MVA.HIVconsv
B/ES/15/12

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

08/10/15

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/15/12
(c)	Date of acknowledgement of notification	26/10/2015
(d)	Title of the project	

AN OPEN LABEL PHASE I TRIAL TO EVALUATE THE SAFETY AND EFFECT OF HIVconsv VACCINES IN COMBINATION WITH HISTONE DEACETYLASE INHIBITOR ROMIDEPSIN ON THE VIRAL REBOUND KINETIC AFTER TREATMENT INTERRUPTION IN EARLY TREATED HIV-1 INFECTED INDIVIDUALS.

(e)	Proposed period of release	From February 2016 until September 2016 (last administration of the GMO)

2. Notifier

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

3. GMO characterization **MVA.HIVconsv**

(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.HIVconsv. 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. To the MVA it has been inserted a transgene coding for the insert HIVconsv in order to induce an HIV-1 specific T cell immune response. The size of MVA.HIVconsv size after the insertion is estimated to be approximately 180 kbp.

(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 that remains localized in the cytoplasm of the cell until the cell destruction.

The OMG MVA.HIVconsv is generated in chicken embryo fibroblasts (CEFs) and purified by sucrose density gradient. The vector of the MVA vaccine is a recombinant live attenuated virus by serial passage in CEF containing six large genomic deletions relative to the parental virus. MVA genome size upon insertion of the coding sequence HIVconsv is estimated to be approximately 180 kbp. The genetic modifications of GMOs MVA.HIVconsv are stable and remain after successive passes in CEFs.

The production of the recombinant virus MVA.HIVconsv is done 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 virus), 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 under cGMP conditions is 24 months when stored at-70ºC and stability tests are repeated for MVA.HIVconsv annually

There is a large experience with the stability of recombinant poxvirus, and recombinant MVA in particular, not only by IDT. 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 HIVconsv.

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

a) Identity by PCR using HIVconF and HIVconR, producing a product of 938 bp specific primers and confirms the presence of the product HIVconsv.

b) Purity by PCR: the absence of specific PCR product wildtype MVA strain. The absence of wild MVA strain (non-recombinant) is verified by PCR reaction using TKF and TKB primers that produce a TK-site specific insertion TK band of 228 bp. Genomic DNA MVA/rMVA serves as template and pVT2 plasmid as positive control in PCR. A negative result indicates no less than 1 per 20,000 recombinant MVA MVA.HIVconsv.

c) Identity by sequencing the entire ORF of HIVconsv identical to the master sequence (2536bp)

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 HIVconsv 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 (X) No (.)
If yes:
- Member State of notification United Kingdom (GB), Kenya and Spain
- Notification number In the clinical trials HIV-CORE001, HIVCORE002, PEACHI-01 (GB), HIVCORE004 (Kenya), the same GMO was notified as 'contained use' in GB.
In Spain, notification number B/ES/12/10.

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.

There is no scientific reason to expect that the use of HIVconsv 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.HIVconsv is attenuated for replication, 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.HIVconsv is not able to survive, establish, spread to other organisms, and is not pathogenic to animals or plants. The chimeric protein-HIVconsv-insert-consists of 14 fragments of the genome of HIV-1 and it is not involved in the pathogenicity of the virus, 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.HIVconsv 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/nul in the context of the proposed measures for use.

First MVA.HIVconsv GMO release in Spain was under B/ES/12/10 notification. There was no incidence nor accident during the release period.

After the evaluation by the National Biosafety Committee, it was considered that in the present knowledge and under use conditions, BCN01 trial was not presenting any risk for human health or the environment. Nevertheless, it was considered necessary to conduct GMO biodistribution studies to ensure non-dissemination in the environment through patients' 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. At the moment, they are conducting such determinations at the University of Oxford, the final results will be forwarded to the National Biosafety Committee along with this notice (estimated November 2015 reporting date).

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	

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

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. It is based on the absence of gene 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

As before

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

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 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.

8. Information concerning reproduction

(a) Generation time in natural ecosystems:
Not applicable. Does not generate 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		

Irrelevant

(b) relevant factors affecting survivability:

We do not expect any survival of the MVA as 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 as a method of physics. Thus, a completely effective elimination is achieved by autoclaving at 121 ° C for 15 minutes.

10. (a) Ways of dissemination

The MVA.HIVconsv, 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)

Same GMO with same characteristics and no additional genetic modification, notification B/ES/12/10.

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, Meisinger-Henschel et al. 2007, 2010 and T. Hanke et al, 2004) making the MVA safe for clinical application because of its limited ability to replicate in human cells.

The transgene encoding the insert HIVconsv has been inserted into the thymidine kinase locus of MVA genome under control of the promoter p7.5in order induce an HIV-specific T-cell immune response.

With all these changes is intended that those cells that are infected with the MVA-HIVconsv can express the immunogen HIVconsv 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

pSC11.HIVconsv

(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 psC11.HIVconsv harbors a b-galactosidase gene as a marker, generating blue colonies on a monolayer of infected cells.

The final construct ChAdV63.HIVconsv includes an epitope for the antibody Pk located at the C-terminus of the immunogen that can be detected by immunofluorescence.

Indication of which antibiotic resistance gene is inserted
…

(e) Constituent fragments of the vector
Recombinant MVA are produced by homologous recombination between MVA-derived genomic DNA and transfected shuttle plasmid containing the passenger gene expression cassette flanked by MVA sequences in CEFs. pSC11.HIVconsv is a co-expression plasmid that directs the insertion of a gene of interest, along with b-galactosidase gene from Escherichia coli in the locus of the thymidine kinase (TK)of the vaccinia virus. It contains the HIVconsv insert sequence, the gene for the B-galactosidase and the promoter 7.5.

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

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

Briefly, CEFs infected with parental MVA at a MOI 1 and transfected with Superfectin (Qiagen) 3 ug pSC11.HIVconsv, which also harbors the gene for the b-galactosidase as a marker. Two days later, the total virus is collected and used to reinfect CEF cells. The rMVAs were subjected to five rounds of plaque purification, after which it was obtained the master virus stock, purified on a cushion of 36% sucrose, titrated and stored at -80 ° C until use.

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

HIVconsv:

The novel immunogen, termed HIVconsv (for conserved), was designed as a chimaeric protein and assembled from the most highly conserved domains among the HIV-1 clade A, B, C and D proteomes (Létourneau *et al*, 2007). It was decided that the HIVconsv gene should be approximately 2.5 kbp in size, making it suitable for most currently used genetic vaccine vectors and likely to support high protein expression.
It encodes 14 of the most conserved regions of HIV-1 genome, each between 27 and 128 aminoacid. (Létoruneau et al, 2007) plus a 15th fragment harbouring an epitope recognized by CD8 + T cells from rhesus macaques (Mamu-A * 01, Allen *et al,* 2000) and mice (H-2D^{d} and L^{d}, Takahashi *et al*, 1998) respectively. Also an epitope of a mAb monclonal PK antibody was added to the C-terminus of the immunogen (Hanke *et al*, 1992) to facilitate detection of protein expression.

(b) Source of each constituent part of the insert

The primary donor transgene sequences HIVconsv are 14 fragments of HIV-1 genome highly conserved between clades A, B, C and D.

1	MEEKAFSPEVIPMFTALSEGATPQDLNTMLNTVGGHQAAMQMLKDTINE EAAEWDR
2	IYKRWIILGLNKIVRMYSPVSILDIRQGPKEPFRDYVDRF
3	ARNCRAPRKKGCWKCGKEGHQMKDCTERQANFLGKIWPS
4	RWKPKMIGGIGGFIKVRQYDQILIEICGHKAIGTVLVGPTPVNIIGRNLLTQI GCTLNFPISPIETVPVKLKPGMDGPKVKQWPLTEEKIKALVEICTEMEKEG KISKIGPENPYNTPVFAIKKKDSTKW
5	RKLVDFRELNKRTQDFWEVQLGIPHPAGLKKKKSVTVLDVGDAYFSVPL DEGFRKYTAFTIPSINNETPGIRYQYNVLPQGWKGSPAIFQSSMTKILEPF RAQNPEIVIYQYMDDLYVGSDLEIGQHR
6	MENRWQVMIVWQVDRMRIRTWKSLVKHH
7	LTEEAELELAENREILKDPVHGVYYDPSKDLIAEIQ
8	YWQATWIPEWEFVNTPPLVKLWYQLEK
9	NVTENFNMWKNDMVDQMHEDIISLWDQSLKPCVKLTP
10	WVPAHKGIGGNEQVDKLVSQGIRKVLFLDGIDKAQ
11	AKEIVASCDKCQLKGEAMHGQVDCSPGIWQLDCTHLEGKVILVAVHVAS GYIEAEVIPAETGQETAYFLLKLA
12	MNKELKKIIGQVRDQAEHLKTAVQMAVFIHNFKRKGGIGGYSAGERI
13	WKGPAKLLWKGEGAVVIQDNSDIKVVPRRKAKIIRDYGKQMAGADCV
14	FLGAAGSTMGAASMTLTVQARQLLSGIVQQQNNLLRAIEAQQHLLQLTV WGIKQ
15	ACTPYDINQMLRGPGRAFVTIPNPLLGLD

The last segment includes a macaque epitope Mamu-A * 01,a murine epitope H-2D^{d} and L^{d} and the mAb PK epitope.

The protein HIVconsv was chemically synthesized by GeneArt (Germany)

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

HIVconsv immunogen function is the induction of HIV-1 specific immune responses

of cytotoxic T cells directed against the regions covered in the HIVconsv insert, which can help to control the HIV-1 infection effectively.
The last segment includes a macaque epitope, a murine epitope and the mAb PK epitope. Their function is to detect the expression of the immunogen in transfected cells as well as in immunogenicity preclinical studies in mice and / or macaques.

(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 timidinic kinase locus and under the control of promoter 7.5.

(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 (HIVconsv) 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 …

HIVconsv chimeric protein is synthesized chemically by the union of 14 fragments of the HIV-1 genome (human immunodeficiency virus type 1) between 27 and 128 aminoacid each.

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 clades A, B, C and D

(vi)	strain	…
(vii)	cultivar/breeding line	…
(viii)	pathovar	…
(ix)	common name	HIV-1, human immunodeficiency virus

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 to an effective immune response, explaining the clinical manifestations resulting from progressive progressive immunosuppression. HIV-1 is an RNA virus, whose main target cells are CD4 + T-helper cells, macrophages and some populations of dendritic cells. Upon entry, begins in the cytoplasm the viral RNA genome retrotransciption, whose double-stranded DNA product is transported to the cell nucleus where it integrates 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 by reduced CD4 levels below 200cels/ul or the appearance of opportunistic infections or AIDS-defining cancer, can go from 6 months to more than 25 years.

Lentiviruses are typically restricted in its 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 asymptomatic in most cases.

The fragments or sequences included in the immunogen HIVconsv 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 HIVconsv is produced by chemical synthesis, not by HIV-1 replication and is not pathogenic, 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.HIVconsv

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.HIVconsv, as 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 …

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 to the destruction of the cell. According to data from clinical trials of other studies for MVA- vectored GMOs, there has been no spread of the vector, which is supposed 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 with serious adverse events.
MVA.HIVconsv 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 that remains localized in the cytoplasm of the cell until the cell destruction.

The GMO MVA.HIVconsv is generated in chicken embryo fibroblasts (CEFs) and purified by sucrose density gradient. The vector of the MVA vaccine is a recombinant live attenuated virus by serial passage in CEF containing six large genomic deletions relative to the parental virus. MVA genome size upon insertion of the coding sequence HIVconsv is estimated to be approximately 180 kbp. The genetic modifications of GMOs MVA.HIVconsv are stable and remain after successive passes in CEFs.

The production of the recombinant virus MVA.HIVconsv is done 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 virus), 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 under cGMP conditions is 24 months when stored at-70ºC and stability tests are repeated for MVA.HIVconsv annually

There is a large experience with the stability of recombinant poxvirus, and recombinant MVA in particular, not only by IDT. 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 HIVconsv.

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

a) Identity by PCR using HIVconF and HIVconR, producing a product of 938 bp specific primers and confirms the presence of the product HIVconsv.

b) Purity by PCR: the absence of specific PCR product wildtype MVA strain. The absence of wild MVA strain (non-recombinant) is verified by PCR reaction using TKF and TKB primers that produce a TK-site specific insertion TK band of 228 bp. Genomic DNA MVA/rMVA serves as template and pVT2 plasmid as positive control in PCR. A negative result indicates no less than 1 per 20,000 recombinant MVA MVA.HIVconsv.

c) Identity by sequencing the entire ORF of HIVconsv identical to the master sequence (2536bp)

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 HIVconsv 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-HIVconsv 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 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

…

(b) Techniques used to identify the GMO

…

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

Most of the methods used in the final characterization of MVA.HIVconsv 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).

After recombination with the plasmid insert HIVconsv 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 HIVconsv. The amplified DNA fragment of appropriate size confirms the identity of the insert.

The immunogenicity of MVA.HIVconsv demonstrated in Balb / c reshus 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.HIVconsv has been developed as a therapeutic vaccine candidate for HIV-1. Its release is necessary to implement the second phase I clinical trial in our country with MVA-HIVconsv in combination with the drug romidepsin in patients with recently HIV-1 infection. In humans, in HIV-CORE001 and HIV-CORE002 clinical trials in United Kingdom, and at present time a phase I/IIa clinical trial in Kenya is also taking place with the same GMO

The present BCN02-Romi study aims to evaluate the safety, as well as the effect on the latent reservoir and viral rebound of MVA.HIVconsv vaccination combined with 3 doses of romidepsin. Two administrations of the GMO will be given, 3 weeks before and 4 weeks after romidepsin infusions. Participants of the BCN02-Romi trial will be the same individuals who previously participated of the BCN01 trial (previous notification). Study population includes HIV-infected individuals with early virological suppression who received ChAdV63.HIVconsv and MVA.HIVconsv vaccines intramuscular administered according to two different vaccination patterns: 0-8 weeks apart or 0-24 weeks apart (in the context of the Chad-MVA.HIVconsv-BCN01 clinical trial).

There should be no potential environmental benefits 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 our geographical location.

3. Information concerning the release and the surrounding area

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

Although the trial involves two recruiting sites, vaccinations with MVA.HIVconsv be held 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: 15m^2
(ii) wider release site (m^2): same and never >15m^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 effect on the flora and fauna is not possible.

4. Method and amount of release

(a) Quantities of GMOs to be released:

A maximum of 24 patients meeting the inclusion and exclusion criteria will be included. They all will receive MVA.HIVconsv in the baseline visit and at week 9.All patients already received the same GMO in the previous BCN01 trial (notification B/ES/12/10).
In total it is estimated to administer a maximum of 96 vials (extra patients counting for loss) with a dose of 2 x 10^8 pfu per vaccination.

(b) Duration of the operation:

Each vaccination takes about few minutes. The recruitment period (and dosing of MVA.HIVconsv) of the 24 participants is estimated to be made over a 6 months. Total follow-up study will last 14 months.

(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 cell product works 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 labeled. The vials will be sent from Germany kept 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. The area used for the preparation for injection should 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 vial will be placed in a sealed bag and kept in a -80ºC freezer until its destruction that will be performed in pool basis at the end of the trial.

In case of accidental contamination, each contaminated surface should be treated using conventional hospital procedures for infected products. All personnel involved in handling the vaccines should be informed that in case of skin contamination should immediately wash skin thoroughly with water and disinfected with iodine locally to 4% and in case of eye contamination, wash and rinse only with water. It should also make 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 Germans Trias i Pujol Hospital.

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.

First MVA.HIVconsv GMO release in Spain was under B/ES/12/10 notification. There was no incidence nor accident during the release period.

After the evaluation by the National Biosafety Committee, it was considered that in the present knowledge and under use conditions, BCN01 trial was not presenting any risk for human health nor for the environment. Nevertheless, it was considered necessary to conduct GMO biodistribution studies to ensure the non-dissemination in the environment trough patients' body fluids. The research group proposed holding the insert PCR detection HIVconsv in urine samples collected at 24 hours and 7 days post vaccination with the GMO MVA.HIVconsv. At the moment, they are conducting such determinations, the final results will be forwarded to the National Biosafety Committee along with this notice (estimated November 2015 reporting date).

In all trials sponsored by University of Oxford (UK) where MVA.HIVconsv has been released, contained use was considered so there are no specific data for environmental MVA.HIVconsv dissemination since no environment dissemination studies were requested. However, seven rMVAs developed at the University of Oxford have been evaluated so far in clinical trials, two with transgenes from HIV (HIVA and HIVconsv). Biodistribution studies performed in mice and monkeys with MVA.HIVA insert and whose production system is comparable to MVA.HIVconsv have shown no detection of GMOs beyond the site of inoculation.

Based on the data from the clinical studies HIV-CORE001, HIV-CORE002, HIV-CORE004 and ChAd-MVA.HIVconsv-BCN01, the following adverse events are expected to occur in some volunteers following vaccination with MVA.HIVconsv:

- Injection site pain
- Injection site tenderness
- Injection site erythema
- Injection site swelling
- Injection site pruritus
- Induration
- Myalgia
- Headache
- Fatigue
- Fever
- Malaise
- Nausea
- Chills
- Vomiting
- Flu symptoms
- Diarrhea
- Sweating
- Anorexia
- Abdominal Pain
- Syncope

These adverse events are expected to be primarily mild in severity, however occasional moderate or severe adverse events have been reported. These adverse events are expected to last for approximately 24-48 hours following vaccination, though adverse events of longer duration have also been reported. The listed adverse reactions are therefore considered 'expected' for the purposes of expedited reporting to regulatory authorities, ethics committees and investigators.

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 HIVconsv.

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 it is a defective virus unable to replicate is not expected any interaction with other organisms in the environment. For the gene encoding HIVconsv 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 and it spreads from the release site, because it has a very limited selection of host and also considering the contained release in the context of the trial. 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

(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	…

Not applicable

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 HIVconsv is a chimeric protein designed exclusively for the induction of specific cellular responses through the union of 14 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, it has not been planned in the current proposal or in the HIV-CORE02 already undergoing, any specific viral detection of MVA.HIVconsv in biological fluids or blood.

There will be monitoring of side effects of treatment during the trial by physical examination, blood tests and urine and communication of adverse events. The safety assessment will be

made over the participation of patients 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 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. Patients will be clinically monitored.

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^2)
… m^2
Not applicable: The GMO is administered only to patients by intramuscular injection the hospital rooms, as described in Section F.

5. Duration of the monitoring

The safety assessment will be made over the participation of patients in the clinical trial. And future extension phases.

6. Frequency of the monitoring

Monitoring visits during which safety is evaluated will be held a week, one month and 2 months 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 labeled medical waste - Group III and decontaminated before disposal

3. (a) Type and amount of waste generated

Vaccine vials, needles, gloves, gowns, masks, bandages / tape (24 patients total)

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. Will be introduced to the following vessels:

Infectious Waste Solids:
Bag should always be in red 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.

Vaccine vial will be placed in a sealed bag and kept in a -80ºC freezer until its destruction at the end of the trial, in a pool basis.

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 on the procedures to act 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, energetic and scrubbed wash with iodine solution with 4% will be done.
In case of eye contact, a wash with saline for a period 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.
Patients 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.