MVA.HTI and ChAdOx1.HTI

B/ES/18/20

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

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/18/20
(c) Date of acknowledgement of notification: 13/07/2018
(d) Title of the project:

A Phase I, randomized, double-blind, placebo-controlled safety, tolerability and immunogenicity study of candidate HIV-1 vaccines DNA.HTI, MVA.HTI and ChAdOx1.HTI in early treated HIV-1 positive individuals (AELIX-002).

(e) Proposed period of release:
ChAdOx1.HTI - Start date: 02/2019 - Finish date: 07/2020

2. Notifier

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

In collaboration with IrsiCaixa AIDS Research Institute
Hospital Universitari Germans Trias i Pujol Carretera de Canyet s/n
08916 Badalona (Barcelona)

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 Environment, 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)

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

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

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

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

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

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

The standard stability (shelf life) allocated to the MVA-based vaccines produced by IDT Biologika under cGMP conditions is 36 months when stored at -70ºC and stability tests are repeated for MVA.HTI annually

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

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

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

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

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

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

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

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

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).

MVA.HTI and ChAdOx1.HTI

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

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

MVA.HTI will be released in the HIVACAR EudraCT clinical trial 2017-000566-30 (pending request) in ES, BE, FR and DK

MVA.HTI and ChAdOx1.HTI will be released in the AELIX-003 EudraCT 2018-002125-30 clinical trial (B / ES / 18/21 - application under evaluation) only in ES.

In addition MVA.HTI has already been released as the initial phase (ie in AELIX-002AB) of the same test that is the subject of this EudraCT file 2017-000532-34 (B / ES / 16/11 and authorized) only in ES.

MVA.HTI and ChAdOx1.HTI

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 -
- Notification number

MVA.HTI and ChAdOx1.HTI will be released in the AELIX-003 EudraCT 2018-002125-30 clinical trial (B / ES / 18/21 - application under evaluation) only in ES.

In addition MVA.HTI has already been released as the initial phase (ie in AELIX-002AB) of the same test that is the subject of this EudraCT file 2017-000532-34 (B / ES / 16/11 and authorized) only in ES.

MVA.HTI and ChAdOx1.HTI

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

Yes (.) No (X)

If yes:

- Member State of notification -
- Notification number -

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

There is no scientific reason to expect that the use of HTI as an insert in the viral vector will change its distribution characteristics, shedding or replicative capacity compared to other inserts used in the same MVA. The MVA has been used extensively in clinical trials, both as direct administration or cell therapy strategies. MVA. HTI and ChAdOx1.HTI are not expected to survive as they are found exclusively in the cytoplasm of the cell and are unable to produce vector particles in human cells outside the site of inoculation.

Both vectors are organisms attenuated for replication, not propagative, so no effect is expected on other humans, flora or fauna near or far to the area of release. Genetically modified viral vaccines are not capable of surviving, establishing, disseminating or displacing other organisms. MVA is not pathogenic to animals or plants while ChAdOx1 is not normally found in humans although traces of infection of other adenovirus serotypes can be found in most people where they cause only a limited pathology such as a cold or sometimes pneumonia, croup, bronchitis and in rare cases diseases such as gastroenteritis, conjunctivitis, cystitis (Wold et al., 2013; Athanasopoulos et al., 2017) (Wold et al., 2013; Athanasopoulos et al., 2017).

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.

With all the above, the probability that any of the GMOs become persistent and invasive in natural habitats is very low. The spontaneous reversion of MVA to vaccinia virus competent for replication has never been documented. Neither has reversion or complementation of adenoviral vectors been observed in clinical trials of gene therapy or vaccination. The consequences of these environmental risks are considered low or highly unlikely, in the context of the proposed measures for the use of vaccine content.

There is a history of the release of highly similar products in Spain, such as the GMO MVA.HIVconsv under file B / ES / 12/10 and B / ES / 15/12. During the release period, no incident or accident was recorded. Biodistribution studies of the GMO were carried out to ensure non-

dissemination in the environment through the bodily fluids of the patients participating in the trial. The research group proposed to perform PCR detection of the HIVconsv insert in urine samples collected at 24 hours and 7 days post vaccination with the MVA.HIVconsv. The results were negative, and no DNA (HIVconsv) was found in any of the urine samples analyzed. Then these results show that a viral shedding is not likely (Edmund Wee, 2015).

There is also a history of the release of highly similar products in Spain, such as the OMG ChAdV63.HIV under file B / ES / 12/09 and B / ES / 12/10, as well as the OMG 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: 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)

ChAdY25

1. Recipient or parental organism characterisation:

ChAdY25

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

(select one only)

(select one only)

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

(specify phylum, class)

other, specify

ChAdY25

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 from chimpanzee feces and was passed 6 times in HEp-2 cells before the initial characterization. The complete sequence of its genome is available as access 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 the hexon and Y25 fiber, as well as the simian adenovirus SAdV-23, are grouped separately with other E-members of human adenovirus.

2. Name

(i)	order and/or higher taxon	Adenoviridae
(ii)	genus	Mastadenovirus
(iii)	species	
(iv)	subspecies	
(v)	strain	Chimpanzee adenovirus Y25
(vi)	pathovar (biotype, ecotype, race, etc.)	
(vii)	common name :	Chimpanzee adenovirus Y25

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 (**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 (.)

(ii) No (**X**)

(iii) Not known (.)

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

Yes (.) No (.)

(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 …

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:

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 the previous section 5a).

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.HTI

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

ChAdOx1.HTI

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, 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:

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 293 M9.S cells in the presence of its deleted genes.

(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

MVA and ChAdY25

(a) ability to form structures enhancing survival or dormancy:

(i)	endospores	(.)	
(ii)	cysts	(.)	
(iii)	sclerotia	(.)	
(iv)	asexual spores (fungi)		(.)
(v)	sexual spores (funghi)		(.)
(vi)	eggs	(.)	
(vii)	pupae	(.)	
(viii)	larvae	(.)	
(ix)	other, specify		

Not applicable

(b) relevant factors affecting survivability:

Survival of the MVA or ChAdY25 is not expected as they are found exclusively in the cytoplasm of the cell and are unable to produce vector particles in human cells outside the site of inoculation. The bioactivity of both at room temperature decays logarithmically. It is susceptible to various chemical agents such as sodium hypochlorite 1% and 2% glutaraldehyde, used as disinfectants, and has shown sensitivity to heat inactivation. A completely effective elimination is achieved by autoclaving at 121°C for 15 minutes.

10. (a) Ways of dissemination

MVA and ChAdY25 remain localized in the cell cytoplasm until the destruction of the cell. According to clinical studies, there has been no spread of the vectors, which are supposed to be exclusively located at the point of injection.

(b) Factors affecting dissemination

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 **MVA.HTI and ChAdOx1.HTI**

(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 (.)

If no, go straight to question 5.

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.

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)

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)

(c)

(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.

ChAdY25

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, specif:	

MVA.HTI
Method: 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
Method: 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.

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

6. Composition of the insert

(a) Composition of the insert

HTI:

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

(8%) and Nef (3%) (see Figure 3).

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.

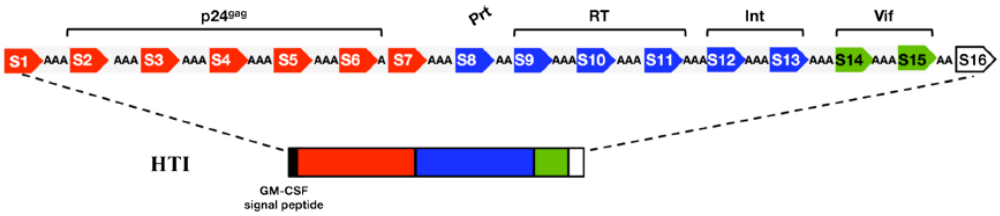

Figure 1: The HTI protein is composed of 16 individual segments arranged linearly and linked via 1–3 alanine amino acid linkers and contains the GM-CSF signal peptide for better secretion. Total length of HTI protein is 529 aa, including alanine linkers

(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 (Mothe et al., 2011)..

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.*,

2015a)

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) (Mothe *et al.*, 2015b).

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

(d) Location of the insert in the host organism

- on a free plasmid (.)
- integrated in the chromosome (X)
- other, specify

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

(e) Does the insert contain parts whose product or function are not known?
Yes (.) No (X)
If yes, specify …

D. Information on the organism(s) from which the insert is derived

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

(a) to which of the following organisms:

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

(b) are the donated sequences involved in any way to the pathogenic or harmful properties of the organism
Yes (.) No (X) Not known (.)

If yes, give the relevant information under Annex III A, point II(A)(11)(d):

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

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

Lentiviruses are typically restricted in their host range, although naturally or

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

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

4. Is the donor organism classified under existing Community rules relating to the protection of human health and the environment, such as Directive 90/679/EEC on the protection of workers from risks to exposure to biological agents at work?

Yes (X) No (.)

If yes, specify

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

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

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

E. Information relating to the genetically modified organism

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 …

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

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.

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

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

Specify: The same as before (section E-a)

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

ChAdOx1.HTI does not have replication capacity in cells that do not express the E1 region of the adenovirus

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

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

Specify …

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

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 and thus reduced compared to wild type adenovirsues and the parenteral strain.

(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 or pathology. The MVA was used as a vaccine against smallpox in the 1970s at the end of the eradication campaign in 120.000 people without serious adverse events. MVA.HTI maintains the same characteristics of pathogenicity as MVA. The effects are limited to those arising from the initial infection of receptive cells locally.

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 (Dicks *et al.*, 2012).

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.

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

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

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

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

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

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

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

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

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

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).

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 above. Pathogenicity of MVA.HTI does not differ from MVA in that there is no known pathogenicity for MVA.

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, 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
There are no techniques planned to detect and identify the GMOs 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 (Mothe *et al.*, 2015b).

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 identity tests are carried out including:

- 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)

The GMOs have been developed as therapeutic vaccine candidates for HIV-1. GMO release is necessary to conclude the phase I clinical trial in Spain with MVA.HTI and ChAdOx1.HTI (in earlier phases of the stud, subjects were also administered MVA.HTI after priming with the plasmid DNA.HTI in subjects uninfected with HIV-1.

The proposed study, “A Phase I, Randomized, Double-Blind, Placebo-Controlled Safety, Tolerability and Immunogenicity Study of Candidate HIV-1 Vaccines DNA.HTI, MVA.HTI and ChAdOx1.HTI in Early Treated HIV-1 Positive Individuals (AELIX-002)”, will assess the safety

and immunogenicity of the GMOs MVA.HTI and ChAdOx1.HTI in a third phase of the study (AELIX-002C) in which participants who already received a placebo or a regimen of DNA.HTI and MVA.HTI in an early phase (AELIX-002A and AELIX-002B phases; identical design) will be boosted with the GMOs. The AELIX-002A and AELIX-002B vaccination schedule included DNA.HTI at 4 mg administered at 0, 4 and 8 week, followed by the administration of MVA.HTI at 2 x 10^8 pfu at weeks 12 and 20. AELIX-002A and AELIX-002B are covered in application B/ES/16/11.

In the third part of the study (AELIX-002C, covered exclusively by this application) the vaccination schedule consists of a dose of 5.0 x 10^{10} Vp of ChAdOx1.HTI in weeks 0 and 12 and a single dose of MVA.HTI in week 24 An important inclusion criterion is that for the transition from AELIX-002A and B to AELIX-2C (they are the same subjects), the participants must have received the last dose of MVA.HTI of the DDDMM regime at least 180 days before starting CCM in AELI-002C. This will minimise possible recombination.

The vaccines were be administered intramuscularly. The total study population includes 45 subjects.

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

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

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

2. Is the site of the release different from the natural habitat or from the ecosystem in which the recipient or parental organism is regularly used, kept or found?

Yes (X) No (.)

If yes, specify …

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.Information concerning the release and the surrounding area

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

The vaccinations will be conducted at the Unidad Polivalente de Investigación Clínica (UPIC), located on the 2nd floor of the maternal building in Hospital Universitari Germans Trias i Pujol, located in Ctra. Canyet, s / n, 08916 Badalona. First, the medication preparation room, type BSL-1 (4.4 m^2) will be used. The administration to patients will be carried out in the adjoining consultations.

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

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

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

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

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

3. Method and amount of release

(a) Quantities of GMOs to be released:

A maximum of 45 subjects (30 treated subjects and 15 subjects treated with placebo) meeting the inclusion and exclusion criteria will be included. Thirty (30) of these will receive MVA.HTI and ChAdOx1.HTI at weeks 0, 12 and 24. The remaining 15 will receive placebo.

In total it is estimated to administer a maximum of 60 vials of ChAdOx1.HTI with a dose of 5.0×10^{10} Vp per vaccination (two administrations; one vial per volunteer per administration) and 30 vials of MVA.HTI at 2×10^{8} pfu per vaccination (one administration; one vial per volunteer per administration).

(b) Duration of the operation:

Each vaccination takes a few minutes. The recruitment period is estimated to require 12 weeks. All patients will be followed 68 weeks after the initial vaccination (from Baseline up to 44 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 GMO is released for clinical use only.

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

GMOs are manufactured according to GMP and packaged in hermetically sealed vials and properly labelled. The vials will be sent under GMP conditions 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 prepare the vaccines use 'universal precautions' and sterile techniques (gloves, masks and disposable gowns). The product shall be prepared under aseptic conditions in a BL-1 room for drug preparation at UPIC in appropriate compliance for the preparation of injectables. GMOs will be thawed at room temperature and drawn up into a 1 mL insulin syringe using a validated closed system transfer device (CSTD). The area used for the preparation for injection will be decontaminated before and after manipulation with Safe Seft® and 70º alcohol

After preparation, GMOs will be administered in a contiguous room at the UPIC. The staff that will administer the product to patients will use 'universal precautions'. 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 digluconate and will be covered properly after the injection. All used material is considered group III sanitary waste and will be managed as such. Particularly, gloves, mask and gown will be discarded specific hermetically sealed yellow containers for group III sanitary waste. Used vials will be returned to pharmacy in closed containers and destroyed following the protocols pharmacy service.

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

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

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

5. 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

1. Name of target organism (if applicable)

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

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

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

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

The possibility of gene transfer to other species is minimal under the conditions of the proposed release to the GMO.

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

ChAdOx1.HTI
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 adenoviruses 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: -
In the case of OMG (ChAdOx1.HTI) is a chimpanzee recombined adenovirus, alive without replication capacity 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 the release modalities that 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.

(a) likely consequences of gene transfer:

None, given that the HTI is a sequence designed exclusively for the induction of specific T cell response through 26 overlapping peptides of the HIV 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.HTI
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
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 during follow-up after the last injection in the study (see details in attached protocol synopsis).

2. Methods for monitoring ecosystem effects

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

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

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

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

5. Duration of the monitoring

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

6. Frequency of the monitoring

Not applicable.

I. Information on post-release and waste treatment

1. Post-release treatment of the site

The virus suspension 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 digluconate 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 and decontaminated before disposal.

3. (a) Type and amount of waste generated

It is expected to generate the following waste: vaccination vials (90 vials in total; 45 people in total recruited, 30 treated with ChAdOx1.HTI twice each and 30 treated once with MVA.HTI), 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.

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.

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.

REFERENCES

Antoine, G, Scheiflinger, F, Dorner, F and Falkner, FG (1998). "The complete genomic sequence of the modified vaccinia Ankara strain: comparison with other orthopoxviruses." Virology **244**(2): 365-396.

Athanasopoulos, T, Munye, MM and Yanez-Munoz, RJ (2017). "Nonintegrating Gene Therapy Vectors." Hematol Oncol Clin North Am **31**(5): 753-770.

Baldo, A, van den Akker, E, Bergmans, HE, Lim, F and Pauwels, K (2013). "General considerations on the biosafety of virus-derived vectors used in gene therapy and vaccination." Curr Gene Ther **13**(6): 385-394.

Bett, AJ, Prevec, L and Graham, FL (1993). "Packaging capacity and stability of human adenovirus type 5 vectors." J Virol **67**(10): 5911-5921.

Borducchi, EN, Cabral, C, Stephenson, KE, Liu, J, Abbink, P, Ng'ang'a, D, Nkolola, JP, Brinkman, AL, Peter, L, Lee, BC, Jimenez, J, Jetton, D, Mondesir, J, Mojta, S, Chandrashekar, A, Molloy, K, Alter, G, Gerold, JM, Hill, AL, Lewis, MG, Pau, MG, Schuitemaker, H, Hesselgesser, J, Geleziunas, R, Kim, JH, Robb, ML, Michael, NL and Barouch, DH (2016). "Ad26/MVA therapeutic vaccination with TLR7 stimulation in SIV-infected rhesus monkeys." Nature **540**(7632): 284-287.

Dicks, MD, Spencer, AJ, Edwards, NJ, Wadell, G, Bojang, K, Gilbert, SC, Hill, AV and Cottingham, MG (2012). "A novel chimpanzee adenovirus vector with low human seroprevalence: improved systems for vector derivation and comparative immunogenicity." PLoS One **7**(7): e40385.

Directive 2000/54/EC. "Directive 2000/54/EC of the European Parliament and of the Council of 18 September 2000 on the protection of workers from risks related to exposure to biological agents at work (seventh individual directive within the meaning of Article 16(1) of Directive 89/391/EEC)" (Last Updated 18/09/2000). European Commission (EC), Brussels, Belgium. Retrieved 27/07/2018 from https://eur-lex.europa.eu/legal-content/EN/TXT/PDF/?uri=CELEX:32000L0054&from=EN.

Drexler, I, Heller, K, Wahren, B, Erfle, V and Sutter, G (1998). "Highly attenuated modified vaccinia virus Ankara replicates in baby hamster kidney cells, a potential host for virus propagation, but not in various human transformed and primary cells." J Gen Virol **79 (Pt 2)**: 347-352.

Edmund Wee (2015). Assessment of shedding of the ChAdV63.HIVconsv and MVA.HIVconsv vaccines after immunization in the urine of vaccine recipients in clinical trial BCN 01. UK, University of Oxford.

EMEA/273974/2005. "Guideline on non-clinical testing for inadvertent germline transmission of gene transfer vectors" (Last Updated 16/11/2006). European Medicines Agency (EMA), London, UK. Retrieved 02/06/2015 from http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/10/WC500003982.pdf.

Ewer, K, Rampling, T, Venkatraman, N, Bowyer, G, Wright, D, Lambe, T, Imoukhuede, EB, Payne, R, Fehling, SK, Strecker, T, Biedenkopf, N, Krahling, V, Tully, CM, Edwards, NJ, Bentley, EM, Samuel, D, Labbe, G, Jin, J, Gibani, M, Minhinnick, A, Wilkie, M, Poulton, I, Lella, N, Roberts, R, Hartnell, F, Bliss, C, Sierra-Davidson, K, Powlson, J, Berrie, E, Tedder, R, Roman, F, De Ryck, I, Nicosia, A, Sullivan, NJ, Stanley, DA, Mbaya, OT, Ledgerwood, JE, Schwartz, RM, Siani, L, Colloca, S, Folgori, A, Di Marco, S, Cortese, R, Wright, E, Becker, S, Graham, BS, Koup, RA, Levine, MM, Volkmann, A, Chaplin, P, Pollard,

AJ, Draper, SJ, Ballou, WR, Lawrie, A, Gilbert, SC and Hill, AV (2016). "A Monovalent Chimpanzee Adenovirus Ebola Vaccine Boosted with MVA." N Engl J Med **374**(17): 1635-1646.

Ferreira, TB, Alves, PM, Aunins, JG and Carrondo, MJ (2005). "Use of adenoviral vectors as veterinary vaccines." Gene Ther **12 Suppl 1**: S73-83.

Goossens, M, Pauwels, K, Willemarck, N and Breyer, D (2013). "Environmental risk assessment of clinical trials involving modified vaccinia virus Ankara (MVA)-based vectors." Curr Gene Ther **13**(6): 413-420.

Hillis, WD and Goodman, R (1969). "Serologic classification of chimpanzee adenoviruses by hemagglutination and hemagglutination inhibition." J Immunol **103**(5): 1089-1095.

Hogg, JC (2000). "Latent adenoviral infection in the pathogenesis of emphysema: the Parker B. Francis Lectureship." Chest **117**(5 Suppl 1): 282S-285S.

Im, EJ and Hanke, T (2004). "MVA as a vector for vaccines against HIV-1." Expert Rev Vaccines **3**(4 Suppl): S89-97.

Kovesdi, I and Hedley, SJ (2010). "Adenoviral producer cells." Viruses **2**(8): 1681-1703.

Le Gall, S, Stamegna, P and Walker, BD (2007). "Portable flanking sequences modulate CTL epitope processing." J Clin Invest **117**(11): 3563-3575.

Mayr, A, Stickl, H, Muller, HK, Danner, K and Singer, H (1978). "[The smallpox vaccination strain MVA: marker, genetic structure, experience gained with the parenteral vaccination and behavior in organisms with a debilitated defence mechanism (author's transl)]." Zentralbl Bakteriol B **167**(5-6): 375-390.

Meisinger-Henschel, C, Schmidt, M, Lukassen, S, Linke, B, Krause, L, Konietzny, S, Goesmann, A, Howley, P, Chaplin, P, Suter, M and Hausmann, J (2007). "Genomic sequence of chorioallantois vaccinia virus Ankara, the ancestor of modified vaccinia virus Ankara." J Gen Virol **88**(Pt 12): 3249-3259.

Meisinger-Henschel, C, Spath, M, Lukassen, S, Wolferstatter, M, Kachelriess, H, Baur, K, Dirmeier, U, Wagner, M, Chaplin, P, Suter, M and Hausmann, J (2010). "Introduction of the six major genomic deletions of modified vaccinia virus Ankara (MVA) into the parental vaccinia virus is not sufficient to reproduce an MVA-like phenotype in cell culture and in mice." J Virol **84**(19): 9907-9919.

Mensah, VA, Gueye, A, Ndiaye, M, Edwards, NJ, Wright, D, Anagnostou, NA, Syll, M, Ndaw, A, Abiola, A, Bliss, C, Gomis, JF, Petersen, I, Ogwang, C, Dieye, T, Viebig, NK, Lawrie, AM, Roberts, R, Nicosia, A, Faye, B, Gaye, O, Leroy, O, Imoukhuede, EB, Ewer, KJ, Bejon, P, Hill, AV, Cisse, B and group, M (2016). "Safety, Immunogenicity and Efficacy of Prime-Boost Vaccination with ChAd63 and MVA Encoding ME-TRAP against Plasmodium falciparum Infection in Adults in Senegal." PLoS One **11**(12): e0167951.

Mothe, B, Climent, N, Plana, M, Rosas, M, Jimenez, JL, Munoz-Fernandez, MA, Puertas, MC, Carrillo, J, Gonzalez, N, Leon, A, Pich, J, Arnaiz, JA, Gatell, JM, Clotet, B, Blanco, J, Alcami, J, Martinez-Picado, J, Alvarez-Fernandez, C, Sanchez-Palomino, S, Guardo, AC, Pena, J, Benito, JM, Rallon, N, Gomez, CE, Perdiguero, B, Garcia-Arriaza, J, Esteban, M, Lopez Bernaldo de Quiros, JC, Brander, C, Garcia, F and Group, R-S (2015a). "Safety and immunogenicity of a modified vaccinia Ankara-based HIV-1 vaccine (MVA-B) in HIV-1-infected patients alone or in combination with a drug to reactivate latent HIV-1." J Antimicrob Chemother **70**(6): 1833-1842.

Mothe, B, Hu, X, Llano, A, Rosati, M, Olvera, A, Kulkarni, V, Valentin, A, Alicea, C, Pilkington, GR,

Sardesai, NY, Rocafort, M, Crespo, M, Carrillo, J, Marco, A, Mullins, JI, Dorrell, L, Hanke, T, Clotet, B, Pavlakis, GN, Felber, BK and Brander, C (2015b). "A human immune data-informed vaccine concept elicits strong and broad T-cell specificities associated with HIV-1 control in mice and macaques." J Transl Med **13**(1): 60.

Mothe, B, Llano, A, Ibarrondo, J, Daniels, M, Miranda, C, Zamarreno, J, Bach, V, Zuniga, R, Perez-Alvarez, S, Berger, CT, Puertas, MC, Martinez-Picado, J, Rolland, M, Farfan, M, Szinger, JJ, Hildebrand, WH, Yang, OO, Sanchez-Merino, V, Brumme, CJ, Brumme, ZL, Heckerman, D, Allen, TM, Mullins, JI, Gomez, G, Goulder, PJ, Walker, BD, Gatell, JM, Clotet, B, Korber, BT, Sanchez, J and Brander, C (2011). "Definition of the viral targets of protective HIV-1-specific T cell responses." J Transl Med **9**: 208.

Rauschhuber, C, Noske, N and Ehrhardt, A (2012). "New insights into stability of recombinant adenovirus vector genomes in mammalian cells." Eur J Cell Biol **91**(1): 2-9.

Shizuya, H, Birren, B, Kim, UJ, Mancino, V, Slepak, T, Tachiiri, Y and Simon, M (1992). "Cloning and stable maintenance of 300-kilobase-pair fragments of human DNA in Escherichia coli using an F-factor-based vector." Proc Natl Acad Sci U S A **89**(18): 8794-8797.

Verheust, C, Goossens, M, Pauwels, K and Breyer, D (2012). "Biosafety aspects of modified vaccinia virus Ankara (MVA)-based vectors used for gene therapy or vaccination." Vaccine **30**(16): 2623-2632.

Wold, WS and Toth, K (2013). "Adenovirus vectors for gene therapy, vaccination and cancer gene therapy." Curr Gene Ther **13**(6): 421-433.

Zhang, SC, Martin, E, Shimada, M, Godfrey, SB, Fricke, J, Locastro, S, Lai, NY, Liebesny, P, Carlson, JM, Brumme, CJ, Ogbechie, OA, Chen, H, Walker, BD, Brumme, ZL, Kavanagh, DG and Le Gall, S (2012). "Aminopeptidase substrate preference affects HIV epitope presentation and predicts immune escape patterns in HIV-infected individuals." J Immunol **188**(12): 5924-5934.