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	Belgium
(b)	Notification number	B/BE/07/BVW3.
(c)	Date of acknowledgement of notification	../../….
(d)	Title of the project	A multi-centre phase I study to evaluate the safety and tolerability of a heterologous prime-boost vaccination with INX102-3697 HBV pDNA/INX-102-00557 HBV MVA in healthy volunteers and HBeAg+ chronic hepatitis patients
(e)	Proposed period of release	From 01/03/2008 until 01/03/2010

2. Notifier

Name of institution or company: GENimmune N.V.

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)

Modified Vaccinia virus Ankara (MVA), strain MVATGN33

(c) Genetic stability – according to Annex IIIa, II, A(10)

Not applicable.

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 (.) No (X)

If yes:
- Member State of notification …
- Notification number B/../../…

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 (.) No (X)

If yes:
- Member State of notification …
- Notification number B/../../…

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

The recipient MVA is a highly attenuated, host-restricted, non-replicating poxvirus strain. It is widely considered as the Vaccinia virus strain of choice for clinical investigation and development for use as a potent vector system in recombinant vaccine development. Recombinant MVA vaccines have been safely administered to humans in clinical trials for melanoma, HIV, and orthopox infections. In the EU, several clinical trials with genetically modified MVA have been conducted. All cases confirmed that the MVA vectors can be safely used and do not lead to uncontrolled dispersal. Due to its attenuation, MVA is generally classified as an organism belonging to biological risk level 1. The genetic modification does not change that.

The GMO will be administered via injection to healthy volunteers and, if found to be safe, to patients with chronic hepatitis B. The transgene is expressed only transiently. The organism does not persist in the subject's body. Release via shedding is not expected.
Potential exposure, except for the subject, is limited to the medical staff involved at the time of injection and is restricted to the hospital room. Although the probability is extremely limited adhering good clinical practice (protective clothing, wound care, waste treatment)

exposure may arise from (accidental) contact with the solution of the investigational product or due to a needle stick injury.
Exposure to other humans, even subject's family members is highly unlikely.

Vaccinia virus is traditionally regarded as a laboratory virus with no natural reservoir. As MVA is unable to replicate effectively in human cells and most mammalian host cells, the risk of dissemination and transmission in humans and the environment is highly reduced.

In summary, the risk assessment for this study shows a very low risk associated with administering the GMO to patients. The risk to other humans is negligible and the risk to the environment is estimated to be practically zero.

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)	Poxviridae
(ii)	genus	Orthopoxvirus
(iii)	species	Vaccinia virus
(iv)	subspecies	
(v)	strain	Modified Vaccinia virus Ankara (MVA), strain MVATGN33
(vi)	pathovar (biotype, ecotype, race, etc.)	
(vii)	common name	

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 (.)

Modified Vaccinia virus Ankara is a laboratory virus with no natural reservoir.

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

(i) Yes (X) *idem as 3.A*

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

Atlantic	..
Mediteranean	..
Boreal	..
Alpine	..
Continental	..
Macaronesian	..

(ii) No (X) *idem as 3.A*

(iii) Not known (.)

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

Yes (X.) No (.)

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

Yes (X) No (.)

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	MVA replicates in primary chicken embryo fibroblasts (CEFs) and baby hamster kidney cells (BHKs) only.

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

5. (a) Detection techniques

Biological markers.
Molecular techniques based on PCR and sequencing.
Specific culture requirements: with the exception of CEFs and BHKs, the MVA virus grows in cell cultures only abortively.

(b) Identification techniques

Restriction analysis of the DNA, PCR analyses and DNA sequencing.

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

Yes (X) No (.)

If yes, specify: /

Due to its attenuation, MVA is generally classified as an organism belonging to biological risk level 1 by the US Centers for Disease Control and Prevention (CDC), the US National Institutes of Health (NIH), the German Central Advisory Committee for Biological Safety (ZKBS) and the Swiss Expert Committee for Biosafety (EFBS). The Dutch Advisory Committee, COGEM, concludes in its advice CGM/030519-06 of 26 May 2003 that MVA can be safely handled under biosafety level 1 containment measures.

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

MVA has been administered to numerous animal species including monkeys, mice, swine, sheep, cattle, horses, and elephants, with no local or systemic adverse effects. The MVA strain has been reported to be replication-incompetent in most mammalian cells, avirulent among normal and immunosuppressed animals and safe among humans. Various human transformed and primary cells were non-permissive or showed very limited MVA replication. Studies by several groups showed that no virus production occurred in patient-derived infected primary human cells.

MVA has been tested in several animal models and in human clinical trials, demonstrating its safety and its ability to protect against the development of poxvirus infections. These studies involved over 120,000 humans, including high-risk patients, and proved that compared to Vaccinia based vaccines, MVA had diminished virulence or infectiousness while it induced a good specific immune response by intradermal, subcutaneous, or intramuscular injection. MVA immunization has been shown to provide protection against a pulmonary Vaccinia virus challenge.

As MVA is a highly attenuated, host-restricted, non-replicating poxvirus strain it is widely considered as the Vaccinia virus strain of choice for clinical investigation and development for use as a potent vector system in recombinant vaccine development. Recombinant MVA vaccines have been safely administered to humans in clinical trials for melanoma, HIV and orthopox infections.

No vectors are known.

8. Information concerning reproduction

(a) Generation time in natural ecosystems:

Vaccinia virus replicates only in the cytoplasm of the host cell, outside of the nucleus. Only in permissive cells MVA completes its replication cycle. In non-permissive cells virion assembly is blocked and only immature viruses accumulate.

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

The GMO is not able to replicate in the study subjects or the environment.

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

(d) Factors affecting reproduction:

MVA can only complete its life cycle in CEFs and BHKs under laboratory conditions.

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, specify	none

(b) relevant factors affecting survivability:

The GMO can only grow in artificial laboratory conditions.

10. (a) Ways of dissemination

Due to the attenuation there is little potential for dissemination. Person-to-person transmission is highly unlikely.

(b) Factors affecting dissemination

No specific factors.

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)

The recipient organism has not been modified before.
Other notifications have been made for trials involving GMOs derived from the same recipient, in particular B/BE/01/B7 and B/BE/02/B7.

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

The insertion is synthetically composed and encodes a hepatitis B virus (HBV) polyepitope. A selection has been made of HBV peptides bearing epitopes that will induce cytotoxic T lymphocyte (CTL) responses aiming at eradicating or at least long term-suppressing the infecting HBV.
The polyepitope was integrated into the MVA genome via homologous recombination.
In the trial, administration of the MVA will be alternated with injections of a plasmid solution containing the same synthetic gene construct.
Upon administration to subjects, the protein is expressed transiently and processed rapidly into individual epitopes. The gene product is designed to elicit immune responses in a broad population, but has no enzymatic activity by itself.

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	(.)
virus	(.)
cosmid	(.)
transposable element	(.)
other, specify	…

(b) Identity of the vector.
Plasmid pTG16997

(c) Host range of the vector
The vector has an ColE1 origin of replication as well as a bacteriophage f1 origin of replication. The plasmid is designed to enable homologous recombination in MVA deletion III.

(d) Presence in the vector of sequences giving a selectable or identifiable phenotype
Yes (X) No (.)

antibiotic resistance (.)
Indication of which antibiotic resistance gene is inserted
…
other, specify: an eGFP/*gpt* expression cassette is present containing a synthetic gene for a fusion protein combining the enhanced green fluorescent protein (eGFP) and the *E. coli* xanthine-guanine phosphoribosyltransferase (GPT) controlled by the early-late Vaccinia virus synthetic p11K7.5K promoter. After viral plaque purification, selection agents are omitted resulting in a rapid elimination of the selection marker by intragenic homologous recombination during few viral amplification and plaque purification steps.

(e) Constituent fragments of the vector
Plasmid pTG16626 is a transfer plasmid vector containing the MVA deletion III flanking sequences (BRG3/BRD3), an empty expression cassette for H5R promoter-driven expression (incl. typical Vaccinia transcription termination signal), an eGFP/*gpt* expression cassette, and sequences required for replication in *E. coli.* The eGFP/*gpt* expression cassette was placed between two homologous sequences (BRG3 and BRD3). In the presence of selective medium, the recombinant MVA maintains this cassette integrated within its genome. After viral plaque purification, selection agents are omitted resulting in a rapid elimination of the selection marker by intragenic homologous recombination during few viral amplification and plaque purification steps.

The coding region for the HBV polyepitope was subcloned into pTG16626 plasmid downstream the early late H5R promoter resulting in the pTG16997 transfer plasmid

(f) Method for introducing the vector into the recipient organism

(i) transformation (.)

(ii)	electroporation	(.)
(iii)	macroinjection	(.)
(iv)	microinjection	(.)
(v)	infection	(.)
(vi)	other, specify	transfection, homologous recombination

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	…..

6. Composition of the insert

(a) Composition of the insert

The insert consists of a Vaccinia promoter, a consensus eukaryotic Ig kappa signal sequence, the HBV polyepitope encoding sequence and a Vaccinia transcription termination sequence.

(b) Source of each constituent part of the insert

The promoter and terminator originate from Vaccinia.
The HBV polyepitope a synthetic gene derived from hepatitis B virus.
Also the consensus eukaryotic Ig kappa signal sequence is a synthetic DNA fragment.

(c) Intended function of each constituent part of the insert in the GMO

The promoter is used to drive expression of the polyepitope.
The eukaryotic Ig kappa signal sequence is meant to target the expressed polyepitope to the endoplasmatic reticulum.
The HBV polyepitope is aimed at inducing cytotoxic T lymphocyte (CTL) and T-Helper lymphocyte (HTL) responses.

(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	(X)
bacterium	(.)
fungus	(.)
animal:	
- mammals	(.)
- insect	(.)
- fish	(.)
- other animal	(.)

specify phylum, class: …

other, specify: man

2. Complete name

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

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

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

If yes, specify the following:

(a) to which of the following organisms:

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

(b) are the donated sequences involved in any way to the pathogenic or harmful properties of the organism

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

If yes, specify: In Directive 2000/54/EC on the protection of workers from risks to exposure to biological agents at work, hepatitis B virus received classification 3**, presenting a limited risk of infection for workers because they are not normally infectious by the airborne route.

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 (.)

If yes, 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) Not known (.)

If yes, specify: …

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

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

If yes, specify: …

(d) is the GMO in any way different from the recipient as far as pathogenicity is concerned?

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

If yes, specify: …

2. Genetic stability of the genetically modified organism

The insertion has occurred on the viral genome, which was confirmed by PCR amplification. Sequence integrity was controlled by DNA sequence analysis of the HBV polyepitope expression cassette.

The MVA pre-master virus seed (PMVS1) was subcultured for 6 consecutive passages by infection of CEF cells. Genetic stability was evaluated on 100 viral clones isolated from the 6th passage of the PMVS1 stock. Two methods were used:

(1) PCR amplification to determine the integrity of the expression and demonstrate the absence of parental (wild-type) MVA and

(2) DNA sequence analysis of the HBV polyepitope expression cassette on 50 viral clones.

Results of the analyses showed that 100% of clones contained only the HBV polyepitope expression cassette with DNA sequences identical to the original sequence, demonstrating genetic stability.

Genetic stability during manufacture of the GMO is demonstrated for each batch.

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

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

(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)

MVA is a highly attenuated, host-restricted, non-replicating poxvirus strain, that has several inherent safety features severely hindering dissemination and transmission. It is widely considered as the Vaccinia virus strain of choice for clinical investigation and development for use as a potent vector system in recombinant vaccine development. Recombinant MVA vaccines have been safely administered to humans in clinical trials for several viral infections.

There is no indication that the GMO itself is toxic, allergenic or pathogenic. The changes that were induced, do not affect the basic toxic or allergenic features. Several toxicity studies (in rabbits and mice) have been performed as part of product development. They all support the safety of the product and did not reveal any indication of toxicity.

4. Description of identification and detection methods

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

The plasmid and the MVA can be traced based on molecular techniques. The methods based on PCR identification are suitable for this purpose. However, given the highly unlikely event that shedding occurs except to a very limited extent at the injection site, no routine tracing and monitoring of the GMO is foreseen. Rather the method can be used in case of an unexpected finding or in case of an emergency.

(b) Techniques used to identify the GMO

PCR and sequencing methods result in clear-cut identifications.

F. Information relating to the release

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

The deliberate release is a an open-label, non-randomized, uncontrolled, multi-centre, phase I study to evaluate the safety, tolerability, and immunogenicity of a heterologous prime-boost vaccination of alternating intramuscular (IM) injections of INX102-3697 HBV pDNA and subcutaneous (SC) injections of INX102-0557 HBV MVA.
The proposed Phase I clinical trial is the first evaluation of the alternating application of the polyepitope vaccine presented via a plasmid and via an MVA vector. The study will consist of a first part with vaccination of healthy volunteers. Conditionally, after an interim safety evaluation, if the vaccine is proven to be safe and tolerable, a second part with vaccination of HBeAg+ patients with chronic hepatitis B will be initiated.

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 parental MVA strain can only grow in laboratory conditions in permissive cells. The GMO will be administered to subjects via subcutaneous (SC) injections. MVA is unable to replicate effectively in human beings and will eventually be eliminated by the person’s cellular processes.

3. Information concerning the release and the surrounding area

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

The trial subjects can live in Brussels, Flanders and Wallonia The administration will potentially take place at the following clinical trial centres:

- Centre Hospitalier Universitaire Brugmann, Brussels,
- Cliniques Universitaires Saint-Luc, Brussels,
- Drug Reseach Unit Ghent (D.R.U.G.) Gent
- SGS Life Science Services - Research Unit Stuivenberg, Antwerpen
- Universitair Ziekenhuis Antwerpen, Antwerpen
- Universitair Ziekenhuis Brussel, Brussels.

- Universitair Ziekenhuis Gasthuisberg, Leuven, and
- Universitair Ziekenhuis Gent, Gent,

Actual participation will depend on recruitment of subjects and fulfilment of all trial conditions, including those related to activities with GMOs.

(b) Size of the site (m^2): … m^2

(i) actual release site (m^2): … m^2
(ii) wider release site (m^2): … m^2

The size of the room at each site where the GMO will be administered to study subjects is not known.

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

Not relevant. The GMO is restricted to laboratory growing conditions. Shedding from treated subjects is not anticipated.

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

Not Applicable. The organism is restricted in its host range to CEFs and BHKs.

4. Method and amount of release

(a) Quantities of GMOs to be released:

A total of 15 healthy volunteers and 15 patients are involved in the studies. Subjects will be injected 5 times with a solution containing either the plasmid or the MVA. Treatments will be separated by a period of 3 weeks. For each formulation one dose is foreseen. At every time point either a total of 4 mg of INX102-3697 HBV pDNA or a total of $2x10^8$ pfu of INX102-0557 HBV MVA will be administered.

(b) Duration of the operation:

Recruitment of the first healthy volunteers is expected to start in March 2008. Completion will depend on availability of patients fulfilling the selection criteria and could take until March 2010. Each subject will be treated during 15 weeks.

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

During the preparatory phase, the plasmid INX102-3697 and MVATG16997 are formulated and packaged in sealed vials and ampoules. The solution can be aspirated directly from the vials by insertion of the injection needle through the cap. The ampoule needs to be opened for aspiration. In both cases, the manipulation is not expected to contribute to a large -if any- release. The release will be limited to the immediate vicinity and will be cleaned up immediately.

An accidental release from the containers is unlikely given the specifications of the glass vials and ampoules. Even in case of an accidental release, the limited quantity of solution is easily kept contained and cleaned (standard detergent or bleach).

Once injected, a limited release from the injection site can be expected. No other routed for release e.g. via body fluids is likely. The injection site is disinfected upon administration and in addition the initial wound dressing is replaced after 4 hours and disposed as hazardous medical waste.

All disposables used during the treatment are collected and inactivated as hazardous medical waste. This includes gloves and wipes used by the medical staff. In addition the medical staff present will be asked to disinfect hands before leaving the treatment room.

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

Not relevant.

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.

A first clinical trial (single centre Phase I study) with INX102-3697 HBV pDNA plasmid expressing the polyepitope was conducted in the USA. The trial evaluated the safety and tolerability of four monthly intramuscular injections at two dose levels, 0.4 mg and 4.0 mg, as assessed by clinical adverse events and dose limiting toxicities (the highest dose is comparable to the dose of INX102-3697 HBV pDNA plasmid that will be provided in the proposed trial). There were no clinically significant changes that could be attributed to the treatment. Study medication was regarded as safe and was well tolerated at both dose levels. Injection site reactions were similar between the two active substance groups whereas placebo had no injection site reaction. All other safety parameters were comparable between verum and placebo and between the two dose levels.

The particular MVA strain has not been tested in a clinical trial setting before. However, several trials have been conducted in the EU and in the rest of the world with modified MVA vectors for similar purpose. All results confirm that MVA is a promising safe vector for medical applications.

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):

The target organisms are a specific group of patients (HBeAg+ patients with chronic hepatitis). In a first part, also healthy volunteers will be enrolled.

(i)	order and/or higher taxon (for animals)	Primates
(ii)	family name for plants	…
(iii)	genus	*Homo*

(iv)	species	*Homo sapiens*
(v)	subspecies	…
(vi)	strain	…
(vii)	cultivar/breeding line	…
(viii)	pathovar	…
(ix)	common name	man

2. Anticipated mechanism and result of interaction between the released GMOs and the target organism (if applicable)

Human patients will be injected with the GMO. The organism will express the HBV polyepitope transiently and trigger cytotoxic T and T-Helper lymphocyte responses. The vaccine is aimed at eradicating the virus.

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

No interactions with other organisms are identified.
In the unlikely case healthy persons (e.g. medical staff, family members) would be exposed, the chances for an clinical effect would be limited as the way of administration and dose would be most likely insufficient.
Exposure to the MVA background could trigger in itself a reaction. As MVA was successfully used for large scale vaccination, it could lead to an immunization reaction, which in itself is not undesirable. Again the dose and frequency will most likely be too low to have a consequence. Furthermore, extensive experience is available on possible side effects.

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

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

Give details:

The GMO is restricted to artificial growing conditions and is able to replicate only in certain host cells (CEFs and BHKs).

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

There is no active dispersal mechanism. MVA is unable to replicate effectively in human cells and most mammalian host cells, which reduces the risk of dissemination and transmission.

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

(i)	order and/or higher taxon (for animals)	No specific interactions with non-target organisms have been identified.
(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:

The HBV polyepitope gene was integrated into the MVA strain via homologous recombination. There are no indications that such mechanism would also occur in nature given already the absence of other MVA or relevant Vaccinia strains in the expected environment.

(b) from other organisms to the GMO:

Since Vaccinia viruses, including MVA, are considered to have no natural reservoir, no transfer is expected. MVA has been used extensively and no transfer of genetic material has been reported.

(c) likely consequences of gene transfer:

In the highly unlikely event that the gene for the HBV polyepitope is transferred to other organisms, it would give no selective advantage to those organisms.

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.):

Information not available.

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

No potential interactions with biogeochemical processes have been identified.

H. Information relating to monitoring

1. Methods for monitoring the GMOs

MVA can be traced based on molecular techniques (PCR amplification). However, no routine tracing and monitoring of the GMO is foreseen as it is very unlikely that shedding occurs.

2. Methods for monitoring ecosystem effects

Not planned.

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

To detect the hypothetical transfer of donated genetic material to other organisms, PCR aimed at the gene for the polyepitope can be used.

4. Size of the monitoring area (m^2)
. m^2

Not relevant.

5. Duration of the monitoring

Not relevant.

6. Frequency of the monitoring

Not relevant.

I. Information on post-release and waste treatment

1. Post-release treatment of the site

The site of release will be limited to the hospital room. All material and/or recipients that have been in contact with the biological material are either stored for return to the sponsor GENimmune or destroyed like hazardous medical waste according to local procedures. For eventual storage precautions will be taken to avoid subsequent release.

The material includes all disposables such as disposable overcoats, gloves, disposable bed covers, cottons, wound dressings, syringes, etc.

2. Post-release treatment of the GMOs

No specific elimination or inactivation is foreseen for material that has been injected in the subject. The presence and expression is transient and will remain confined to the subject.
Material that may contain traces of or that has been in contact with the biological material will be collected and disposed of as hazardous medical waste.

3. (a) Type and amount of waste generated

Disposable materials that have been exposed to the GMO are empty product containers, disposable overcoat; gloves; covering material for hospital bed/treatment table, disposable wipes, injection needles & syringes, bandage.

(b) Treatment of waste

Disposable items will be collected and inactivated as hazardous medical waste by incineration or other validated method.

J. Information on emergency response plans

1. Methods and procedures for controlling the dissemination of the GMO(s) in case of unexpected spread

   Unexpected spread would mainly be limited to accidental opening of the packaged materials, releasing the solution with the GMO. However this will be a very small quantity that can easily be kept contained, with standard alcohol-based disinfection solutions and recovered using standard spillage procedures. Following recovery, the area should be treated with a standard detergent (soap) or bleach. All material collected should be handled and disposed of as hazardous medical waste.

2. Methods for removal of the GMO(s) of the areas potentially affected

   Idem.

3. Methods for disposal or sanitation of plants, animals, soils, etc. that could be exposed during or after the spread

   Idem. No specific sanitation measures are foreseen.

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

   The clinical trial protocol foresees action in case the subject would react adversely to the treatment.
   The trial is conducted in a limited number of humans and these are enrolled in a staggered way. Should any time an undesirable effect be observed, then the treatment can be stopped and treated subjects can be followed in more detail implementing additional measures specific for the observed effect.