ChAdV63.HIVconsv
B/ES/12/09

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

January 12th 2012

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/12/09
(c)	Date of acknowledgement of notification	31/01/2012
(d)	Title of the project	

Safety and Immunogenicity of ChAdV63.HIVconsv and MVA.HIVconsv candidate HIV-1 vaccines in recently HIV-1 infected individuals with early viral suppression after initiation of antiretroviral therapy (HAART)

(e)	Proposed period of release	From June 2012 until June 2013 (last expected administration of the GMO).

2. Notifier

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

3. GMO characterisation **ChAdV63.HIVconsv**

(a) Indicate whether the GMO is a:

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

specify phylum, class …

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

The final GMO ChAdV63.HIVconsv is a life recombinant replication-incompetent chimpanzee adenovirus (ChAdV63) that harbors the codifying sequence for the HIV-1 specific T-cell immunogen HIVconsv.

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

ChAdV-63 is stable adenovirus unable to integrate their DNA in the host genome. Genetic stability is verified in different steps of the process of production to demonstrate the integrity of the vector and the insert (restriction pattern and sequencing of the genome), purity, biological potency and safety (analysis of the replicative competent adenoviruses) on the primary viral stock (PVS), working viral stock (WVS) and the final filtered product. The working virus stock is obtained at passage 3, purified by two CsCl gradient centrifugations, titred and stored at -80C until use. Individual purified lots are packaged in glass and stored at -80C until use.

PCR analyses are used to demonstrate the presence of the HIVconsv antigen coding sequence and absence as well as to rule out the introduction of mutations or deletions.

Parental adenoviruses are stable in nature. Four products have been made using the same basic AdChV-63 vector backbone so far, expressing ME-TRAP, MSP1, AMA1 and HIVconsv. The first manufactured ChAdV63-63-vectored product was AdCh63 ME-TRAP made in February 2007. The clinical lot has been retested in plaque assays to measure virus infectivity as a determinant of potency showing stability annually since then.

An initial shelf life of 12 months has been assigned to the ChAdV63.HIVconsv vaccine when stored at -80C, the intended storage temperature. The stability of the clinical batch will be monitored on an annual basis, by testing for virus titre (infectivity)

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

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

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

Yes (X) No (.)
If yes:
- Member State of notification United Kingdom (GB)
- - *Notification number* ***In the clinical trial HIV-CORE002; Eudract: 2010-018439-16, the same GMO was notified as 'contained use' in GB.***

Please use the following country codes:
Austria AT; Belgium BE; Germany DE; Denmark DK; Spain ES; Finland FI; France FR; United Kingdom GB; Greece GR; Ireland IE; Iceland IS; Italy IT; Luxembourg LU; Netherlands NL; Norway NO; Portugal PT; Sweden SE

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

Yes (.) No (X)

If yes:

- Member State of notification …
- Notification number B/../../…

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

There is no available data on the environmental impact of the release of the ChAdV63.HIVconsv, as the first in humans clinical trial using the GMO is being undergone at this time in GB. However, there are no scientific reasons to suspect that the insert HIVconsv in the viral vector could modify the characteristics of distribution, sheeding or replicative capacity in comparison with other inserts used in the same adenoviral vector, or in other human adenovirus. Therefore, the chances of ChAdV63.HIVconsv becoming persistent and being invasive in natural environment are low based on:

The potential environmental impact could become from the following:
-Breakage of a vaccine vial during handling or transport
-Accidental spillage during immunisation procedures
-Leakage grom the vaccination site after immunisation
-Incorrect disposal of dressings used to cover the immunisation site.

The only characteristic of the GMO vaccine which might result in potential hazard to the environment is the potential transfer of genetic material between the vaccine and other organisms. However, ChAdV63 is a non-replicative, non-propagative vector. The GMO vaccine is not able to survive, establish, disseminate and/or displace other organisms, and is not pathogenic to animals or plants. The products of gene expression –the insert HIVconsv- is constituted by 14 fragments of HIV-1 genome which are not implied in the pathogenicity of the virus; moreover, it does not contain any native entire protein. Therefore it is non-hazardous and no harmful effects on other organisms are envisaged.

The consequence of the hazard is considered negligible due to the control measures incorporated into the procedures proposed for the release.

Defective recombinant adenovirus have been used extensively in clinical trials, either through direct administration or cell therapy strategies (contained in the cells) The majority of the studies have not detected viral release in biological samples (sputum, saliva, urine, feces) and whenever detected through urine or saliva, it disappears in few days from administration. It is important to highlight that in any case adenovirus detected had replicative capacity that suggested occurrence of *in vivo* recombination phenomena (Grace, 2000)

In a previous study developed in Ireland to assess the potential environmental impact of a GMO based on ChAd63 expressing a malaria protein (CS), the risk was established as

low/acceptable. There is significant literature on safety of vaccines using these specific vectors in animals and humans in the field of malaria. It is not expected that the ChAdV63.HIVconsv has an environmental impact superior to other previously used adenoviruses.

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

1. Recipient or parental organism characterisation:

Chimpanzee adenovirus 63

(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)	**Adenoviridae**
(ii)	genus	**Mastadenovirus**
(iii)	species	…
(iv)	subspecies	…
(v)	strain	**Serotype 63 from chimpanzee adenovirus with deletions in the E1 and E3 regions and substitution of the native E1 by the ORF6 from human adenovirus 5 (HAdV-5)**
(vi)	pathovar (biotype, ecotype, race, etc.)	…
(vii)	common name	**Chimpanzee adenovirus 63, ChAd63**

3. Geographical distribution of the organism

(a) Indigenous to, or otherwise established in, the country where the notification is made:

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

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

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

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

(ii) No (X)
(iii) Not known (.)

(c) Is it frequently used in the country where the notification is made?
Yes (.) No (X)

(d) Is it frequently kept in the country where the notification is made?
Yes (.) No (X)

4. Natural habitat of the organism

(a) If the organism is a microorganism

water (.)
soil, free-living (.)
soil in association with plant-root systems (.)
in association with plant leaf/stem systems (.)
other, specify …

The main host of adenoviruses is human and minimal infectious dose is 150 ufp through intranasal or fecal-oral transmission. It has a worldwide distribution without seasonal incidence. The host of chimpanzee adenoviruses is the chimpanzee; it produces same kind of infections in apes.

The ChAdV63 virus is a replication-incompetent chimpanzee adenovirus modified in the laboratory. It is not found in natural ecosystems. It can be grown in human cells HEK-293 (cell line transformed with human adenovirus 5 specially used for the propagation of viruses with deletions of key genes for replication, such as the E1 and E3)

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

5. (a) Detection techniques
The infectivity of the virus is assayed using an anti-hexon antibody that has been shown to react with the AdChV-63 hexon protein. HEK 293 cells are infected with

virus and fixed after 48 hours. The fixed samples are then stained using a cross-reactive hexon antibody. Transduced cells expressing adenovirus hexon protein are then visualised and counted. The titre of the virus is expressed in infectious units per millilitre.

(b) Identification techniques
As before

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

Yes (X) No (.)

If yes, specify

The ChAdV63 is replication-deficient and a chimpanzee species and therefore considered not to be pathogenic. Replication-deficient adenoviruses lacking EI are classified as Biological Safety Level 1.

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

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

If yes:

(a) to which of the following organisms:

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

(b) give the relevant information specified under Annex III A, point II. (A)(11)(d) of Directive 2001/18/EC

Adenoviruses are classified as Class 2 under Directive 93/88/EEC due its limited pathogenicity. Human adenoviruses commonly cause asymptomatic infection in humans, although they can cause respiratory tract infections, gastrointestinal discomfort or eye infections. They are more common in children. The incubation period is between 1 and 10 days. The majority of the population is seropositive for more than one subspecies of adenovirus and can quickly produce neutralizing antibodies. The principal host is human, and the minimal infectious dose of 150 plaque-forming units intranasally. Normally, the host enters the respiratory tract or the eyes through aerosols produced by infected individuals. Most infections are minor in nature and self-limiting. Adenoviruses are usually not integrated and do not persist in lymphoid tissues.

Adenovirus infectious in nonhuman primates (NHP) are also predominantly subclinical, except for some cases of pneumonia in immunosuppressed SIV-infected animals. Defective recombinant adenoviruses have been widely used in clinical trials, both as direct administration or cell therapy strategies (contained in the

interior of cells). The non-replicative adenoviruses lacking the E1 are classified as biosafety level class 1.

The vector of the vaccine, ChAdV63 is replication-deficient and a chimpanzee species and therefore considered not to be pathogenic. It does not present a risk of integration or activation of latent provirus.

8. Information concerning reproduction

(a) Generation time in natural ecosystems:
Not applicable. Does not generate in natural ecosystems
(b) Generation time in the ecosystem where the release will take place:
Not applicable. Will not generate effectively
(c) Way of reproduction: Sexual .. Asexual
Not applicable
(c) Factors affecting reproduction:
Not applicable

9. Survivability

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

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

(b) relevant factors affecting survivability:

None expected survival of ChAdV-63 given its lack of replication capacity. The bioactivity of adenovirus decays at room temperature logarithmically. Adenoviruses are susceptible to different chemical agents such as sodium hypochlorite 1% and 2% glutaraldehyde, used as disinfectants, and has shown sensitivity to heat inactivation. Thus, a completely effective elimination is achieved by autoclaving at 121 ° C for 15 minutes.

10. (a) Ways of dissemination

The minimal infectious dose of adenovirus is 150 plaque forming units intranasally.
Is transmitted directly by oral contact or droplets of Pflügge; indirectly by handkerchiefs, eating utensils and other items recently contaminated with respiratory secretions of an infected person, it exists the possibility of spread via fecal-oral route.

However, as mentioned before, the GMO ChAdV63.HIVconsv is modified to be non-replicative and therefore non-pathogenic. Its spread is supposed to be limited at the point of injection.

(b) Factors affecting dissemination

The ability to spread is dependent on the dose, aerosol formation and the proximity of the contact as well as the safety measures taken in the research environment stated 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)

Not applicable. This is the first use in our country. In GB, in the clinical trial HIV-CORE002, EudraCT: 2010-018439-16, the same GMO was notified as 'contained use'

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

ChAdV63.HIVconsv is a non-replicating defective recombinant adenovirus. The vector ChAdV63 system was obtained by introducing deletions into the E1 and E3 regions of the ChAd63 genome, and substituting the native E4 region with human adenovirus type 5 (AdH5) open-reading frame (ORF) 6 (Okairos, unpublished) so it is not competent to replicate. The ORF coding for the immunogen HIVconsv was inserted under the control of the human cytomegalovirus intermediate/early promoter and the bovine growth hormone polyadenylation signal, with its expression is controllable through the bacterial tetracycline-sensitive repressor (Stanton *et al*, 2008). The transgene expression cassette was inserted into the E1 of the ChAd63 backbone by homologous recombination in *E. coli* strain 5183. The resulting ChAdV63.HIVconsv virus was rescued inan HEK293 cell derivative line expressing the *tet* repressor, designated Procell-92 (Okairos, unpublished) by plasmid transfecting the pChAdV63.HIVconsv pre-Adeno plasmid DNA and was then furher amplified by serial passaging.

With all these modifications is intended that those cells that are infected with the immunogen can express HIVconsv for activation of HIV-1 specific immune responses against the HIV-1 virus, but there is no viral replication.

3. (a) Has a vector been used in the process of modification?

Yes (X) No (.)

If no, go straight to question 5.

(b) If yes, is the vector wholly or partially present in the modified organism?
Yes (X) No (.)

If no, go straight to question 5.

4. If the answer to 3(b) is yes, supply the following information

(a) Type of vector

plasmid	(X)
bacteriophage	(.)
virus	(.)
cosmid	(.)
transposable element	(.)
other, specify	…

(b) Identity of the vector

The modification of the vector where the chimpanzee adenovirus 63 has been cloned includes the following:

1. Virus isolation and amplification in HEK 293 cells
2. Cloning into the plasmid pChAdV63 virus
3. E1 gene deletion
4. E3 gene deletion
5. Substitution of the native E4 region by the ORF 6 of the human adenovirus type 5

(c) Host range of the vector
The plasmid will replicate in laboratory strain of E.coli. The final adenovirus vector can then only replicate in cells which express E1 (HEK 293 for instance)

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

antibiotic resistance (.)
other, specify … E1 and E3 deletions in the genome of ChAdV63, substitution of the native E4 region by the ORF6 of human adenovirus type 5.

The final construct ChAdV63.HIVconsv includes an epitope for the antibody Pk located at the C-terminus of the immunogen that can be detected by immunofluorescence in HEK293 after transfection.

Indication of which antibiotic resistance gene is inserted
…

(e) Constituent fragments of the vector

ChAdV63 with deletions in E1 and E3 and replacement of native E4 by ORF 6 of the human adenovirus type 5.

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

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

5. If the answer to question B.3(a) and (b) is no, what was the method used in the process of modification?

(i)	transformation	(.)
(ii)	microinjection	(.)
(iii)	microencapsulation	(.)
(iv)	macroinjection	(.)
(v)	other, specify …	

6. Composition of the insert

(a) Composition of the insert

1. Substitution the native E4 gene by the ORF 6 of the human adenovirus type 5
2. Insertion of the transgene encoding the immunogen HIVconsv.

The novel immunogen, termed HIVconsv (for conserved), was designed as a chimaeric protein and assembled from the most highly conserved domains among the HIV-1 clade A, B, C and D proteomes (Létourneau *et al*, 2007). It was decided that the HIVconsv gene should be approximately 2.5 kbp in size, making it suitable for most currently used genetic vaccine vectors and likely to support high protein expression.
It encodes 14 of the most conserved regions of HIV-1 genome, each between 27 and 128 aminoacid. (Létoruneau et al, 2007) plus a 15th fragment harbouring an epitope recognized by CD8 + T cells from rhesus macaques (Mamu-A * 01, Allen *et al*, 2000) and mice (H-2D^{d} and L^{d}, Takahashi *et al*, 1998) respectively. Also an epitope of a mAb monclonal PK antibody was added to the C-terminus of the immunogen (Hanke *et al*, 1992) to facilitate detection of protein expression. HIVconsv gene transcription is controlled by the CMV promoter and a polyadenylated signal of BGH with its expression is controllable through the bacterial tetracycline-sensitive repressor (Stanton *et al*, 2008). The expression cassette was inserted into the E1 region by homologous recombination in E.Coli strain 5183.

(b) Source of each constituent part of the insert

1. ORF6 of human adenovirus type 5
2. The primary donor transgene sequences HIVconsv are 14 fragments of HIV-1 genome highly conserved between clades A, B, C and D.

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

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

The protein HIVconsv was chemically synthesized by GeneArt (Germany)

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

Both the CMV promoter and the BGH polyadenylation sequence are dedicated to promote the expression of the gene of interest, allowing the recognition by the RNA polymerase for transcription and increasing the stability of mRNA molecules synthesized.
HIVconsv immunogen function is the induction of HIV-1 specific immune responses of cytotoxic T cells directed against the regions covered in the HIVconsv insert, which can help to control the HIV-1 infection effectively.
The last segment includes a macaque epitope, a murine epitope and the mAb PK epitope. Their function is to detect the expression of the immunogen in transfected cells as well as in immunogenicity preclinical studies in mice and / or macaques.

(d) Location of the insert in the host organism

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

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

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

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

1. Indicate whether it is a:

viroid	(.)
RNA virus	(X)
DNA virus	(.)
bacterium	(.)
fungus	(.)
animal	
- mammals	(.)
- insect	(.)
- fish	(.)
- other animal	(.)
(specify phylum, class)	…
other, specify	…

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

2. Complete name

(i)	order and/or higher taxon (for animals)	…
(ii)	family name for plants	Retroviridae
(iii)	genus	*Lentivirus*
(iv)	species	Human
(v)	subspecies	fragments from clades A, B, C and D
(vi)	strain	…
(vii)	cultivar/breeding line	…
(viii)	pathovar	…
(ix)	common name	HIV-1, human immunodeficiency virus

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

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

If yes, specify the following:

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

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

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

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

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

Yes (X) No (.)

If yes, specify

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

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

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

E. Information relating to the genetically modified organism

ChAdV63.HIVconsv

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

(a) is the GMO different from the recipient as far as survivability is concerned?
Yes (X) No (.) Not known (.)
Specify …
ChAdV63.HIVconsv cannot replicate in cells which do not express the adenoviral E1 regions.

(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 (.) Not known (.)
Specify …
ChAdV63.HIVconsv cannot replicate in cells which do not express the adenoviral E1 regions.

(c) is the GMO in any way different from the recipient as far as dissemination is concerned?
Yes (X) No (.) Not known (.)
Specify …

Since the replication capacity of ChAdV63.HIVconsv replication is restricted to the HEK293 cell line, this greatly limits their ability to spread, since unlike the wild type human or chimpanzee adenoviruses, the infection can not be transmitted through its natural host.

(d) is the GMO in any way different from the recipient as far as pathogenicity is concerned?
Yes (X) No (.) Not known (.)
Specify …

ChAdV63.HIVconsv can not replicate, therefore it is not pathogenic.
The inability to replicate from the originally infected cells prevents from spreading to other cells, which completely changes the pathogenicity. Therefore, no forms of adenoviral disease have been described in patients treated with adenoviral vectors in the context of clinical trials.

2. Genetic stability of the genetically modified organism

The virus is stable. Tests are performed to test the genetic stability in different steps of the final batch production through vector analysis as well as testing the integrity of the insert (restriction pattern and sequencing of the complete viral genome), purity, potency and biological safety (competent replicative adenovirus analysis), both the primary viral stock (PVS), the working virus stock (WVS) obtained at passage 3

purified by two CsCl gradient centrifugations and the formulated and filtered final product.

PCR sequencing confirms the presence of the antigen encoding for the HIVconsv insert and rules out the production of mutations or deletions.

The parental ChAdV-63 has proven to be stable. So far, four GMO using the same ChAdV-63 original vector have been produced with the same cloning strategy and production (expressing the inserts ME-TRAP, MSP1, AMA1 and HIVconsv). The first ChAdV63.ME-TRAP has demonstrated stability from 2007 to the present (annual measurements).

Stability controls of the ChAdV63.HIVconsv batch prepared for preclinical toxicology and stability analyses showed no degradation of the material in plaque formation assays over a period of 9 months. It has been assigned an initial shelf life of 12 months when stored at -80 ° C. The stability of the clinical trial batch is monitored annually by titrating the virus in plaque forming assays which measure both infectivity and potency.

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

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

(a) to which of the following organisms?

humans (.)
animals (.)
plants (.)
other …

(b) give the relevant information specified under Annex III A, point II(A)(11)(d) and II(C)(2)(i)

Same as before.
The final GMO ChAdV63.HIVconsv is a replication-deficient and a chimpanzee species and therefore considered not to be pathogenic.

Adenoviruses are classified as Class 2 under Directive 93/88/EEC due its limited pathogenicity. Human adenoviruses commonly cause asymptomatic infection in humans, although they can cause respiratory tract infections, gastrointestinal discomfort or eye infections. They are more common in children. The incubation period is between 1 and 10 days. The majority of the population is seropositive for more than one subspecies of adenovirus and can quickly produce neutralizing antibodies. The principal host is human, and the minimal infectious dose of 150 plaque-forming units intranasally. Normally, the host enters the respiratory tract or the eyes through aerosols produced by infected individuals. Most infections are minor in nature and self-limiting. Adenoviruses are usually not integrated and do not persist in lymphoid tissues.

Adenovirus infectious in nonhuman primates (NHP) are also predominantly subclinical, except for some cases of pneumonia in immunosuppressed SIV-infected animals. Defective recombinant adenoviruses have been widely used in clinical trials, both as direct administration or cell therapy strategies (contained in the interior of cells). The non-replicative adenoviruses lacking the E1 are classified as biosafety level class 1.

The vector of the vaccine, ChAdV63 is replication-deficient and a chimpanzee species and therefore considered not to be pathogenic. It does not present a risk of integration or activation of latent provirus.

4. Description of identification and detection methods

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

…

(b) Techniques used to identify the GMO

…

There are any technique planned to detect and identify the GMO in the environment in the context of the clinical trial.

Most of the methods used in the final characterization of ChAdV63.HIVconsv during development, control processes, production and release tests are standardized methods previously used and validated (as appropriate for the use of the material in early clinical phases) for different AdCh63 and other viral constructs. Briefly, after cloning the insert of interest in the vector, the DNA is extracted from the virus sample. By PCR, DNA specific sequences are amplified. PCR primers are designed in such ways that are unique to the transgene HIVconsv. The amplification of a DNA fragment of appropriate size confirms the identity of the insert.
The expression of the HIVconsv protein from the ChAdV63.HIVconsv is demonstrated by immunofluorescence in transiently transfected HEK293 T cells using the Pk antibody epitope located at the C-terminus of the protein.
The immunogenicity of ChAdV63.HIVconsv is demonstrated in Balb/C and reshus macaques.

F. Information relating to the release

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

The GMO ChAdV63.HIVconsv has been developed as a therapeutic vaccine candidate for HIV-1. Its release is necessary to implement the first phase I clinical trial in our country with ChAdV63.HIVconsv vaccine candidate in patients with recently HIV-1 infection. Its first use in humans (Phase I in healthy individuals) is taking place at present time in United Kingdom.

The present study, the trial (EudraCT 2011-000846-39) aims to evaluate the safety and immunogenicity of ChAdV63.HIVconsv and MVA.HIVconsv vaccine candidates administered intramuscularly in two different vaccination schedules: 0-8 weeks or 0-24 weeks, in patients recently infected with HIV-1 with early virologic suppression at 6 months after initiation of antiretroviral treatment regimen with Tenofovir / Emtricitabine and Raltegravir. If security is acceptable and exploratory immunogenicity studies are promising we will move forward into a phase II trial.

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

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

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

Yes (X) No (.)

If yes, specify … ChAdV63 chimpanzee adenoviruses have not been described in our geographical location.

3. Information concerning the release and the surrounding area

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

HIV units from 2 university hospitals:
-Hospital Universitario Germans Trias i Pujol, Crta Canyet s/n, 08916, Badalona
-Hospital Clínic, Villarroel, 17, 08036 Barcelona.

(b) Size of the site (m^2): … m^2

(i) actual release site (m^2): Room of Day care hospital of the center: 15m^2

(ii) wider release site (m^2): same and never >15m^2

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

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

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

Not applicable, the effect on the flora and fauna is not possible.

4. Method and amount of release

(a) Quantities of GMOs to be released:

20 patients meeting the inclusion and exclusion criteria will be included. The first 10 patients will be assigned to the vaccination in the 0-24 weeks schedule (Branch A). The following 10 patients will be assigned to the vaccination in the 0-8 weeks schedule (Branch B).

In total it is estimated to administer a maximum of 20 vials (extra patients counting for loss) with a dose of 5 x 10^{10} vp (viral particles) per vial.

(b) Duration of the operation:

Each vaccination takes about few minutes. The recruitment period (and dosing of ChAdV63.HIVconsv) of the 20 participants is estimated to be made over a year. Total follow-up study will last 72 weeks.

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

The GMO is released for clinical use only
The personnel involved in the preparation of cell product works according to the conditions specified in the standards of Good Clinical Practice and Good Manufacturing Practices. GMP Laboratory located in Oxford, Great Britain, prepared and packaged the product in vials hermetically sealed and properly labeled. The vials will be imported from Britain kept at-80C and stored until use in the units of the participating pharmacy. The administration is under the responsibility of the investigator, according to a clinical protocol and respecting the rules of Good Clinical Practice.
The clinical staff who administer the vaccines use 'universal precautions' and sterile techniques (gloves, masks and disposable gowns). The product shall be prepared under aseptic conditions, in appropriate compliance to the preparation of injectables. The area used for the preparation for injection should be decontaminated before and after manipulation with standard disinfecting solution (eg, chlorine> 1.6 ° Cl, eg, 5 grams of active chlorine per liter of water)

The site of inoculation will be covered properly. The location (Day Hospital rooms) will be cleaned with sodium hypochlorite diluted to 1% immediately after the administration. The removal of contaminated material will be held in hermetically sealed yellow containers or in a special thick red bag with a sticker labeled medical waste - GROUP III.

All transfers of preparation should be undertaken using a closed container. Furthermore, employees will follow the clinic or hospital policy recommended standard for handling live virus vaccines.

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

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

Not applicable. All immunizations are going to be performed at the HIV unit of the hospitals involved.

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 same GMO is being used at present time in the first Phase I (HIV-CORE 002) in healthy individuals in United Kingdom. In GB the GMO has been listed as contained use. HIV-CORE 002 (EudraCT: 2010-018439-16) is a single-blind randomized placebo-controlled trial evaluating the safety of HIVconsv delivered by different regimens in healthy individuals: as 3 prime DNA formulations followed by a boost using ChAdV63 and MVA (DDDCM and DDDMC) or as a prime with ChAdV63 followed by boost with MVA (CM). So far (October 4, 2011), two individuals have received $5x10^9$ vp of ChAdV63.HIVconsv and eight individuals have received $5x10^{10}$ vp without significant adverse effects and excellent tolerability. In total, 24 individuals are planned to receive ChAdV63.HIVconsv at doses of $5x10^{10}$ vp (such as those used in the our proposed study). Vaccinations are still ongoing at present.
It has not been previously used in our country.

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

1. Name of target organism (if applicable)

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

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

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

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

The possibility of gene transfer to other species is minimal under the conditions of the proposed release to the GMO. As it is a defective virus unable to replicate is not expected any interaction with other organisms in the environment.

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

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

Give details
…

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

As it is a defective virus unable to replicate, as well as delivery that is expected in this study, it is unlikely that the GMO can be released to the ecosystem and its spread from the release site.

6. Complete name of non-target organisms which (taking into account the nature of the receiving environment) may be unintentionally significantly harmed by the release of the GMO

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

Not applicable

7. Likelihood of genetic exchange in vivo

(a) from the GMO to other organisms in the release ecosystem:
Highly unlikely

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

(c) likely consequences of gene transfer:

Nil, because the HIVconsv is a chimeric protein designed exclusively for the induction of specific cellular responses through the union of 14 fragments of the HIV-1 genome, so it is not pathogenic.

8. Give references to relevant results (if available) from studies of the behaviour and characteristics of the GMO and its ecological impact carried out in stimulated natural environments (e.g. microcosms, etc.):
None

9. Possible environmentally significant interactions with biogeochemical processes (if different from the recipient or parental organism)
Not applicable

H. Information relating to monitoring

1. Methods for monitoring the GMOs

Defective recombinant adenoviruses have been widely used in clinical trials, both as direct administration or through cell therapy strategies (contained in the interior of cells). Most studies have not detected viral release in biological samples (sputum, saliva, urine, feces) and the few that have detected the GMO in the urine or saliva, usually disappear after two days of administration. It is important to note that in no case were detected adenoviruses able to replicate to indicate the occurrence of recombination events *in vivo* (Grace, 2000).

Based on this information, it has not been planned in the current proposal or in the HIV-CORE02 already undergoing, any specific viral detection of ChAdV63.HIVconsv in biological fluids or blood.

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

2. Methods for monitoring ecosystem effects

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

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

Same as above

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

5. Duration of the monitoring

The safety assessment will be made over the participation of patients in the clinical trial and up to 48 weeks after the last injection.

6. Frequency of the monitoring

Monitoring visits during which safety is evaluated will be held a week after vaccination and then every 12 weeks until final follow up. Addition monitoring visits will be conducted during each injection of the GMO.

I. Information on post-release and waste treatment

1. Post-release treatment of the site

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

2. Post-release treatment of the GMOs

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

3. (a) Type and amount of waste generated

Vaccine vials, needles, gloves, gowns, masks, bandages / tape (20 patients total)

3. (b) Treatment of waste

The remaining waste material in contact with the GMO, are considered specific medical waste (Group III), and managed as such. Will be introduced to the following vessels:

A. Infectious Waste Solids.
Bag should always be in red as Medical Waste Group III.

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

J. Information on emergency response plans

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

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

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

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

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

Not applicable

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

In the case of skin contact, energetic and scrubbed wash with iodine solution with 4% will be done.
In case of eye contact, a wash with saline for a period not less than 15 minutes will be made. The subject will be evaluated by an ophthalmologist as soon as possible.
In case of accidental puncture, immediate wash with plenty of soap and water will be performed, and then the puncture site will be disinfected with iodine solution to 9-12% for at least 5 minutes with sodium hypochlorite solution of 10 g / l.
Patients included in the clinical trial will be monitored as provided by the protocol according to standards of good clinical practice. Adverse events will be registered and reported according detailed procedures in the protocol.
Due to the risk management procedures of accidental environmental release is very low.
In addition being the GMO a virus without replicative capacity, environmental risk consequent to accidental release is considered minimal.