BN-IMMUNOTHERAPEUTICS

PROSTVAC-VF

PART C:

SUMMARY NOTIFICATION INFORMATION FORMAT (SNIF) FOR RELEASES OF GENETICALLY MODIFIED ORGANISMS OTHER THAN HIGHER PLANTS IN ACCORDANCE WITH ARTICLE 11 OF DIRECTIVE 2001/18/EC

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A. General Information

1. Details of Notification

Member state of notification: France

Notification number: **B/FR/12/GT01**

Date of acknowledgement of notification: 09/03/2012

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"

Proposed period of release: The enrollment of study BNIT-PRV-301 is anticipated to begin in the EU in the first half of 2012. The active treatment period for this study is approximately 5 months. The date of final release will be once approximately 5 patients have completed the active treatment period at the designated France sites.

2. Notifier

BN ImmunoTherapeutics, Inc.
2425 Garcia Avenue
Mountain View, CA 94043
USA
Contact: Heidi Petersen
Sr. Director, Regulatory Affairs
(650) 681-4656

3. GMO characterization

a. Indicate whether the GMO is a:

Viroid	☐	
RNA virus	☐	
DNA virus	☒	Vaccinia virus and Fowlpox virus
Bacterium	☐	
Fungus	☐	
Animal	☐	
Other (please specify)	☐	

b. Identity of the GMO (genus and species)

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: (1) PROSTVAC-V: Recombinant vaccinia virus that contains a modified gene encoding human prostate-specific antigen (PSA) and genes encoding three human immunological costimulatory molecules: B7.1, intracellular adhesion molecule-1 (ICAM-1), and leukocyte function-associated antigen-3 (LFA-3) (or TRIad of COstimulatory Molecules, TRICOM™); and (2) PROSTVAC-F: Recombinant fowlpox virus that co-expresses the same four human genes as PROSTVAC-V.

PROSTVAC-V:	Genus: Orthopox Virus Species: Vaccinia
PROSTVAC-F :	Genus: Avipox Virus Species: Fowlpox

c. Genetic 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.

4. Is the same GMO release planned elsewhere in the Community (in conformity with Article 5 (1))?

Yes ☒ **No** ☐

If yes, insert the country code(s): AT, BE, CZ, DE, DK, EE, ES, FR, LT, NL, PL, UK

5. Has the same GMO been notified for release elsewhere in the Community by the same notifier?

Yes ☐ **No** ☒

If yes – Member State of notification:

6. Has the same GMO been notified for release or placing on the market outside the Community by the same notifier?

Yes ☒ **No** ☐

If yes – specify countries: AUSTRALIA, CANADA, CHILE, ICELAND, ISRAEL, MEXICO, PANAMA, RUSSIA, SWITZERLAND and the USA

7. Summary of the potential environmental impact of the release of the GMOs

PROSTVAC-V and PROSTVAC-F are recombinant poxviruses that each co-expresses human prostate-specific antigen and human co-stimulatory molecules. PROSTVAC-V and PROSTVAC-F are derived from vaccine strains of vaccinia and fowlpox, respectively.

The release of PROSTVAC-V and PROSTVAC-F as described in this application is not expected to result in adverse environmental impact. Data that support this assessment include the following:

- Comparablity of parental and recombinant viruses. 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.

- Minimal risk of gene transfer. Poxviral replication takes place entirely in the cytoplasm; thus, PROSTVAC V/F DNA is extra-chromosomal and is not integrated. As a result, it is not subject to events that could lead to rearrangement or recombination in the subjects participating in the study. Poxviruses are cleared from the host within several days for PROSTVAC-F and weeks for PROSTVAC-V.

- Minimal risk of viral shedding. Viral shedding studies of PROSTVAC and of related poxviruses generated using the same parental virus as PROSTVAC-V indicate that viral shedding occurs transiently at the site of vaccination. Poxviral shedding from sites other than the site of vaccination is rare and has not been reported for any recombinant poxvirus. Subcutaneous vaccination, which is the intended route of administration of PROSTVAC-V, reduces the frequency of viral shedding relative to the conventional route of scarification used for vaccinia virus as smallpox vaccine. Viral shedding at the vaccination site is contained by bandaging, further minimizing release into the environment.

- Minimal risk of contact transmission. Contact transmission of vaccinia-based smallpox vaccine is rare. No secondary transmission of recombinant poxviruses, including PROSTVAC-V and PROSTVAC-F, has been reported in humans. Risk of transmission is reduced by use of universal precautions by healthcare workers and education of patients in proper hygiene and proper care of the vaccination site.

- Minimal risk of environmental persistence. Although poxviruses are relatively stable at sub-freezing temperatures, they 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.

B. Information Relating To The Recipient Or Parental Organisms From Which The GMO Is Derived

1. Indicate whether the recipient or parental organism is a

Viroid	☐
RNA virus	☐
DNA virus	☒
Bacterium	☐
Fungus	☐
Animal	☐
Other (please specify)	☐

2. Complete name

PROSTVAC-V

Recipient Virus:	Vaccinia virus
Family:	Poxviridae; Subfamily: Chordopoxvirinae
Genus:	Orthopox virus
Species:	Vaccinia
Subspecies:	Not applicable
Strain:	New York City Board of Health Vaccine (NYCBH)
Pathovar:	Not applicable
Common name:	Not applicable

PROSTVAC-F

Recipient Virus:	Fowlpox virus
Family:	Poxviridae; Subfamily: Chordopoxvirinae
Genus:	Avipox virus
Species:	Fowlpox
Subspecies:	Not applicable
Strain:	POXVAX-TC, a tissue culture-adapted vaccine strain
Pathovar:	Not applicable
Common name:	Not applicable

3. Geographical distribution of the organism

a. Indigenous to the France

Vaccinia vaccine:	☐ **Yes**	☒ **No**	☐ **Not known**
Fowlpox virus vaccine:	☒ **Yes**	☐ **No**	☐ **Not known**

b. Indigenous to other EC countries

Vaccinia vaccine:	☐ **Yes**	☒ **No**	☐ **Not known**
Fowlpox virus vaccine:	☒ **Yes**	☐ **No**	☐ **Not known**

c. If Yes, indicate the type of ecosystem in which it is found

Atlantic ☒
Mediterranean ☒
Arctic ☒
Continental ☒

d. Is it regularly used in the France**?**

Vaccinia vaccine:	☐ **Yes**	☒ **No**	☐ **Not known**
Fowlpox virus vaccine:	☒ **Yes**	☐ **No**	☐ **Not known**

d. Is it regularly kept in the France**?**

Vaccinia vaccine:	☐ **Yes**	☒ **No**	☐ **Not known**
Fowlpox virus vaccine:	☒ **Yes**	☐ **No**	☐ **Not known**

4. Natural habitat of the organism

a. If the organism is a micro-organism

Water ☐
Soil, free-living ☐
Soil in association with plant-root systems ☐
In association with plant-leaf/stem systems ☐
In association with animals ☒

Avipoxviruses are distributed worldwide. The virus species fowlpox virus infects and causes disease in poultry. POXVAC-TC, the parental virus for PROSTVAC-F, is a vaccine strain of fowlpox virus that is used as a vaccine for the prevention of fowlpox infection in poultry.

Other (specify) ☒

Vaccinia (NYCBH strain), the parental virus for PROSTVAC-V, is a vaccine for the prevention of smallpox infection in humans. It has no known animal reservoirs.

b. If the organism is an animal: natural habitat or usual agro-ecosystem – Not applicable.

5a. Detection techniques

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.

5b. Identification techniques

See 5a.

6. Is the recipient organism classified under existing Community rules relating to the protection of human health and/or the environment?

Vaccinia virus: ACDP class 2

Fowlpox virus: Biosafety Level 1 (as defined by the United States Center for Disease Control and Prevention, Laboratory Biosafety Level Criteria).

7. Is the recipient organism pathogenic or harmful in any other way (including its extracellular products) either living or dead?

Yes ☒ **No** ☐

If yes,

a. To which of the following organisms:

☒ **Humans** ☒ **Animals** ☐ **Plants** ☐ **Others**

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

Vaccinia Virus (PROSTVAC-V)

Normal reactions to vaccinia (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).

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.

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.

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 and no toxic or allergenic effects were reported.

8. Information concerning reproduction

a. Generation time in natural ecosystems

Vaccinia:

Not applicable; vaccinia virus has no known natural animal reservoirs.

Fowlpox :

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.

b. Generation time in the ecosystems where the release will take place

Release of PROSTVAC-VF will comprise vaccination of volunteers at licensed healthcare facilities. In these volunteers, PROSTVAC-V (vaccinia virus) will cause 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. PROSTVAC-F (fowlpox virus) does not replicate in humans.

c. Way of reproduction

☐ **Sexual** ☐ **Asexual** ☒ **Other -** Viral

d. Factors affecting reproduction

Vaccinia and fowlpox viruses are rapidly inactivated by a number of disinfectants. In addition to chemical agents, these viruses are inactivated by exposure to ultraviolet light and by exposure to 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.

9. Survivability

a. Ability to form structures enhancing survival or dormancy

i.	**endospores**	☐	
ii.	**cysts**	☐	
iii.	**Sclerotia**	☐	
iv.	**asexual spores (fungi)**	☐	
v.	**sexual spores (fungi)**	☐	
vi.	**eggs**	☐	
vii.	**pupae**	☐	
viii.	**larvae**	☐	
ix.	**Other, please specify**	☒	None

b. Relevant factors affecting survivability

Vaccinia and fowlpox 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. Additionally, poxviruses are readily inactivated by a number of detergents.

10a. Ways of dissemination

Transmission of vaccinia virus requires close contact. Contact transmission of vaccinia virus as smallpox vaccine is rare, occurring in one to three per 50,000 vaccinees in human studies. Contact transmission of recombinant vaccinia virus, including PROSTVAC-V, has not been reported in human studies. Transmission of recombinant vaccinia virus between animals in close contact has been demonstrated after oral vaccination but not after subcutaneous vaccination.

Contact transmission of recombinant avipox virus, including PROSTVAC-F, has not been reported in human studies. Transmission of recombinant vaccines based on vaccine strains of avipox virus is rare in permissive avian species. Transmission of nonpathogenic fowlpox virus has been observed only in chickens and is rare even in this species, which supports productive replication of fowlpox virus.

10b. Factors affecting dissemination

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. Fowlpox virus is host-range restricted *in vivo* to certain avian species, and the fowlpox vaccine strain used for PROSTVAC-F is non-virulent.

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

11. Previous genetic modifications of the recipient or parental organism already notified for release in France (give notification numbers)

None.

C. Information Relating To The Genetic Modification

1. Type of genetic modification

- **a) insertion of genetic material** ☒
- **b) deletion of genetic material** ☒
- **c) base substitution** ☐
- **d) cell fusion** ☐
- **e) other, specify** ☐

2. Intended result of the genetic modification

The intended result of the genetic modification was the generation of recombinant vaccinia and fowlpox viruses with utility for the treatment of prostate cancer.

3(a) Has a vector been used in the process of modification? – Yes.

3(b) If yes, is the vector wholly or partially present in the modified organism? – Yes.

4. If the answer to 3(b) is yes, supply the following information:

a. Type of vector: plasmid, bacteriophage, virus, cosmid, transposable element, or other – Plasmid.

b. Identity of the vector

PROSTVAC-V: Plasmid vector pT2240

PROSTVAC-F: Plasmid vector pT2246

For both plasmid vectors, the plasmid backbone, including the bacterial origin of replication and the ampicillin resistance gene, was derived from the commercially available plasmid vector pUC8.

c. Host range of the vector – *Escherichia coli.*

d. Presence in the vector of sequences giving a selectable or identifiable phenoytype, antibiotic resistance, or other. Indication of which antibiotic resistance gene is inserted.

The plasmid backbone contains the ampicillin resistance gene to allow for selection in bacterial cells; however, the ampicillin resistance gene is not present in the final GMO.

e. Constituent fragments of the vector

The plasmid vectors used for the insertion of genes into vaccinia and fowlpox virus contain 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 vaccinia or fowlpox genome, which direct insertion of foreign sequences into this region via homologous recombination;
- the *E. coli lacZ* gene, flanked by repeated sequences;
- a chimeric gene comprising the vaccinia 40K transcriptional promoter linked to the modified PSA 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.

f. Method for introducing the vector into the recipient organism: transformation, electroporation, macroinjection, microinjection, infection, or other?

Using the calcium phosphate precipitation method, the plasmid vector 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.

5. If the answer to questions 3(a) and (b) is no, what was the method used to introduce the insert into the recipient/parental cell?

Not applicable.

6. Information on the insert

a. Composition of the insert

In both PROSTVAC-V and PROSTVAC-F, the insert comprises the coding sequences of the four human transgenes (PSA, B7.1, ICAM-1, and LFA-3) together with their associated transcriptional control regions.

b. Source of each constitutent part of the insert

Constituent parts of the insert are listed in section 4e, above. Source of each component is as follows:

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. 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. 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.

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. The PSA gene was modified by *in vitro* mutagenesis to express full-length protein containing one altered epitope that has been shown to enhance immunogenicity. This mutation changed the encoded amino acid at position 155 from isoleucine to leucine.

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.

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.

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.

c. Intended function of each constituent part of the insert in the GMO

The chimeric gene comprising the vaccinia 40K transcriptional promoter linked to the PSA gene directs the expression of the tumor-associated prostate-specific antigen in human cells. The expressed PSA antigen is processed and expressed on the surface of antigen-presenting cells (APCs) within the major histocompatability complex (MHC).

The three chimeric genes that comprise TRICOM (i.e., 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; and a chimeric gene comprising the sE/L transcriptional promoter linked to the B7.1 gene) direct the expression of these three human costimulatory molecules.

Vaccination with PROSTVAC-V results in the simultaneous expression by infected cells of PSA epitopes in combination with the TRICOM 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.

d. Location of the insert in the host organism

☐ **on a free plasmid** ☒ **Integrated in the chromosome** ☐ **Other**

e. Does the insert contain parts whose products or functions are not known? – No

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D. Information On The Organism From Which The Insert Is Derived (Donor)

1. Indicate whether it is a:

- ☐ **viroid**
- ☐ **RNA virus**
- ☐ **DNA virus**
- ☐ **bacterium**
- ☐ **fungus**
- ☐ **animal**
- ☒ **other (please specify) –** human

2. Complete name

Order:	Primates
Family:	Hominidae
Genus:	Homo
Species:	Sapiens
Subspecies:	Not applicable
Strain:	Not applicable
Cultivar/Breeding line:	Not applicable
Common name:	Human

3. Is the organism pathogenic or harmful in any other way (including its extracellular products), either living or dead? – No.

4. Is the donor organism classified under existing Community rules relating to the protection of human health and the environment? – No.

5. Do the donor and recipient organism exchange genetic material naturally? – No

E. Information Relating To The Genetically Modified Organism

1. Genetic traits and phenotypic characteristics of the recipient or parental organism which have been changed as a result of the genetic modification

a. Is the GMO different from the recipient as far as survivability is concerned? If yes, please specify. – No

b. Is the GMO in any way different from the recipient as far as mode and/or rate of reproduction is concerned? If yes, please specify. – No

c. Is the GMO in any way different from the recipient as far as dissemination is concerned? If yes, please specify. – No

d. Is the GMO in any way different from the recipient as far as pathogenicity is concerned? – No

2. Genetic stability of the genetically modified organism

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.

3. Is the GMO pathogenic or harmful in any other way (including its extracellular products), either living or dead?

Yes; PROSTVAC-V only.

a. To which of the following organisms:

☒ **Humans** ☒ **Animals** ☐ **Plants** ☐ **Others**

b. Give the details on 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)

Normal reactions to vaccination with PROSTVAC-V 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.

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. 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.

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.

c. In relation to human health -

i. The toxic or allergenic effects of the non-viable organism and/or its metabolic products,

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

ii. The product hazards

Product hazards are summarized in section v below. No other product hazards are known from studies to date.

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

PROSTVAC-V and PROSTVAC-F are comparable to their parental viruses with respect to pathogenicity.

iv. The capacity of the organism for colonisation,

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-VF 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.

v. If the organism is pathogenic to humans who are immunocompetent –

(a) Diseases caused and mechanisms of pathogenicity, including invasiveness and virulence

The most common adverse events (AEs) related to PROSTVAC-V and PROSTVAC-F observed to date have been injection site reactions, all of which were ≤ Grade 2 severity. The most common systemic AEs attributed to PROSTVAC-V and PROSTVAC-F administration were fatigue, nausea/vomiting, fever, chills, arthralgia and dizziness.

Reactions and Complications Associated with Vaccinia Vaccination

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).

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. Retrospective analysis of 2002 – 2004 vaccinations suggests an incidence of ~50 cases per 1,000,000. The rash appears within a week after vaccination and resolves within a week. Most instances do not require specific therapy.

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).

Finally, recent vaccination campaigns in the US revealed a higher than historically observed incidence of myopericarditis in vaccinees. Myo/pericarditis has been long associated with a number of viral infections, although there are very few reports of confirmed viremia.

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. Vaccinia keratitis is the most serious consequence of autoinoculation, since lesions on the cornea threaten eyesight. 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.

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. The rate of contact vaccinia in 2002 – 2004 was <10 cases per 100,000.

Safety of Fowlpox Vaccination

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 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..

(b) Communicability

PROSTVAC-V (Vaccinia)

Vaccinia virus may be transmitted by direct contact with virus shed from the vaccination site or with contaminated dressings or other infectious material. Epidemiologic evidence for airborne or droplet spread of vaccinia virus spread is scant, and contact transmission of vaccinia-based smallpox vaccine is rare.

PROSTVAC-F (Fowlpox)

Fowlpox virus does not replicate in human cells. Consequently, viral shedding in humans is limited and appears to be confined to the vaccination site. Human-to-human contract transmission of recombinant fowlpox virus has not been observed.

(c) Infective dose

Under the proposed release, each patient will receive one immunization with 2 x 10^8 infectious units (Inf. U.) of PROSTVAC-V Week 1, followed by six immunizations with 1 x 10^9 Inf. U. of PROSTVAC-F administered in Weeks 3, 5, 9, 13, 17, and 21.

(d) 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. The insertion of human genes into the genome of fowlpox to generate PROSTVAC-F does not alter this host range.

(e) 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.

(f) 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.

Fowlpox virus does not replicate in human cells. Consequently, 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.

(g) Biological stability

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. Stability of fowlpox virus *in vivo* is not relevant to this application, since fowlpox virus does not replicate in mammalian cells.

(h) Antibiotic-resistance patterns

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

(i) Allerginicity

PROSTVAC-V and PROSTVAC-F have not been shown to be allergenic in any preclinical or clinical studies to date.

(j) Availability of appropriate therapies

For some very rare complications of vaccinia infection, early administration of vaccinia immune globulin (VIG) is advised. 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 is working on securing a necessary supply of VIG for the countries where it is not available internally.

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.

4. Description of identification and detection methods

a. Techniques used to detect the GMO in the environment

Detection and identification of PROSTVAC-V and PROSTVAC-F may be accomplished using the following assays: (1) quantitative FACS assay to measure titers of infectious virus; (2) Polymerase Chain Reaction (PCR) of inserted genes and recombination junctions for virus identity; and (3) transgene expression by Western Blot. However, 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.

b. Techniques used to identify the GMO – See 4a, above

F. Information Relating To The Release

1. Purpose 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 that 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. This trial will be conducted globally.

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

2. Is the site of the release different from the natural habitat or from the ecosystem in which the recipient organism is regularly used, kept or found?

☒ **Yes** ☐ **No**

The site of release will be licensed healthcare facilities. PROSTVAC-V and PROSTVAC-F are genetically engineered viruses and thus do not exist in nature. With respect to the parental viruses, vaccinia viruses are laboratory strains that have no known animal reservior. The parental virus used to generate PROSTVAC-F is a vaccine strain of fowlpox virus that is used on poultry farms.

3. Information concerning the release and the surrounding area

a. Geographical location (administrative region and where appropriate, grid reference).

The following are clinical study sites within the France where study vaccine will be administered:

Site 1: Aude Flechon

Centre Léon Bérard - Centre Régional de Lutte Contre le Cancer Lyon et Rhône-Alpes
Cancérologie Médicale
28 rue Laennec
69008 Lyon
France

b. Size of the site (m^2)

i. **Actual release site (m^2) –** Not applicable; the study vaccine will be administered at licensed healthcare facilities listed above.

ii. **Wider release area (m^2) –** Not applicable; the study vaccine will be administered at licensed healthcare facilities.

c. Proximity to internationally recognized biotypes or protected areas (including drinking water reservoirs), which could be affected.

Not applicable; the study vaccine will be administered at licensed healthcare facilities. It is not anticipated that the study vaccine or any waste associated with study procedures will affect the surrounding ecosystem.

d. Flora and fauna, including crops, livestock and migratory species, which may potentially interact with the GMO.

Not applicable; the study vaccine will be administered at licensed healthcare facilities. It is not anticipated that the study vaccine or any waste associated with study procedures will affect the surrounding ecosystem.

4. Method and amount of release

a. Quantities of GMOs to be released.

Under the proposed France release, each of the approximately enrolled patients will receive one immunization with 2 x 10^8 infectious units (Inf. U.) of PROSTVAC-V Week 1, followed by six immunizations with 1 x 10^9 Inf. U. of PROSTVAC-F administered in Weeks 3, 5, 9, 13, 17, and 21.

A central storage and distribution depot for study vaccine and placebo for 13 participating EU countries [Austria, Belgium, Czech Republic, Estonia, France, Germany, Iceland, Lithuania, Poland, Spain, Denmark, Netherlands, and the United Kingdom] and also including Israel, Russia and Switzerland will be located in Craigavon, Northern Ireland, United Kingdom. This depot 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.

b. Duration of the operation.

The enrollment of study BNIT-PRV-301 is anticipated to begin in the EU in the first half of 2012. The active treatment period for this study is approximately 5 months. The date of final release will be once approximately 5 patients have completed the active treatment period at the designated France sites.

c. Methods and procedures to avoid and/or minimise the spread of the GMOs beyond the site of the release.

To avoid the spread of the GMO, procedures will be in place to (1) control containment both during transport and at the clinical sites and (2) minimize the potential of secondary transmission to vulnerable populations through exclusion criteria defined in the study protocol. In addition, patients will be educated as to the care of the injection site, including proper bandage changing, bathing, possible side effects, and minimization of contact with vulnerable populations, in order to further decrease the potential for spread and/or environmental exposure.

5. Short description of average environmental conditions (weather, temperature, etc.) – Not applicable

6. Relevant data regarding previous releases carried out with the same GMO, if any, specially related to the potential environmental and human health impacts from the release

Nonclinical Studies of PROSTVAC-VF

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. 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 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. No evidence for contact transmission was observed in any clinical trial. The most common adverse reactions were injection site reactions, all of which were ≤ Grade 2 severity and resolved without sequelae. The most common systemic AEs attributed to PROSTVAC-V and PROSTVAC-F administration were fatigue, nausea/vomiting, fever, chills, arthralgia and dizziness. Laboratory evaluations likewise did not reveal any untoward effects of treatment. In summary, no adverse environmental or human health impacts were observed in connection with these trials.

G. Interactions Of The GMO With The Environment And Potential Impact On The Environment

1. Complete name of target organisms

Order and Higher Taxon:	Primates
Family:	Hominidae
Genus:	Homo
Species:	Sapiens
Subspecies:	Not applicable
Strain:	Not applicable
Pathovar:	Not applicable
Common name:	Human

2. Anticipated mechanism and result of interaction between the released GMOs and the target organism.

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.

3. Other potentially significant interactions with other organisms in the environment.

None.

4. Is post-release selection for the GMO likely to occur? If Yes, give details

☐ **Yes** ☒ **No**

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*, and there is no evidence to suggest that post-release selection for the GMO will occur.

5. Type of ecosystems in which the GMO could be disseminated from the site of release and in which it could become established

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.

6. Complete name of non-target organisms which may be affected unwittingly

The most likely non-target organisms which may be accidentally exposed to the GMO are human clinic staff members or close patient contacts.

Order and Higher Taxon:	Primates
Family:	Hominidae
Genus:	Homo
Species:	Sapiens
Subspecies:	Not applicable
Strain:	Not applicable
Pathovar:	Not applicable
Common name:	Human

7. Likelihood of genetic exchange in vivo

a. From the GMO to other organisms in the release ecosystem

The potential for gene transfer from PROSTVAC-VF to other species, including humans, is extremely low. The poxvirus life cycle is carried out in the cytoplasm; poxviruses do not integrate into the genome of the infected cell. The physical segregation between host and viral genomes renders recombination 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, although the likelihood of coninfection of host organisms with both recombinant and wild type viruses is extremely remote. Further, such recombination would not be expected to alter the virulence, growth properties, or environmental persistence of the viruses. Recombination with other viral genomes is also unlikely due to the lack of homology between different families of viruses. 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.

b. From other organisms to the GMO

See response to 7a, above.

c. Likely consequences of gene transfer

See response to 7a, above.

8. *Give references to relevant results from studies of the behaviour and characteristics of the GMO and its ecological impact carried out in simulated natural environments (eg microcosms, etc).*

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

9. Possible environmentally significant interactions with biogeochemical processes (if different from the recipient or parental organism)

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

H. Information Relating To Monitoring

1. Methods for monitoring the GMOs.

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. With respect to safety, 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. Additionally, patients will be monitored with respect to primary endpoint (overall survival) as well as secondary and exploratory endpoints.

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.

2. Methods for monitoring ecosystem effects.

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.

3. Methods for detecting transfer of the donated genetic material from the GMO 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.

4. Spatial extent of the monitoring area (m^2).

Not applicable; no specific viral detection/monitoring relative to PROSTVAC-V/F is planned.

5. Duration of the monitoring.

Not applicable; no specific viral detection/monitoring relative to PROSTVAC-V/F is planned.

6. Frequency of the monitoring.

Not applicable; no specific viral detection/monitoring relative to PROSTVAC-V/F is planned.

I. Information On Post-Release And Waste Treatment

1. Post-release treatment of the site

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 returned to ALMAC Clinical Services central storage depot in the UK or disposed of at the clinical site upon authorization from BNIT.

2. Post-release treatment of the GMOs

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

3a. Type and amount of waste generated

Based on the current protocol, approximately 25 - 30 subjects will be recruited in England in up to 10 sites over an estimated one-year recruiting period. Each dose of PROSTVAC is supplied in borosilicate (2R) glass vials, which are sealed with rubber stoppers and aluminium-plastic closures. Based on the packaging configuration and for some waste or resupply, up to 72 spent vials of PROSTVAC-V, 132 spent vials of PROSTVAC-F, and 204 spent vials of empty fowlpox vector could be generated as waste. 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.

3b. Treatment of waste

Waste generated during the course of the study (spent vials, syringes, needles, dressings, etc) will be destroyed on site, following normal site procedures for disposal of infectious biomedical waste. At the conclusion of the Treatment phase of the study, all study medication will be either (1) destroyed on site, following normal site procedures for disposal of infectious biomedical waste; (2) destroyed by a licensed facility contracted by the site, or (3) returned to the Sponsor or designee following final reconciliation. At the conclusion of the Treatment phase of the study, an overall summary of all study drug received, unused, partially used, wasted, and returned will be prepared.

J. Information On Emergency Response Plans

1. Methods and procedures for controlling GMOs 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 will be used. Vaccinia and fowlpox are enveloped viruses and are susceptible to detergents and clorox-based disinfectants. Contaminated materials will be placed in biohazard safety bags and disposed of as biohazard waste. Surfaces in contact with vaccinia will be thoroughly cleaned with an appropriate disinfectant and cleaning materials will be disposed of as biohazard. Sites of skin contact will be cleaned with standard detergents appropriate for hand washing.

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.

2. Methods for decontamination of the areas affected.

Any unexpected release or spills will be decontaminated using detergent-based cleaners or 10% Clorox.

3. Methods for disposal or sanitation of plants, animals, soils, etc. that were 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.

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

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.