

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

**A. General information**

1. Details of notification

- (a) Member State of notification Germany  
(b) Notification number B/DE/06/PEI210  
(c) Date of acknowledgement of notification 18/08/2006  
(d) Title of the project  
HIV-POL-001: Phase I, Open, Sequential Vaccination Study on Safety and Tolerability of  
two different doses of recombinant MVA-HIV polytope vaccine (MVA-mBN32) in HIV  
negative 18-50 year old Healthy Volunteers  
(e) Proposed period of release From 23/08/2006 until 12/11/2007

2. Notifier

Name of institution or company: Bavarian Nordic GmbH  
Fraunhoferstr. 13  
82152 Martinsried  
Germany

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)

Family: Poxviridae  
Subfamily: Chordapoxviridae  
Genus: Orthopoxviruses  
Species: Vaccinia

Strain: MVA-BN<sup>®</sup> (Modified Vaccinia Virus Ankara – Bavarian Nordic)

- (c) Genetic stability – according to Annex IIIa, II, A(10)  
MVA-BN<sup>®</sup> was subjected to several passages in Chicken Embryo Fibroblasts (CEF) cells during generation of recombinant MVA-mBN32 virus.  
Genetic stability was verified by polymerase chain reaction (PCR) amplification, sequencing and reverse transcriptase PCR (RT-PCR) of the recombinant inserts. Genetic stability of the recombinant inserts was verified in MVA-mBN32 Master Virus Bank (used as primary inoculums for GMP production), MVA-mBN32 Master Seed Virus and MVA-mBN32 Investigational Medicinal Product. Absence of wild type MVA-BN virus was also shown in all preparations.

In addition the stability or retention of the attenuated profile of the recombinant vaccine was verified in comparison to MVA-BN<sup>®</sup> and a replication positive MVA strain (MVA-I721).

4. Is the same GMO release planned elsewhere in the Community (in conformity with Article 6(1)), by the same notifier?

Yes (.) No (X)  
If yes, insert the country code(s) ...

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

Yes (X) No (.)  
If yes:  
- Member State of notification DE  
- Notification number PEI 1197/01, EudraCT no. 2004-001266-41

**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:  
- Member State of notification USA  
- Notification number IND 13243

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

MVA originates from the dermal Vaccinia Virus Ankara strain (Chorioallantois Vaccinia Virus Ankara, CVA). It was attenuated by over 570 continuous passages in primary CEF cells resulting in the loss of approximately 26 kb of its genome (Mayr et al. 1975, Meyer et al., 1991, Meisinger-Henschel et al., 2007). The deletions affect a number of virulence and host range genes as well as the gene for the Type A inclusion bodies (Rosel et al., 1986) (Altenburger et al., 1989) (Meyer et al., 1991) (Antoine et al., 1998). As a consequence, MVA exhibits a severely restricted host range, and fails to replicate in the majority of mammalian cells, including human cells, therefore neither potentially infectious skin lesions nor subsequent viral shedding is expected to occur.

The GMO MVA-mBN32 will be administered to subjects in a clinical environment. Only authorized and well educated persons are allowed to handle and administer the product.

Given the very limited host range of the vector, there is little or no likelihood of survival outside the clinical and laboratory environment.

**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

- (i) order and/or higher taxon (for animals) not applicable
- (ii) genus Orthopoxviruses
- (iii) species Vaccinia
- (iv) subspecies not applicable
- (v) strain (MVA-BN) Modified Vaccinia Virus  
Ankara – Bavarian Nordic
- (vi) pathovar (biotype, ecotype, race, etc.) not applicable
- (vii) common name Recombinant MVA-mBN32 HIV  
Vaccine

3. Geographical distribution of the organism

(a) Indigenous to, or otherwise established in, the country where the notification is made:  
Yes (.) No (X) Not known (.)

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

- (i) Yes (.)

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

- Atlantic ..
- Mediterranean ..

Boreal ..  
Alpine ..  
Continental ..  
Macaronesian ..

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

(c) Is it frequently used in the country where the notification is made?  
Yes (.) No (X)

(d) Is it frequently kept in the country where the notification is made?  
Yes (.) No (X)

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 (.)  
other, specify

No natural host known: MVA originates from the dermal Vaccinia Virus Ankara strain (Chorioallantois Vaccinia Virus Ankara, CVA). It was attenuated by over 570 continuous passages in primary CEF cells resulting in the loss of approximately 26 kb of its genome (Mayr et al. 1975, Meyer et al., 1991, Meisinger-Henschel et al., 2007). MVA-BN<sup>®</sup> is a further attenuated MVA strain, which has lost its ability to replicate in most mammalian cell types, including almost all human cell lines and is safe in severely immune compromised animals (AGR129 mice) (Chaplin et al., 2002).

(b) If the organism is an animal: natural habitat or usual agroecosystem:  
...

5. (a) Detection techniques  
Culture on CEF cells

(b) Identification techniques  
PCR, Sequencing

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

Yes (.) No (.)

If yes, specify

Related to vaccine against smallpox (BSL 1)

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

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

If yes:

(a) to which of the following organisms:

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

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

...

## 8. Information concerning reproduction

(a) Generation time in natural ecosystems:

...

(b) Generation time in the ecosystem where the release will take place:

...

(c) Way of reproduction:                      Sexual                      ..                      Asexual                      ..

(c) Factors affecting reproduction:

MVA originates from the dermal Vaccinia Virus Ankara strain (Chorioallantois Vaccinia Virus Ankara, CVA). It was attenuated by over 570 continuous passages in primary CEF cells resulting in the loss of approximately 26 kb of its genome (Mayr et al. 1975, Meyer et al., 1991, Meisinger-Henschel et al., 2007). The deletions affect a number of virulence and host range genes as well as the gene for the Type A inclusion bodies (Rosel et al., 1986) (Altenburger et al., 1989) (Meyer et al., 1991) (Antoine et al., 1998). As a consequence, MVA exhibits a severely restricted host range, and fails to replicate in the majority of mammalian cells, including human cells. MVA-BN<sup>®</sup> is a further attenuated MVA strain, which has lost its ability to replicate in most mammalian cell types, including almost all human cell lines and is safe in severely immune compromised animals (AGR129 mice) (Chaplin et al., 2002).

## 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 ...

(b) relevant factors affecting survivability:

Temperature, UV irradiation and chemical disinfection

10. (a) Ways of dissemination
- (b) Factors affecting dissemination  
The GMO MVA-mBN32 will be administered to subjects in a clinical environment. Only authorized and well educated persons are allowed to handle and administer the product. Given the very limited host range of the vector, there is little or no likelihood of survival outside the clinical and laboratory environment.

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)  
Not applicable.

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

1. Type of the genetic modification

- (i) insertion of genetic material  (X)
- (ii) deletion of genetic material  (.)
- (iii) base substitution  (.)
- (iv) cell fusion  (.)
- (v) others, specify ...

2. Intended outcome of the genetic modification

MVA-mBN32 should induce an immune response against HIV-genes. The composition of MVA-mBN32 is intended to induce HTL as well as CTL immune responses.

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  (.) No  (X)

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  (.)
- bacteriophage  (.)
- virus  (.)
- cosmid  (.)
- transposable element  (.)
- other, specify ...

(b) Identity of the vector

- ...
- (c) Host range of the vector  
...
- (d) Presence in the vector of sequences giving a selectable or identifiable phenotype  
 Yes (.) No (.)  
 antibiotic resistance (.)  
 other, specify ...
- Indication of which antibiotic resistance gene is inserted  
 ...
- (e) Constituent fragments of the vector  
 ...
- (f) Method for introducing the vector into the recipient organism
- (i) transformation (.)  
 (ii) electroporation (.)  
 (iii) macroinjection (.)  
 (iv) microinjection (.)  
 (v) infection (.)  
 (vi) other, specify ...

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 (X)  
 (ii) microinjection (.)  
 (iii) microencapsulation (.)  
 (iv) macroinjection (.)  
 (v) other, specify ...

6. Composition of the insert

(a) Composition of the insert

A HIV CTL epitope (HIV-1 CTL 1090) was cloned into the deletion site II of the MVA-BN<sup>®</sup> genome. It contains a synthetic DNA fragment encoding 21 conserved CTL epitopes derived from structural and regulatory/accessory proteins of HIV-1 (Gag, Pol, Vpr, Nef, Rev, and Env) which are supertype binding peptides of HLA-A2, -A3, and -B7 HLA superfamily Class I molecules. It also contains the synthetic universal HTL epitope PADRE<sup>®</sup>. The insert is placed under the control of the vaccinia virus early/late promoter pr7.5.

A HIV HTL epitope (HIV-1 HTL 1043) was cloned into the intergenic region (IGR) I4L/I5L of the MVA-BN<sup>®</sup> genome. It contains a synthetic DNA fragment encoding 18 HTL epitopes derived from structural and regulatory/accessory proteins of HIV-1 (Gag, Pol, Vpr, Nef, Rev, and Env) that bind to multiple HLA-DR Class II molecules. The insert is placed under the control of the vaccinia virus early/late promoter pr7.5.

- (b) Source of each constituent part of the insert  
 21 conserved CTL epitopes derived from structural and regulatory/accessory proteins of HIV-1 (Gag, Pol, Vpr, Nef, Rev, and Env)  
 18 HTL epitopes derived from structural and regulatory/accessory proteins of HIV-1 (Gag, Pol, Vpr, Nef, Rev, and Env)  
 Vaccinia virus early/late promoter pr7.5
- (c) Intended function of each constituent part of the insert in the GMO  
 MVA-mBN32 should induce an immune response against HIV-genes. The composition of MVA-mBN32 is intended to induce HTL as well as CTL immune responses.
- (d) Location of the insert in the host organism
- on a free plasmid
  - integrated in the chromosome
  - other, specify ...
- (e) Does the insert contain parts whose product or function are not known?  
 Yes  No   
 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 ...

2. Complete name

- (i) order and/or higher taxon (for animals) not applicable
- (ii) family name for plants not applicable
- (iii) genus Retroviridae
- (iv) species Human Immunodeficiency Virus type I
- (v) subspecies not applicable
- (vi) strain ...
- (vii) cultivar/breeding line not applicable

(viii) pathovar not applicable  
(ix) common name HIV-1

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

Yes  No  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

If yes, specify Risk class BSL 3

5. Do the donor and recipient organism exchange genetic material naturally?

Yes  No  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  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  Unknown

Specify ...

(c) is the GMO in any way different from the recipient as far as dissemination is concerned?

Yes  No  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

Genetic stability was verified by polymerase chain reaction (PCR) amplification, sequencing and reverse transcriptase PCR (RT-PCR) of the recombinant inserts. Genetic stability of the recombinant inserts was verified in MVA-mBN32 Master Virus Bank (used as primary inoculums for GMP production), MVA-mBN32 Master Seed Virus and MVA-mBN32 Investigational Medicinal Product. Absence of wild type MVA-BN virus was also shown in all preparations.

In addition the stability or retention of the attenuated profile of the recombinant vaccine was verified in comparison to MVA-BN<sup>®</sup> and a replication positive MVA strain (MVA-I721).

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

Yes (.) No (X) Unknown (.)

(a) to which of the following organisms?

humans (.)

animals (.)

plants (.)

other ...

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

...

4. Description of identification and detection methods

(a) Techniques used to detect the GMO in the environment

PCR

(b) Techniques used to identify the GMO

PCR, Sequencing

**F. Information relating to the release**

1. Purpose of the release (including any significant potential environmental benefits that may be expected)

Release of the GMO is performed as part of a phase I clinical study to evaluate the safety of MVA-mBN32 and its potential to induce immune response against HIV-infections.

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 natural habitat of the MVA virus is not known

3. Information concerning the release and the surrounding area

(a) Geographical location (administrative region and where appropriate grid reference):  
Germany, Munich, Ludwig-Maximilian-University (LMU)

(b) Size of the site (m<sup>2</sup>): LMU, thousands m<sup>2</sup>  
(i) actual release site (m<sup>2</sup>): a room inside, 10-15 m<sup>2</sup>  
(ii) wider release site (m<sup>2</sup>): LMU, thousands m<sup>2</sup>

(c) Proximity to internationally recognised biotopes or protected areas (including drinking water reservoirs), which could be affected:  
Not applicable

(d) Flora and fauna including crops, livestock and migratory species which may potentially interact with the GMO  
Not applicable

4. Method and amount of release

(a) Quantities of GMOs to be released:  
36 patients: 18 patients receiving  $1 \times 10^7$  TCID<sub>50</sub>/dose/vaccination (3 vaccinations)  
and 18 patients receiving  $1 \times 10^8$  TCID<sub>50</sub>/dose/vaccination (3 vaccinations)

(b) Duration of the operation:  
Vaccination normally takes only a few minutes. Vaccinations were scheduled at week 0, week 4 and week 16.

(c) Methods and procedures to avoid and/or minimise the spread of the GMOs beyond the site of the release  
Waste will be collected and autoclaved before disposal.

5. Short description of average environmental conditions (weather, temperature, etc.)  
Indoor conditions, room temperature

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.  
Not applicable

**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) Not applicable  
(ii) family name for plants Not applicable

(iii)	genus	Not applicable
(iv)	species	Homo
(v)	subspecies	sapiens
(vi)	strain	Not applicable
(vii)	cultivar/breeding line	Not applicable
(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)

MVA-mBN32 should induce an immune response against HIV genes. The composition of MVA-mBN32 is intended to induce HTL as well as CTL immune responses.

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

Not applicable

4. Is post-release selection such as increased competitiveness, increased invasiveness for the GMO likely to occur?

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

Give details

...

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

Given the very limited host range of the vector, there is little or no likelihood of survival outside the clinical and laboratory environment.

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

Not applicable

(i)	order and/or higher taxon (for animals)	...
(ii)	family name for plants	...
(iii)	genus	...
(iv)	species	...
(v)	subspecies	...
(vi)	strain	...
(vii)	cultivar/breeding line	...
(viii)	pathovar	...
(ix)	common name	...

7. Likelihood of genetic exchange in vivo

(a) from the GMO to other organisms in the release ecosystem:

Given the very limited host range of the vector no genetic exchange is expected.

(b) from other organisms to the GMO:

Given the very limited host range of the vector no genetic exchange is expected.

- (c) likely consequences of gene transfer:  
Not applicable

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.):

Not available

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

Not applicable

## **H. Information relating to monitoring**

1. Methods for monitoring the GMOs

The GMO itself will not be monitored, but the patients will be monitored during the clinical trial.

The study started with a variable screening period of minimum 1 day and maximum 21 days (V1-V2). At V2 (Day 0) eligible subjects were enrolled to the open treatment period lasting 18 weeks (V2-V7) during which each subject received three vaccinations, one at Week 0 (V2), at Week 4 (V4) and at Week 16 (V6), either with  $1 \times 10^7$  TCID<sub>50</sub> (Group 1) or with  $1 \times 10^8$  TCID<sub>50</sub> (Group 2). A control visit took place two weeks after each vaccination (V3, V5 and V7 = end of treatment period). To collect long-term post-vaccination safety data subjects were asked to return for a follow-up examination 22 weeks after V7 (V8 = final study visit)

2. Methods for monitoring ecosystem effects

Not applicable

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

Not applicable

4. Size of the monitoring area (m<sup>2</sup>)

Not applicable

5. Duration of the monitoring

Not applicable

6. Frequency of the monitoring

Not applicable

## **I. Information on post-release and waste treatment**

1. Post-release treatment of the site

All material used in contact with the recombinant MVA-mBN32 will be autoclaved before disposal. Unused vials will have to be returned to Bavarian Nordic. Surfaces will be cleaned according to standard procedures.

2. Post-release treatment of the GMOs

All material used in contact with the recombinant MVA-mBN32 will be autoclaved before disposal. Unused vials will have to be returned to Bavarian Nordic.

3. (a) Type and amount of waste generated  
Vials, syringes, needles, gloves
3. (b) Treatment of waste  
All material used in contact with the recombinant MVA-mBN32 will be autoclaved before disposal.

**J. Information on emergency response plans**

1. Methods and procedures for controlling the dissemination of the GMO(s) in case of unexpected spread  
Blood and vaccine that has been spilled during any of the vaccination procedures will be cleaned using absorbent material and alcohol. All materials used during the cleaning procedures will be destructed according to procedures for destruction of hospital waste.
2. Methods for removal of the GMO(s) of the areas potentially affected  
Blood and vaccine that has been spilled during any of the vaccination procedures will be cleaned using absorbent material and alcohol.
3. Methods for disposal or sanitation of plants, animals, soils, etc. that could be exposed during or after the spread  
Not applicable.
4. Plans for protecting human health and the environment in the event of an undesirable effect  
Patients will be monitored for the occurrence of adverse events and serious adverse events (SAE) according to the clinical protocol. Each SAE will be recorded and assessed by the hospital staff, the study sponsor and Health Authorities will be notified.