CRL 1740, American Type Culture Collection (ATCC), Rockville, MD), which originated from a metastatic lesion of a prostatic adenocarcinoma (*Horoszewicz, 1983*). Nucleotide sequence analysis indicated that the sequence of this cDNA was identical to the published sequence with the exception of a single nucleotide change resulting in a change in amino acid residue 658 from asparagine to tyrosine (*Lundwall, 1987*). The PSA gene was then modified by *in vitro* mutagenesis to express full-length protein containing one altered epitope that has been shown to enhance immunogenicity (*Terasawa, 2002*). This mutation changed the encoded amino acid at position 155 from isoleucine to leucine.

The gene encoding LFA-3 was isolated by PCR amplification of Human Spleen Quick-Clone cDNA (Clontech Inc.) using the published sequence (*Wallner, 1987*). The gene encoding ICAM-1 was isolated by PCR amplification of cDNA reverse-transcribed from RNA from an Epstein-Barr Virus-transformed B cell line derived from a healthy male, using the published sequence (*Staunton, 1988*). The gene encoding B7.1 was isolated at the National Cancer Institute by PCR amplification of cDNA derived from RNA from the human Raji cell line (ATCC # CCL 86), using the published sequence (*Chen, 1992*).

The sE/L promoter was isolated as a 60 bp Hind III-Sal I fragment from pJS-8, a derivative of pSC65 (*Chakrabarti, 1997*). The remaining vaccinia promoter sequences were isolated from genomic DNA prepared TBC-Wy or from the vaccinia WR strain (*Panicali, 1981*).

Additional details on the genes inserted into the genomes of PROSTVAC-V and PROSTVAC-F may be found in Item 20, below.

Characteristics Of The Vector

15. The nature and source of the vector.

As described more fully in Items 19 and 20 below, the insertion of foreign genes into the poxvirus genome is accomplished using a plasmid transfer vector that contains the expression cassettes flanked by additional poxvirus sequences that direct recombination within a specified genome region. The starting plasmid used to generate the plasmid transfer vectors was derived from the plasmid vector pUC8 (*Viera, 1982*).

16. The sequence of transposons, vectors and other non-coding genetic segments used to construct the genetically modified organisms and to make the introduced vector and insert function in those organisms.

As described more fully in Items 19 and 20 below, human genes were inserted into the parental vaccinia and fowlpox genomes by homologous DNA recombination within infected cells using plasmid transfer vectors that contain the expression cassettes flanked by additional poxvirus sequences that direct recombination within a specified genome region. The genetic structures of the plasmid transfer vectors used in the generation of PROSTVAC-V and PROSTVAC-F, as well as the origins of each vector component are described in detail in Item 20 below.

17. The frequency of mobilisation, genetic transfer capabilities and/or methods of determination of the inserted vector.

Not applicable. Homologous recombination between poxvirus DNA and the plasmid vector results in insertion of the heterologous genes into the poxviral genome; the original plasmid transfer vector is not part of the GMO.

18. The degree to which the vector is limited to the DNA required to perform the intended function.

The plasmid vectors used in the generation of PROSTVAC-V and PROSTVAC-F each contain DNA sequences encoding the PSA and TRICOM genes together with the poxviral regulatory sequences (promoters) that direct their expression in poxvirus-infected cells. These gene sequences are flanked by genomic regions that allow homologous recombination between the transfer plasmid and the vaccinia or fowlpox genomes, respectively. The backbone of each transfer plasmid includes a bacterial origin of replication and the ampicillin resistance gene, which allow the selection and propagation of the plasmids in bacterial cells; however, only the PSA and TRICOM genes, together with the poxviral regulatory sequences are present in the final recombinant viruses.

Characteristics of the Modified Organisms

19. The methods used for the modification

As described briefly in Items 17 and 18 above, the generation of recombinant pox viruses is accomplished via homologous recombination, within an infected cell, between pox virus DNA and a plasmid vector that carries the heterologous sequences to be inserted. The plasmid vector contains one or more chimeric genes, each comprising a pox virus promoter linked to a protein coding sequence; the gene sequences to be inserted are flanked by viral sequences from a non-essential region of the pox virus genome. The plasmid is transfected into primary chicken embryo fibroblast (CEF) cells infected with the parental pox virus, and recombination between pox virus sequences on the plasmid and the corresponding DNA in the viral genome results in the insertion into the viral genome of the chimeric genes on the plasmid.

20. The methods used

a. To construct inserts and introduce them into the recipient organism

PROSTVAC-V

Plasmid Vector. The plasmid vector used for insertion of the PSA and TRICOM genes into the parental vaccinia virus genome by recombination contains the following elements:

- a prokaryotic origin of replication to allow amplification of the vector in a bacterial host;
- the gene encoding resistance to the antibiotic ampicillin, to permit selection of prokaryotic host cells that contain the plasmid;
- DNA sequences homologous to the Hind III F region of the vaccinia genome, which direct insertion of foreign sequences into this region via homologous recombination; this flanking sequence also includes the vaccinia F13L gene encoding the 37K envelope protein;
- a chimeric gene comprising the vaccinia 40K transcriptional promoter linked to the PSA gene;
- a second chimeric gene comprising the vaccinia 30K transcriptional promoter linked to the LFA-3 gene;
- a third chimeric gene comprising the vaccinia I3 transcriptional promoter linked to the ICAM-1 gene;
- a fourth chimeric gene comprising the sE/L transcriptional promoter linked to the B7.1 gene.

Plasmid Backbone. The plasmid backbone, including the bacterial origin of replication and the ampicillin resistance gene, was derived from the plasmid vector pUC8 (*Viera, 1982*) by deletion of a 442 base pair (bp) Hae II fragment containing the pUC8 polylinkers and *lac*Z gene. A linker containing a single Hind III site was inserted in the unique Nde I site in this vector to facilitate additional cloning.

Flanking Sequences. The vaccinia Hind III F sequences and the vaccinia promoter sequences were isolated from genomic DNA prepared from TBC-Wy or from the vaccinia WR strain (*Panicali, 1981*). Sequences from the Hind III F region, including the F13L gene, that flank the PSA and TRICOM genes, comprise vaccinia nucleotide sequence 43961-45163 (GenBank accession number M35027; *Goebel, 1990*) upstream of the 40K-PSA(L155) sequence and vaccinia nucleotide sequence 43192-43944 downstream of the sE/L-B7.1 sequence.

Transcriptional Promoters. The 40K promoter element was isolated as a 161 bp Dra I - FnuD II fragment from the vaccinia virus Hind III H region (*Rosel, 1986*) The 30K (M2L) promoter element was isolated as a 415 bp Sal I-Rsa I fragment from the Hind III M region of the vaccinia genome (*Goebel, 1990*). The I3 promoter element was isolated by polymerase chain reaction (PCR) amplification of a 201 bp sequence immediately 5' to the translation initiation codon of the I3 gene (*Schmitt, 1988*).

PSA gene. The gene encoding PSA was isolated at the National Cancer Institute by polymerase chain reaction amplification of cDNA derived from RNA from the human LNCaP cell line (CRL 1740, American Type Culture Collection (ATCC), Rockville, MD), which originated from a metastatic lesion of a prostatic adenocarcinoma (*Horoszewicz, 1983*). Nucleotide sequence analysis indicated that the sequence of this cDNA was identical to the published sequence with the exception of a single nucleotide change resulting in a change in amino acid residue 658 from asparagine to tyrosine (*Lundwall, 1987*). The PSA gene was then modified by *in vitro* mutagenesis to express full-length protein containing one altered epitope that has been shown to enhance immunogenicity (*Terasawa, 2002*). This mutation changed the encoded amino acid at position 155 from isoleucine to leucine. The modified PSA gene was contained on a 786 bp fragment which consists of the entire coding sequence for PSA (*Lundwall, 1987*).

LFA-3 gene. The gene encoding LFA-3 was isolated at the National Cancer Institute by PCR amplification of Human Spleen Quick-Clone cDNA (Clontech Inc.) using the published sequence (*Wallner, 1987*). The gene was contained on a 753 bp fragment which consists of the entire coding sequence for LFA-3.

ICAM-1 gene. The gene encoding ICAM-1 was isolated at the National Cancer Institute by PCR amplification of cDNA reverse-transcribed from RNA from an Epstein-Barr Virus-transformed B cell line derived from a healthy male, using the published sequence (*Staunton, 1988*). The gene was contained on a 1599 bp fragment which consists of the entire coding sequence for ICAM-1.

B7.1 gene. The gene encoding B7.1 was isolated at the National Cancer Institute by PCR amplification of cDNA derived from RNA from the human Raji cell line (ATCC # CCL 86), using the published sequence (*Chen, 1992*). The gene was contained on an 867 bp fragment which consists of the entire coding sequence for B7.1.

The structure of the plasmid transfer vector was verified by restriction endonuclease digestion using BamH I, EcoR I, and Sac I. In addition, the products of digestion with these enzymes were subjected to Southern blot analysis using labeled probes corresponding to the PSA, B7.1, ICAM-1, and LFA-3 genes and to the vaccinia Hind III F sequences. The DNA fragments visualized by these methods were of the predicted sizes, and the presence of the PSA, B7.1, ICAM-1, and LFA-3 genes was unequivocally demonstrated, thus confirming the predicted structure of the plasmid.

Generation of Recombinant Virus. The PROSTVAC-V recombinant virus was generated using standard recombination techniques. CEF cells were infected with the parental vaccinia virus at a multiplicity of infection (MOI) of 0.1 pfu/cell. Using the calcium phosphate precipitation method (*Graham, 1973*) cells were then transfected with the plasmid transfer vector pT2240. After 48 hours, infected cells were harvested and progeny virus was released by three rounds of freezing and thawing.

A derivative of TBC-Wy was created to facilitate the isolation of recombinant virus. This derivative virus, designated TBC-WyΔ37, was generated from TBC-Wy by replacement of the F13L gene with the *E. coli gpt* gene and the *lac*Z gene using a procedure similar to that described in Blasco & Moss (*1991*). Deletion of the F13L gene results in a virus defective in in the formation of pox viral plaques on infected cells *in vitro*. Expression of the *lacZ* gene by TBC-WyΔ37 results in the production of ß-galactosidase.

PROSTVAC-V was generated using TBC-WyΔ37by the method described by Blasco & Moss (*1995*). CEF cells were infected with TBC-WyΔ37 and transfected with pT2240. Recombination between the plasmid vector and the viral DNA restores the F13L (37K) gene of the parental TBC-Wy; thus, the desired recombinant virus possesses the ability to form plaques on a permissive cell line. In addition, in recombinant viruses, the *gpt* gene and the *lac*Z gene are replaced by the four chimeric transgenes described above.

Recombinant progeny viruses were identified by their ability to form plaques and their colorless appearance in the presence of Bluo-Gal™, the histochemical substrate for β-galactosidase. After recombination, CEF cells were infected with progeny virus until distinct plaques were visible. Plaques were picked from the cell monolayer and their progeny were further propagated. Repeated rounds of plaque isolation and replating resulted in the purification of the desired recombinant, which was then amplified on CEF cells to generate a recombinant seed stock.

PROSTVAC-F

Plasmid Vector. The plasmid vector used for insertion of the PSA and TRICOM genes into the parental fowlpox virus genome by homologous recombination contains the following elements:

- a prokaryotic origin of replication to allow amplification of the vector in a bacterial host;
- the gene encoding resistance to the antibiotic ampicillin, to permit selection of prokaryotic host cells that contain the plasmid;
- DNA sequences homologous to the BamH I J region of the fowlpox genome, which direct insertion of foreign sequences into this region via homologous recombination;
- the *E. coli lac*Z gene, flanked by repeated sequences;
- a chimeric gene comprising the vaccinia 40K transcriptional promoter linked to the PSA(L155) gene;
- a chimeric gene comprising the vaccinia 30K transcriptional promoter linked to the LFA-3 gene;
- a chimeric gene comprising the vaccinia I3 transcriptional promoter linked to the ICAM-1 gene;
- a chimeric gene comprising the sE/L transcriptional promoter linked to the B7.1 gene.

Plasmid backbone. The plasmid backbone was identical to that used for PROSTVAC-V

Flanking Sequences. The fowlpox BamH I J (*Jenkins, 1991*) sequences were isolated from genomic DNA prepared from the POXVAC-TC vaccine strain (Schering Corporation) of fowlpox virus. Sequences from the BamH I J region that flank the PSA(L155), LFA-3, ICAM-1, B7.1 and *lac*Z genes include an 850 bp BamH I - Bgl II fragment upstream of the 40K-lacZ sequence and a 750 bp Bgl II - Xba I fragment downstream of the sE/L-B7.1 sequence.

Transcriptional Promoters. The 40K, 30K (M2L), and I3 promoter element were identical to those used for PROSTVAC-V. The sE/L promoter was isolated as a 60 bp Hind III-Sal I fragment from pJS-8, a derivative of pSC65 (*Chakrabarti, 1997*).

Sequences for Transient Selection. The *E. coli lac*Z gene was isolated as a 3100 bp BamH I fragment from pDP500 (*Panicali, 1986*). The *lac*Z gene is flanked by repeated sequences for transient *lac*Z selection. The repeated sequence consists of 219 bp from the 3' end of the left flanker of the fowlpox BamH I J insertion site plus 161 bp from the vaccinia 40K promoter.

PSA gene. The gene encoding modified PSA was isolated as described for PROSTVAC-V

LFA-3 gene. The gene encoding LFA-3 was isolated as described for PROSTVAC-V.

ICAM-1 gene. The gene encoding ICAM-1 was isolated as described for PROSTVAC-V.

B7.1 gene. The gene encoding B7.1 was isolated as described for PROSTVAC-V.

The structure of the plasmid transfer vector was verified by restriction endonuclease digestion using BamH I, EcoR I, and EcoR V. In addition, the products of digestion with these enzymes were subjected to Southern blot analysis using labeled probes corresponding to the PSA, B7.1, ICAM-1, and LFA-3 genes and to the fowlpox BamH I J sequences. The DNA fragments visualized by these methods were of the predicted sizes, and the presence of the PSA, B7.1, ICAM-1, and LFA-3 genes was unequivocally demonstrated, thus confirming the predicted structure of the plasmid.

Generation of Recombinant Virus. The PROSTVAC-F recombinant virus was generated using standard recombination techniques. CEF cells were infected with the parental fowlpox virus. Using the calcium phosphate precipitation method (*Graham, 1973*) cells were then transfected with the plasmid transfer vector pT2246. Infected cells were harvested and progeny virus was released by three rounds of freezing and thawing.

A chromogenic assay for β-galactosidase was used to identify and isolate recombinant viruses containing the *lac*Z, PSA and TRICOM genes. This method takes advantage of the ability of fowlpox virus to form distinct plaques when grown on CEF cell monolayers. Cells were infected with progeny virus until distinct plaques were visible, at which time the plaques were overlaid Bluo-Gal to allow visualization of plaques expressing the lacZ gene. Viral plaques expressing *lac*Z appeared blue against a clear background. Positive plaques were picked from the cell monolayer and their progeny were further subjected to several rounds of plaque purification.

The *E. coli lac*Z gene was flanked by repeated sequences. Because repeated sequences are unstable in pox viruses (*Moss, 1981*), upon amplification the purified "blue" recombinant viruses spontaneously gave rise to white plaques from which the *lac*Z gene has been deleted. The white plaques were selected and plaque-purified. The purified recombinant pox virus contained only the desired genes encoding PSA and TRICOM. Positive recombinants were amplified on CEF cells to produce a seed stock. The seed stock was then subjected to titration, sterility testing, and genomic and protein expression analysis.

21. The description of any insert or vector construction

Detailed in Item 20 above.

22. The purity of the insert from any unknown sequence and information on the degree to which the inserted sequence is limited to the DNA required to perform the intended function

For both PROSTVAC-V and PROSTVAC-F, the DNA sequence of the inserted transgenes and associated transcriptional control regions, together with approximately 800-900 base pairs of genomic viral sequences on either side of the inserted genes was determined. Both PROSTVAC-V and PROSTVAC-F exhibited the expected nucleotide sequences; the coding sequences of the four inserted genes and their associated transcriptional control regions are identical to the predicted sequences. The transgenes are inserted at the expected site in the viral genome. No unexpected rearrangements were detected. Portions of the vector manipulated during its derivation conformed to predicted sequence. The inserted sequences are limited to the four human genes and the associated poxvirus transcriptional control regions required for their express in the recombinant poxviruses.

23. The methods and criteria used for selection

The methods and criteria used to select the recombinant pox viruses PROSTVAC-V and PROSTVAC-F are detailed in item 20, above. Confirmation of identity, genomic structure, and protein expression were performed as described below.

Confirmation of the identity and genomic structure of PROSTVAC-V and PROSTVAC-F was accomplished by restriction enzyme digestion using three different enzymes and Southern blot hybridization using vaccinia-specific or fowlpox-specfic probes, respectively, as well as PSA-specific, LFA-3-specific, ICAM-1-specific, and B7.1-specific probes. Analysis of each recombinant virus was conducted in parallel with the parental virus and the plasmid used for homologous recombination.

In addition, sequence analysis was performed on the inserted foreign genes, on the recombination junctions, and on the regions 200 base pairs upstream and downstream of the gene insert. Recombinant virus DNA sequences were compared to the sequence of the plasmid vector used to generate the recombinant.

Western blot analysis, using antibodies specific for PSA and the three TRICOM proteins was used to examine the molecular weight and identity of the proteins expressed by the recombinant viruses.

24. The sequence, functional identity and location of the altered, inserted or delected nucleic acid segments in question and, in particular, any known harmful sequence.

There are no harmful traits associated with the human genes inserted into PROSTVAC-V and PROSTVAC-F.

In PROSTVAC-V, the human PSA and TRICOM genes were inserted into an intergenic region between open reading frames F12L and F13L in the Hind F region of the genome. No vaccinia genes are interrupted by this insertion.

In PROSTVAC-F, the human PSA and TRICOM genes were inserted into the FPV426 gene in the Bam HI J region of the genome. The absence of this gene, FPV246, which has homology to the ankyrin repeat gene family, is not predicted to have an effect on the properties of fowlpox virus.

The identity and function of the inserted genes is described in detail in Item 23, above.

Characteristics of the Genetically Modified Organisms in Their Final Form

25. The description of genetic traits or phenotypic characteristics and in particular any new traits and characteristics which may be expressed or no longer expressed

PROSTVAC-V and PROSTVAC-F are vaccinia and fowlpox viruses, respectively, into which have been inserted genes encoding modified human PSA and the three human immunoregulatory molecules B7.1, ICAM-1, and LFA-3. Both PROSTVAC-V and PROSTVAC-F express the four inserted human genes.

No vaccinia genes are interrupted by the insertion of human genes into PROSTVAC-V; in PROSTVAC-F, a single gene, FPV246, is inactivated by the gene insertion. The inactivation of FPV246, which has homology to the ankyrin repeat gene family not predicted to have an effect on the properties of fowlpox virus.

Thus, with the exception of their ability to express the human PSA and TRICOM genes, the genetic traits or phenotypic characteristics of PROSTVAC-V and PROSTVAC-F are essentially those of parental vaccine strains of vaccinia virus and fowlpox virus.

26. The structure and amount of any vector or donor nucleic acid remaining in the final construction of the modified organisms.

The only exogenous genes present in PROSTVAC-V and PROSTVAC-F are genes encoding human PSA, B7.1, LFA-3, and ICAM-1, together with the poxviral regulatory sequences (promoters) that direct their expression in poxvirus-infected cells.

27. The stability of the organism in terms of genetic traits

The entire genome of the Working Seed Virus (WSV) and the entire genome of one production lot of PROSTVAC-V and PROSTVAC-F are sequenced. In addition, for each production lot, identity is demonstrated by PCR, Western blot, and restriction site analysis. In aggregate this testing provides verification of genetic stability.In addition, a comparability assessment of the full genomes Phase 2 and Phase 3 IMP material was completed. These data further support the genetic stability of PROSTVAC-V and PROSTVAC-F.

28. The rate and level of expression of the new genetic material in the organisms and the method and sensitivity of measurement of that rate and level.

Rate of expression of the new genetic material has not been measured.

29. The activity of the gene product

The presumed mode of action of PROSTVAC-V/F involves the induction of anti-PSA immune responses that result in the eradication of PSA-expressing tumor cells. PROSTVAC-V and PROSTVAC-F are therapeutic vaccines designed to deliver the tumor-associated antigen PSA to antigen-presenting cells (APCs), where it is then processed and expressed on the APC surface within the major histocompatability complex (MHC), leading to T-cell activation (*Essajee, 2004; Schlom, 2008*). In order to enhance the immunogenicity of the expressed PSA, PROSTVAC-V and PROSTVAC-F also express three human costimulatory molecules, designated TRICOM. Vaccination with PROSTVAC-V results in the simultaneous expression by infected cells of PSA epitopes in combination with costimulatory molecules. The co-expression of PSA in the context of the TRICOM molecules is expected to enhance the T cell immune response to PSA. These immune responses to PSA are then boosted by multiple immunizations with the corresponding recombinant fowlpox virus, PROSTVAC-F. Data describing the activity of PROSTVAC gene products are reported in section 32.

30. The description of identification and detection techniques, including techniques for the identification and detection of the inserted sequence and vector

Confirmation of the identity and genomic structure of the recombinant viruses is accomplished by (1) PCR amplification of the inserted genes and flanking regions; (2) FACS assay using antibodies specific for PSA, B7.7, ICAM-1, LFA-3, and the vector to examine the co-expression of all insert-derived antigen in host cells ; and (3) Western blot analysis using antibodies specific for PSA, B7.7, ICAM-1and LFA-3 to examine the molecular weight and identity of the polypeptides expressed by the recombinant viruses in cell lines. Analysis is conducted in parallel with analysis of PROSTVAC-V and F reference standards.

31. The sensitivity, reliability (in quantitative terms) and specificity of detection and identification techniques

Descriptions of the PCR, Western blot,and FACS assays are provided in item 8, above.

32. The history of previous releases or uses of the organisms

Nonclinical studies of PROSTVAC and related vaccines

PROSTVAC-V and PROSTVAC-F have been evaluated in a number of non-clinical studies to establish their safety for clinical use. In addition, substantial non-clinical data on the safety and efficacy of related pox-virus vaccines have been evaluated and further support the safety of PROSTVAC-V and PROSTVAC-F.

Non-clinical studies conducted on PROSTVAC-V and/or PROSTVAC-F are listed in **Table A1-1; Table A1-2** lists non-clinical studies conducted on poxviral vaccines that contain one or more components similar to those that comprise the PROSTVAC-V and PROSTVAC-F vaccines.

Table A1- 1: PROSTVAC-V and PROSTVAC-F Non-clinical Studies

Study	Test Product	Species
PHARMACOLOGY		
Biodistribution	PROSTVAC-F/ TRICOM	mouse
Immunogenicity and anti-tumor activity	PROSTVAC-V/TRICOM PROSTVAC-F/TRICOM	mouse
TOXICOLOGY		
Neurovirulence and Blood Brain Barrier crossing	PROSTVAC-V Dryvax smallpox vaccine	mouse
Safety	Group 1: PBS/10% Glycerol Group 2: rV-PROSTVAC/TRICOM and rF-PROSTVAC/TRICOM, Group 3: rV-PROSTVAC/TRICOM and rF-PROSTVAC/TRICOM and GM-CSF Group 4: rV-CEA(6D)/TRICOM + rV-MUC-1 and rF-CEA(6D)/TRICOM + rF-MUC-1/TRICOM and GM-CSF Group 5: rV-CEA(6D)/TRICOM + rV-MUC-1 and rF-CEA(6D)/TRICOM + rF-MUC-1/TRICOM Group 6: GM-CSF	non-human primate

Table A1-2: Supportive Non-clinical Studies on Related Poxviral Vaccines

Test Product	Species	Study or Publication
PHARMACOLOGY		
rV-CEA/TRICOM and rF-CEA/TRICOM	*in vitro*	Hodge JW, Sabzevari H, Yafal AG, *et al.* A triad of costimulatory molecules synergize to amplify T-cell activation. *Cancer Res*. 1999;59:5800-7.
TRICOM	*in vitro*	Shankar P, Schlom J, and Hodge JW. Enhanced activation of rhesus T-cells by vectors encoding a triad of costimulatory molecules (B7-1, ICAM-1, LFA-3). *Vaccine*. 2001;20(5-6):744-55.
rF-TRICOM	*in vitro*	Zhu M, Terasawa H, Gulley J, *et al.* Enhanced activation of human T-cells via avipox vector-mediated hyperexpression of a triad of costimulatory molecules in human dendritic cells. *Cancer Res*. 2001;61:3725-34.
rF-TRICOM rV-TRICOM	*in vitro*	Hodge JW, Rad AN, Grosenbach DW, *et al.* Enhanced activation of T-cells by dendritic cells engineered to hyperexpress a triad of costimulatory molecules. *J Natl Cancer Inst*. 2000;92:1228-39. Rad AN, Schlom J, and Hodge JW. Vector-driven hyperexpression of a triad of costimulatory molecules confers enhanced T-cell stimulatory capacity to DC precursors. *Crit Rev Oncol Hematol*. 2001;39:43-57. Hodge JW, Grosenbach DW, Rad AN, *et al.* Enhancing the potency of peptide-pulsed antigen presenting cells by vector-driven hyperexpression of a triad of costimulatory molecules. *Vaccine*. 2001;19:3552-67.
rV-CEA/muTRICOM rV-CEA vaccinia virus	Mouse	Hodge JW, Sabzevari H, Yafal AG, *et al.* A triad of costimulatory molecules synergize to amplify T-cell activation. *Cancer Res*. 1999;59:5800-7.
rV-B7.1 rV-CEA	Mouse	Hodge JW, McLaughlin JP, Abrams SI, *et al.* Admixture of a recombinant vaccinia virus containing the gene for the costimulatory molecule B7 and a recombinant vaccinia virus containing a tumor-associated antigen gene results in enhanced specific T-cell responses and antitumor immunity. *Cancer Res*. 1995;55:3598-3603.
Vaccinia vector encoding both CEA and B7.1	Mouse	Kalus RM, Kantor JA, Gritz L, *et al.* The use of combination vaccinia vaccines and dual-gene vaccinia vaccines to enhance antigen-specific T-cell immunity via T-cell costimulation. *Vaccine*. 1999;17:893-903.
rV-CEA, rV-CEA/B7.1, rV-CEA/muTRICOM	CEA-transgenic mouse	Hodge JW, Sabzevari H, Yafal AG, *et al.* A triad of costimulatory molecules synergize to amplify T-cell activation. *Cancer Res*. 1999;59:5800-7
rV-CEA/muTRICOM plus GM-CSF and IL-2	CEA-transgenic mice with CEA-positive liver carcinoma metastases	Grosenbach DW, Barrientos JC, Schlom J, and Hodge JW. Synergy of vaccine strategies to amplify antigen-specific immune responses and antitumor effects. *Cancer Res*. 2001;61:4497-4505.

Test Product	Species	Study or Publication
rV-PSA	Monkey	Karr JF, Kantor JA, Hand PH, *et al.* The presence of prostate-specific antigen-related genes in primates and the expression of recombinant human prostate-specific antigen in a transfected murine cell line. *Cancer Res.* 1995;55:2455-62. Lundwall A and Lilja H. Molecular cloning of human prostate specific antigen cDNA. *FEBS Lett.* 1987;214:317-22. Hodge SW, Schlom J, Donohue SJ, *et al.* A recombinant vaccinia virus expressing human prostate-specific antigen (PSA): safety and immunogenicity in a non-human primate. *Int J Cancer*. 1995;63:231-7.
TOXICOLOGY		
rV-muB7.1 Wyeth vaccinia virus Hanks buffered saline	Mouse	Freund YR, Mirsalis JC, Fairchild DG, *et al.* Vaccination with a recombinant vaccinia vaccine containing the B7-1 co-stimulatory molecule causes no significant toxicity and enhances T-cell-mediated cytotoxicity. *Int J Cancer*. 2000;85(4):508-17.
rV-PSA Wyeth vaccinia virus	Mouse	Repeat dose study of rV-PSA
rV-CEA, rV-CEA/muTRICOM or wild-type vector followed by rF-CEA, rF-CEA/muTRICOM or wild-type vector	Mouse	Repeat dose study in mice with rV-CEA/TRICOM and rF-CEA/TRICOM
rV-CEA, rV-CEA/muTRICOM or wild-type vector followed by rF-CEA, rF-CEA/muTRICOM or wild-type vector	human CEA-transgenic mouse	Repeat dose study in mice with rV-CEA/TRICOM and rF-CEA/TRICOM
rV-PSA FDA Reference Smallpox Vaccine	Rabbit	Dermotopic properties of rV-PSA
rV-PSA Wyeth vaccina virus	Monkey	Hodge SW, Schlom J, Donohue SJ, *et al.* A recombinant vaccinia virus expressing human prostate-specific antigen (PSA): safety and immunogenicity in a non-human primate. *Int J Cancer*. 1995;63:231-7.

Summary of Nonclinical Studies

PROSTVAC-V and -F, and related pox virus vaccines have been tested in mouse, rabbit and non-human primate models as well as in a number of *in vitro* experiments. Notably, PROSTVAC was shown to induce robust anti-PSA humoral as well as cell mediated immunity in mouse, which translated into anti-tumor efficacy in a PSA-tumor model; in addition, anti-tumor activity provided by related pox virus vaccines targeting different antigens has also been reported in numerous publications (**Table A1-2**). No biologically significant changes or signs of untoward toxicological effects were noted in either rodent or non-human primate safety studies.

Clinical Studies of PROSTVAC-V/F

PROSTVAC-V/F is the product of more than 15 years of poxviral vaccine development and evaluation by the US National Cancer Instititue (NCI) and the former Therion Biologics Corporation (Therion). Since 1991, ten recombinant vaccinia-based vaccines and eight recombinant fowlpox-based vaccines produced by Therion for the treatment of various cancers have been evaluated in human clinical trials sponsored the NCI. Over 1,000 cancer patients, most with metastatic disease, have been treated to date with these various poxvirus-based vaccines in NCI-sponsored or Therion-sponsored clinical trials.

The PROSTVAC-V and PROSTVAC-F products were preceded by a number of Phase 1 and Phase 2 clinical studies on earlier vaccinia and fowlpox constructs that expressed PSA. Clinical evaluations of early versions of these PSA-containing poxviral vectors involved over 250 patients. The initial constructs tested were vaccinia-PSA, in three Phase 1 studies totaling 81 patients (*Sanda, 1999; Eder, 2000; Gulley, 2002*). Later, a prime boost regimen was devised with boosting with fowlpox-PSA containing vectors, in two studies, one with 64 patients (*Kaufman, 2004*) and another unpublished study of approximately 16 patients. The NCI subsequently began a series of trials using additional admixed vectors encoding a single costimulatory molecule (B7.1/CD80). These studies involved 94 patients (*Gulley, 2005; Arlen, 2005; Madan, 2008*). No significant safety issues were identified in these early studies.

PROSTVAC-V and PROSTVAC-F have been evaluated in eight clinical trials in the United States under two separate INDs. These agents have been administered to over 300 men up to a maximum dose of 2×10^8 plaque-forming units (pfu) of PROSTVAC-V and 1×10^9 pfu of PROSTVAC-F. Investigation of PROSTVAC-V and PROSTVAC-F was initiated by Therion in 2002 under BB-IND 10428 (now BB-MF 10428). Therion conducted a Phase 1 trial evaluating the safety and immunogenicity of PROSTVAC-V and PROSTVAC-F in ten patients, and a randomized, placebo-controlled Phase 2 trial evaluating the safety and efficacy (as defined by progression-free survival) of PROSTVAC-V and PROSTVAC-F in 122 men (125 enrolled, 122 received drug) with castration-resistant metastatic prostate cancer (mCRPC).

The NCI initiated its own ongoing investigations of PROSTVAC-V and PROSTVAC-F, alone and in combination with other therapies, in 2003, under NCI BB-IND 10915. These clinical

trials cover a range of study designs evaluating PROSTVAC-V and PROSTVAC-F in early and late-stage disease and in combination with other therapies. To date, the NCI has conducted two Phase 1 studies, one Phase 1/2 study and three Phase 2 studies in the United States; no dose-limiting adverse effects have been noted in any of these trials.

Therion completed the randomized Phase 2 study through approximately one year of follow-up. However, in 2006, Therion went out of business. In early 2007, the NCI, which had collaborated with Therion to develop PROSTVAC-V and PROSTVAC-F for the treatment of prostate cancer, acquired all rights to the PROSTVAC-V and PROSTVAC-F technology and to the regulatory files previously owned by Therion. In 2008, BNIT acquired the Therion regulatory files and rights to the PROSTVAC-V and PROSTVAC-F technology from the NCI in a license agreement, and a collaborative research and development agreement (CRADA). BNIT conducted and completed the final overall survival analysis for the Phase 2 trial.

Summaries of Individual Clinical Studies with the GMO Conducted by the NCI

The NCI studies outlined in **Table A1-3** evaluate PROSTVAC-V and PROSTVAC-F under a variety of study designs alone and in combination with various therapies. These are all small studies designed to evaluate the broad potential utility of PROSTVAC-V and PROSTVAC-F for the treatment of prostate cancer, and they are reviewed primarily to further support the safety of this product.

Note that in the NCI clinical trials, PROSTVAC-V and PROSTVAC-F are termed, respectively, PROSTVAC-V/TRICOM and PROSTVAC-F/TRICOM, and alternatively termed (Recombinant Vaccinia-PSA(L155)/TRICOM™ and Recombinant Fowlpox-PSA(L155)/TRICOM™).

Table A1- 3: PROSTVAC-V and PROSTVAC-F Clinical Studies Conducted by the NCI Under BB-IND 10915

Protocol No.	Study Type	Study Title	N	Study Duration and Status
5911	Efficacy, Safety	A Phase 1/II Pilot Study of Sequential Vaccinations with rFowlpox-PSA (L155)-TRICOM (PROSTVAC-F/TRICOM) Alone, or in combination with r Vaccinia-PSA (L155)-TRICOM (PROSTVAC-V/TRICOM), and the Role of GM-CSF, in Men with Prostate Cancer	47	57 days with long term extension ------ Complete
6066	Feasibility	Phase 1 Feasibility Study of Intraprostatic PSA-Based Vaccine in Men with Prostate Cancer and Local Failure Following Radiotherapy or Cryotherapy or Clinical Progression on Androgen Deprivation Therapy in the Absence of Local Definitive Therapy	21	85 days ------ Ongoing, closed to accrual
7207	Efficacy in combination with an Anti-CTLA-4 antibody	Phase 1 Trial of a PSA Based Vaccine and an Anti-CTLA-4 Antibody in Adults with Metastatic Androgen Independent Prostate Cancer	30	Long Term ------ Ongoing, closed to accrual
E9802	Efficacy and Safety	A Phase II Study of PROSTVAC-V (Vaccinia)/TRICOM and PROSVAC-F (Fowlpox)/TRICOM with GM-CSF in Patients with PSA Progression After Local Therapy for Prostate Cancer	50	Long Term ------ Ongoing, Closed to accrual
7354	Efficacy in combination with Flutamide	Randomized Phase II Trial Combining Vaccine Therapy with PROSTVAC/TRICOM and Flutamide vs. Flutamide Alone in Men with Androgen Insensitive, Non-Metastatic (D0.5) Prostate Cancer	24	Long Term ------ Ongoing
7678	Efficacy in combination with Quadramet	A Randomized Phase 2.5 Study of 153Sm-EDTMP (Quadramet) With or Without a PSA/TRICOM Vaccine in Men with Androgen-Insensitive Metastatic Prostate Cancer	17	Long Term ------ Ongoing

33. In relation to human health

a. the toxic or allergenic effects of the non-viable organisms and/or their metabolic products

PROSTVAC-VF has previously been administered to over 300 subjects in eight Phase 1 and 2 clinical studies (see Item 32) and no toxic or allergenic effects were reported.

b. the comparison of the organisms to the donor, recipient or (where appropriate) parental organism regarding pathogenicity

As described in Item 5, PROSTVAC-V and PROSTVAC-F are comparable to their parental viruses with respect to pathogenicity.

c. the capacity of the organisms for colonization

Replication and transcription of members of the *Pox* family of viruses occurs in the cytosol of infected cells, with virally encoded enzymes driving these processes. PROSTVAC V/F DNA is extra-chromosomal and is not integrated. Poxviruses are cleared from the host within several days for PROSTVAC-F and weeks for PROSTVAC-V. Thus, colonization by PROSTVAC-V/F does not occur.

d. if the organisms are pathogenic to humans who are immunocompetent

i Diseases caused and mechanisms of pathogenicity including invasiveness and virulence

The most common AEs related to PROSTVAC-V and PROSTVAC-F observed to date have been injection site reactions, all of which were ≤ Grade 2 severity. Typical AEs historically seen with intradermal administration (scarification) of vaccinia virus vaccines (vesicles, pustules, and scaring) have not been observed with PROSTVAC-V. PROSTVAC-V immunization is subcutaneously administered, which greatly reduces injection site reactions, and skin surface wound formation/viral shedding. The most common systemic AEs attributed to PROSTVAC-V and PROSTVAC-F administration were fatigue, nausea/vomiting, fever, chills, arthralgia and dizziness.

<u>Reactions and Complications Associated with Smallpox Vaccinia Vaccination</u>

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 the 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 smallpox vaccination are expected and considered to be normal: fever, myalgia, headache, fatigue, chills, nausea, soreness and erythema at the vaccination site, local lymphadenopathy. Satellite lesions around the vaccination site have been reported as well as local edema. These symptoms are self-limiting, last for around three weeks after vaccination and rarely are a cause for serious concern (*Frey, 2002; Fulginiti, 2003*). Mild adverse reactions that can occur post vaccination are bacterial superinfection of vaccination site, erythema multiforme and generalized vaccinia. Superinfection is a rare event with incidence from 0.14 to 55 cases per million according to different reports (Vellozzi, 2004).

Erythema multiforme (EM) most often presents as papules, plaques or urticaria which may be symmetrical and may involve palms and soles. EM resolves spontaneously and requires no special care. A development of Stevens-Johnson syndrome with mucosal involvement is extremely rare, with only one case noted in the 2003-2004 vaccination campaign in the US (<1 per 1,000,000) (*Fulginiti, 2003; Neff, 2008*).

Generalized vaccinia results from viremic spread of vaccinia virus from the vaccination site. It presents as generalized rash which behaves like the vaccination site lesion, progressing through papular, vesicular, pustular and scab-forming stages. The incidence is difficult to assess, since historically there was no strict definition to distinguish generalized vaccinia from other conditions where rash is a dominant symptom (severe chickenpox, smallpox, eczema vaccinatum, EM). Retrospective analysis of 2002 – 2004 vaccinations suggests an incidence of ~50 cases per 1,000,000 (*Bryant-Genevier, 2006*). The rash appears within a week after vaccination and resolves within a week. Most instances do not require specific therapy (*Fulginiti, 2003*).

Some of the post-vaccinia adverse events, although very rare, are serious and potentially life-threatening. They include progressive vaccinia (PV), eczema vaccinatum (EV) and postvaccinial encephalitis (PVE).

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

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 dermatis or other eczematous skin condition. Historically it occurred at a rate of ~ 1 case per 25,000 vaccinations. EV can occur in a vaccine recipient as well as in susceptible individuals in close contact. Two cases of EV from transmission have been recently reported; both in children of recently vaccinated US military personnel (*Lederman, 2009; Vora, 2008*). In the military vaccination program in the US there were no reports of EV among 450,239 vaccinees, probably due to careful screening for contraindications (*Grabenstein, 2003*). Review of civilian vaccinations did not detect any cases of EV (*Velozzi, 2005*). 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.PVE has higher prevalence and mortality rate in children compared to adults. Higher historical rates were reported in Europe compared to US. Variability is attributed to differences in case definitions, clinical evaluations and differences in vaccine strains used by different countries (*Sejvar, 2005*). Pathogenesis is still under investigation, although several compelling theories focus on autoimmune mechanism. Aside from vaccinia, measles and rabies vaccines have known association with PVE, as well as other viral and bacterial infections (*Benneto, 2004; Menge, 2007*). Review of 2002 – 2004 vaccinations in US reported 3 cases of PVE for the rate of 5 per 1 000 000.

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

Review of data from 2002 – 2004 vaccinations in US reported ~ 1 case of autoinoculation per 6,500 vaccinations with 17% of ocular cases, none with corneal involvement (*Neff, 2008*). Vaccinia keratitis is the most serious consequence of autoinoculation, since lesions on the cornea threaten eyesight. Diseased or injured conjunctiva and cornea may increase the risk of this complication. Vaccinia keratitis will respond to treatment with topical antiviral agents and

interferon, and can be prevented with use of occlusive bandages over scarification site and patient education (*Fulginiti, 2003*).

Transmission of vaccinia to close contacts is another known complication. Contact vaccinia may manifest as PV, EV or accidental infection of the eye, mouth, or genital areas. Review of several national and state surveys between 1962 and 1968 gives a frequency for EV at 8 - 27 per 1,000,000, and for accidental infections at 3 - 44 per 1,000,000 (*Neff, 2002*). The rate of contact vaccinia in 2002 – 2004 was <10 cases per 100,000. Education of vaccinees in proper care for the vaccination site, proper hand hygiene, and avoidance of contact with at-risk individuals seems to be a reasonable and effective prophylactic against accidental contact with vaccinia.

Severe adverse reactions associated with the use of traditional smallpox vaccines have not been observed in prior clinical studies of PROSTVAC or related PSA (or other tumor antigen)-containing vaccinia virus vaccines administered to vaccinia pre-immune cancer patients. The use of attenuated vaccinia virus, diligent screening for exclusion of patients with known risk factors and thorough education of potential patients and study personnel in proper hygiene and would care should reduce the risk of development of these reactions.

Safety of Fowlpox Vaccination

Fowlpox virus is a member of the genus Avipox, which is evolutionarily divergent from vaccinia virus and serologically non-crossreactive (*Taylor, 1988; Beukema, 2006*). Immune responses to vaccinia 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.

Fowlpox virus has been investigated and used in vaccine design for at least two decades. As with vaccinia 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 (HIV, malaria, cancer) have been tested in both animals and humans. No safety concerns have been raised and the adverse events associated with the use of fowlpox vectors have been limited to mild injection site reactions (*Beukema, 2006; Essagee, 2004; Webster 2006*).

ii Communicability

Vaccinia

Vaccinia virus may be transmitted by direct contact with virus shed from the vaccination site or with contaminated dressings or other infectious material. Vaccinia is shed from the primary vaccination lesion of humans and guinea pigs from approximately the third day to the end of the third week after vaccination (*Cooney, 1991; Friedman, 1962*); shedding from other sites is rare, and is generally associated with more virulent strains of smallpox vaccine or with complications after vaccination (*Cummings, 2008; Frey, 2002; Kim, 2005; Klote, 2005; Koplan, 1975*). Clinical studies of recombinant vaccinia viruses, including PROSTVAC-V, have demonstrated viral shedding only at the site of vaccination (*Brysiowicz, 1996; Cooney, 1991; Graham, 1992; Mukherhjee, 2000; Scholl, 2000; Scholl, 2003)*.

Limited data are available on viral shedding after vaccination of vaccinia-experienced (previously vaccinated) individuals, but shedding from revaccination sites seems to be shorter by about1 week and possibly of lower titer than shedding from primary vaccination sites (*Seaman, 2010*). This finding is significant, as all study subjects are required to have had prior smallpox vaccination. Viral shedding is further reduced in individuals that are vaccinated by the subcutaneous route (*Henderson, 1939; Cherry, 1977; Connor, 1977*), which is the route that will be used for the administration of PROSTVAC-V. Bandaging contains the virus at the vaccination site, further minimizing release into the environment.

That airborne or droplet spread of vaccinia virus from the respiratory tract of healthy vaccinees occurs is doubtful (*Lane, 2003; Neff, 2003; Talbot, 2007*). Although the virus can be found in the bloodstream and pharynx of patients with adverse events involving vigorous viral replication and/or abnormal host defenses, particularly eczema vaccinatum and progressive vaccinia (*Lane, 2003*), epidemiologic evidence for airborne spread is scant.

Contact transmission of vaccinia-based smallpox vaccine is rare (*Neff, 2002; CDC, 2004*). No secondary transmission of recombinant poxviruses, including PROSTVAC-V and PROSTVAC-F, has been reported in humans. However, PROSTVAC-V and PROSTVAC-F are live viruses and, as such, retain the potential for transmission. Consequently, healthcare personnel who have direct contact with contaminated dressings or other infectious material from participants in clinical studies are instructed to adhere to appropriate infection control measures. In addition, study exclusion criteria exclude subjects from participation if they have close or household contact with individuals at risk for developing serious adverse reactions. Use of appropriate infection control measures, such as covering the vaccination site (including bandages) and washing hands after contact with the vaccination site, will prevent transmission.

Fowlpox virus does not replicate in human cells. Consequently, viral shedding in humans is limited and appears to be confined to the vaccination site. Fowlpox is not known to cause disease in healthy adult humans and is of minimal potential hazard under ordinary conditions of

use. They can be handled safely in the laboratory without special apparatus or equipment, using techniques generally acceptable for nonpathogenic material.

iii Infective dose

The minimum infectious dose of vaccinia and fowlpox viruses is unknown (*Savona, 2006*). Under the proposed release, each patient will receive one immunization with 2×10^8 infectious units (Inf. U.) of PROSTVAC-V Week 1, followed by six immunizations with 1×10^9 Inf. U. of PROSTVAC-F administered in Weeks 3, 5, 9, 13, 17, and 21.

iv Host range and possibility of alteration

Vaccinia can infect warm-blooded vertebrates such as mammals, rodents, and birds. The insertion of human genes into the genome of vaccinia to generate PROSTVAC-V does not alter this host range.

Fowlpox virus replication *in vivo* is restricted to certain avian species. Fowlpox virus replicates in chickens, turkeys, and pigeons, but not in quail, ducks, or canaries (*Tripathy, 1984; McMillen, 1994*). The insertion of human genes into the genome of fowlpox to generate PROSTVAC-F does not alter this host range.

v Possibility of survival outside of human host

Poxviruses cannot propagate without a permissive host organism. Poxviruses have the capacity to survive for considerable periods in dried material such as detached vaccination scabs. They are also are relatively stable when stored frozen or lyophilized under carefully controlled conditions. However stability decreases significantly as temperature is increased. Under normal environmental conditions, PROSTVAC-V and PROSTVAC-F are expected to lose viability within days or weeks. In addition, poxviruses are readily inactivated by a number of common disinfectants and cleaning agents.

vi Presence of vectors or means of dissemination

The primary means of dissemination of vaccinia is via direct contact with the infection site or with virus-contaminated materials. Contact transmission of vaccinia-based smallpox vaccine is rare (*Neff, 2002; CDC, 2004*). Airborne or droplet spread of vaccinia virus from the respiratory tract of healthy vaccinees has not been definitively demonstrated (*Lane, 2003; Neff, 2003; Talbot, 2007*). Although the virus can be found in the bloodstream and pharynx of patients with adverse events involving vigorous viral replication and/or abnormal host defenses, particularly eczema vaccinatum and progressive vaccinia (*Lane, 2003*), epidemiologic evidence for airborne spread is scant.

Fowlpox virus does not replicate in human cellsConsequently, viral shedding in humans is limited and appears to be confined to the vaccination site. The probability of transmission to non-target

organisms is therefore very low. In permissive avian species, transmission of recombinant vaccines based on vaccine strains of avipox virus is rare (*McMillen, 1994; Paoletti, 1995*).

vii Biological stability

The evaluation of genetic stability of PROSTVAC-V and PROSTVAC-F at several stages of the production process is described in Item 11. In terms of stability *in vivo*, vaccinia virus causes a transient infection in susceptible hosts, 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. *In vivo* stability of fowlpox virus *in vivo* is not relevant to this application, since fowlpox virus does not replicate in mammalian cells.

viii Antibiotic resistance patterns

Not applicable.

ix Allerginicity

PROSTVAC-V and PROSTVAC-F have not been shown to be allergenic in any preclinical or clinical studies to date. However, based on the manufacturing procedures used to produce PROSTVAC-V and PROSTVAC-F, patients with known allergy to eggs, egg products or aminoglycoside antibiotics (for example, gentamicin or tobramycin) are excluded from the study. In addition, patients should remain in the clinic for at least 30 minutes following administration of the PROSTVAC for observation for signs of adverse reactions.

x Availability of appropriate therapies

For some very rare complications of vaccinia infection, early administration of vaccinia immune globulin (VIG) is advised. Recognition of clinical symptoms compatible with eczema vaccinatum, severe generalized vaccinia, progressive vaccinia, and some cases of auto-inoculation should prompt consideration of VIG therapy. The effectiveness of VIG therapy appears to be time-dependent. VIG is of no benefit in the treatment of post-vaccinial encephalitis, and is **contraindicated** for the treatment of vaccinial keratitis. VIG is available in United States through CDC and in several other countries through appropriate health authorities. Despite the very low risk for complications that require VIG administration, BNIT has secured a necessary supply of VIG for the countries where it is not available internally.

Although there is no recognized alternative to VIG in treating severe complications resulting from vaccinia vaccination, *in vitro* and animal model data in several poxvirus infections models demonstrate the activity of cidofovir at clinically relevant doses. Subjects who experience severe vaccinia complications may be treated with cidofovir. Treatment with cidofovir will be recommended primarily after clinical failure following treatment with vaccinia immune globulin. Cidofovir is generally available through hospital pharmacies.

e. Other Product Hazards

No other product hazards are known from studies to date.

PART III
CONDITIONS OF RELEASE

The Release

34. The description of the proposed deliberate release, including the purpose or purposes and foreseen products of the release.

PROSTVAC-V and PROSTVAC-F are used in a prime-boost vaccination regimen to optimize immune responses against prostate cancer tumor cells.

The proposed Phase 3 trial is a, double-blind, randomized, placebo-controlled trial is being conducted to evaluate PROSTVAC-V/F with and without adjuvant GM-CSF for the treatment of men with asymptomatic or minimally symptomatic metastatic castration-resistant prostate cancer (mCRPC). This trial will be conducted globally.

Patients will be randomized with equal probability to one of three double-blind arms of 400 patients each:

- Arm V+G: PROSTVAC-V/F + adjuvant GM-CSF
- Arm V: PROSTVAC-V/F + GM-CSF Placebo
- Arm P : PROSTVAC-V/F Placebo + GM-CSF Placebo

The trial interventions will consist of 1 subcutaneous (sc) immunization with PROSTVAC-V (2×10^8 Inf. U/dose) or placebo in Week 1, followed by six immunizations with PROSTVAC-F (1×10^9 Inf. U//dose) or placebo administered in Weeks 3, 5, 9, 13, 17, and 21. Each immunization will be accompanied by administration of sc GM-CSF or placebo on the day of immunization and for the subsequent three days (sc injection within 5 mm of the original PROSTVAC-V/F or placebo injection site). The Treatment phase of this trial is six months including an End-of-Treatment visit at Week 25. During the Treatment phase, patients will continue to be treated with vaccination therapy through PSA and/or radiological progression.

The primary objective of this study is to ascertain whether the survival of patients randomized to receive PROSTVAC (with or without GM-CSF) is superior to that of patients randomized to receive the placebo control.

Exploratory endpoints will also be included and are outlined below:

- To model secondary radiological disease progression over the first year of study participation. To ascertain whether a greater proportion of patients randomized to either Arm V+G or Arm V remain event-free (radiological progression) six months post End-of-Treatment visit (using scans at End-of-Treatment visit as a new baseline),

as compared to the patients randomized to Arm P. This analysis will include all patients, and separately the HLA-A2-expressing subgroup.

- To assess the role of post-progression anti-cancer therapies as an alternative explanation for observed survival differences.
- To ascertain whether the survival of HLA-A2-expressing patients randomized to Arm V+G, or Arm V is superior to that from HLA-A2-expressing-patients randomized to Arm P.
- To ascertain whether a greater proportion of HLA-A2 expressing patients randomized to Arm V+G or to Arm V remain event-free at six months as compared to the HLA-A2 expressing patients randomized to Arm P.
- To compare arms with respect to immune response to immunizing antigen (PSA), as well as to non-vaccine containing prostate antigens, and tumor-associated antigens, and assess whether immune responses are prognostic and/or predictive.
- To evaluate for baseline biomarkers which are prognostic and/or predictive of long term survival outcome.
- To compare arms with respect to vaccine effects on circulating tumor cell (CTC) levels (US sites only).

This proposed Phase 3 study will provide the primary basis of the efficacy claim in the planned BLA in the US and MAA in the EU for PROSTVAC.

35. The foreseen dates of the release and time planning of the experiment including frequency and duration of releases.

Enrollment of study BNIT-PRV-301 began in the EU on 20th July 2012 and it is anticipated to be completed by December 2013.

36. The preparation of the site before the release.

All principal investigators and sub-investigators participating in the study will be qualified by education, training and experience to assume responsibility for the proper conduct of the trial according to the guidelines outlined in International Conference on Harmonisation (ICH) E6 - Good Clinical Practices. Clinical sites where the study is to be conducted will be thoroughly evaluated prior to the initiation of the study to ensure that the facilities are sufficient for storing and administering the vaccine, as well as having the appropriate facilities for the collection and storage of human specimens. Additionally, all clinical site personnel involved in the handling or administration of study vaccine will be trained according to the study protocol, and all supportive documentation, including study specific laboratory and clinical trial material manuals. A thorough study-specific training will occur prior to the initiation of the study via a formal investigator meeting and/or on-site study initiation visit.

37. The size of the site.

The study vaccine will be administered at licensed healthcare facilities where there are standard facility controls in place for administration of GMO vaccines, collection and processing of blood and serum samples, and all equipment and staff required for the clinical evaluation of study subjects. Clinical site staff will be instructed to follow universal precautions for the prevention of transmission of infectious agents in healthcare settings, such as the World Health Organization Standard Precautions. No risk related to PROSTVAC-related waste is anticipated. Clinical study sites will be instructed to follow normal site procedures for disposal of biomedical or infectious waste.

The study is not expected to have any effect on the local population other than for those subjects enrolled on the study and those individuals with close contact with the study subjects. There are projected to be 12 clinical sites in England. It is also projected that an average of approximately 3 patients per site will be enrolled per year.

The following are clinical study sites in England where study vaccine will be administered:

Site 1

The GMO will be administered Queen Elizabeth Hospital, Queen Elizabeth Medical Centre, Edgbaston, Birmingham, B15 2TH. The national grid appendices of this building are SP044839. The population of Birmingham is approximately 1 082 000 inhabitants.

Site 2

The GMO will be administered at Mount Vernon Centre for Cancer Treatment, Mount Vernon Hospital, Rickmansworth Road, Northwood, Middlesex, HA6 2RN. The national grid appendices of this building are TQ077918. The population of Northwood is approximately 25 000 inhabitants.

Site 3

The GMO will be administered at The Christie Hospital, Wilmslow Road, Withington, Manchester, M20 4BX. The national grid appendices of this building are SJ848925. The population of Manchester is approximately 510 000 inhabitants.

Site 4

The GMO will be administered at Bristol Haematology & Oncology Centre, Horfield Road, Bristol, BS2 8ED. The national grid appendices of this building are ST585734. The population of Bristol is approximately 430 000 inhabitants.

Site 5

The GMO will be administered at Taunton & Somerset NHS Foundation Trust, Musgrove Park Hospital, Parkfield Drive, Taunton, Somerset, TA1 5DA. The national grid appendices of this building are ST215242. The population of Taunton is approximately 62 000 inhabitants.

Site 6

The GMO will be administered at St. Bartholomew's Hospital, West Smithfield, London, EC1A 7BE. The national grid appendices of this building are TQ318814. The population of London is approximately 8 175 000 inhabitants.

Site 7

The GMO will be administered at Guy's Hospital, Great Maze Pond, London, SE1 9RT. The national grid appendices of this building are TQ327801. The population of London is approximately 8 175 000 inhabitants.

Site 8

The GMO will be administered at Sarah Cannon Research UK, 93 Harley Street, London, W1G 6AD. The national grid appendices of this building are TQ285818. The population of London is approximately 8 175 000 inhabitants.

Site 9

The GMO will be administered at The Royal Marsden NHS Foundation Trust, Department of Clinical Oncology (Urology), Fulham Road, London, SW3 6JJ. The national grid appendices of this building are TQ269784. The population of London is approximately 8 175 000 inhabitants.

Site 10

The GMO will be administered at Royal Derby Hospital, Uttoxeter Road, Derby, DE22 3NE. The national grid appendices of this building are SK327348. The population of Derby is approximately 242 000 inhabitants.

Site 11

The GMO will be administered at Queen's Hospital, Rom Valley Way, Romford, Essex, RM7 0AG. The national grid appendices of this building are TQ511877. The population of Romford is approximately 250 000 inhabitants.

Site 12

The GMO will be administered at Royal Sussex County Hospital - Sussex Cancer Centre, Eastern Road, Brighton, BN2 5BE. The national grid appendices of this building are TQ326039. The population of Brighton is approximately 156 000 inhabitants.

38. The methods to be used for the release.

PROSTVAC-V and PROSTVAC-F are frozen preparations of live recombinant vaccinia and fowlpox vaccines that are supplied in borosilicate (2R) glass vials sealed with rubber stoppers and aluminium-plastic closures. Each vial of PROSTVAC-V contains a single dose (2×10^8 infectious units (Inf. U.) per 0.50 mL) of PROSTVAC-V in PBS/10% glycerol. Each vial of PROSTVAC-F contains a single dose (1×10^9 Inf. U.per 0.50mL) of PROSTVAC-F in PBS/10% glycerol. The drug product contains no adjuvants or preservatives.

PROSTVAC-V/F will be administered to study subjects by subcutaneous injection. The site of the PROSTVAC-V or placebo vaccination will be covered with a sterile, nonadherent dressing such as a Telfa bandage until the scab, if any, falls off naturally. For the PROSTVAC-F or placebo, the injection site will be covered with a plain adhesive bandage until a scab (if any) forms or for patient comfort.

39. The quantity of organisms to be released.

A central storage and distribution depot for study vaccine and placebo for 10 participating EU countries [Belgium, , Denmark, Estonia, France, Germany, Netherlands Poland, Slovakia, Spain and the United Kingdom] and also including Iceland, Israel, and Russia will be located in Craigavon, Northern Ireland, United Kingdom at the following location:

Company name	ALMAC Clinical Services
Address	9 Charlestown Road Seagoe Industrial Estate Craigavon BT63 5PW United Kingdom

Study vaccine will be packaged as individual-use vials, with 6 vials to a carton. Cartons sent to the drug distribution depot will be bundled for shipping into 10-carton packs. From the depot, individual sites or secondary depots (Russia, Israel) will be supplied over a period of approximately two years with enough IMP to service the enrolled patient population for the entire dosing period (~5 months per patient). It is anticipated that this will require multiple shipments.

Sites will initially be supplied with one carton of PROSTVAC-V, one carton of PROSTVAC-V placebo, 2 cartons of PROSTVAC-F, and 2 cartons of PROSTVAC-F placebo. Sites will be resupplied when the IVRS resupply trigger of 6 remaining vials of PROSTVAC-F/placebo or 3 remaining vials of PROSTVAC-V/placebo is met.

The primary EU depot (ALMAC Clinical Services, Craigavon, Northern Ireland) will receive shipments of study vaccine estimated to contain a total of 1,800 vials of PROSTVAC-V; 1,800 vials of PROSTVAC-V placebo; 6,002 vials of PROSTVAC-F, and 3,024 vials of PROSTVAC-F placebo. It should be noted that PROSTVAC-V and PROSTVAC-F placebo are the same construct: empty fowlpox vector.

40. The disturbance of the site, including the type and method of cultivation, mining, irrigation, or other activities.

Not applicable. Protocol BNIT-PRV-301 is not an agricultural study.

41. The worker protection measures taken during the release.

All principal investigators and sub-investigators participating in the study will be qualified by education, training and experience to assume responsibility for the proper conduct of the trial according to the guidelines outlined in International Conference on Harmonisation (ICH) E6 - Good Clinical Practices. Clinical sites where the study is to be conducted will be thoroughly evaluated prior to the initiation of the study to ensure that the facilities are sufficient for storing and administering the vaccine, as well as having the appropriate facilities for the collection and storage of human specimens.

Additionally, all clinical site personnel involved in the handling or administration of study vaccine will be trained according to the study protocol, and all supportive documentation, including study specific laboratory and clinical trial material manuals. A thorough study-specific training will occur prior to the initiation of the study via a formal local investigator meeting and/or on-site study initiation visit.

The risk of transmission of recombinant viruses to exposed healthcare workers is very low. There have been no cases of transmission to healthcare personnel in any of the studies with PROSTVAC-V/F or PROSTVAC-V/F-related vaccinia-based vaccines. As reported for the handling of vaccinia strains found in smallpox vaccines, if appropriate infection-control precautions are observed, healthcare workers are probably at less risk of infection than laboratory workers because of the smaller volume of lower titer of virus in clinical specimens as compared with laboratory material (*Garner, 1983; Bolyard, 1998*). However, because of the potential for transmission of vaccinia or recombinant vaccinia viruses, healthcare personnel who have direct contact with contaminated dressings or other infectious material from participants in clinical studies will receive detailed instruction on proper prophylactic measures. BNIT does not recommend prophylactic smallpox vaccination for healthcare workers conducting the study, since vaccinations would put such workers at a real risk of post-vaccination complications. The most critical preventive measure is proper handling of the vaccine and thorough hand hygiene after any contact with vaccination site or bandage materials. In fact, there were no cases of contact transmission in health care setting or from civilian vaccinees to their contacts reported during the US vaccination campaign (MMWR May 9, 20003/52(18);419-420).

Procedures for preparation of the vaccine are described in the clinical protocol. PROSTVAC-V classified as a group 2 biological agent according to the European Economic Community (EEC) classification for the protection of workers with biological agents {Directive 2000/54/EC}. Loading of syringes may be performed using standard aseptic methods in a clinical pharmacy or clinical laboratory. These recommendations are the same as those for administering smallpox vaccine with bifurcated needles. BNIT recommends routine use of standard universal precautions when directly handling the vaccine, including the wearing of a lab coat, eye protection, and gloves.

PROSTVAC-F is classified as BSL1 and because it cannot replicate in mammalian cells, no special precautions beyond standard, universal precautions for infectious materials are required. Thus, standard universal precautions as dictated by the WHO (WHO, 2006) are adequate in dealings with PROSTVAC-F and should be followed at any clinical trial site in accordance to ICH/GCP.

In case of spills, poxviruses are readily inactivated by a number of detergents and can easily be contained. Material Safety Data sheets will be provided with each product and study staff will be provided with specific instructions to address spills, including information on containment, personal protective equipment, disinfection, and disposal procedures.

42. The post-release treatment of the site.

All transport of PROSTVAC will be done according to EU guidelines for the transport of GMOs and IATA Transportation Regulations. PROSTVAC vaccine or placebo will be transported to the clinical study site as cartons of 6 individual, frozen vials. Study vaccine will be stored at -70°C or below in the original outer package and will be stored in a secure location with limited access. Following administration, used study vaccine materials will be placed immediately into sealed bags and retained for accountability. Upon reconciliation and accountability, used study materials will be destroyed by the clinical site following institutional procedures for the disposal of biohazardous material. All unused study vaccine will be disposed of at the clinical site upon authorization from BNIT.

43. The techniques foreseen for elimination or inactivation of the organisms at the end of the experiment.

All vaccine materials, including vials, syringes, and other biomedical waste generated during the conduct of the study will be discarded into appropriate biohazard waste containers and disposed of at the study site according to site procedures.

44. Information on, and the results of, previous releases of the organisms and in particular, releases on a different scale or into different ecosystems.

Preclinical studies and clinical studies using PROSTVAC or related vaccines are listed in Item 31.

PROSTVAC-V/F and related pox virus vaccines have been tested in mouse, rabbit and non-human primate models as well as in a number of *in vitro* experiments. Recombinant vaccinia and fowlpox vaccines elicited immune responses to antigens presented and showed substantial evidence of anti-tumor activity in murine models. No biologically significant changes or signs of untoward toxicological effects were noted in either rodent or non-human primate safety studies.

In clinical studies with PROSTVAC-V/F and related vaccines, adverse reactions were 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.

All clinical studies have been performed in standard healthcare facilities using standard procedures and controls as outlined throughout this application.

The Environment (Both on the Site and in the Wider Environment)

45. The geographical location and national grid reference of the site onto which the release will be made, or the foreseen areas of use of the product.

A table of principal investigators and addresses of the clinical sites in England to be used in the study is provided below.

	Principal Investigator	Address	National Grid Reference
1	Prof. Nicholas James MD, MB BS, FRCR, FRCP, PhD, MRCP.	Queen Elizabeth Hospital Queen Elizabeth Medical Centre, Edgbaston, Birmingham, B15 2TH	SP044839
2	Prof. Peter Hoskin M.D. MB BS, FRCR, FRCP, PhD, MRCP	Mount Vernon Centre for Cancer Treatment, Mount Vernon Hospital, Rickmansworth Road, Northwood, Middlesex, HA6 2RN	TQ077918
3	Tony Elliott M.D. MB CHB, FRCR, PhD	The Christie Hospital, Wilmslow Road, Withington, Manchester, M20 4BX	SJ848925
4	Amit Bahl M.D. MB BS, FRCR, FRCP, MRCP	Bristol Haematology & Oncology Centre, Horfield Road, Bristol, BS2 8ED	ST585734
5	John Graham M.DGraham MB CHB, FRCR, FRCP.	Taunton & Somerset NHS Foundation Trust Musgrove Park Hospital, Parkfield Drive, Taunton, Somerset, TA1 5DA	ST215242
6	Jonathan Shamash MD, MB CHB, FRCP, MRCP	St Bartholomew's Hospital, West Smithfield, London, EC1A 7BE	TQ318814
7	Simon Chowdhury MA, MD, PhD, MRCP, MB BS	Guy's Hospital Great Maze Pond, London, SE1 9RT	TQ327801
8	Simon Chowdhury MA, MD,	Sarah Cannon Research UK, 93 Harley Street,	TQ285818

	PhD, MRCP, MB BS	London, W1G 6AD	
9	Vincent Khoo MD, MB BS, FRCR, PhD	The Royal Marsden NHS Foundation Trust Department of Clinical Oncology (Urology), Fulham Road London, SW3 6JJ	TQ269784
10	Prabir Chakraborti MD, MB BS, MRCP	Derby Hospitals NHS Foundation Trust Royal Derby Hospital, Uttoxeter Road, Derby, DE22 3NE	SK327348
11	Stephanie Gibbs MD, MB BS, MRCP, FRCR	Barking, Havering & Redbridge University Hospitals NHS Trust (BHRUT) Queen's Hospital, Rom Valley Way, Romford, RM7 0AG	TQ511877
12	Angus Robinson MD, MB BS, MRCP, FRCR	Brighton & Sussex University Hospitals NHS Trust Royal Sussex County Hospital - Sussex Cancer Centre, Eastern Road, Brighton, BN2 5BE	TQ326039

46. The physical or biological proximity of the site to humans and other significant biota.

The study vaccine will be administered at licensed healthcare facilities where there are standard facility controls in place for administration of GMO vaccines, collection and processing of blood and serum samples, and all equipment and staff required for the clinical evaluation of study subjects. Clinical site staff will be instructed to follow universal precautions for the prevention of transmission of infectious agents in healthcare settings, *e.g.*, the World Health Organization Standard Precautions. It is not anticipated that the study vaccine or any waste associated with study procedures will affect the surrounding ecosystem.

47. The proximity to significant biotopes or protected areas.

The study vaccine will be administered at licensed healthcare facilities where there are standard facility controls in place for administration of GMO vaccines, collection and processing of blood and serum samples, and all equipment and staff required for the clinical evaluation of study subjects.

Clinical site staff will be instructed to follow universal precautions for the prevention of transmission of infectious agents in healthcare settings, *e.g*., the World Health Organization Standard Precautions. It is not anticipated that the study vaccine or any waste associated with study procedures will affect the surrounding ecosystem.

48. The size of local human population.

An indication of local human populations is provided in Item 37.

49. The local economic activities which are based on the natural resources of the area.

The study vaccine will be administered at licensed healthcare facilities where there are standard facility controls in place for administration of GMO vaccines, collection and processing of blood and serum samples, and all equipment and staff required for the clinical evaluation of study subjects. Clinical site staff will be instructed to follow universal precautions for the prevention of transmission of infectious agents in healthcare settings, *e.g*., the World Health Organization Standard Precautions. .The study is not expected to have any effect on the local population other than for those subjects enrolled on the study and those individuals with close contact with the study subjects.

50. The distance to the nearest drinking water supply zone areas and/or areas protected for environmental purposes.

The study vaccine will be administered at licensed healthcare facilities where there are standard facility controls in place for administration of GMO vaccines, collection and processing of blood and serum samples, and all equipment and staff required for the clinical evaluation of study subjects. Clinical site staff will be instructed to follow universal precautions for the prevention of transmission of infectious agents in healthcare settings, e.g., the World Health Organization Standard Precautions. .It is not anticipated that the study vaccine or any waste associated with study procedures will affect the surrounding ecosystem.

51. The climatic characteristics of the region or regions likely to be affected.

The study vaccine will be administered at standard healthcare facilities where it is not anticipated that the study vaccine or any waste associated with study procedures will affect the surrounding ecosystem.

52. The geographical, geological and pedological characteristics.

A complete list of study sites and their locations is provided in Item 37. The study vaccine will be administered at licensed healthcare facilities where there are standard facility controls in place for administration of GMO vaccines, collection and processing of blood and serum samples, and all equipment and staff required for the clinical evaluation of study subjects. Clinical site staff will be instructed to follow universal precautions for the prevention of transmission of infectious

agents in healthcare settings, e.g., the World Health Organization Standard Precautions. It is not anticipated that the study vaccine or any waste associated with study procedures will affect the surrounding ecosystem.

53. The flora and fauna, including crops, livestock and migratory species.

The study vaccine will be administered at licensed healthcare facilities where there are standard facility controls in place for administration of GMO vaccines, collection and processing of blood and serum samples, and all equipment and staff required for the clinical evaluation of study subjects. Clinical site staff will be instructed to follow universal precautions for the prevention of transmission of infectious agents in healthcare settings, e.g., the World Health Organization Standard Precautions. It is not anticipated that the study vaccine or any waste associated with study procedures will affect the surrounding ecosystem.

PROSTVAC-V and PROSTVAC-F cannot infect plant or insect cells. Vaccinia host range is limited to certain warm-blooded vertebrates; fowlpox host range is limited to certain avian species.

54. The description of target and non-target ecosystems likely to be affected.

The study vaccine will be administered at licensed healthcare facilities where there are standard facility controls in place for administration of GMO vaccines, collection and processing of blood and serum samples, and all equipment and staff required for the clinical evaluation of study subjects. Clinical site staff will be instructed to follow universal precautions for the prevention of transmission of infectious agents in healthcare settings, *e.g.*, the World Health Organization Standard Precautions. It is not anticipated that the study vaccine or any waste associated with study procedures will affect the surrounding ecosystem

55. The comparison of the natural habitat of the recipient organisms with the proposed site or sites of release.

Regarding natural habitats of the parental organisms, vaccinia virus has no known natural habitat and the origins of vaccinia virus in nature and as a vaccine are unknown. Avipox viruses are distributed in nature worldwide. It is not anticipated that the study vaccine would have any advantage with respect to growth or survivability as compared to parental vaccines in the natural habitat.

56. Any known planned developments or changes in land use in the region which could influence the environmental impact of the release.

Not applicable. No developments or changes in land use in the region are anticipated.

PART IV
THE ORGANISMS AND THE ENVIRONMENT

Characteristics Affecting Survival, etc

57. The biological features which affect survival, multiplication and dispersal.

The potential for escape, dispersal, or establishment of PROSTVAC-V or PROSTVAC-F in the environment is low. Poxviruses cannot reproduce in the absence of a susceptible host cell. Vaccinia virus replication is restricted to certain warm-blooded vertebrate hosts. Vaccinia virus has no known natural animal reservoirs, although buffalo poxvirus in India has been proposed to be a subspecies of vaccinia virus and Cantagalo virus in humans and cattle in Brazil is reported to be a vaccinia-like virus (*Damaso, 2000*). Fowlpox virus is host-range restricted *in vivo* to certain avian species, and the fowlpox vaccine strain used for PROSTVAC-F is non-virulent.

Unlike viruses such as retroviruses, poxviruses undergo a transient infection in a host organism. The poxvirus replication cycle occurs in the cytoplasm of infected cells. There is no interaction with the nucleus and no integration of viral genes into the host genome. Therefore, the risk of poxvirus persistence by integration into the host chromosome is very low, unlike other viruses, such as retroviruses, which integrate and establish permanent infections in the host. Latent infection of humans with vaccinia virus has not been observed (*Fenner, 1988*).

Pox viruses are not capable of forming spores or generating other specialist structures to enhance environmental survival.

Pox viruses are relatively stable at sub-freezing temperatures but lose viability at higher temperatures. Additionally, poxviruses are readily inactivated by a number of detergents; thus, accidental spills can be contained and are not likely to result in spread of PROSTVAC-V or PROSTVAC-F in the environment. The general environment is not likely to support propagation of these viruses, which require specific eukaryotic cells for replication, and the viruses decay at ambient temperatures (see Item 58, below).

PROSTVAC-V and PROSTVAC-F are comparable to their corresponding nonrecombinant parental viruses with respect to growth characteristics and stability in the environment. The added human transgenes have not fundamentally altered the inherent properties of the viruses. Therefore, PROSTVAC-V and PROSTVAC-F have not acquired any known phenotypic properties that would increase their risk to the environment beyond those associated with the use of the corresponding nonrecombinant parental viruses.

58. The known or predicted environmental conditions which may affect survival, multiplication and dissemination, including wind, water, soil, temperature, pH.

PROSTVAC-V

Vaccinia virus is relatively stable when stored at low temperatures. This contributed to the success of the smallpox vaccination program in developed countries where refrigeration was readily accessible. However, vaccine potency rapidly declined after storage at ambient temperatures, resulting in a high rate of vaccination failures in developing countries in the early twentieth century. Major efforts were required to develop a dried vaccine that would be stable at ambient temperatures (*Fenner, 1988*).

There are several published studies on the recovery of non-recombinant vaccinia virus under various laboratory conditions designed to mimic environmental exposure. Virus survival was evaluated, for example on fabric and food (*Sidwell, 1966; Pastoret, 1996; Essbauer, 2007*); dried or in liquid (including saline, drinking water, sterile-filtered lake or river water, stormwater, stormwater supplemented with fetal calf serum or mixed with potting soil) *(Mahnel, 1977; Mahnel, 1987; Essbauer, 2007*); under different conditions of relative humidity, temperature or pH (*Mahl, 1975; Newman 2003*); exposed to air or in airtight containers (*Mahnel, 1987*). There are many differences among these published experiments, including different vaccinia strains, preparation techniques, different starting virus titers, and viral titration methods. However, there are clear data in all of these studies that show first, that vaccinia is a relatively stable virus under a variety of laboratory conditions, and second, that stability decreases significantly when the virus is exposed to ultraviolet light (*McDevitt, 2007*) or as temperature and/or humidity are increased.

Specific stability studies have been performed on the non-recombinant vaccinia parental virus (TBC-Wy) and on a vaccinia recombinant similar to PROSTVAC-V, designated PANVAC-V (*unpublished data*). PANVAC-V is a recombinant vaccinia virus that, like PROSTVAC-V, expresses the three TRICOM costimulatory molecules; in addition, it expresses two additional tumor-associated antigens (*Petrulio, 2006; Madan, 2007*). Stability of PANVAC-V at dried or in water at various temperatures was compared to that of the non-recombinant parental virus, TBC-Wy. These studies demonstrated that the recombinant virus is comparable to the corresponding non-recombinant parental virus. Both viruses lost viability over a period of weeks when stored in water at 25°C. The same viruses lose viability over a period of days when stored dried at 25°C. These results are consistent with published reports discussed above that demonstrate that poxviruses are stable at low temperatures but are less stable at higher temperatures without special treatment such as lyophilization under controlled conditions.

PROSTVAC-F

There are few published studies on the persistence of avipox viruses in the environment. However, persistence in the environment, adverse sequellae, and other environmental issues have not been reported as a result of the use of licensed recombinant fowlpox and canarypox virus-based products, including veterinary vaccines against Newcastle Disease Virus, avian influenza virus, rabies, feline leukemia virus, canine distemper, and West Nile virus. As part of the licensure procedure, the USDA announced Findings of No Significant Impact with respect to the likelihood of an adverse environmental event using these vaccines (*Payne, 1994*; *Payne, 1995*; *Payne, 1996*; *Federal Register, 1994, 1997a, 1997b, 2003, 2004*).

Specific stability studies have been performed on the non-recombinant fowlpox parental virus (TBC-FPV) and on a fowlpox recombinant similar to PROSTVAC-F, designated PANVAC-F (*unpublished data*). PANVAC-F is a recombinant vaccinia virus that, like PROSTVAC-F, expresses the three TRICOM costimulatory molecules; in addition, it expresses two additional tumor-associated antigens (*Petrulio, 2006; Madan, 2007*). Stability of PANVAC-F at dried or in water at various temperatures was compared to that of the non-recombinant parental virus, TBC-FPV. These studies demonstrated that the recombinant virus is comparable to the corresponding non-recombinant parental virus. Both viruses lost viability over a period of weeks when stored in water at 25°C. The same viruses lose viability over a period of days when stored dried at 25°C. These results indicate that PROSTVAC-F is stable at low temperatures but are less stable at higher temperatures without special treatment such as lyophilization under controlled conditions.

59. The sensitivity to specific agents.

Vaccinia and fowlpox viruses are rapidly inactivated by a number of disinfectants (*Erterpi, 2009*). For example, one study showed that fowlpox virus and vaccinia virus are both inactivated within 1 minute by the following disinfectants: 70% ethanol, 50% isopropyl alcohol, 0.5% sodium hypochlorite, 30% formaldehyde, 10% benzalkonium chloride, a mixture of 6.67% cetyltrimethylammonium chloride and 3.33% benzalkonium chloride, and a mixture of 1.75% iodine and 10% polyethyleneglycol nonylphenyl ether. (*Chambers, 2011*)

In addition to chemical agents, PROSTVAC-V and PROSTVAC-F are inactivated by exposure to ultraviolet light (*Sagriponti, 2011*) and by exposure increasing temperatures. For example, when stored at 25°C, viruses lose viability over a period of weeks when stored in water, and over a period of days when stored dried.

For PROSTVAC-V and PROSTVAC-F, BNIT has qualified and approved the disinfectants Klercide CR-Biocide A and Perform Sterile Concentrated Oxy for product changeover and for surface disinfection, respectively.

Interactions with the Environment

60. The predicted habitat of the organism.

PROSTVAC-V and PROSTVAC-F are genetically engineered viruses and thus do not exist in nature. The habitat of the parental viruses has been previously discussed (see Item 9). Vaccinia virus has no known natural animal reservoirs, although buffalopox virus has been proposed to be a subspecies of vaccinia virus, and several strains of vaccinia virus have been isolated throughout Brazil. Avipoxviruses (APV) are distributed worldwide. The virus species fowlpox virus infects and causes disease in poultry. The parental fowlpox used for the generation of PROSTVAC-F is a non-virulent, vaccine strain.

61. The studies of the behaviour and characteristics of the organisms and their ecological impact carried out in simulated natural environments, such as microcosms, growth rooms and greenhouses.

No studies have been conducted on the ecological impact of PROSTVAC on simulated natural environments.

62. The capability of post-release transfer of genetic material-

a. from the genetically modified organisms into organisms in affected ecosystems

There is minimal potential for gene transfer to other species under the proposed release of the GMO. PROSTVAC-V and PROSTVAC-F cannot infect microbes, insects, cold-blooded vertebrates, or plant cells. The GMO will be released in a hospital examination room and is unlikely to come in contact with other animal species. Furthermore, no dissemination of PROSTVAC-V or PROSTVAC-F outside the injection site has been shown in humans injected by the subcutaneous route.

Recombination between the DNA genome of PROSTVAC-V or PROSTVAC-F with other DNA genomes, such as the human genome or other viral genomes in the infected host cell, is improbable for the following reasons. The poxvirus life cycle is carried out in the cytoplasm (*Moss, 1996*); poxviruses do not integrate into the genome of the infected cell. Therefore, the risk of poxvirus persistence by integration into the host chromosome is very low. In non-avian species susceptible to infection by PROSTVAC-F, few opportunities for genetic recombination with animal poxviruses could occur, since the level of replication that the vector DNA undergoes *in vivo* is low, and limited to cells infected by the inoculum (no generation of infectious particles).

Recombination with other viral genomes is unlikely due to the lack of homology between different families of viruses.

The human genes expressed in PROSTVAC-V and PROSTVAC-F share homology with their counterparts in the human genome; however the physical segregation between host and viral genomes renders recombination between PROSTVAC-V or PROSTVAC-F and the human genome an unlikely event.

Recombination between PROSTVAC-V or PROSTVAC-F and a wild-type vaccinia or fowlpox virus in an infected host organism is theoretically possible. Recombination between modified poxvirus and other poxviruses is dependent on DNA replication, co-localization of replication and DNA homology between the two viruses. The likelihood of recombination between PROSTVAC-V and wild type vaccinia *in vivo* is extremely low because vaccinia is not normally found in nature. Recombination between PROSTVAC-F and a wild or vaccine strain of fowlpox would require release of PROSTVAC-F into an environment containing poultry. Such release is highly unlikely under the proposed conditions of release.

b. from indigenous organisms to the genetically modified organisms.

Recombination between the DNA genome of indigenous organisms to the genetically modified organisms is unlikely for reasons described in 61a, above. The poxvirus life cycle is carried out in the cytoplasm (*Moss, 1996*); consequently, the physical segregation between host and viral genomes renders recombination with PROSTVAC-V or PROSTVAC-F improbable.

63. The likelihood of post-release selection leading to the expression of unexpected or undesirable traits in the genetically modified organisms.

There is no evidence that selection leading to the expression of unexpected or undesirable traits in PROSTVAC-V or PROSTVAC-F would occur under the conditions of release.

64. The measures employed to ensure and to verify genetic stability, the description of genetic traits which may prevent or minimise dispersal of genetic material and methods to verify genetic stability.

Genetic stability of PROSTVAC-V/F following administration under the conditions of this release will not be monitored. As stated above the DNA dose not incorporate into host cell genome and is cleared along with infected host cells shortly after administration.

The GMO is limited in its ability to disperse in the environment. Under normal environmental conditions pox viruses lose viability within days or weeks.

65. The routes of biological dispersal, known or potential modes of interaction with the disseminating agent, including inhalation, ingestion, surface contact, burrowing.

Following administration of PROSTVAC, the route of subsequent biological dispersal to the environment or to non-target organisms could occur through direct contact with the vaccination site or indirect contact with contaminated surfaces or objects.

Vaccinia viral shedding is transient and occurs primarily at the vaccination site; it has not been demonstrated to occur at other sites, including nose, throat, urine, and feces (*Cummings, 2008; Frey, 2002; Kim,2005; Klote, 2005; Koplan,1975*). The frequency of viral shedding is reduced after subcutaneous vaccination with recombinant vaccinia virus, which is the intended route of administration of PROSTVAC-V (*Henderson, 1939; Cherry, 1977; Connor, 1977*); furthermore, bandaging contains the virus at the vaccination site (*Talbot, 2006*).

Recombinant avipoxviral shedding has not been detected in blood, saliva, urine, or rectal swabs after vaccination (*Bleijs, 2005)*. Viral DNA has been detected at the injection site, but the presence of live virus was not assessed. Therefore, avipox viral shedding in humans, if it occurs, appears to be confined to the vaccination site.

66. The description of ecosystems to which the organisms could be disseminated.

The dissemination and impact of PROSTVAC-V/F on ecosystems is limited because dissemination requires close contact with the vaccination site or indirect contact with contaminated surfaces or objects. The study will be conducted at standard healthcare facilities. It is not anticipated that the study vaccine or any waste associated with study procedures will affect the surrounding ecosystem.

Potential Environmental Impact

67. The potential for excessive population increase of the organisms in the environment.

PROSTVAC-V and PROSTVAC-F are labile viruses that cannot replicate outside permissive cells. Therefore, they cannot increase in number without contact with permissive cells or host.

68. The competitive advantage of the organisms in relation to the unmodified recipient or parental organisms.

PROSTVAC-V and PROSTVAC-F have not been shown to display a competitive advantage over their unmodified parental viruses with respect to replication *in vitro*.

69. The identification and description of the target organisms.

PROSTVAC-V/F will be administered to men aged 18 years or older with asymptomatic or minimally symptomatic, metastatic CRPC. Patients will have progressive disease following androgen suppression or blockade therapy and withdrawal, or surgical castration, and will be chemotherapy naïve for metastatic prostate cancer. Patients must be vaccinia-experienced (previous smallpox vaccination).

70. The anticipated mechanism and result of interaction between the released organisms and the target organisms.

PROSTVAC-V/F is a viral vector-based product that is administered in seven subcutaneous vaccinations, over a five month period. It is intended to generate immune responses to prostate-specific antigen and prostate cancer cells. It uses poxviral vectors to introduce modified PSA to the patient in an inflammatory and immunogenic manner to break self-tolerance, which generates immune responses directed against prostate cancer cells. The vaccine appears to induce a chronic active immunomodulatory action, and to slow overall disease progression.

71. The identification and description of non-target organisms which may be affected.

The potential for PROSTVAC-V/F interaction with other organisms in the environment would be limited to viral shedding and secondary transmission via direct contact with the vaccination site or indirect contact with contaminated surfaces or objects. The most likely non-target organisms would include health care workers or contacts of vaccinees. In addition, environmental release could theoretically affect animals susceptible to pox virus infection, including certain warm blooded vertebrates such as mammals, rodents, or and birds. Neither PROSTVAC-V nor PROSTVAC-F is known to infect cold-blooded vertebrates such as fish, amphibians, and reptiles (*Essbauer, 2001*); thus, there is no known risk to aquatic animals from any potential environmental release. Additionally, neither virus infects microbes or plants.

The extent of exposure to non-target species is expected to be limited by the fact that vaccine administration occurs in a clinical site under controlled conditions. The administration of PROSTVAC-V/F via the subcutaneous route, the use of bandaging, which contains virus at the vaccination site, and comprehensive education of healthcare providers and patients all serve to minimize exposure to non-target species. Although the potential theoretically exists for secondary transmission of the virus to non-target organisms, multiple studies indicate that transmission to non-target organisms, either human-to-animal or human-to-animal, is rare except perhaps in the case of transmission of vaccinia by vaccinated or infected animal handlers to cattle.

To date there has never been a published report of transmission of vaccinia virus from vaccine recipients to health-care personnel (*CDC, 2001*). Furthermore, hundreds of individuals have been vaccinated with PROSTVAC-V/F or with related recombinant poxvirus vaccines, and no evidence of contact transmission has been noted in any clinical trial to date.

72. The likelihood of post-release shifts in biological interactions or in the host range.

Vaccinia can infect warm-blooded vertebrates such as mammals, rodents, and birds. Fowlpox virus infects certain avian species including chickens, turkeys, and pigeons. The host range of PROSTVAC-V and PROSTVAC-F is identical to that of their parental viruses.

There is no reason to believe that the host range of PROSTVAC-V or PROSTVAC-F would be altered under the conditions of this release. A s indicated above, the likelihood of release into the general environment is remote. The intrinsic mutation rate of poxviruses has not been precisely determined but is probably similar to that of other systems with proofreading DNA polymerases (*Knipe, 2007*). Shifts in biological interactions or in the host range would require rapid genetic changes in the GMO; as this is not expected to occur, the likelihood of post-release shifts in biological interactions or host range is negligible.

73. The known or predicted effects on non-target organisms in the environment and the impact on population levels of competitors, prey, hosts, symbionts, predators, parasites and pathogens.

The extent of exposure to non-target organisms is expected to be limited by the fact that vaccine administration occurs in a clinical site under controlled conditions. The administration of PROSTVAC-V/F via the subcutaneous route, the use of bandaging, which contains virus at the vaccination site, and comprehensive education of healthcare providers and patients all serve to minimize exposure to non-target species. Although the potential exists for secondary transmission of vaccinia virus to non-target organisms, multiple studies indicate that transmission to non-target organisms, either human-to-animal or animal-to-animal, is unlikely except perhaps in the case of transmission of vaccinated or infected animal handlers to cattle.

74. The known or predicted involvement in biogeochemical processes.

Not applicable. Neither vaccinia nor fowlpox virus have been shown, and are not anticipated to, have any involvement in biogeochemical processes.

75. Any other potentially significant interactions with the environment.

PROSTVAC-V and PROSTVAC-F are live viruses that require a host cell to replicate. They can only remain viable following infection and proliferation in an appropriate host organism. Other than potential host organisms, no environmental niches or habitats would be affected, either directly or indirectly, by exposure to PROSTVAC-V/F.

PART V
MONITORING, CONTROL, WASTE TREATMENT AND EMERGENCY RESPONSE PLANS

Monitoring Techniques

76. Methods for tracing the organisms and for monitoring their effects.

Overall Study Monitoring and Record Retention

The study will be monitored by BNIT or its designee on a regular basis throughout the study period in accordance with general monitoring principles set forth in ICH E5. All study documents (subject files, signed informed consent forms, copies of case report forms, Study File Notebook, study vaccine accountability records, etc) must be kept secured until disposal is authorized by the Sponsor, which will be no sooner than two years after approval of the study drug for marketing in an ICH region (United States, Japan, Europe), and until there are no more pending or contemplated marketing applications in an ICH region or at least two years have elapsed since the formal discontinuation of the clinical development program for the study drug. These documents may need to be retained for longer periods in some countries due to local regulatory requirements.

Primary Endpoints

The primary endpoint for the study is overall survival. Expected median survival is approximately 2 years. Approximately 10-20% of patients would be expected to be surviving at 4 years post study. All patients will be followed for vital status until study closure or death.

Twelve hundred (1200) patients are planned to be enrolled in a 3 arm study. There are 2 comparisons, PROSTVAC-V/F plus GM-CSF versus control, and PROSTVAC-V/F alone versus control. The final analysis will be triggered when 534 events (deaths) occur per comparison. The trial is powered to determine a 20% reduction in the risk of death (critical HR 0.82) with criteria for success of P=0.025.

Secondary Endpoints

The secondary endpoints include the following:

- Efficacy: To ascertain whether a greater proportion of patients randomized to one or both PROSTVAC-V/F arms remain event-free at six months as compared to the patients randomized to placebo
- Safety: To further characterize the safety and tolerability of PROSTVAC immunotherapy, characterize type and number of AEs compared to placebo
- Exploratory

- To model secondary radiological disease progression over the first year of study participation. To ascertain whether a greater proportion of patients randomized to either Arm V+G or Arm V remain event-free (radiological progression) six months post End-of-Treatment visit (using scans at End-of-Treatment visit as a new baseline), as compared to the patients randomized to Arm P. This analysis will include all patients, and separately the HLA-A2-expressing subgroup.
- To assess the role of post-progression anti-cancer therapies as an alternative explanation for observed survival differences.
- To ascertain whether the survival of HLA-A2-expressing patients randomized to Arm V+G, or Arm V is superior to that from HLA-A2-expressing-patients randomized to Arm P.
- To ascertain whether a greater proportion of HLA-A2 expressing patients randomized to Arm V+G or to Arm V remain event-free at six months as compared to the HLA-A2 expressing patients randomized to Arm P.
- To compare arms with respect to immune response to immunizing antigen (PSA), as well as to non-vaccine containing prostate antigens, and tumor-associated antigens, and assess whether immune responses are prognostic and/or predictive.
- To evaluate for baseline biomarkers which are prognostic and/or predictive of long term survival outcome.
- To compare arms with respect to vaccine effects on circulating tumor cell (CTC) levels (US sites only).

Safety Assessment and Monitoring

Patients will be followed during the Treatment phase of the study for any signs or symptoms of treatment-emergent toxicity by means of a focused physical exam, hematology, serum chemistry panels, EKG, and recording of AEs and concomitant medications. All SAEs will be immediately reviewed by the Sponsor and CRO medical monitors. In addition, this study will employ a Data Monitoring Committee.

Immune Response Assessment and Monitoring

Serum samples will be assayed for antibody development to the vectors and the insert (PSA). In addition, serum will be assayed for development of antibodies to other prostate cell antigens, as well as other tumor-associated antigens. Serum will also be profiled for changes in cytokine and chemokine expression, changes in circulating tumor markers, and circulating nucleic acid tumor markers. Paxgene tubes will yield total whole blood RNA and DNA for analysis of select gene expression by quantitative PCR, as well as enable analysis of gene arrays to detect changes in overall patterns of gene expression. Serum samples are very sensitive for detecting appropriate

vaccine induced anti-viral titers. Antibody responses to PSA are difficult to detect, as PSA is a self antigen, and high circulating levels of PSA in the blood of prostate cancer patients may bind and clear any induced antibody.

Selected sites will collect research samples of peripheral blood mononuclear cells (PBMCs) for immune function assays. PBMCs will be assessed for PSA antigen-specific responses by ELISPOT, flow cytometric, and T-cell culture assays. Determinant spread to other prostate antigens and tumor-associated antigens will also be assessed. All evaluations will be performed at laboratories using standard procedures for these assays. Only US sites within a narrow geographic range of a processing laboratory will participate in this portion of the protocol. Cellular assays are difficult to standardize, and because of strong self tolerance anti-PSA responses are rare (1/100,000 T cells range). However, anti-tumor associated antigen responses are frequently induced post PROSTVAC immunization, and are monitored.

Viral Shedding Assessment and Monitoring

The shedding of poxviruses (including PROSTVAC-V/F) in patients has been evaluated (*Arlen 2007; Hepburn 2004*). These studies have demonstrated that vaccinia viral shedding occurs transiently at the site of vaccination. Bandaging contains the virus at the vaccination site. Viral shedding does not occur at other sites, including urine, throat and blood, during the normal course of vaccination with smallpox vaccine. Viral shedding in urine and throat is rare and is associated with complications after vaccination or with smallpox vaccine strains that are more pathogenic than the New York City Board of Health strain used to derive PROSTVAC-V. Moreover, subcutaneous vaccination, the intended route of administration for PROSTVAC-V, reduces the frequency of viral shedding. Recombinant vaccinia virus was detected at the site of vaccination, up to 14 days post treatment. It was not detected in urine or saliva after vaccination with doses up to and including the dose intended for clinical use of PROSTVAC-V (*Arlen 2007*). Similarly, shedding of avipox viruses after vaccination of non-avian species is confined to the vaccination site and is additionally limited due to lack of viral replication. No secondary transmission of recombinant poxviruses, including PROSTVAC-V and PROSTVAC-F, has been reported in humans. Consequently, no specific viral detection/monitoring relative to PROSTVAC-V/F is scheduled in the present proposal.

77. Specificity (to identify the organisms and to distinguish them from the donor, recipient or the parental organisms), sensitivity and reliability of the monitoring techniques.

No specific viral detection/monitoring relative to PROSTVAC-V/F is scheduled in the present proposal.

78. Techniques for detecting transfer of the donated genetic material to other organisms.

As noted previously, there is minimal risk of gene exchange between the GMO and other organisms. Therefore, no monitoring of other organisms is planned.

79. Duration and frequency of the monitoring.

No specific viral detection/monitoring relative to PROSTVAC-V/F is planned.

Control of the Release

80. Methods and procedures to avoid and/or minimise the spread of the organisms beyond the site of release or the designated area for use.

Procedures are in place to avoid and/or minimize the spread of the GMO by controlled containment during transport and at the clinical sites and by minimizing the potential of secondary transmission to vulnerable populations through exclusion criteria defined in the study protocol.

All principal investigators and sub-investigators participating in the study will be qualified by education, training and experience to assume responsibility for the proper conduct of the trial according to the guidelines outlined in International Conference on harmonization (ICH) E6-Good Clinical Practices. Clinical sites where the study is to be conducted will be thoroughly evaluated prior to the initiation of the study to ensure that the facilities are sufficient for storing and administering the vaccine, as well as having the appropriate facilities for the collection, processing and storage of human specimens. All clinical site personnel involved in the handling or administration of study vaccine will be trained according to the study protocol, and all supportive documentation, including study specific laboratory and clinical trial material manuals. A thorough study-specific training will occur prior to the initiation of the study via a formal local investigator meeting and/or on-site study initiation visit.

Clinical site staff responsible for administering PROSTVAC-V/F, collecting clinical samples, or conducting clinical evaluation of study subjects will be instructed to follow the World Health Organization (WHO) universal precautions for the prevention of transmission of infectious agents in healthcare settings (*WHO Standard Precautions, 2006*).

Strict accountability of all doses of PROSTVAC-V/F imported into the UK will be maintained at all times. PROSTVAC-V/F study vaccines and placebo will be shipped from BNIT's contract manufacturer in Germany directly to ALMAC Clinical Services, Craigavon, Northern Ireland from which distribution to clinical sites will occur. Upon receipt at the clinical site, the shipment will be promptly inspected and processed immediately. Study vaccine will be stored at -70°C or below in the original outer package in a secure location with limited access.

All unused study vaccine will be disposed of at the clinical site upon authorization of BNIT according to the site's standard destruction policies for infectious medical waste, or sent to a licensed infectious medical waste destruction facility according to site SOPs. Accurate study vaccine accountability records will be maintained by site personnel. Following administration, used study vaccine vials will be placed immediately into locked containers or sealed bags and according to site SOPs, either retained for accountability or sent for immediate destruction. All sample collections will be performed by study site personnel who have been trained appropriately.

The other possible route of release of PROSTVAC is via viral shedding during the clinical trials.

Vaccinia viral shedding is transient and occurs primarily at the vaccination site; it has not been demonstrated to occur at other sites, including nose, throat, urine, and feces. The frequency of viral shedding is reduced after subcutaneous vaccination with recombinant vaccinia virus, which is the intended route of administration of PROSTVAC-V; furthermore, bandaging contains the virus at the vaccination site.

Recombinant avipoxviral shedding has not been detected in blood, saliva, urine, or rectal swabs after vaccination. Viral DNA has been detected at the injection site, but the presence of live virus was not assessed. Therefore, avipox viral shedding in humans, if it occurs, appears to be confined to the vaccination site.

No secondary transmission of recombinant poxviruses, including PROSTVAC-V and PROSTVAC-F, to contacts has been reported in humans.

The study exclusion criteria outlined in the protocol exclude subjects from participation if they have the potential to come into contact with individuals considered to be at risk for secondary transmission of PROSTVAC-V/F should a subject shed vaccine virus. These exclusion criteria provide a guideline for the extent of contact that should be avoided to minimize the risk of transmission to these populations. The exclusion criteria, together with education of vaccinees in proper care for the vaccination site, proper hand hygiene, and avoidance of contact with at-risk individuals, offer reasonable and effective prophylactic against accidental contact with vaccinia.

81. Methods and procedures to protect the site from intrusion by unauthorised individuals.

Study vaccine and subject data will be stored in a secure location with limited access to clinical study staff. Study sites will otherwise follow standard daily operating procedures for security measures. Clinical study sites will follow applicable local and national regulations regarding the maintenance of confidential patient information during the conduct of a clinical trial.

82. Methods and procedures to prevent other organisms from entering the site.

All staff working in the facility will use personal protective equipment (laboratory coat, apron, safety glasses, and disposable gloves) as appropriate and will follow local documented procedures for Infection Control, which should minimise the risk of other organisms entering the facility.

Waste Treatment

83. Type of waste generated.

Each dose of PROSTVAC is supplied in borosilicate (2R) glass vials, which are sealed with rubber stoppers and aluminium-plastic closures. In addition to vials, other waste generated includes syringes and needles used for vaccine administration and for collection of blood samples, dressings, and other standard supplies required for physical and medical examination of subjects.

84. Expected amount of waste.

Based on the current protocol, approximately 36 subjects will be recruited in England in up to 12 sites over an estimated one-year recruiting period. Each site will receive one 6-vial carton of PROSTVAC-V, one carton of PROSTVAC-V placebo (empty fowlpox vector), two 6-vial cartons of PROSTVAC-F, and two cartons of PROSTVAC-F placebo (empty fowlpox vector) as initial supply. Each subject will receive a maximum of one dose of PROSTVAC-V (2×10^8 Inf. U.) or placebo, and six doses of PROSTVAC-F (1×10^9 Inf. U.) or placebo. A small overage (12 vials of each of the four component vaccine products will be held for emergency resupply. Therefore, allowing for the packaging configuration and for some waste or resupply, up to 90 vials of PROSTVAC-V and 162 vials of PROSTVAC-F, and 246 vials of empty fowlpox vector could be generated as waste.

Patients will be provided with injection site care ‘kits’ containing instruction sheets, disposable gloves, absorbent toweling, alcohol swabs, nonadherent, Telfa-type bandages, band-aids and zip-lock biohazard bags for disposal of used bandages and gloves. Patients will be instructed to return the zip-lock biohazard bag containing all used supplies at their next clinic visit.

The Investigator or his/her designee must maintain accurate records of dates, quantities and the lot numbers of all study drug received, to whom dispensed (patient-by-patient accounting), and accounts of any product accidentally wasted or intentionally destroyed. The Investigator or designee must retain all used, unused, partially used, wasted, or expired study drug until the study monitor has confirmed accountability unless the institution has a policy of immediate disposal/destruction for used experimental products.

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

contracted by the site. At the conclusion of the Treatment phase of the study, an overall summary of all study drug received, unused, partially used, and destroyed will be prepared.

For any investigational product that is not destroyed at the clinical site upon authorization from BNIT according to the site's standard destruction policies for infectious medical waste, or sent to a licensed infectious medical waste destruction facility according to site SOPs, it will be returned to Almac, the distributor for all the countries listed in Section 39. The total product to be stored at Almac for distribution to the UK and 13 other countries is listed in Section 39.

85. Possible risks.

All clinical study sites are licensed healthcare facilities and have standard facility controls in place for administration of vaccines, collection and processing of clinical specimens, and clinical evaluation of study subjects. Clinical site staff will be instructed to follow the World Health Organization (WHO) universal precautions for the prevention of transmission of infectious agents in healthcare settings (*WHO Standard Precautions, 2006*).

No risk related to PROSTVAC-related waste is anticipated. Clinical study sites will be instructed to follow normal site procedures for disposal of infectious biomedical waste. Patients will be provided with zip-lock biohazard bags for disposal of used bandages and gloves and will be instructed to return the zip-lock biohazard bag containing all used supplies at their next clinic visit.

86. Description of treatment envisaged.

Clinical study sites will be instructed to follow normal site procedures for disposal of infectious biomedical waste.

Emergency Response Plans

87. Methods and procedures for controlling the organisms in case of unexpected spread.

In the event that the contents of the vaccine vial are accidentally released and come in contact with shipping materials, exposed skin, clothing or laboratory surfaces, standard safety precautions should be used. Vaccinia and fowlpox are enveloped viruses and are susceptible to detergents and clorox-based disinfectants. Contaminated materials should be placed in biohazard safety bags and disposed of as biohazard waste. Surfaces in contact with vaccinia should be thoroughly cleaned with an appropriate disinfectant and cleaning materials should be disposed of as biohazard. Sites of skin contact should be cleaned with standard detergents appropriate for hand washing.

Accidental release could also potentially occur by direct contact. The Centers for Disease Control noted in a 10-state survey of smallpox vaccinations that the risk of transmission to contacts was 27 infections per million vaccinations, and 44% of these occurred in children <5

years old. Therefore, the precautions of injection site care, including covering the site with a dressing, covering it with a layer of clothes (e.g., long pants or sleeves), subcutaneous injection (rather than dermal scarification), and careful hand washing, are critical. The vaccine should not be given to subjects who are primary care-givers for children ≤ 3 years old.

Accidental transmission of vaccinia virus to a clinic staff member or a member of the patient's family or friends will be reported on a modified SAE form and the event will be followed by the Principal Investigator until resolved. All such events will be summarized in the annual safety update to the appropriate regulatory authorities. Any accidental or suspected secondary transmission will be reported immediately to the Sponsor's Medical Monitor and to the CRO or designee. All such events will be summarized for the Data Monitoring Committee (DMC) and forwarded prior to the next scheduled meeting.

PROSTVAC-F does not replicate in human cells; therefore the risk of horizontal transmission is extremely low. This risk will be further mitigated through the use standard biosafety precautions such as those that are used when handling human blood or tissue samples. In addition, it should be noted that in extensive clinical experience with related viral vaccines, horizontal transmission has not been reported.

88. Methods, such as eradication of the organisms, for decontamination of the areas affected.

In case of accidental release, the source of the spill or leak should be contained. Absorbent material should be used to absorb liquid from contaminated surface. Absorbent materials must be disposed of in biohazard bags. The contaminated surface should be cleaned with detergent-based cleaners or 10% Clorox. Cleaning materials must be disposed of in biohazard bags. Individuals involved in clean up should wear protective clothing including gloves, eye protection and laboratory coat.

89. Methods for disposal or sanitation of plants, animals, soils and any other thing exposed during or after the spread.

Administration of PROSTVAC-V/F will occur only within contained clinical sites. It is therefore not anticipated that PROSTVAC-V or PROSTVAC-F will come into direct contact with any plants, animals or soils. Additionally, neither PROSTVAC-V nor PROSTVAC-F is capable of infecting microbes or plants.

90. Methods for the isolation of the areas affected by the spread.

PROSTVAC-V/F vaccines will be supplied in individual dose vials. Each vial of PROSTVAC-V contains a single dose (2×10^8 infectious units (Inf. U.) per 0.50 mL) of PROSTVAC-V in PBS/10% glycerol. Each vial of PROSTVAC-F contains a single dose (1×10^9 infectious units (Inf. U.) per 0.50 mL) of PROSTVAC-F in PBS/10% glycerol. The amount of vaccine stored at

each clinical study site and resultant waste will be quite low. Additionally, any unexpected release or spills can be decontaminated quickly using detergent-based cleaners or 10% Clorox. It is therefore not anticipated that isolation procedures will be required.

91. Plans for protecting human health and the environment in case of the occurrence of an undesirable effect.

As described, extensive procedural controls are in place for the transport, storage, administration, disposal, and monitoring of PROSTVAC-V/F treatment for the duration of the clinical study. Should any unexpected undesirable effect occur, BNIT will follow standard procedures of assessment of the effect and decisions regarding study continuance.

PART VI
INFORMATION ON METHODOLOGY

Information on the viral titration assays, PCR analysis, FACS analysis, and Western blot analysis are provided in Items 8 of this application. Information on the methods used for construction of the transfer plasmids used to generate PROSTVAC-V and PROSTVAC-F are included in Item 20 of this application.