PART 1 (COUNCIL DECISION 2002/813/EC)

SUMMARY NOTIFICATION INFORMATION FORMAT FOR THE RELEASE OF GENETICALLY MODIFIED ORGANISMS OTHER THAN HIGHER PLANTS IN ACCORDANCE WITH ARTICLE 11 OF DIRECTIVE 2001/18/EC

In order to tick one or several possibilities, please use crosses (meaning x or X) into the space provided as (.)

A. General information

1. Details of notification

(a)	Member State of notification	Netherlands
(b)	Notification number	B/NL/14/002
(c)	Date of acknowledgement of notification	TBC
(d)	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"
(e)	Proposed period of release	The enrollment of study BNIT-PRV-301 began in the EU on 20th July 2012. The active treatment period for this study is approximately 5 months. The date of final release will be once approximately 40 patients have completed the active treatment period at the designated Netherlands sites.

2. Notifier

Name of institution or company: Erasmus MC - Centrumlocatie
's-Gravendijkwal 230
3015 CE Rotterdam, the Netherlands

3. GMO characterisation

(a) Indicate whether the GMO is a:

viroid	(.)
RNA virus	(.)
DNA virus	(X)
bacterium	(.)
fungus	(.)
animal	
- mammals	(.)
- insect	(.)
- fish	(.)

- other animal (.)

specify phylum, class …

(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 contains 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 – according to Annex IIIa, II, A(10)
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 6(1)), by the same notifier?
Yes (X) No (.)
If yes, insert the country code(s) **BE, DE, DK, EE, ES, FR, PL, and the UK**

5. Has the same GMO been notified for release elsewhere in the Community by the same notifier?
Yes (X) No (.)
If yes:
BE - B/BE/11/BVW2

DE - TBC

EE – B/EE/12/01

ES - B/ES/12/14

FR – B/FR/12/GT01

PL -TBC

UK

England – 11/R44/01 and 12/R44/01

Scotland – REF: 12/R44/01/S

Wales - 11/R44/01(W)

Northern Ireland – REF: 12/R44/01/NI

Please use the following country codes:
Austria AT; Belgium BE; Germany DE; Denmark DK; Spain ES; Finland FI; France FR; United Kingdom GB; Greece GR; Ireland IE; Iceland IS; Italy IT; Luxembourg LU; Netherlands NL; Norway NO; Portugal PT; Sweden SE

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

Yes (X) No (.)

If yes:
AUSTRALIA, CANADA, ICELAND, ISRAEL, PUERTO RICO, RUSSIA and the USA

AUSTRALIA – DIR116

CANADA – EAU-666, EAU-667, EAU-668

ICELAND – B/IS/12/01

7. Summary of the potential environmental impact of the release of the GMOs.
PROSTVAC-V and PROSTVAC-F are recombinant poxviruses that each contain 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:

- Comparability 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 and PROSTVAC-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 (**Arlen, 2007**). 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 (**Eterpi, 2009**); 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 (**Fenner, 1988**).

B. Information relating to the recipient or parental organism from which the GMO is derived

1. Recipient or parental organism characterisation:

(a) Indicate whether the recipient or parental organism is a:

(select one only)

viroid (.)

RNA virus (.)
DNA virus (X)
bacterium (.)
fungus (.)
animal
- mammals (.)
- insect (.)
- fish (.)
- other animal (.)
(specify phylum, class) …

other, specify …

2. 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:	rilimogene galvacirepvec

PROSTVAC-F

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

3. Geographical distribution of the organism

(a) Indigenous to, or otherwise established in, the country where the notification is made:

Vaccinia vaccine:	Yes	(.)	No	(X)	Not known	(.)
Fowlpox virus vaccine:	Yes	(X)	No	(.)	Not known	(.)

(b) Indigenous to, or otherwise established in, other EC countries:

Vaccinia vaccine:	Yes	(.)	No	(X)	Not known	(.)

Fowlpox virus vaccine: Yes (X) No (.) Not known (.)

(c) If yes, indicate the type of ecosystem in which it is found:

Atlantic	X
Mediteranean	X
Boreal	..
Alpine	..
Continental	X
Macaronesian	..
Arctic	X

(ii) No (.)
(iii) Not known (.)

(c) Is it frequently used in the country where the notification is made?

Vaccinia vaccine: Yes (.) No (X) Not known (.)
Fowlpox virus vaccine: Yes (X) No (.) Not known (.)

(d) Is it frequently kept in the country where the notification is made?

Vaccinia vaccine: Yes (.) No (X) Not known (.)
Fowlpox virus vaccine: Yes (X) No (.) Not known (.)

4. Natural habitat of the organism

(a) If the organism is a microorganism

water	(.)
soil, free-living	(.)
soil in association with plant-root systems	(.)
in association with plant leaf/stem systems	(.)
In association with animals	(X)

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 tissue culture-adapted vaccine strain of fowlpox virus that is used as a vaccine for the prevention of fowlpox infection in poultry.

Other (specify) (X)

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 agroecosystem: Not applicable.

…

5. (a) Detection techniques

The techniques listed in Section E.4.a for PROSTVAC-V and PROSTVAC-F are vector-specific; therefore they can be employed for identification and detection of the vaccinia and fowlpox vector backbones.

(b) 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 significantly pathogenic or harmful in any other way (including its extracellular products), either living or dead?

Yes (X) No (.) Not known (.)

If yes:

(a) to which of the following organisms:

humans	(X)
animals	(X)
plants	(.)
other	(.)

(b) give the relevant information specified under Annex III A, point II. (A)(11)(d) of Directive 2001/18/EC
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

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 vaccinia virus

[New York City Board of Health (NYCBH) strain] used by Wyeth Pharmaceuticals to produce the licensed Dryvax® Smallpox Vaccine. The NYCBH strain has been associated with the lowest incidence of clinical complications following immunization (**Fenner, 1988).**

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 (**Moss, 1996**). Thus, vaccinia is incapable of colonizing the host organisms that it infects. 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.

Normal reactions to vaccinia (smallpox) vaccination are mild and self-limited, and may include fever, myalgia, headache, fatigue, chills, nausea, soreness and erythema at the vaccination site, and 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). Please note that these very rare but serious adverse reactions (PV, EV, and PVE) are known to be associated with traditional smallpox vaccinations using the dermal scarification technique in vaccinia-naïve subjects. These reactions have not been observed in prior clinical studies of PROSTVAC-V or other vaccinia-based vaccines. The risk of these rare events is thought to be further reduced for PROSTVAC-V due to the subcutaneous administration of PROSTVAC-V rather than by the traditional dermal scarification method and restricting administration of PROSTVAC-V to previously-vaccinated individuals only.

Fowlpox Virus

The parental fowlpox 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. The POXVAC-TC strain is non-virulent (**Fenner, 1988**). Thus, no untoward effects on the environment, other bird species, or animal handlers have been reported from the use of POXVAC-TC.

Fowlpox virus does not replicate productively in human cells, although the virus mediates a limited infection, with early viral gene expression. Late gene expression is blocked, and no infectious particles are produced. Productive replication of the parental fowlpox virus (TBC-FPV) used to generate

PROSTVAC-F has only been demonstrated in vivo in avian species. Fowlpox virus has a highly restricted host range, limited in vivo to chickens, turkeys, and pigeons (**Tripathy, 1984; McMillen, 1994**). Replication of fowlpox virus has not been demonstrated in quail, ducks, or canaries. Productive replication in vitro has not been observed in human cells, monkey cells, and in most mammalian cells tested (**Somogyi, 1993**). A single exception is a hamster cell line, BHK-21 cells, which is semi-permissive for the growth of fowlpox virus (**Weli, 2004; Weli, 2005**).

Fowlpox virus is not 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, fowlpox virus does not infect microbes or plants.

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 (TBC-FPV) 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 ecosystem where the release will take place:
Release of PROSTVAC-V and PROSTVAC-F consists of vaccination of clinical trial subjects at licensed healthcare facilities. In these clinical trial subjects, 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

(c) Factors affecting reproduction:

Vaccinia and fowlpox viruses are rapidly inactivated by a number of disinfectants (**Eterpi, 2009**). In addition to chemical agents, these viruses are inactivated by exposure to ultraviolet light (**Sagripanti, 2011**) and by exposure to increasing temperatures (**Mahl, 1975**). 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 (funghi)		(.)
(vi)	eggs		(.)
(vii)	pupae		(.)
(viii)	larvae		(.)
(ix)	other, specify	(X)	None

(b) relevant factors affecting survivability:
Vaccinia and fowlpox are live viruses and do not form spores or other structures. Survivability is dependent upon the ability to replicate within a host cell. 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.

Poxviruses have the capacity to survive for considerable periods in dried material such as detached vaccination scabs (**Sidwell, 1966**). They are also relatively stable when stored frozen or lyophilized under carefully controlled conditions. However stability decreases significantly as temperature is increased (**Mahl, 1975**). 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 (**Eterpi, 2009**).

10. (a) Ways of dissemination
The potential for dissemination of the vaccinia virus to non-target species is limited to secondary transmission via direct contact with an infection site or with contaminated surfaces or objects. Contact transmission of vaccinia virus as smallpox vaccine is rare, occurring in one to three per 50,000 vaccinees in human studies. It is important to note that these numbers are within the context of the dermal scarification mode of administration. PROSTVAC-V and PROSTVAC-F are administered via subcutaneous injection, which further minimizes any risk for

transmission. Please also note that contact transmission of recombinant vaccinia virus, including PROSTVAC-V, has not been reported in human studies.

The potential for dissemination of the fowlpox virus to non-target species is limited to secondary transmission via direct contact with an infection site or with contaminated surfaces or objects. It is important to note that 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 even rare in permissive avian species.

(b) Factors affecting dissemination
Based on the conditions of this release, the potential for dissemination or establishment of vaccinia and fowlpox viruses in the environment is low.

The extent of exposure to non-target species is expected to be limited by several factors:

(1) vaccine is subject to controlled containment during transport and at the clinical sites;

(2) vaccine administration occurs in a clinical site under controlled conditions;

(3) shedding of vaccinia virus is expected to be confined to the vaccination site;

(4) there is no evidence of fowlpox viral shedding in humans as the virus does not replicate in mammalian cells;

(5) bandaging contains virus at the vaccination site;

(6) comprehensive education of healthcare providers and subjects minimizes potential for environmental release;

(7) productive replication of fowlpox virus is restricted to chickens, turkeys, and pigeons (**Tripathy, 1984; McMillen, 1994**).

11. Previous genetic modifications of the recipient or parental organism already notified for release in the country where the notification is made (give notification numbers)
None.

C. **Information relating to the genetic modification**

1. Type of the genetic modification

(i)	insertion of genetic material	(X)
(ii)	deletion of genetic material	(X)
(iii)	base substitution	(.)
(iv)	cell fusion	(.)

(v) others, specify …

2. Intended outcome 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 (X) No (.)

If no, go straight to question 5.

(b) If yes, is the vector wholly or partially present in the modified organism?
Yes (X) No (.)

If no, go straight to question 5.

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

(a) Type of vector

plasmid		(X)
bacteriophage		(X)
virus		(X)
cosmid		(X)
transposable element		(X)
other, specify	(X)	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 phenotype
Yes (X) No (.)

antibiotic resistance	(X)	See explanation below.
other, specify	…	

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 lac*Z gene, flanked by repeated sequences (PROSTVAC-F only);
- 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

(i) transformation (.)
(ii) electroporation (.)
(iii) macroinjection (.)
(iv) microinjection (.)
(v) infection (.)
(vi) other, specify …

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 question B.3(a) and (b) is no, what was the method used in the process of modification?

(i) transformation (.)
(ii) microinjection (.)
(iii) microencapsulation (.)
(iv) macroinjection (.)
(v) other, specify …

Not applicable.

6. Composition of the insert

(a) Composition of the insert
In both PROSTVAC-V and PROSTVAC-F, the insert is comprised of 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 constituent part of the insert
Constituent parts of the insert are listed in section 4e, above. The 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. The sE/L promoter was isolated as a 60 bp Hind III-Sal I fragment from pJS-8, a derivative of pSC65.

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 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/F 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 (X)
- other, specify …

(e) Does the insert contain parts whose product or function are not known?

Yes (.) No (X)

If yes, specify …

D. Information on the organism(s) from which the insert is derived

1. Indicate whether it is a:

viroid (.)
RNA virus (.)
DNA virus (.)

bacterium (.)
fungus (.)
animal
- mammals (.)
- insect (.)
- fish (.)
- other animal (.)
(specify phylum, class) …
other, specify (X) Human

2. Complete name

(i)	order and/or higher taxon (for animals)	Primates
(ii)	family name for plants	Hominidae
(iii)	genus	Homo
(iv)	species	Sapiens
(v)	subspecies	Not applicable
(vi)	strain	Not applicable
(vii)	cultivar/breeding line	Not applicable
(viii)	pathovar	…
(ix)	common name	Human

3. Is the organism significantly pathogenic or harmful in any other way (including its extracellular products), either living or dead?
Yes (.) No (X) Not known (.)
If yes, specify the following:

(b) to which of the following organisms:

humans (.)
animals (.)
plants (.)
other ..

(b) are the donated sequences involved in any way to the pathogenic or harmful properties of the organism
Yes (.) No (.) Not known (.)

If yes, give the relevant information under Annex III A, point II(A)(11)(d):
…

4. Is the donor organism classified under existing Community rules relating to the protection of human health and the environment, such as Directive 90/679/EEC on the protection of workers from risks to exposure to biological agents at work?
Yes (.) No (X)

If yes, specify …

5. Do the donor and recipient organism exchange genetic material naturally?
Yes (.) No (X) Not known (.)

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?
Yes (.) No (X) Not known (.)
Specify …

(b) is the GMO in any way different from the recipient as far as mode and/or rate of reproduction is concerned?
Yes (.) No (X) Unknown (.)
Specify …

(c) is the GMO in any way different from the recipient as far as dissemination is concerned?
Yes (.) No (X) Not known (.)
Specify …

(d) is the GMO in any way different from the recipient as far as pathogenicity is concerned?
Yes (.) No (X) Not known (.)
Specify …

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, the genetic stability of PROSTVAC-V and PROSTVAC-F is demonstrated by the analysis of the PCR-amplicons encompassing the whole insert sequences and restriction site analysis of the whole genomes for each production lot as stated in the response to Section E.4.a.

3. Is the GMO significantly pathogenic or harmful in any way (including its extracellular products), either living or dead?
Yes (X, PROSTVAC-V only) No (.) Unknown (.)

(a) to which of the following organisms?

humans	(X)
animals	(X)
plants	(.)
other	…

(b) give the relevant information specified under Annex III A, point II(A)(11)(d) and II(C)(2)(i)

PROSTVAC-V

PROSTVAC-V is comparable to its parental virus with respect to pathogenicity, host range, and ability to colonise other organisms. Please refer to Section B.7.b for details regarding the pathogenicity of the vaccinia 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 with PROSTVAC-V. In addition, PROSTVAC-V has previously been administered to over 300 subjects in eight phase 1 and 2 clinical studies 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. Further details regarding effects in humans are summarized in the response to section E.3.c.v.(a) below.

PROSTVAC-F

PROSTVAC-F is comparable to its parental virus with respect to pathogenicity, host range, and ability to colonise other organisms. Please refer to Section B.7.b for details regarding the pathogenicity of the fowlpox virus:

Fowlpox virus-based vaccines such as PROSTVAC-F have been tested in both animals and humans. No safety concerns related to the use of recombinant fowlpox-based vaccines in humans 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**).

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 with PROSTVAC-F. 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. The most common AEs related to PROSTVAC-F observed to date have been injection site reactions, all of which were ≤ Grade 2 severity.

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) Strain identity by whole genome sequencing (2) Strain identity by whole genome restriction site analysis (3) Recombinant strain identity by Polymerase Chain Reaction (PCR) of inserted human genes and vector recombination junctions and (4) Microorganism detection by Quantitative PCR (qPCR) targeting a recombinant strain specific genome region. 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 and PROSTVAC-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 (including any significant potential environmental benefits that may be expected)
PROSTVAC-V and PROSTVAC-F are used in a prime-boost vaccination regimen to optimize immune responses against prostate cancer tumor cells.

The 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 is currently being 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 (empty vector fowlpox). This 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 or parental organism is regularly used, kept or found?
Yes (X) No (.)
If yes, specify
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 reservoir. 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):

Site 1: Erasmus MC – Centrumlocatie, 's-Gravendijkwal 230, 3015 CE Rotterdam, the Netherlands

(b) Size of the site (m^2): … 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 site (m^2): Not applicable; the study vaccine will be administered at licensed healthcare facilities.

(c) Proximity to internationally recognised biotopes 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 Netherlands release, each of the approximately 40 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 9 participating EU countries [Belgium, Denmark, Estonia, France, Germany, Netherlands, Poland, , Spain, and the United Kingdom] and also including Iceland, Israel and Russia 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 began in the EU on 20th July 2012. The active treatment period for this study is approximately 5 months. The date of final release will be once approximately 40 patients have completed the active treatment period at the designated Netherlands 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, health care providers and 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 PROSTVAC-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 $\leq$ 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, if significantly different from the recipient or parent organism

1. Name of target organism (if applicable)

(i)	order and/or higher taxon (for animals)	Primates
(ii)	family name for plants	Hominidae
(iii)	genus	Homo
(iv)	species	Sapiens
(v)	subspecies	Not applicable
(vi)	strain	Not applicable
(vii)	cultivar/breeding line	…
(viii)	pathovar	Not applicable
(ix)	common name	Human

2. Anticipated mechanism and result of interaction between the released GMOs and the target organism (if applicable)
PROSTVAC-V and PROSTVAC-F are viral vector-based investigational vaccines that are administered in seven subcutaneous vaccinations, over a five month period. They are intended to generate immune responses to prostate-specific antigen and prostate cancer cells. They use poxviral vectors to introduce modified PSA to the patient in an immunogenic manner to break self-tolerance, which generates immune responses directed against prostate cancer cells. The vaccines appear to induce a chronic active immunomodulatory action, and to slow overall disease progression.

3. Any other potentially significant interactions with other organisms in the environment
None.

4. Is post-release selection such as increased competitiveness, increased invasiveness for the GMO likely to occur?
Yes (.) No (X) Not known (.)
Give details
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. Types of ecosystems to 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 and PROSTVAC-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 licensed

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 (taking into account the nature of the receiving environment) may be unintentionally significantly harmed by the release of the GMO

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

(i)	order and/or higher taxon (for animals)	Primates
(ii)	family name for plants	Hominidae
(iii)	genus	Homo
(iv)	species	Sapiens
(v)	subspecies	Not applicable
(vi)	strain	Not applicable
(vii)	cultivar/breeding line	…
(viii)	pathovar	Not applicable
(ix)	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 either PROSTVAC-V or PROSTVAC-F 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 infection 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 (if available) from studies of the behaviour and characteristics of the GMO and its ecological impact carried out in stimulated natural environments (e.g. microcosms, etc.):
No studies have been conducted on the ecological impact of PROSTVAC-V or PROSTVAC-F 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. Size of the monitoring area (m^2)

   Not applicable; no specific viral detection/monitoring relative to PROSTVAC-V or PROSTVAC-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 disposed of at the site upon authorization of BNIT according to Appendix 8 and 9 of Regeling GGO.

2. Post-release treatment of the GMOs

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

3. (a) Type and amount of waste generated

Based on the current protocol, approximately 40 subjects will be recruited in the Netherlands 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 126 spent vials of PROSTVAC-V, 354 spent vials of PROSTVAC-F, and 276 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.

3. (b) 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 destroyed on site following normal site procedures for disposal of infectious biomedical waste or destroyed 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 wasted will be prepared.

J. Information on emergency response plans

1. Methods and procedures for controlling the dissemination of the GMO(s) 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. The genetic modifications made to these viruses

do not alter this characteristic. 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 removal of the GMO(s) of the areas potentially 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 could be exposed during or after the spread
Administration of PROSTVAC-V/F will occur only within licensed healthcare facilities. 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 the event of an undesirable effect
In order to protect human health and the environment, extensive procedural controls are in place for the transport, storage, administration, disposal, and monitoring of PROSTVAC-V/F 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.