MVA.HTI

B/ES/16/11

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

18/10/2016

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: Spain
(b) Notification number: B/ES/16/11
(c) Date of acknowledgement of notification: 21/10/2016
(d) Title of the project:

A phase I randomized, placebo-controlled trial, to evaluate the safety, tolerability and immunogenicity of experimental HIV-1 vaccines, DNA.HTI and MVA.HTI administered in HIV-1 negative volunteer adults (Aelix-oo1).

(e) Proposed period of release: Start date Aelix-001: 01/02/2017- Finish date: 28/02/2018

2. Notifier

Name of institution or company:
Aelix Therapeutics in collaboration with IrsiCaixa AIDS Research Institute
Hospital Universitari Germans Trias i Pujol Carretera de Canyet s/n
08916 Badalona (Barcelona)

3. GMO characterization MVA.HTI

(a) Indicate whether the GMO is a:

viroid	(.)
RNA virus	(.)
DNA virus	(X)
bacterium	(.)
fungus	(.)

animal

- mammals (.)
- insect (.)
- fish (.)
- other animal (.) specify phylum, class

other, specify (kingdom, phylum and class)

(b) Identity of the GMO (genus and species)

The genetically modified organism (GMO) used in the trial is the MVA.HTI. The MVA vector is a modified vaccinia Ankara virus, live recombinant, attenuated by serial passages in cultured chicken embryo fibroblasts (CEF) with contains six large deletions from the parental virus genome. Within the MVA has been inserted a transgene coding for the insert HTI in order to induce an HIV-1 specific T cell immune response. The size of MVA.HTI after the insertion is estimated to be approximately 179.6 kp.

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

MVA is a genetically stable virus unable to integrate its DNA into the host genome and which remains localized in the cytoplasm of the cell until the cell destruction.

The GMO MVA.HTI is generated in chicken embryo fibroblasts (CEFs) and purified by sucrose cushion centrifugation. The MVA vector is a recombinant live attenuated virus by serial passage in CEF containing six large genomic deletions relative to the parental virus. The MVA genome size after insertion of the coding sequence HTI is estimated to be approximately 179.6 kp. The genetic modifications of GMOs MVA.HTI are stable and remain after successive passages in CEFs.

The production of the recombinant virus MVA.HTI is carried out by the German company IDT Biologika and is based on a system of 'seed virus', in which a 'master seed virus' (MSV) and a working virus (WSV) is prepared. All the preparation, verification of the genetic stability and MSV and WSV storage is done at ITD Biologika under cGMP conditions and according to EU regulations.

Genetic stability is verified in various steps of the production process, through integrity analysis of the vector and insert (restriction pattern and sequencing of the insert), purity, biological potency and safety (analysis of the absence of the parental virus), both on the initial inoculum produced by Dr. Tomas Hanke, the WSV, the MSV and the final product.

The standard stability (shelf life) allocated to the MVA-based vaccines produced by IDT Biologika under cGMP conditions is 24 months when stored at -70ºC and stability tests are repeated for MVA.HTI annually

There is a large experience with the stability of recombinant poxvirus, and recombinant MVA in particular, not only by IDT Biologika. To date, University of Oxford has developed seven recombinant MVA that have entered clinical trials, two with transgenes derived from HIV-1

(HIVA and HIVconsv) The stability data of two closely related products (MVA85A and MVA.HIVA), which had undergone the same manufacturing process showed stability over a period of 6 years. The transgenes HIVA and 85A are 1584 bp and 1107 bp in size respectively, and thus similar to the size of the gene HTI (1,578 pb).

Within the identification and validation tests to confirm the overall integrity of the genome MVA.HTI (hence its genetic stability) are included:

a. The presence of the intact HTI ORF in the MVA.HTI is checked by a PCR reaction using primers J1312 and J1205, which yield a 331 bp product.

b. Purity by PCR: the absence of wild type MVA (non-recombinant or MVA.RFP) is checked by a PCR reaction using specific primers, which yield a 688 bp RPF-specific band. The plasmid pRFP serves as the positive control in this PCR.

c. Identity by sequencing the entire ORF of HTI identical to the master sequence.

Monitoring the stability of the clinical batches, as outlined in the Investigational Medicinal Product Dossier (IMPD), is performed periodically using infectivity assays in CEFs. The virus titre is expressed in plaque forming units per millilitre (pfu/ml). Tests are performed to assess the genetic stability in different parts of the final batch production. PCR amplification and subsequent sequencing serves to confirm the presence of the insert coding for HTI antigen and to reject the production of mutations or deletions.

Stability tests (qualification) are complemented by *in-vivo* potency tests measuring immunogenicity in mice

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 -

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 -

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

There is no scientific reason to expect that the use of HTI as an insert in the viral vector will change its distribution characteristics, shedding or replicative capacity compared to other inserts used in the same MVA. The MVA has been used extensively in clinical trials, both as direct administration or cell therapy strategies. MVA is not expected to survive as it is found exclusively in the cytoplasm of the cell and is unable to produce vector particles in human cells outside the site of inoculation. The possibility of gene transfer to other species is minimal under the conditions of the proposed release. The MVA.HTI is attenuated for replication and does not spread so we do not expect any impairment to other humans, flora or fauna, near or far to the release area.

Genetically modified viral vaccine MVA.HTI is not able to survive, establish, spread to other organisms, and is not pathogenic to animals or plants. The chimeric HTI-insert consists of 16 fragments of the genome of HIV-1 which are not involved in the pathogenicity of the virus. HTI also does not contain whole native proteins so that it is not functionally active, it is not dangerous and has no harmful effects for other organisms.

Therefore, the MVA.HTI would unlikely become persistent and invasive in natural habitats. It has never been documented spontaneous reversion of the MVA to the replication competent vaccinia virus (VV). The consequences of the environmental risk are considered low/nil in the context of the proposed measures for use.

There is a background and experience with products highly similar to MVA.HTI that have been released in Spain; MVA.HIVconsv GMO under B/ES/12/10 and B/ES/15/12 notification. There was no incidence nor accident during the release period. GMO biodistribution studies have been performed to ensure non-dissemination in the environment through a vaccinees's body fluids. The research group proposed detecting HIVconsv by PCR in urine samples collected at 24 hours and 7 days post vaccination with the GMO MVA.HIVconsv. The results showed that no MVA.HIVconsv vaccine DNA was detected in any of the analyzed urine samples. With this results it was demonstrated that there is no evidence of vaccine shedding (Edmund Wee, 2015).

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

1. Recipient or parental organism characterisation: modified vaccinia Ankara virus (MVA).

(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/Chordopoxviridae
(ii)	genus	Orthopoxvirus
(iii)	species	Vaccinia virus
(iv)	subspecies	-
(v)	strain	Modified vaccinia Ankara virus.
	pathovar (biotype, ecotype, race, etc.)	-
(vi)	common name	MVA

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	..
Mediteranean	..
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	…

The MVA is a recombinant live vaccinia virus, attenuated, with limited ability to replicate in human cells. It is not found in natural ecosystems. It replicates well in avian cells (chicken embryo fibroblasts or CEF) and baby hamster, but poorly in most mammalian cells (Mayr *et al.*, 1978; Drexler *et al.*, 1998) and it is unable spread in normal human cells.

(b) If the organism is an animal: natural habitat or usual agroecosystem:
Not applicable.

5. (a) Detection techniques

The identity of MVA can be confirmed by PCR based on the absence of genes deleted from the wildtype vaccinia virus, specific from the MVA strain.

MVA virus infectivity is measured by the average of 3 independent titrations in chicken embryo fibroblasts. The virus titre expressed in plaque forming units per milliliter (pfu/ml).

(b) Identification techniques

The same as in previous 5a) section.

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

MVA is classified as Biological Safety Level 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

MVA are classified as Biological Safety Level 1 due to its limited pathogenicity.

The immune response generated after infection with the parental vaccinia virus protects individuals against smallpox; for this reason it was used as a vaccine for smallpox. The vaccinia virus infection is very mild and usually asymptomatic in healthy individuals but can cause a mild rash and fever. However, sometimes there are some complications and side effects, and the likelihood of this happening is significantly higher in immunocompromised persons. The MVA however, that was used as a vaccine against smallpox in the 1970s to the end of the eradication campaign in 120,000 people did not produce any serious adverse event.

With the global eradication of smallpox, routine vaccination with vaccinia virus is no longer performed. However, after the Anthrax bioterrorism attack in October 2001, the U.S. government has done everything possible to improve preparedness for accidental or intentional release of vaccinia virus. Initially, it began with attempts to vaccinate a large number of potential emergencies and health workers. There were also funds for the development and production of a new smallpox vaccines and the development of therapies antipoxvirus. Some laboratory researchers, health workers, first aid, and military personnel are still being vaccinated. The vaccinia virus vaccine is only available in the United States through Center for Disease Control and Prevention.

MVA presents no risk of integration or activation of latent provirus, since the vector is found exclusively in the cytoplasm and is highly unlikely that there will be a significant spread of infectious particles outside the injection site.

8. Information concerning reproduction

(a) Generation time in natural ecosystems:
Not applicable. Does not replicate in natural ecosystems. It replicates well in avian cells (chicken embryo fibroblasts or CEF) and baby hamster, but poorly in most mammalian cells (Mayr *et al.*, 1978; Drexler *et al.*, 1998) and it is unable spread in normal human cells.

(b) Generation time in the ecosystem where the release will take place:
Not applicable. Will not generate effectively.

(c) Way of reproduction: Sexual Asexual
Not applicable

(c) Factors affecting reproduction:
Not applicable

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		

Not applicable

(b) relevant factors affecting survivability:

Survival of the MVA is not expected as it is found exclusively in the cytoplasm of the cell and is unable to produce vector particles in human cells outside the site of inoculation. The bioactivity of MVA at room temperature decays logarithmically. It is susceptible to various chemical agents such as sodium hypochlorite 1% and 2% glutaraldehyde, used as disinfectants, and has shown sensitivity to heat inactivation. A completely effective elimination is achieved by autoclaving at 121°C for 15 minutes.

10. (a) Ways of dissemination

The MVA.HTI, like the parental MVA and other MVA-vectored GMOs, remains localized in the cell cytoplasm until the destruction of the cell. According to clinical studies, there has been no spread of the vector, which is supposed to be located at the point of injection.

(b) Factors affecting dissemination

Irrelevant

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 viral vector MVA has already been used in numerous clinical trials (NCT01712425, NCT02099994, NCT01151319, NCT00982579 and NCT01371175) along with other candidates ChAdV63.HIVconsv, pSG2.HIVconsv pTHr.HIVA DNA and DNA.

In Spain a highly similar GMO product to that proposed, MVA.HIVAconsv, has already been notified, B/ES/12/10, B/ES/15/12 and has been used in various clinical trials, including individuals with existing HIV infection

Other GMOs with the same host organism, MVA, have been notified in Spain (B/ES/08/46 and B/ES/09/63).

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 modified Ankara vaccinia virus (MVA) is a recombinant live vaccinia virus, attenuated by serial passages in cultured chicken embryo fibroblasts (CEF) that contains six large genomic deletions from the parental virus (15% of the parental genome), including cytokine receptor genes. Among the mutated genes are included mainstream membrane proteins (ORF F5L), and between deleted regions, there are classical poxviral immune evasion genes, virulence genes and two out of five host genes (Antoine *et al.*, 1998; Im *et al.*, 2004; Meisinger-Henschel *et al.*, 2007; Meisinger-Henschel *et al.*, 2010) making the MVA safe for clinical application because of its limited ability to replicate in human cells.

The transgene encoding the insert HTI has been inserted into the thymidine kinase locus of MVA genome under control of the modified H5 promoter in order induce an HIV-1-specific T-cell immune response. With all these changes is intended that cells that are infected with the MVA-HTI can express the immunogen HTI for activation of immune responses against HIV-1 virus.

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 p3055

(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 (.)

other, specify p3055 harbors a green fluorescent protein (GFP) gene as a marker. The parental MVA also includes red fluorescent protein (RFP). After recombination, there is a transient virus with both GFP and RFP, which then spontaneously resolved into a colourless, recombining both markers out.

Indication of which antibiotic resistance gene is inserted
Not applicable.

(e) Constituent fragments of the vector
P3055 is a co-expression plasmid that directs the insertion of a gene of interest in the locus of the thymidine kinase of the vaccinia virus, along with modified H5 promoter and green fluorescent protein (GFP) gene. Therefore, the constituent fragments of the vector are: thymidine kinase gene, modified H5 promoter, green fluorescent protein (GFP) gene and HTI insert.

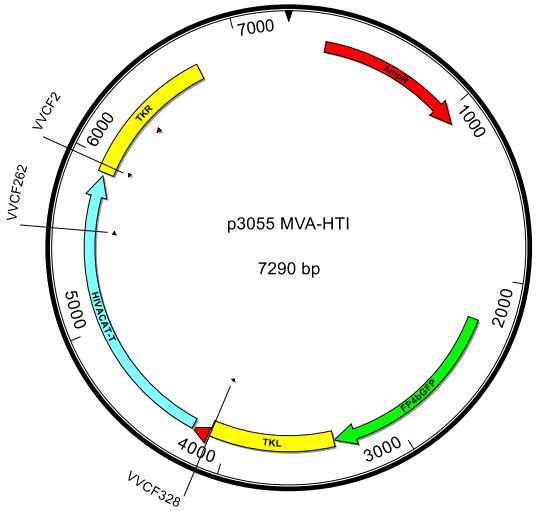

Figure 1. Plasmid p3055 construct.

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

(i)	transformation	(.)
(ii)	electroporation	(.)
(iii)	macroinjection	(.)
(iv)	microinjection	(.)
(v)	infection	(.)
(vi)	other, specif: homologous recombination	

Briefly, CEFs were infected with parental MVA at a MOI 1 and transfected with Superfectin (Qiagen) 3 µg of DNA.HTI (plasmid p3055), which also harbors the GFP gene as a marker. Two days later, the total virus was collected and used to

reinfect CEF cells. The rMVAs were subjected to five rounds of plaque purification, after which the master virus stock was obtained, purified on a cushion of 36% sucrose, titrated and stored at -65 ° C until use.

A PCR was performed in order to confirm identity (the presence of the antigen in question) and purity (lack of red virus contamination in the final stock).

5. If the answer to question B.3(a) and (b) is no, what was the method used in the process of modification? Not applicable.

transformation	(.)
microinjection	(.)
microencapsulation	(.)
macroinjection	(.)
other, specify …	

6. Composition of the insert

(a) Composition of the insert

HTI:

The novel immunogen, termed HTI was designed as an immunogen for T cells. HTI is a sequence consisting of 16 continuous segments of HIV-1, each between 11 and 78 amino acids in length, and encoding critical targets of viral proteins Gag (45%), Pol (44%), Vif (8%) and Nef (3%) (see Figure 2).

Design of the HTI was based on identification of beneficial T cell targets in HIV clade B infection. In these analyses, 232 untreated HIV clade B infected individuals were screened and regions of the viral proteome that were predominantly targeted by subjects with superior HIV control were identified (Mothe et al., 2011). Of the 410 18mer overlapping peptides (OLP) spanning the entire viral proteome, 26 OLP were identified. These beneficial OLPs were located in the HIV Gag (n=10), Pol (n=12), Vif (n=3) and Nef (n=1) proteins of the virus (Table 1)

The HTI fragment contains alanine linkers between the segments (one, two or three alanine residues, as shown in the figure), and are included in order to induce preferential proteolytic cleavage between these segments, thereby avoiding premature epitope digestion (Le Gall *et al.*, 2007; Zhang *et al.*, 2012).

Furthermore, the HTI fragment contains a human granulocyte-macrophage colony-stimulating factor (GM-CSF) signal peptide (AA 1-17; Genbank NP_000749 Nr) in the N-terminus to improve translocation to the endoplasmic reticulum.

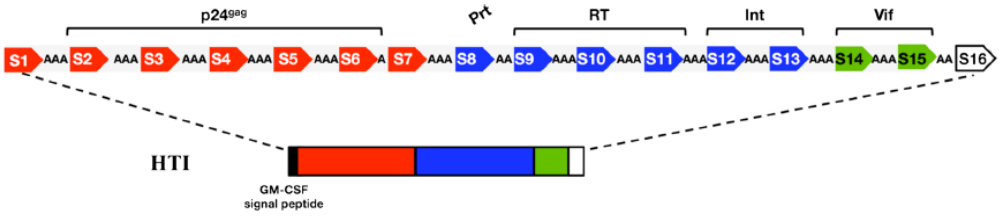

Figure 2: The HTI protein is composed of 16 individual segments arranged linearly and linked via 1–3 alanine amino acid linkers and contains the GM-CSF signal peptide for better secretion. Total length of HTI protein is 529 aa, including alanine linkers

(b) Source of each constituent part of the insert

HTI transgene sequences consist of 16 fragments corresponding to critical targets of the HIV-1 specific T cell response, identified in HIV-1 subtype B infected individuals. Below is depicted a list of overlapping peptides identified and incorporated into the final design of the T cell immunogen, HTI.

Table 1: List of identified beneficial overlapping peptides (OLP, PR > 1) incorporated in the final T-cell immunogen design, based on previous analysis (Mothe *et al.*, 2011; Mothe *et al.*, 2015)

OLP no.	Protein	Subunit	OLP clade B cons sequence
3	Gag	p17	EKIRLRPGGKKKYKLKHI
6	Gag	p17	ASRELERFAVNPGLL
7	Gag	p17	ERFAVNPGLLETSEGCR
10	Gag	p17	QLQPSLQTGSEELRSLY
12	Gag	p17	SLYNTVATLYCVHQRIEV
23	Gag	p24	AFSPEVIPMFSALSEGA
31	Gag	p24	IAPGQMREPRGSDIA
34	Gag	p24	STLQEQIGWMTNNPPIPV
48	Gag	p24	ACQGVGGPGHKARVLAEA
60	Gag	p15	GKIWPSHKGRPGNFLQSR
75	Nef	-	WLEAQEEEEVGFPVRPQV
159	Pol	Prt	KMIGGIGGFIKVRQYDQI
160	Pol	Prt	FIKVRQYDQILIEICGHK
161	Pol	Prt	QILIEICGHKAIGTVLV
163	Pol	Prt	LVGPTPVNIIGRNLLTQI
171	Pol	RT	LVEICTEMEKEGKISKI
195	Pol	RT	LRWGFTTPDKKHQKEPPF
196	Pol	RT	DKKHQKEPPFLWMGYELH
210	Pol	RT	EIQKQGQGQWTYQIY
269	Pol	Int	TKELQKQITKIQNFRVYY
270	Pol	Int	TKIQNFRVYYRDSRDPLW
271	Pol	Int	YYRDSRDPLWKGPAKLLW
276	Pol	Int	KIIRDYGKQMAGDDCVA
405	Vif	-	VKHHMYISGKAKGWFYRH
406	Vif	-	GKAKGWFYRHHYESTHPR
424	Vif	-	TKLTEDRWNKPQKTKGHR

The expression-optimized (RNA/codon-optimized) HTI gene was synthesized by GeneArt (Invitrogen).

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

The engineered continuous HTI has a total length of 529 aa and included a high density of both, CD4 and CD8 T cell epitopes across all protein subunits restricted by 42 different HLA alleles (n=55 well-characterized optimal defined CTL epitopes, and 6 targets in Gag most frequently targeted by CD4 T helper (Table 1).

Therefore, the function of the immunogen HTI is the induction of an immune response of HIV-1 specific T cells directed against the regions included in the insert HTI, which can control HIV-1 effectively.

(d) Location of the insert in the host organism

- on a free plasmid (.)
- integrated in the chromosome (X)
- other, specify integrated in the genome of the MVA at thymidine kinase locus and under the control of modified H5 promoter.

(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

The following information is related to the organism from which the inserted transgene (HTI) belongs, the human immunodeficiency virus or HIV-1.

1. Indicate whether it is a:

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

(specify phylum, class) .

other, specify:

HTI is a sequence of 16 fragments of the HIV-1 genome (human immunodeficiency virus type 1) each between 11 and 78 amino acids in length.

2. Complete name

(i) order and/or higher taxon (for animals) …
(ii) family name for plants Retroviridae
(iii) genus *Lentivirus*
(iv) species Human
(v) subspecies fragments from clade B, and with clade C analogy.

(vi) strain
(vii) cultivar/breeding line
(viii) pathovar

(ix) common name Human immunodeficiency virus, VIH-1

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):

HIV-1 directly infects and destroys cells that are critical for an effective immune response, explaining the clinical manifestations resulting from progressive immunodeficiency. HIV-1 is an RNA virus, whose main target cells are CD4+ T-helper cells, macrophages and some populations of dendritic cells. Upon entry, the viral RNA genome retrotransciption starts in the cytoplasm, from where double-stranded DNA products are transported to the cell nucleus where they integrate into the chromosomal DNA of the infected cell, a step necessary for the efficient synthesis of viral RNA and consequent production of new infectious viral particles. *Lentiviruses* like HIV-1, are unique among retroviruses to generate pre-integration products that can be transported to the nucleus interface of resting cells in G1 phase.

In vivo infections with HIV-1 are limited to humans and chimpanzees and its transmission is by contact with blood, sex or vertical transmission from mother to child during pregnancy and childbirth. The course of the disease in humans varies greatly among infected individuals. The time between infection and the development of AIDS - defined as reduced CD4 levels below 200 cells/µl or the appearance of opportunistic infections or AIDS-defining cancers, can reach from 6 months to more than 25 years.

Lentiviruses are typically restricted in their host range, although naturally or experimentally induced cross-species infections have been documented. However, in chimpanzees, the only non-human primate capable of becoming infected with HIV-1, no immunodeficiency or long-term illness is seen. During primary infection with HIV-1, the circulating virus can be isolated for several weeks intermittently, but is then resolved asymptomatically in most cases.

The fragments or sequences included in the immunogen HTI are not involved in the pathogenic properties of the virus.

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

The human immunodeficiency virus (HIV-1) is classified as Biosafety Level Class 3 *D. However, the HTI is produced by chemical synthesis, not by HIV-1 replication, so it does not have any safety classification.

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

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

E. Information relating to the genetically modified organism

MVA.HTI

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 …

The MVA.HTI, as a MVA, is a modified vaccinia virus, live recombinant, attenuated with limited ability to replicate in human cells.

(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 (.)

Specify: The same as before

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

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

Specify …

The MVA has extremely limited capacity to spread, as it remains localized in the cell cytoplasm until the destruction of the cell. According to data from clinical trials of other studies with MVA-vectored GMOs, there has been no spread of the vector, which is believed to be located at the point of injection.

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

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

Specify …

The MVA was used as a vaccine against smallpox in the 1970s at the end of the eradication campaign in 120.000 people without serious adverse events. MVA.HTI

maintains the same characteristics of pathogenicity as MVA. The effects are limited to those arising from the initial infection of receptive cells locally.

2. Genetic stability of the genetically modified organism

MVA is a genetically stable virus unable to integrate its DNA into the host genome and which remains localized in the cytoplasm of the cell until the cell destruction.

The GMO MVA.HTI is generated in chicken embryo fibroblasts (CEFs) and purified by a sucrose density gradient. The MVA vaccine vector is a recombinant live virus attenuated by serial passage in CEF containing six large genomic deletions relative to the parental virus. MVA genome size upon insertion of the coding sequence HTI is estimated to be approximately 179.6 kp. The genetic modifications of MVA.HTI are stable and remain after successive passes in CEFs.

The production of the recombinant virus MVA.HTI was performed by the German company IDT Biologika and is based on a system of 'seed virus', in which a 'master seed virus' (MSV) and a working virus (WSV) is prepared. All the preparation, verification of the genetic stability and MSV and WSV storage is done at ITD under cGMP conditions and according to EU regulations.

Genetic stability is verified in various steps of the production process, through integrity analysis of the vector and insert (restriction pattern and sequencing of the insert), purity, biological potency and safety (analysis of the absence of the parental virus), both on the initial inoculum produced by Dr. Tomas Hanke, the WSV, the MSV and the final product. The standard stability (shelf life) allocated to the MVA-based vaccines produced by IDT Biologika under cGMP conditions is 24 months when stored at -70ºC and stability tests are repeated for MVA.HTI annually.

There is a large experience with the stability of recombinant poxvirus, and recombinant MVA in particular, not only by IDT Biologika. To date, University of Oxford has developed seven recombinant MVA that have entered clinical trials, two with transgenes derived from HIV-1 (HIVA and HIVconsv). The stability data of two closely related products (MVA85A and MVA.HIVA), which had undergone the same manufacturing process showed stability over a period of 6 years. The transgenes HIVA and 85A are 1584 bp and 1107 bp in size respectively, and thus similar to the size of the gene HTI (1578 pb).

Within the identification and validation program to confirm the overall integrity of the MVA.HTI genome (and hence its genetic stability) are included the following tests:

a. The presence of the intact HTI ORF in the MVA.HTI is checked by a PCR reaction using primers J1312 and J1205, which yield a 331 bp product.

b. Purity by PCR: the absence of wild type MVA (non-recombinant or MVA.RFP) is checked by a PCR reaction using specific primers, which yield a 688 bp RPF-specific band. The plasmid pRFP serves as the positive control in this PCR.

c. Identity by sequencing the entire ORF of HTI identical to the master sequence.

Monitoring the stability of the clinical batches, as outlined in the IMPD, is performed periodically using infectivity assays in CEFs. The virus titre is expressed in plaque forming units per millilitre (pfu/ml). Tests are performed to assess the genetic stability in different parts of the final batch production. PCR amplification and subsequent sequencing serves to confirm the presence of the insert coding for HTI antigen and to reject the production of mutations or deletions.

Stability tests (qualification) are complemented by *in vivo* potency tests measuring immunogenicity in mice

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)

Same as stated before for MVA. Pathogenicity of MVA-HTI does not differ from MVA.

The MVA are classified as Biological Safety Level 1 due to its limited pathogenicity.

The immune response generated after infection with the parental vaccinia virus protects individuals against smallpox; for this reason it was used as a vaccine for smallpox. The vaccinia virus infection is very mild and usually asymptomatic in healthy individuals but can cause a mild rash and fever. However, sometimes there are some complications and side effects, and the likelihood of this happening is significantly higher in immunocompromised persons. The MVA however, that was used as a vaccine against smallpox in the 1970s to the end of the eradication campaign in 120,000 people did not produce any serious adverse event.

With the global eradication of smallpox, routine vaccination with vaccinia virus is no longer performed. However, after the Anthrax bioterrorism attack in October 2001, the U.S. government has done everything possible to improve preparedness for accidental or intentional release of vaccinia virus. Initially, it began with attempts to vaccinate a large number of potential emergencies and health workers. There were also funds for the development and production of a new smallpox vaccines and the development of therapies antipoxvirus. Some laboratory researchers, health workers, first aid, and military personnel are still being vaccinated. The vaccinia virus vaccine is only available in the United States through CDC.

MVA presents no risk of integration or activation of latent provirus, since the vector is found exclusively in the cytoplasm and is highly unlikely that there will be a significant spread of infectious particles outside the injection site.

4. Description of identification and detection methods

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

There are no techniques planned to detect and identify the GMO in the environment in the context of the clinical trial.

(b) Techniques used to identify the GMO

Most of the methods used in the final characterization of MVA.HTI during development, control processes, production and release tests are standardized methods previously used and validated (as appropriate for the use of the material in early clinical phases) for different MVAs.

The identity of MVA can be confirmed by PCR. It is based on the absence of deleted genes from wildtype vaccinia virus, specific of MVA strain. MVA virus infectivity is measured by the average of 3 independent titrations in chicken embryo fibroblasts. The virus titer expressed in plaque forming units per milliliter (pfu/ml).

Within the identification and validation program to confirm the overall integrity of the MVA.HTI genome (and hence its genetic stability) are included the following tests:

a. The presence of the intact HTI ORF in the MVA.HTI is checked by a PCR reaction using primers J1312 and J1205, which yield a 331 bp product.

b. Purity by PCR: the absence of wild type MVA (non-recombinant or MVA.RFP) is checked by a PCR reaction using specific primers, which yield a 688 bp RPF-specific band. The plasmid pRFP serves as the positive control in this PCR.

c. Identity by sequencing the entire ORF of HTI identical to the master sequence.

After recombination with the plasmid insert HTI transfer, the DNA is extracted from the virus sample. By PCR, DNA sequences are amplified. PCR primers are designed in such a way that is unique to the transgene HTI. The amplified DNA fragment of appropriate size confirms the identity of the insert.

The immunogenicity of MVA.HTI was demonstrated in mice and Rhesus macaques.

F. Information relating to the release

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

The GMO MVA.HTI has been developed as a therapeutic vaccine candidate for HIV-1. Its release is necessary to implement the phase I clinical trial in Spain with MVA-HTI in combination with the plasmid DNA.HTI in subjects uninfected with HIV-1.

The proposed study, "A phase I randomized double-blind, placebo-controlled, dose-escalation study to evaluate the safety and immunogenicity of two candidate HIV-1 Vaccines, DNA.HTI and MVA.HTI administered in combination to healthy, low risk, HIV-1 negative adults", will assess the safety and immunogenicity of the GMO (MVA.HTI) in combination with a DNA.HTI. The vaccination schedule includes three different doses of DNA.HTI (1, 4 and 8 mg in three cohorts) administered at 0, 4 and 8 week, followed by the administration of MVA.HTI (2×10^8 pfu) at weeks 16 and 20. The heterologous vaccine will be administered intramuscularly. The total study population will include 26 healthy volunteers.

Phase I/IIa clinical trials using highly similar candidates, such as MVA.HTI or MVA.HIVA, have been completed (NCT01712425, NCT02099994, NCT01151319, NCT00982579 and NCT01371175) along with other candidates ChAdV63.HIVconsv, DNA and DNA pSG2.HIVconsv pTHr.HIVA.

There should be no potential environmental impact of the release of GMOs during the clinical trial.

The details of the trial design and its objectives are described in the attached protocol.

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 … MVA does not exist in any geographical location.

3. Information concerning the release and the surrounding area

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

The vaccinations with MVA.HTI will be conducted at the Unidad Polivalente de Investigación Clínica (UPIC), located on the 2nd floor of the maternal building in Hospital Universitari Germans Trias i Pujol, located in Ctra. Canyet, s / n, 08916 Badalona.

(b) Size of the site (m^2): … m^2
(i) actual release site (m^2): Room of UPIC of the center: 22m^2
(ii) wider release site (m^2): same and never >22m^2

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

Not applicable, the effect on these areas is not possible.

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

Not applicable, the effects on flora and fauna are not possible.

4. Method and amount of release

(a) Quantities of GMOs to be released:

A maximum of 26 subjects (20 treated subjects and 6 subjects treated with placebo) meeting the inclusion and exclusion criteria will be included. Twenty (20) of these will receive MVA.HTI at weeks 16 and 20, the remaining 6 placebo.

In total it is estimated to administer a maximum of 40 vials with a dose of 2 x 10^8 pfu per vaccination (two administrations; one vial per volunteer per administration).

(b) Duration of the operation:

Each vaccination takes a few minutes. The recruitment period is estimated to be made over 12 weeks. All individuals will be followed 32 weeks after the initial vaccination (20 weeks of treatment and 12 weeks after the last vaccination).

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

The GMO is released for clinical use only.

The personnel involved in the preparation of the product will work according to the conditions specified in the standards of Good Clinical Practice and Good

Manufacturing Practices. GMP fabrication laboratory (IDT Biologika GmbH) located in Germany, prepared and packaged the product in hermetically sealed vials and properly labelled. The vials will be sent from Germany at -80ºC and stored until use in the Pharmacy Service of the Germans Trias i Pujol Hospital. The administration is under the responsibility of the investigator, according to a clinical protocol and respecting the rules of Good Clinical Practice.

The staff who administer the vaccines use 'universal precautions' and sterile techniques (gloves, masks and disposable gowns). The product shall be prepared under aseptic conditions, in appropriate compliance to the preparation of injectables. MVA.HTI will be thawed at room temperature and drawn up into a 1 mL insulin syringe in aseptic conditions (in a BL2 hood) and using a validated closed system transfer device (CSTD, BD PhaSealTM). The area used for the preparation for injection will be decontaminated before and after manipulation with bleach and 70º alcohol. The site of inoculation will be covered properly.

The location (UPIC) will be cleaned with sodium hypochlorite diluted to 1% immediately after the administration. Used material is considered group III sanitary waste and will be managed as such. Particularly, gloves, mask and gown will be discarded in a red bag and needles in specific yellow containers. Used vials will be destroyed following the protocols pharmacy service.

In case of accidental contamination, each contaminated surface will be treated using conventional hospital procedures for infected products. All personnel involved in handling the vaccines will be informed that in case of skin contamination they should immediately wash skin thoroughly with water and disinfected with iodine locally to 4% and in case of eye contamination, wash and rinse with water only. The affected worker should also undergo an evaluation by an ophthalmologist as soon as possible. There is no biological test designed specifically for personnel handling this GMO.

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

Not applicable. All immunizations are going to be performed in UPIC, in Hospital Germans Trias i Pujol.

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.

There were no previous MVA.HTI releases.
However, there are highly similar GMO that were previously released (B/ES/12/10 and B/ES/15/12).

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	Sapiens
(vi)	strain	-
(vii)	cultivar/breeding line	-
(viii)	pathovar	-
(ix)	common name	Human

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

Development of HIV-1 specific cytotoxic T cell responses directed against the regions included in the immunogen HTI.

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

The possibility of gene transfer to other species is minimal under the conditions of the proposed release to the GMO. As MVA is a defective virus unable to replicate, interaction with other organisms in the environment are expected.

For the gene that encodes HTI to be transferred to other species of poxvirus, the susceptible cells would need to be infected by a poxvirus and also be transduced by the vector, which is extremely unlikely.

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

It is unlikely that the GMO can be released to the ecosystem if it spreads from the release site, because it has a very limited host range and the release in the context of the trial is highly restricted.

In the unlikely event of involuntary administration to other organisms, the further spread would be unlikely, because several studies have demonstrated that MVA is avirulent in laboratory immunocompetent and immunocompromised animals as well as in primary human cell cultures.

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:
Highly unlikely

(b) from other organisms to the GMO:
Highly unlikely

(a) likely consequences of gene transfer:
Nil, because the HTI is a sequence designed exclusively for the induction of specific cellular responses through the union of 16 fragments of the HIV-1 genome, so it is not pathogenic.

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.):
None

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 MVA has been used extensively in clinical trials, both as direct administration or cell therapy strategies (contained in the interior of cells). Based on this information, no specific detection of MVA.HTI in biological fluids or blood has been planned.

There will be monitoring of side effects of treatment during the trial by physical examination, blood tests and communication of adverse events. The safety assessment will be ensured by monitoring subjects while in the clinical trial and up to 48 weeks after the last injection in the study (see details in attached protocol).

2. Methods for monitoring ecosystem effects

Not planned, as the GMO is not found naturally in the environment and it is non-replicative so there is no chance that an impact on the ecosystem of the GMO with infectivity will be seen. Subjects will be clinically monitored.

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

Unplanned since this event is highly unlikely (see previous sections).

4. Size of the monitoring area (m^2)
… m^2
Not applicable: The GMO is administered only to subjects by intramuscular injection in the ambulatory, as described in Section F.

5. Duration of the monitoring

The safety assessment will be made during the participation of subjects in the clinical trial and follow-up.

6. Frequency of the monitoring

Monitoring visits during which safety is evaluated will be held at 24 hours (in placebo and MVA.HTI-treated subjects, after a week, after 4 weeks and after 12 weeks after vaccination.

I. Information on post-release and waste treatment

1. Post-release treatment of the site

The injection site will be covered with a plaster or bandage. The release site is cleaned with sodium hypochlorite diluted to 1%, and GMP approved disinfectants for use immediately after liberation.

2. Post-release treatment of the GMOs

The transfer of the material used for the preparation and injection of GMOs will be held in hermetically sealed yellow containers or in a special thick red bag with a sticker labelled medical waste - Group III and decontaminated before disposal.

3. (a) Type and amount of waste generated

Vaccine vials, needles, closed BD Phaseal system, gloves, gowns, masks, bandages/tape (26 subjects)

3. (b) Treatment of waste

The remaining waste material in contact with the GMO are considered specific sanitary waste (Group III) and managed as such and will be placed to the following vessels:

Infectious Waste Solids:
Bag should always be labelled as Medical Waste Group III.

Waste sharps:
Be deposited in special containers, rigid, leak proof yellow, adequate in size and shape to the use to which they will provide.

The vaccine vial attached to the BD Phaseal system will be deposited in waste sharp containers.

The withdrawal and the final closing of both the bags and containers will be carried out by appropriately trained staff and following the appropriate protective measures.

J. Information on emergency response plans

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

In case of contamination the personnel involved in the preparation, packaging or product management will notify the principal investigator and the Service of Occupational Health and Safety. All staff will be instructed about the procedures to follow in case of accidental release.

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

The place in which the release occurs will be cleaned with diluted sodium hypochlorite 1%.

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

In the case of skin contact, scrub wash with iodine solution with 4% will be done.

In case of eye contact, a wash with saline for a period of not less than 15 minutes will be made. The subject will be evaluated by an ophthalmologist as soon as possible.

In case of accidental puncture, immediate wash with plenty of soap and water will be performed, and then the puncture site will be disinfected with iodine solution to 9-12% for at

least 5 minutes with sodium hypochlorite solution of 10 g/l.

Subjects included in the clinical trial will be monitored as provided by the protocol according to standards of good clinical practice. Adverse events will be registered and reported according detailed procedures in the protocol.

Due to the risk management procedures of accidental environmental release is very low. In addition being the GMO a virus without replicative capacity, environmental risk consequent to accidental release is considered minimal.

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