MVA.HTI and ChAdOx1.HTI
B/ES/18/21

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

B/ES/18/21

17 of August 2018

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/18/21
(c) Date of acknowledgement of notification: 13-07-2018
(d) Title of the project:
Phase IIa, randomized, double blind, placebo-controlled, first-in-human study of the safety, tolerability and immunogenicity of candidate vaccines MVA.HTI and ChAdOx1.HTI alone or administered sequentially with GS-9620 in HIV-1positive patients treated early (AELIX-003).
(e) Proposed period of release Start date: 30/09/2018 - Finish date: 30/06/2020

2. Notifier

Name of institution or company: AELIX Therapeutics SL
Baldiri Reixac 4-8,
Barcelona, Spain, 08028

3. GMO characterization

The genetically modified organisms (GMOs) to be used in the assay are:

- **MVA.HTI**
- **ChAdOx1.HTI**

The GMO **MVA.HTI** has already been released in the clinical trial " A Phase I, randomized, double-blind, placebo-controlled safety, tolerability and immunogenicity study of candidate HIV-1 vaccines DNA.HTI and MVA.HTI in early treated HIV-1 positive individuals" (AELIX-002).The file number for this release is B/ES/16/11.

Following the advice of the Spanish Ministry of Agriculture and Fisheries, Food and Environme, the information related only to **MVA.HTI** will not be repeated to a full extension in all the sections of this dossier. All the information is accessible to the reviewers in file B/ ES / 16/11.

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)

One of 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.

Genetic stability is verified in various steps of the production process.

ChAdOx1.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)

Family: Adenoviridae
Genus: Mastadenovirus
Species: attenuated chimpanzee adenovirus, without replication capacity, derived from serotype Y25.

The genetically modified organism (GMO) to be used in the clinical trial is ChAdOx1.HTI, a live, recombinant, chimpanzee adenovirus, with no capacity for replication due to deletions and genomic modifications.

In the ChAdOx1.HTI, the transgene that codes for the HTI insert has been inserted with the aim of inducing a specific T-cell HIV-1 immune response.

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

Once administered, ChAdOx1.HTI is directed and located in the nucleus of the host cell, however, it does not integrate its DNA into the host's genome (Rauschhuber *et al.*, 2012; Athanasopoulos *et al.*, 2017). ChAdOx1.HTI remains localized in the cell cytoplasm until the cell is destroyed (Rauschhuber *et al.*, 2012; Athanasopoulos *et al.*, 2017). The integration of adenovirus DNA into the genome is an extremely rare event that has only been observed in some cultures of human primary cell lines (Hogg, 2000). According to European Medicines Agency (EMA) guidelines, adenoviral vectors are considered non-integrating vectors (EMEA/273974/2005, 2006).

ChAdOx1.HTI is a genetically stable virus that is unable to integrate its DNA into the host genome and remains localized in the cell cytoplasm until the cell is destroyed (Rauschhuber *et al.*, 2012; Athanasopoulos *et al.*, 2017).

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

ChAdOx1.HTI and MVA.HTI

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

If yes, insert the country code(s) MVA.HTI: ES, DK, FR, BE; ChAdOx1.HTI: ES

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

MVA.HTI and ChAdOx1.HTI

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

If yes:

- Member State of notification ES
- Notification number MVA.HTI – AELIX-002: B/ES/16/11; MVA.HTI and ChAdOx1.HTI: B/ES/18/21

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

ChAdOx1.HTI and MVA.HTI

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

If yes:

- Member State of notification
- Notification number

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

MVA.HTI

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.

ChAdOx1.HTI

There is no scientific reason to suppose that the use of the HTI transgene as an insert in the viral vector ChAdOx1 modifies the distribution characteristics, "shedding" or replicative capacity with respect to other inserts used in similar viral vectors such as ChAd155 and ChAd63. Chimpanzee adenoviruses have been used in clinical trials as an advantageous alternative to human adenoviruses due to their high capacity to generate an immune response, the possibility of inserting longer sequences and the lack of immunity against the vector due to non-exposure previous.

No survival of ChAdOx1.HTI is expected since 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 in the proposed release conditions. The product is also controlled for the emergence of replication competent viruses during production as well as other adventitious agents.

In addition, the ChAdOx1.HTI is replication incompetent so no effect on humans, flora or fauna near or far to the release zone are expected. The genetically modified ChAdOx1.HTI vaccine is not capable of surviving, establishing, spreading or displacing other organisms, and is not pathogenic to animals or plants. Adenoviruses most commonly cause respiratory illness. The illnesses can range from the common cold to pneumonia, croup, and bronchitis. Depending on the type, in rare cases adenoviruses can also cause other illnesses such as gastroenteritis, conjunctivitis, cystitis.

The chimeric protein -HTI -the transgene- is constituted by 16 fragments of the HIV-1 genome not involved in the pathogenicity of the virus; It also does not contain entire native proteins, so it is not functionally active, it is not dangerous and it does not have harmful effects for other organisms.

Therefore, the probability that ChAdOx1.HTI becomes persistent and invasive in natural habitats is very low.

There are precedents for the release of highly similar products in Spain, such as GMO ChAdV63.HIV under file B/ES/12/09 and B/ES/12/10, as well as the GMO ChAd155-RSV under file B/ES/16/07. The chimeric protein HTI-transgene is also being used in clinical

trials in Spain, also for experimental vaccines against HIV-1 (GMO MVA.HTI) under the file B/ES/16/11. During the release period, no incident or accident was recorded.

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

MVA

1. Recipient or parental organism characterisation:

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

(select one only)

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

(specify phylum, class)

other, specify

2. Name

(i)	order and/or higher taxon (for animals)	Poxviridae/Chordopoxviridae
(ii)	genus	Orthopoxvirus
(iii)	species	Vaccinia virus
(iv)	subspecies	
(v)	strain	Modified vaccinia Ankara virus
(vi)	pathovar (biotype, ecotype, race, etc.)	
(vii)	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**)

ChAdY25
The receptor or parental organism, on which the genetic modifications have been made, is the chimpanzee adenovirus Y25 (ChAdY25). Serotype Y25 (ChAdY25), which is the wild serotype, is considered the receptor (parenteral virus) of the product. AdChAdY25 was originally obtained from William Hillis, John Hopkins University of Medicine (Hillis *et al.*, 1969; Dicks *et al.*, 2012). ChAdOx1 is serotype Y25 after being genetically modified and incapacitated for replication.

1. Recipient or parental organism characterisation:

(b) 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

Chimpanzee adenovirus Y25 was originally obtained from William Hillis, John Hopkins University of Medicine (Hillis *et al.*, 1969; Dicks *et al.*, 2012). The original isolate was found in chimpanzee feces and was passed 6 times in HEp-2 cells before the initial characterization. The complete sequence of its genome is available from GenBank no. JN254802, as well as the phylogenetic relationship with other serotypes (by comparison of hexon and fiber proteins) and the main components of the capsid exposed to the surface.

ChAdY25 is phylogenetically grouped with the human adenovirus E (HAdV), HAdV-4. The DNA sequences of ChAdY25 hexon and fiber, as well as the simian adenovirus SAdV-23, are grouped separately with other E-members of human adenovirus.

2. Name

(viii)	order and/or higher taxon		Adenoviridae
(ix)	genus		Mastadenovirus
(x)	species		Subgroup of simian adenovirus E
(xi)	subspecies		
(xii)	strain		Chimpanzee adenovirus Y25
(xiii)	pathovar (biotype, ecotype, race, etc.)		
(xiv)	common name	:	Chimpanzee adenovirus Y25

3. Geographical distribution of the organism

MVA and ChAdY25

(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 (**X**)

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 (.)

(e) 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	

MVA

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.

ChAdY25

Adenoviruses have a worldwide distribution. Wild-type adenoviruses have been detected in waters around the world, including waste water, river water, drinking water, oceans and swimming pools. Humans and animals are the natural reservoirs for wild-type adenoviruses.

The natural host of the ChAdY25 wild-type adenovirus is the chimpanzee. The parental adenovirus is not found in the natural ecosystem outside its natural host. The parent organism was isolated from the faeces of a chimpanzee and genetically modified. It was created and maintained in laboratories due to its inability to replicate (Hillis *et al.*, 1969; Dicks *et al.*, 2012).

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

MVA and ChAdY25

Not applicable.

5. (a) Detection techniques

MVA

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).

ChAdY25

Adenoviruses are detected by PCR according to generic sequences of chimpanzee adenovirus or specific for serotype Y25. They can also be detected with immunocytochemistry assays using anti-hexon antibodies.

(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

MVA is classified as Biological Safety Level 1 (Verheust *et al.*, 2012; Goossens *et al.*, 2013).

ChAdY25

In terms of potential hazard classification, the human adenovirus is considered as a Biological Agent of Type 2, according to the classification of the European Economic Community (EEC) for the protection of workers
with biological agents (Directive 2000/54 / EC, 2000). The group 2 designation applies to biological agents that can cause human diseases and can be a hazard to workers, which is unlikely to spread to the community and for which prophylaxis or effective treatment is generally available. However, many adenoviral vectors such as our parent organism have been constructed by deletion of the E1 region that encodes key genes required for viral growth. Without the genes of the E1 region, the adenoviral vectors are unable to produce infection in humans.

Similar chimpanzee vectors have already been used in two previous notifications for voluntary release of recombinant adenoviruses in clinical trials (B/ES/12/09).

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

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.

ChAdY25

Human adenoviruses commonly cause asymptomatic infection in human, although they can also cause respiratory tract, gastrointestinal infections, discomfort or eye infections of varying severity (Wold *et al.*, 2013; Athanasopoulos *et al.*, 2017). They are more common in children and in the immunocompromised population. The incubation period varies from 1 to 10 days. The majority of the population is seropositive for more than one subspecies of adenovirus and can rapidly produce neutralizing antibodies.

Normally, the virus is infected through the respiratory tract or the eyes through aerosols produced by infected people. Most infections are mild in nature. Adenoviruses are rarely integrated into the genome of host cells and do not persist in lymphoid tissues. Adenovirus infections in non-human primates (NHP) are also predominantly mild.

Chimpanzee adenoviruses are increasingly used in clinical trials as an advantageous alternative to human adenoviruses because of their great ability to generate an immune response, the possibility of inserting longer sequences and the lack of immunity against the vector due to the no previous exposure.
In terms of potential hazard classification, the human adenovirus is considered a type II biological agent, according to the classification of the European Economic Community (EEC) for the protection of workers with biological agents (Directive 2000/54/EC, 2000). Group II designation applies to biological agents that can cause human disease and can be a hazard to workers, which is unlikely to spread to the community and for which

prophylaxis or effective treatment is generally available. However, as previously mentioned, many adenoviral vectors, such as our parent organism, have been constructed by deletion of the E1 region that encodes key genes required for viral growth. Without the genes of the E1 region, the adenoviral vectors are unable to produce infection in humans.
Similar chimpanzee vectors have already been used in two previous notifications for the voluntary release of recombinant adenoviruses in clinical trials (B/ES/12/09 and B/ES/16/07).

8. Information concerning reproduction

(a) Generation time in natural ecosystems:
(b)

MVA

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.

ChAdY25

. It replicates in HEK 293cells in the presence of its deleted genes.

(c) 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
(d) 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:
(c)

MVA

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.

ChAdY25

No survival of ChAdY25 is expected since 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.

10. (a) Ways of dissemination

MVA

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.

ChAdY25

ChAdY25 remains localized in the cell cytoplasm until the cell is destroyed. According to data from non-clinical and clinical studies of highly similar products, dissemination of the vector outside the injection site has not been observed.

(b) Factors affecting dissemination

MVA

Irrelevant

ChAdY25

The capacity of propagation depends on the dose, the formation of aerosols and the proximity of contact as well as the security measures taken in the research environment established in the protocol.

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)

MVA

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).

ChAdY25
In Spain, two GMOs similar to the one proposed in this document (ChAd155-RSV and ChAdV63.HIVconsv) have already been notified, with dossiers B / ES / 16/07 and B / ES / 12/09, and have been released in different clinical trials.

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

MVA.HTI
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.

ChAdOx1.HTI
The objective of the project is to develop an immunotherapeutic product (sometimes also called "therapeutic vaccine") for the treatment of HIV infection, in order to achieve a functional cure.

The transgene encoding the HTI insert has been inserted into the locus of the E1 region, after it has been deleted. With these modifications it is intended that those cells that are infected by ChAdOx1.HTI can express the HTI immunogen for the activation of immune responses against the 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 (.)

ChAdOx1.HTI

The recombinant expression cassette with the HTI antigens and the GMO capsid genes (fiber and hexon) will be included in the final GMO. The other genes are auxiliary and will only be used for the correct packaging of the GMO.

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

MVA.HTI

plasmid p3055

ChAdOx1.HTI

plasmid pBACe3.6

Plasmid SAdV-Y25 ΔE1

Plasmid pC255 (SAdV-Y25 ΔE1 + HTI)

(c) Host range of the vector

MVA.HTI

Escherichia coli

ChAdOx1.HTI

Escherichia coli

HEK 293 cells

The plasmid replicates in the *E. coli* laboratory strain.

The final adenovirus vector can only be replicated in cells expressing E1 (such as HEK 293)

(d) Presence in the vector of sequences giving a selectable or identifiable phenotype

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

antibiotic resistance (.)
other, specify

MVA.HTI
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.

ChAdOx1.HTI
The E1 and E3 regions of the ChAdOx1 genome have been deleted, and the native E4 region has been modified by the insertion of the E4Orf4, Orf6 and Orf6 / 7 regions of the human adenovirus type 5.

Indication of which antibiotic resistance gene is inserted

Not applicable.

(e) Constituent fragments of the vector

MVA.HTI
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.

ChAdOx1.HTI
ChAdY25, after having undergone all genetic modifications, is called ChAdOx1. Thus, it is in ChAdOx1 where the HTI antigen will be inserted.

ChAdOx1 is the Y25 serotype after having been genetically modified and incapacitated for replication. To carry out such modifications a Bacterial Artificial Chromosome vector (BAC) was used as a tool ("rescue vector") containing the wild ChAdY25 genome (plasmid pBAC e3 .6). ChAdOx1, was created from pBAC e3.6 using standard molecular recombination techniques. The resulting genome was cloned into a BAC vector pBAC e3.6 ΔE1 clone. Finally, the HTI expression cassette was inserted into pBAC e3.6 ΔE1 clone to make plasmid BAC pC255. The plasmid pC255 directs the insertion of a gene of interest, in this case the gene deficient for the replication of chimp adenovirus ChAdOx1 with the insert of HTI antigens.

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

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

MVA.HTI
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 harbours 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).

ChAdOx1.HTI
Transfection.
The batches are produced by transfecting linearized pC255 in commercial HEK293A T-REx® cells, which stably express the tetracycline repressor protein (Tet), using lipofectamine.

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.

(i)	transformation	(.)
(ii)	microinjection	(.)
(iii)	microencapsulation	(.)
(iv)	macroinjection	(.)
(v)	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 1).

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.

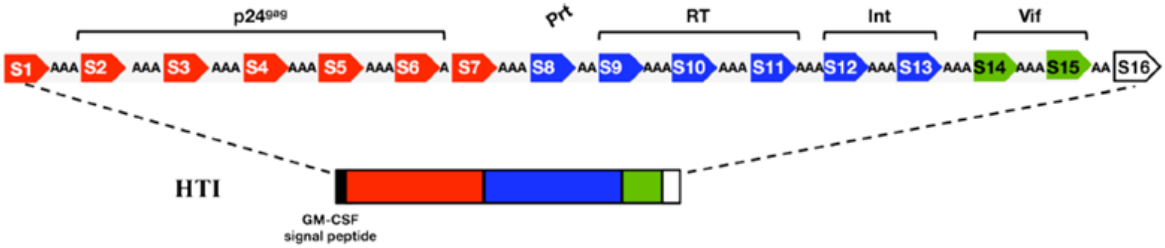

Figure 1. The HTI protein
The HTI protein is composed of 16 individual segments arranged linearly and linked via 1–3 alanine amino acid linkers and contains the GM-CSF signal peptide for better secretion. Total length of HTI protein is 529 aa, including alanine linkers

(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.

(e) Location of the insert in the host organism

- on a free plasmid (.)

- integrated in the chromosome (**X**)
- other, specify

MVA.HTI
Integrated in the genome of the MVA at thymidine kinase locus and under the control of modified H5 promoter.

ChAdOx1.HTI
Integrated into the ChAdOx1 genome under the control of the modified H5 promoter.

(f) 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, so it applies equally to the two GMOs (ChAdOx1.HTI and MVA.HTI).

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:

(b) 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

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

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

E. Information relating to the genetically modified organism

1. Genetic traits and phenotypic characteristics of the recipient or parental organism which have been changed as a result of the genetic modification

MVA.HTI

(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.

ChAdOx1.HTI

(a) is the GMO different from the recipient as far as survivability is concerned?

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

Specify

Although the survival and stability of ChAdOx1.HTI is similar to the original ChAdY25 virus, ChAdOx1.HTI does not have replication capacity in cells that do not express the E1 region of the adenovirus. Therefore, ChAdOx1.HTI is replication incompetent.

MVA.HTI

(b) is the GMO in any way different from the recipient as far as mode and/or rate of reproduction is concerned?

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

Specify The same as before (section E-a)

ChAdOx1.HTI

(b) is the GMO in any way different from the recipient as far as mode and/or rate of reproduction is concerned?

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

Specify

ChAdOx1.HTI is a recombinant virus, completely deficient for replication because the E1 region has been replaced by the expression cassette for HTI antigens.

MVA.HTI

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

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

MVA has an extremely limited capacity to spread, as it is replication defective 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.

ChAdOx1.HTI

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

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

Since ChAdOx1.HTI is deficient for replication and its genome remains localized in the cell cytoplasm until the destruction of the cell, its dissemination is limited.

MVA.HTI

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

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

MVA causes no known human disease. 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.

ChAdOx1.HTI

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

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

ChAdOx1.HTI cannot replicate, therefore, it is not pathogenic. The inability to replicate from the originally infected cells prevents it from spreading to other cells, which completely changes the pathogenicity.

2. Genetic stability of the genetically modified organism

MVA.HTI

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.

ChAdOx1.HTI

Once administered, ChAdOx1.HTI is directed and located in the nucleus of the host cell, however, it does not integrate its DNA into the host's genome (Rauschhuber *et al.*, 2012; Athanasopoulos *et al.*, 2017). The integration of adenovirus DNA into the genome is an extremely rare event that has only been observed in some cultures of human primary cell line. According to EMA guidelines, adenoviral vectors are considered non-integrating vectors (EMEA/273974/2005, 2006).

ChAdOx1.HTI is a genetically stable virus that is unable to integrate its DNA into the host genome and remains localized in the cell cytoplasm until the cell is destroyed (Rauschhuber *et al.*, 2012; Athanasopoulos *et al.*, 2017).

Genetic stability is verified in different steps of the production process.

During the manufacture of ChAdOx1.HTI different studies are carried out in each of the production steps:

- Western blot to confirm correct size of the transgene protein.
- Full sequencing of the transgene.
- Full sequencing of the vector.
- qPCR to confirm the presence of the hexon.
- Analysis of the vector with restriction enzymes.

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)

MVA.HTI

Same as stated before for MVA. Pathogenicity of MVA-HTI does not differ from MVA and MVA is non-pathogenic. MVAs 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.

ChAdOx1.HTI

As already mentioned above in sections B.7. (b) and D.3. (b) and according to Directive 2000/54 / EC (Directive 2000/54/EC, 2000), adenoviruses are classified as biological agents of type 2 due to their limited pathogenicity.
Human adenoviruses commonly cause asymptomatic infection in
human, although they can also cause respiratory tract, gastrointestinal infections, discomfort or eye infections of varying severity. They are more common in children and in the immunocompromised population. The incubation period varies from 1 to 10 days. The majority of the population is seropositive for more than one subspecies of adenovirus and can rapidly produce neutralizing antibodies.
Normally, the virus is infected through the respiratory tract or the eyes through aerosols produced by infected people. Most infections are mild in nature. Adenoviruses are rarely integrated into the genome of host cells and do not persist in lymphoid tissues.
Adenovirus infections in non-human primates (NHP) are also predominantly mild.
Adenoviruses disabled for replication have been widely used in clinical trials.
Adenoviruses lacking the E1 region were classified as class 1 biosecurity biological agents.
The fragments or sequences included in the immunogenic HTI do not participate in the pathogenic properties of the virus.

4. Description of identification and detection methods

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

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

ChAdOx1.HTI
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

MVA.HTI
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 millilitre (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.

ChAdOx1.HTI
Adenoviruses are detected by PCR according to generic sequences of chimpanzee adenovirus or specific for ChAdOx1.HTI. They can also be detected with immunocytochemistry assays using anti-hexon antibodies.
During the manufacture of ChAdOx1.HTI different studies are carried out in each of the production steps:

-Western blot to confirm correct size of the transgene protein.

-Full sequencing of the transgene.
-Full sequencing of the vector.
-qPCR to confirm the presence of the hexon.
-Analysis of the vector with restriction enzymes.

F. Information relating to the release

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

ChAdOx1.HTI
The purpose of the proposed trial is to study the safety and immunogenicity of both GMOs, as well as to investigate the ability of both, alone, together, or in sequence with GS-9620, to reduce the viral reservoir.
The vaccination schedule consists of a dose of 5.0 x 10^{10} Vp of ChAdOx1.HTI at weeks 0 and 12 and a dose of 2 x 10^{8} pfu of MVA.HTI at weeks 24 and 36. The administration will be performed intramuscularly. In weeks 26, 28, 30, 32, 34, 38, 40, 42, 44 and 46, 8 mg (oral) of GS-9620 will be administered (Vesatolimod, not a vaccine) (Lanford *et al.*, 2013; Bam *et al.*, 2017).

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
ChAdOx1 does not exist naturally in our ecosystem.
The natural host of the ChAdY25 wild-type adenovirus is the chimpanzee. The parental adenovirus is not found in the natural ecosystem outside its natural host. The parent organism was isolated from the faeces of a chimpanzee and genetically modified.

3. Information concerning the release and the surrounding area

(a) Geographical location (administrative region and where appropriate grid reference):
MVA.HTI and ChAdOx1.HTI
The preparation of doses and vaccinations with MVA.HTI and ChAdOx1.HTI will be carried out in 9 hospitals in Spain:

- Germans Trias i Pujol University Hospital (HUGTP) (Barcelona).
- University Hospital Vall d'Hebrón (HUVDH) (Barcelona).
- Ramón y Cajal University Hospital (HURyC) (Madrid).
- University Hospital La Paz (HUP) (Madrid)
- Virgen del Rocío University Hospital (HUVR) (Seville)
- H. Clinic (HC) (Barcelona)
- H.U. Gregorio Marañon (HUGM) (Madrid)
- H. U de Bellvitge (HUB) (Barcelona)
- H.U. October 12 (HU12O) (Madrid)

(b) Size of the site (m^2):

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

ChAdOx1.HTI and MVA.HTI

Table 2. Actual área of GMO reléase

	Real liberation area(m^2)	Total (m^2)
H.U. Germans Trias i Pujol	4.41	
H.U. Vall d'Hebrón	70.37	
H. Ramón y Cajal	12	
H.U. La Paz	50	
H.U. Virgen del Rocío	120	
H. Clínic		
H.U. Gregorio Marañon		
H. U de Bellvitge		
H.U. 12 de Octubre		
		256.78

Table 3. Maximum area of GMO release

	Maximum liberation area (m^2)	Total (m^2)
H.U. Germans Trias i Pujol	22	
H.U. Vall d'Hebrón	150	
H.U. Ramón y Cajal	12	
H.U. La Paz	50	
H.U. Virgen del Rocío	120	
H. Clínic		
H.U. Gregorio Marañon	190	
H. U de Bellvitge		
H.U. 12 de Octubre	250	
		794

(d) 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.

(e) 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 90 people will be included. In total, 60 patients will receive ChAdOx1 and MVA.HTI:

- 50 subjects will receive the treatment of ChAdOx1 and MVA.HTI (+ GS-9620)
- 10 patients ChAdOx1.HTI and MVA.HTI

	Number of patients	Number of Doses	Volume /dose	Concentration/dose (Vp)	Concentration/dose (pfu)
ChAdOx1.HTI	60	2	0.5 mL	5.0 X 10^{10} Vp	
MVA.HTI	60	2	0.5 mL		2 x 10^{8} pfu
TOTAL	60	240	120 mL	Total Vp of ChAdOx1.HTI = 6 X 10^{12} Vp	Total pfu of MVA.HTI = 24 X 10^{9} pfu

(b) Duration of the operation:

Each vaccination lasts a few minutes.
All patients will be followed 84 weeks after the initial vaccination (from Baseline up to 48 weeks after last vaccination). A period of 8 weeks will be allowed from the screening to the baseline visit (first vaccination).

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

The GMOs will be released only for clinical use.

The personnel involved in the preparation of the product work according to the conditions specified in the standards of Good Clinical Practices and Good Manufacturing Practices.
The manufacturing laboratory (ReiThera / Advent) located in Italy, prepares and packages the product in hermetically sealed vials and labelled appropriately. The vials will be sent from Italy maintained at -80ºC and stored until their use in the Pharmacy Service by the different hospital centres. The administration is carried out under the responsibility of the researcher, in accordance with a clinical protocol and respecting the Good Clinical Practice Guidelines.

The products will be prepared in aseptic conditions: the personnel that will prepare the vaccines will use sterile techniques (gloves, masks and disposable gowns). The product will be prepared under aseptic conditions, in compliance with the preparation of injectable in a closed room with BSL-1 characteristics. The solution will be transferred to a 1 ml syringe of insulin under aseptic conditions using a validated closed system transfer device (CSTD). The area used to the preparation for your injection should be decontaminated before and after handling with Safe Seft® disinfectant soap and 70ºC alcohol.
All transport of the product is carried out using a closed container of the pharmacy service to the place of administration of the product.

The vaccination site of the patients (deltoid area) is cleaned with 2% chlorhexidine digluconate until it is evaporated before injection and appropriately covered after administration. The personnel that will administer the product to the patients will use 'universal precautions', that is, use of disposable gloves and gowns, as well as dispose of the contaminated material immediately after the administration according to the management of sanitary waste Group III.

After administration, all surfaces are cleaned with antiseptic soap containing 4% chlorhexidine digluconate before and after use. The material used during the preparation and administration is considered sanitary waste Group III and will be managed as such. Specifically, the sizes (if used), gloves, gown, syringes and needles will be disposed of in specific yellow hermetic containers for Group III sanitary waste available both in the preparation room and in the administration office. The vials used will be returned to the pharmacy service in closed containers for destruction at the end of the study following the protocols of the pharmacy service.

In case of accidental contamination, each contaminated surface should be treated in accordance with conventional hospital procedures established by the clinical trial promoter for infected products. All personnel involved in the handling of the product should be informed that in case of skin contamination the skin should be immediately washed thoroughly with water and disinfected locally with 4% iodine, and in case of eye contamination, it is recommended to wash and rinse only with water. An evaluation by the ophthalmologist should be made as soon as possible. No specific biological analysis is planned for the personnel that manages the product.

5. Short description of average environmental conditions (weather, temperature, etc.)
Not applicable. All preparations and immunizations are going to be performed in hospitals.

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.

The GMO MVA.HTI has already been released in the clinical trial " A Phase I, randomized, double-blind, placebo-controlled safety, tolerability and immunogenicity study of candidate HIV-1 vaccines DNA.HTI and MVA.HTI in early treated HIV-1 positive individuals" (AELIX-002).The file number for this release is B/ES/16/11. This study is also the subject of a variation to include the vector ChAdOx1.HTI described in the current document, which has a new file number; B/ES/18/20. No accidents, spills, unintended exposures or environmental risks/threats were reported.

There are no previous releases of ChAdOx1.HTI. However, there are previous releases of GMOs with highly similar inserts (B/ES/12/10 and B/ES/15/12).Similar chimpanzee vectors have also already been used in two previous notifications for the voluntary release of recombinant adenoviruses in clinical trials (B/ES/12/09 and B/ES/16/07).

G. Interactions of the GMO with the environment and potential impact on the environment, if significantly different from the recipient or parent organism

MVA.HTI and ChAdOx1

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 proposed release conditions of GMOs. GMOs will be administered in the hospital units mentioned above and are unlikely to come into contact with other animal species.

Since ChAdOx1.HTI is inoculated to subjects who will also be inoculated with MVA.HTI after ChAdOx1.HTI, it is possible that the HTI-encoded gene is transferred from one genome to another by homologous recombination, either deleted from one of them or duplicated in the other one. The consequence of this unlikely event would be innocuous, since first, neither of the two GMO (MVA.HTI and ChAdOx1.HTI) has replication capacity and second, the fragments or sequences included in the HTI immunogen do not participate in the pathogenic properties of the virus

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

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

Give details

The GMO (ChAdOx1.HTI) is a recombinant chimpanzee adenovirus, alive without capacity for replication due to deletions and genomic modifications. Therefore, ChAdOx1.HTI should have reduced competitiveness and invasiveness compared to the ChAdY25 virus.

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

Given the characteristics of the attenuation and / or modifications that affect the replication capacity, as well as risk management measuresthat are foreseen in this study, it is not probable that the GMOs can be released to the ecosystem nor can they be disseminated from

the place of release. In the case of MVAs, these have been widely used in clinical trials, both in direct administration and in cell therapy strategies (contained inside cells).

In the unlikely event of involuntary administration to organisms other than the target, subsequent dissemination would be unlikely due to the inability of both to replicate.

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. As previously mentioned in section G.3, since ChAdOx1.HTI is inoculated to subjects who will also be inoculated with MVA.HTI, it is possible that the HTI region is transferred from the genome to one another by homologous recombination, or deleted. of one of them or duplicated in the other.

The possibility of recombination with other viruses can not be excluded since ChAdOx1.HTI could, theoretically, exchange its genetic material with a wild-type adenovirus if the same cell was co-infected by both. In this case, ChAdOx1.HTI could reacquire its capacity for replication. The probability of this happening is extremely low because ChAdOx1.HTI is unable to replicate, so the infection would involve a very limited number of viral particles, which would also be quickly eliminated by the immune system. For all this, in the hypothetical case that a possible horizontal transmission acurrates, they would not have any effect on the health of the persons in contact with the vaccinated subject. In addition, individuals who have been naturally exposed to wild type adenoviruses develop neutralizing antibodies that constitute an additional barrier that protects against the possible horizontal transmission of adenoviral vectors.

In the same way, in case of co-infection with a wild-type adenovirus, it could be the case that the HTI gene is transferred to the replication-competent wild-type adenovirus. It would

require co-localization / co-infection of the same cells in the same host, which is very unlikely, especially in humans, because ChAdOx1.HTI is a chimpanzee adenovirus and the homology is minor.

(c) 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

MVA.HTI
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.

ChAdOx1
Chimpanzee adenoviruses are increasingly used in clinical trials as an advantageous alternative to human adenoviruses because of their great ability to generate an immune response, the possibility of inserting longer sequences and the lack of immunity against the vector due to the no previous exposure. No survival of ChAdOx1.HTI is expected since 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 in the proposed release conditions. No specific detection of MVA.HTI in biological fluids or blood has been planned.

MVA.HTI and ChAdOx1
Due to the knowledge acquired in previous clinical trials and other strategies in cell therapy, no specific viral detection related to MVA.HTI or ChAdOx1.HTI in biological fluids or blood during the proposed clinical trial has been planned (Borducchi *et al.*, 2016; Ewer *et al.*, 2016; Mensah *et al.*, 2016).

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 60 weeks after the last injection in the study (see details in attached protocol).

2. Methods for monitoring ecosystem effects

Not planned, as the GMOs are not found naturally in the environment and they are non-replicative so there is no chance that an impact on the ecosystem of the GMOs with infectivity will be seen.

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)

Not applicable: The GMOs are administered only to subjects by intramuscular injection in the ambulatory.

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

Not applicable.

I. Information on post-release and waste treatment

1. Post-release treatment of the site

The GMO virus suspensions will be transferred to a 1 mL syringe of insulin under aseptic conditions by a validated closed system transfer device (CSTD). The preparation site will be cleaned with Safe Seft® and 70º alcohol (disinfectants approved by GMP use) before and after the release. All surfaces where the product will be in contact will be with antiseptic soap containing 4% chlorhexidine digluconate before and after the administration.

The physical site of inoculation (deltoid region) will be cleaned with 2% clorhexidine d igluconate and will be covered properly after the injection with a plaster or bandage.

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 with a sticker labelled medical waste - Group III.

3. (a) Type and amount of waste generated

It is expected to generate the following waste: vaccination vials (240 vials in total; 90 people in total recruited, 60 treated with MVA.HTI and ChAdOx1, twice each) , sizes, syringes, needles, closed systems (CSTD), gloves, gowns, masks, bandages / plaster.

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 (specific sanitary waste Group III) .

The vaccine syringe attached to the CSTD system and needle will be deposited in waste sharp containers.
Used vaccine vials (attached to the CSTD adaptors) will be returned to the pharmacy service for its final destruction at the end of the trial following their specific SOPs.

The withdrawal and the final closing of 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.

The place where the release occurs will be cleaned with sodium hypochlorite diluted at 1%. In the case of contact with the skin, vigorous washing will be carried out and subsequently disinfected with a 4% iodine solution. In case of contact with the eyes, it will be washed with saline for a time not less than 15 minutes. The subject will have to be evaluated by an

ophthalmologist in the shortest possible time. In case of an accidental puncture, an abundant wash with soap and water will be carried out immediately and then the puncture area will be disinfected with 9-12% Iodine solution for at least 5 minutes or with 10 g / L sodium hypochlorite solution.

The volunteers included in the clinical trial will be monitored as planned by the protocol according to standards of good clinical practice. Each adverse event will be notified to the principal investigator and regulatory agencies.

Due to the risk management measures, the risk of accidental environmental release is very low. In addition, as it is a virus without replicative capacity, the environmental risk resulting from accidental release can be considered minimal.

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

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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