SUMMARY NOTIFICATION INFORMATION FORMAT FOR THE RELEASE OF GENETICALLY MODIFIED ORGANISMS OTHER THAN HIGHER PLANTS IN ACCORDANCE WITH ARTICLE 11 OF DIRECTIVE 2011/18/EC

Study code #THV01-11-01

EudraCT #2011-006260-52

Sponsor:	THERAVECTYS Pasteur Biotop building 28 rue du Dr ROUX 75015 Paris - FRANCE
Investigational products:	Two lentiviral vectored vaccines
Products code:	THV01-1; THV01-2
Therapeutic indication:	HIV-1, clade B
Version number:	1.0
Release date:	23/12/2011

Abbreviations

DNA	Deoxyribonucleic acid
GMO	Genetically modified organism
HAART	Highly active antiretroviral therapy
HIV	Human immunodeficiency virus
LTR	Long terminal repeat
RNA	Ribonucleic acid
VSV.G	G protein of the vesicular stomatitis virus
WPRE	Woodchuck hepatitis virus posttranscriptional regulatory element

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PART 1 (COUNCIL DECISION 2002/813/EC)

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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 France

(b) Notification number B/FR/12/GT02

(c) Date of acknowledgement of notification 27/03/2012

(d) Title of the project

"A multi-center, randomized, double-blind, placebo-controlled Phase I/II trial to compare the safety, tolerability and immunogenicity of the therapeutic THV01 vaccination at $5.0X10^6$ TU, $5.0X10^7$ TU or $5.0X10^8$ TU doses to placebo in HIV-1 clade B infected patients under highly active antiretroviral therapy (HAART)"

(e) Proposed period of release (planned date for study completion) From 01/01/2012 until 31/07/2013

2. Notifier

Name of institution or company:

THERAVECTYS
Pasteur Biotop building
28 rue du Dr ROUX
75015 Paris - FRANCE

3. GMO characterisation

(a) Indicate whether the GMO is a:

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

- mammals (.)
- insect (.)
- fish (.)
- other animal (.)

specify phylum, class …

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

The THV01 investigational vaccine comprises two GMOs, THV01-1 and THV01-2, to be injected successively in HIV-infected patients.

THV01-1 and THV01-2 are two live recombinant viral vectored vaccines derived from the NL4-3 strain of the HIV-1. They are non-replicative and self-inactivating vectors due to a deletion of the U3 region in the 3' and 5' LTR. They are non-pathogenic as all accessory genes from the parental virus (HIV) have been removed.

Both GMOs encode the same HIV transgene, composed of clustered peptides and epitopes of the HIV-1 clade B, Gag, Pol and Nef proteins under the regulation of a THERAVECTYS patented human promoter. This transgene is non-functional.

The GMOs are pseudotyped by two different serotypes of the G protein of the vesicular stomatitis virus (VSV.G).

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

The GMOs are non-replicative; therefore their genetic stability is expected to be higher than replication competent GMO.

Genetic stability has been assessed:

- on the starting materials of the clinical batches: the identity of all plasmids used for the transient tri-transfection has been controlled by full length sequencing (i.e. the plasmids encoding the provirus; the plasmid encoding structural and enzymatic proteins and the 2 plasmids encoding the VSV-G proteins– as two different serotypes are used for THV01-1 and THV01-2);
- on the final drug products with identity of the GMOs demonstrated by transgene identity and integrity assessment. This is performed by sequencing the extracted DNA from transduced cells: cells have been transduced by the GMOs; then PCR on full vector length (from their 5' LTR to their 3' LTR) were performed followed by a sequencing of the amplified sequence.

Taken together, these tests guaranty genetic stability of the GMOs.

The GMOs are to be stored at -80°C +/- 10°C. Indeed, at this temperature, stability of the GMOs as detailed in the ICH Q1A (R2) "note for guidance on stability testing" and ICH Q5C "note for guidance on quality of biotechnological products" (i.e. the physical, chemical, biological and efficacy) is of at least 9 months (stability studies ongoing) whereas they are stable at 4°C for at least 30 hours and only a few hours at room temperature as assessed by previous studies (stability studies ongoing with the preclinical and clinical batches).

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) BE

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

Yes (.) No X

If yes:

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

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.

The impact of the THV01-1 and THV01-2 GMOs release on the environment is estimated to be very low as:

- Biodistribution studies -performed under GLP conditions- demonstrated that using the clinical administration route (intramuscular injection), the vectors remained localized at the injection site and draining lymph nodes without dissemination in blood (no integrated vector sequence detected) and urine (no vector sequences detected): **no GMOs shedding was detected**. Of note, **no vector sequence was detected in the gonads**.
- **No toxicity is expected following injection of the GMOs**. Indeed, even following injection of the maximal injectable dose, no relevant toxicity was detected during the nonclinical studies.
- **The risk of recombination leading to replication-competent vectors is almost inexistent** as the GMOs are depleted from the promoter sequence of the U3 region in the 3'- and 5'-LTRs, rendering the expression of the HIV transgene dependant of an internal promoter. Moreover, the probability for the GMOs to encounter wild-type HIV particles *in vivo*, leading to formation of replication competent viruses, is low because the intended targeted population is patients that have an undetectable level of viraemia (<50copies mL^{-1} using standard commercial assays) and thus very low amount of circulating viruses. Finally, there is a very low sequences homology between the GMOs and the wild-type virus and every effort have been made so that the probability of recombination is low (such as the use of a transgene sequence codon-optimized for human use and shuffling the HIV sequence compared to the wild type).

  Nevertheless, to assess whether replication competent vector has been generated during the manufacturing process, a specific test has been implemented on the drug product and is

part of the release tests. This test will be performed by a CRO that has expertise in the field (Texcell, Evry).

- **The risk of insertional mutagenesis leading possibly to activation of proto-oncogene or disruption of tumor suppressor gene leading to oncogenesis is very low** as the lentiviruses have been shown to not integrate preferentially near transcription start regions and as the promoter selected by THERAVECTYS is a human promoter devoid of an enhancer sequence. In addition, the GMOs will induce a cellular immune response that will lead to elimination of the transduced cells from the host.
- The GMOs will be administered to subjects in a clinical environment. Only authorized and well trained persons will be allowed to handle and administer the product. In addition, at all times, the GMOs will remain in closed vials except just prior injection when they will be sucked up in syringe. The vials are filled with 0.5mL extractable volume which represents 0.65mL of total GMO volume. Therefore, **the risk of accidental spread is considered to be almost inexistent**.
- Stability studies demonstrated that the GMOs are susceptible to common disinfectants or solutions used for decontamination (such as Hexanios G+R, bleach, ethanol 70% etc.) as well as UV light, irradiation, autoclaving and incineration. **The risk of environmental persistence is therefore very low**.

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

1. Recipient or parental organism characterisation:

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

(select one only)

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

other, specify …

2. Name

(i)	order and/or higher taxon (for animals)	Retroviruses
(ii)	genus	Lentivirus
(iii)	species	Human immunodeficiency virus (HIV)
(iv)	subspecies	HIV-1
(v)	strain	vector derived from the NL4-3 strain
(vi)	pathovar (biotype, ecotype, race, etc.)	…
(vii)	common name	Lentiviral vectors

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 (.) No X

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

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

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

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

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

4. Natural habitat of the organism

The parental organism is the Human immunodeficiency virus, NL4-3 strain. It is a replication-competent Human virus that can be transmitted during sexual contact, during direct blood contact (sheering needles, blood transfusions) or from mother to child (before or during birth or through breast milk). It is unable to infect other host than Human.

The recipient organism is not naturally found in the environment.

(a) If the organism is a microorganism
Not applicable

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

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

Not applicable

5. (a) Detection techniques

PARENTAL ORGANISM

Detection of an HIV infection in Human is usually done in two steps: a screening for HIV followed by a confirmation using another technique.

Detection technique for diagnosis: ELISA
Enzyme immunoassay that detects HIV antibodies (generally antibodies against the p24 HIV protein i.e. the capsid) is the most used assay to diagnose an HIV infection: it is easy, gives an answer quickly and its sensitivity and specificity is > 99%. Detecting the p24 protein instead of p24 antibodies allows diagnosing HIV infection only one week after it occurred.

Detection techniques used for confirmation
Western-blot
Western blot (WB) tests are generally performed to confirm ELISA results using serum, urine or saliva loaded on a gel; then, detection of the presence of several HIV-1 proteins is made using antibodies that target these proteins. They are not used as an initial HIV screening test because they give much higher false positive results than ELISA.

Immunohistochemistry/immunocytochemistry assay

The necessity of having a microscope makes this technique less commonly used than ELISA and WB assays.

HIV-1 nucleic acid assays

Quantification of HIV DNA or RNA in body fluids can be done using methods such as polymerase chain reaction (PCR), branched DNA (bDNA) testing, nucleic acid sequence-based amplification (NASBA) and nucleic acid testing (NAT). The most commonly used of these assays is the PCR: a DNA probe is used to detect HIV-1 proviral DNA after its amplification. In the reverse transcriptase PCR (RT-PCR), the first step is reverse transcription of the proviral DNA into RNA. These tests have a great sensitivity: levels of HIV RNA as low as 5-40 copies/mL can be detected.

Flow cytometry analysis

During the course of the infection, numeration of the lymphocytes can be performed by flow cytometry with labelled antibodies targeting various lymphocyte subpopulations (CD3, CD4, CD8, CD45…).

RECIPIENT ORGANISM

The recipient organism could be detected using the ELISA assays described in the section above.

(b) Identification techniques

Cf 5 (a) above

As the detection techniques are specific to the HIV, they also provide information regarding identification.

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

Yes (.) No X

If yes, specify

Human immunodeficiency viruses are classified as class 3 under the Directive 2000/54/EC ("protection of workers from risks related to exposure of biological agents at work").

However, the derived lentiviral vectors developed by the notifier are non-replicative, self-inactivating and non-pathogenic.

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

Yes (.) No X Not known (.)

If yes:

(a) to which of the following organisms:

humans (.)
animals (.)

plants (.)
other (.)

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

The GMOs developed by the notifier are not pathogenic or harmful. Indeed, extensive toxicity studies in rats, performed under GLP conditions, demonstrated that no toxicity was raised following injections of the maximal injectable dose of the THV01-1 or THV01-2; of note, macrotoxicity as well as microtoxicity was monitored. The lentiviral vectors do not migrate to vital organs or to gonads as they localized only at the injection site and draining lymph node.

In addition, all integrated vector sequences are eliminated within about 60 days.

The recipient organism is unable to replicate due to deletion of the promoter sequence in both LTR extremities.

Finally, there is no possibility of host to host transmission.

Therefore, the recipient organism is not considered to be pathogenic or harmful.

8. Information concerning reproduction

Not applicable for the recipient as it is a lentiviral vector not competent for replication. Parental organism is HIV.

(a) Generation time in natural ecosystems:

(b) Generation time in the ecosystem where the release will take place:

(c) Way of reproduction: Sexual .. Asexual ..

(c) Factors affecting reproduction:
…

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 …

(b) relevant factors affecting survivability:

HIV survivability outside Human body and at room temperature is of a few minutes. It can be destroyed by numerous chemical agents such as commercial detergents.

Survivability of the lentiviral vectors is ≥ 9 months at ≤ -70°C; ≥ 30 hours at 4°C. Lentiviral vectors can be destroyed using chemical agents such as bleach, Hexanios G+R, ethanol 70%, by irradiation, by autoclaving (121°C for 20min) and by incineration.

10. (a) Ways of dissemination

Dissemination of the HIV occurs through sexual or blood contact or during mother-to-child transmission.

Dissemination of the recipient organism is not expected as **no shedding has been detected** in body fluids: no integrated DNA sequences detected in blood using a reliable and sensitive qPCR (5-10 copies per ng of DNA tested) and no GMO RNA sequences detected in urine. In addition, the GMOs remained at the injection site and draining lymph node following intramuscular injection and they are not competent for replication and survivability at temperature above 4°C is quite low (few hours). **The potential for escape, dispersion or establishment of the lentiviral vectors developed by the notifier are consequently negligible.**

(b) Factors affecting dissemination

Not applicable for the THV01-1 and THV01-2 GMOs.

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)
…, B/../../…

Not applicable as this will be the first-in-human of the GMOs.

C. Information relating to the genetic modification

1. Type of the genetic modification

(i)	insertion of genetic material	X
(ii)	deletion of genetic material	X
(iii)	base substitution	(.)
(iv)	cell fusion	(.)
(v)	others, specify …	

2. Intended outcome of the genetic modification

The starting point of the modifications was to develop lentiviral vectors derived from the HIV that will be safe to be used as vaccines, taking advantage of the lentiviral vectors' ability to transduce non-mitotic cells. By targeting dendritic cells, this will indeed lead to triggering a cellular immune response, mandatory for an efficient control of numerous diseases such as HIV infection.

The GMOs are intended for the treatment of HIV-1 (clade B) infected patients. They are aimed at eliciting a cellular immune response that will enable patients to stop taking their therapies for a sustainable period of time.

The intended outcomes of the genetic modification are:

- to guaranty the safety of the lentiviral vectored vaccines in Human (deletion of the promoter and enhancer sequences in the U3 region of the 3' and 5' LTR; deletion of the HIV accessory genes that could be pathogenic);
- to broader the vector tropism and increase its stability during the manufacturing process (modification of the pseudotyping envelope);
- to generate a cellular immune response in host (insertion of HIV transgene derived from clustered epitopes from Gag, Pol and Nef).

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

Yes X No (.)

If no, go straight to question 5.

(b) If yes, is the vector wholly or partially present in the modified organism?

Yes X No (.)

If no, go straight to question 5.

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

(a) Type of vector

plasmid	X
bacteriophage	(.)
virus	(.)
cosmid	(.)

transposable element (.)
other, specify …

(b) Identity of the vector

- Plasmid encoding the provirus: pFlap-H01-W. This plasmid is derived from the pRRLhPGK.GFP.SIN-18, from which the entire proviral sequence has been extracted and cloned inside a pVAX1 plasmid backbone (Invitrogen Life technologies), to produce the kanamycin resistant pFLAP lentivector generation.

- Plasmid encoding the structural and enzymatic proteins: pGP encoding the *gag-pol-tat-rev* genes from the HIV-1 (alternative name used by the manufacturer: pThV-GP-N). This plasmid is derived from the pCMVΔR8.74, from which the NL4.3 derived *gag* pol gene has been replaced by the NDK (clade D) *gag pol* gene.

- Plasmids encoding the pseudotyping proteins: pEnv1 and pEnv2 (alternative name used by the manufacturer: pThV-Env1 and pThV-Env2). The VSV-G genes have been codon optimized for expression in human cells, and these genes have been cloned into pVAX1 plasmids (Invitrogen, life technologies).

- Cells used for the production of the GMOs: 293T cells. This cell line was bought from Genethon (MCB-293T.516 BPF) and a dCB was made. The CMO (Henogen Novasep) then produced a certified BPF HEK 293T WCB.

(c) Host range of the vector

Escherichia coli (for the plasmids)

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

antibiotic resistance
other, specify Antigen coding for the P24 HIV-1 protein

Indication of which antibiotic resistance gene is inserted

Antibiotic resistance genes are present in the plasmids used to generate the GMOs (ampicilline for the pGP and kanamycine for the pFlap-H01-W, pEnv1 and pEnv2).

However, **they are not contained in the insert of the GMOs**.

(e) Constituent fragments of the vector

i. **pFlap-H01-W**

Plasmid encoding the provirus (non-pathogenic recombinant proviral DNA derived from HIV-1, strain NL4-3), in which an expression cassette is cloned. This plasmid encompasses:

- Coding sequences: The insert contains the transgene developed by the notifier, the promoter for transgene expression and sequences added to increase the

transgene expression and to allow the lentiviral vector to transduce all cell types including non-mitotic ones.

- The transgene: The transgene is composed of cluster of epitopes of the HIV-1 Gag, Pol and Nef proteins. The Gag fragment is depleted of its myristillation site and of the matrix sequence; the Pol and Nef fragments are short polypeptides devoid of any functionality and known as cytotoxic T lymphocytes epitopes. The sequence has been codon-optimized for human use.
- Promoter: The promoter is a human promoter patented by THERAVECTYS. It is devoid of any enhancer sequence and is inducible at a basal level in all cells.

▪ Non coding sequences and expression signals:

- the Long Terminal Repeat sequences (LTR) with the whole *cis*-active elements for the 5'LTR (U3-R-U5) and the deleted one for the 3'LTR hence lacking the promoter region (ΔU3-R-U5), for the transcription and integration mechanisms;
- the encapsidation sequences (SD and 5'Gag);
- the central PolyPurine Tract/Central Termination Site for the nuclear translocation of the vectors;
- WPRE-THV: Woodchuck hepatitis virus Post transcriptional Regulatory Element. This WPRE sequence has been modified by the notifier and is deleted of the X protein 60 first amino acids and from the related promoter.

ii. **pGP**

Plasmid coding for the structural lentiviral proteins (GAG, POL, TAT and REV) derived from HIV-1 and used in *trans* for the encapsidation of the lentiviral provirus. This plasmid encompasses:

▪ Coding sequences:

Polycistronic gene *gag-pol-tat-rev*, coding for the structural (matrix MA, capsid CA and nucleocapsid NC), enzymatic (protease PR, integrase IN and reverse transcriptase RT) and regulatory (TAT and REV) proteins.

▪ Non coding sequences and expression signals:

- Minimal promoter from the cytomegalovirus (CMV) for the transcription initiation;
- Polyadenylation signal from the insuline gene for the transcription termination;
- HIV-1 Rev responsive Element (RRE) participating to the nuclear export of the packaging RNA.

iii. **pEnv1 and pEnv2**

Plasmids coding for glycoproteins G from the Vesicular Stomatitis Virus (VSV-G) Indiana strain (pEnv1) and New-Jersey strain (pEnv2), used for the pseudotyping of the lentiviral vectors

- Coding sequences:
  - Codon optimized VSV-G gene for an optimal expression in human cells
- Non Coding sequences and expression signals:
  - Minimal promoter from the cytomegalovirus (CMV) for the transcription initiation;
  - Polyadenylation signal for the transcription termination.

iv. 293T cells

293T cells are human kidney embryonic cells, immortalized by insertion of the adenoviral E1A and E1B genes, and transformed with the SV40T antigen, allowing the preservation and the replication in episomal forms of plasmids bearing the SV40 origin of replication.

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

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

Transient tri-transfection of the pFlap-H01-W, pGP and the pEnv1 or pEnv2 in 293T cells.

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

The insert contains the transgene developed by the notifier, the promoter for transgene expression and sequences added to increase the transgene expression,

increase the stability of the RNA and to allow the lentiviral vector to transduce all cell types including non-mitotic ones.

The transgene
The transgene is composed of cluster of epitopes of the HIV-1 Gag, Pol and Nef proteins. The Gag fragment is depleted of its myristillation site and of the matrix sequence; the Pol and Nef fragments are short polypeptides devoid of any functionality and known as cytotoxic T lymphocytes epitopes.

The sequence has been codon-optimized for human use.

Promoter
The promoter is a human promoter patented by THERAVECTYS. It is devoid of any enhancer sequence and is inducible at a basal level in all cells.

Non coding sequences and expression signals

- Long terminal repeats (LTRs)

The Long Terminal Repeat sequences (LTR) with the whole *cis*-active elements for the 5'LTR (U3-R-U5) and the deleted one for the 3'LTR hence lacking the promoter region (ΔU3-R-U5), for the transcription and integration mechanisms.

- cPPT/CTS

The central PolyPurine Tract/Central Termination Site for the nuclear translocation of the vectors.

- WPRE-THV (woodchuck hepatitis virus post transcriptional regulatory element)

  This WPRE sequence has been modified by THERAVECTYS and is deleted of the X protein 60 first amino acids and from the related promoter.

- Encapsidation sequences

  The encapsidation sequences (SD and 5'Gag).

- BGH polyadenylation site

  Bovine Growth Hormone polyadenylation signal, to stabilize the RNA.

(c) Source of each constituent part of the insert

The plasmids were produced by Plasmid factory and are high quality grade plasmids.

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

The transgene
The transgene will be expressed and processed into epitopes exposed at the cells' surface. Its intended function is to generate a cellular immune response in the host which will enable the patient to stop taking its antiretroviral therapies for a sustainable period of time while still controlling viral replication.

Promoter
Presence of an internal promoter is mandatory as the LTR regions have been deleted of the HIV promoter.

Long terminal repeats
LTRs are mandatory for reverse transcription of the RNA encoded by the GMOs into DNA that will integrate into the host genome.

cPPT/CTS

The cPPT/CTS sequences will enable formation of the Flap sequence during reverse transcription following transduction. The Flap is a DNA triplex formed during reverse transcription of the HIV. It is mandatory for the infection of non-dividing cells. Vectors that incorporate the Flap are therefore able to transduce all kind of cells, even non mitotic ones. The intended function of the Flap in the GMOs presented here is to enable them to transduce the dendritic cells, non-mitotic cells and the most potent of the antigen presenting cells that re the dendritic cells.

WPRE-THV

The woodchuck hepatitis post regulatory element (WPRE) is a post transcriptional element known to increase the expression of a transgene. As there is still some debate regarding the safety of the protein X sequence encoded, the GMOs incorporate a version of the WPRE sequence deleted from this sequence.

(d) Location of the insert in the host organism

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

The insert is localized in the lentiviral vector sequence.

(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

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	…

2. Complete name

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

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):

The donated sequence is composed of clustered epitopes of HIV proteins, non-functional.

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

Human immunodeficiency viruses are classified as class 3 under the Directive 200/54/EC.

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

Yes (.) No X Not known (.)

Donor organism and recipient organism both derived from HIV.

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

GMO is the lentiviral vector developed by the notifier with the insert incorporated. This does not modify the survivability.

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

Yes (.) No X Unknown (.)

Specify

Cf 1a above.

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

Yes (.) No X Not known (.)

Specify

Cf 1a above.

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

Yes (.) No X Not known (.)

Specify

Both the GMOs and the recipient are not pathogenic.

2. Genetic stability of the genetically modified organism

The GMOs are non-replicative; therefore their genetic stability is expected to be higher than replication competent GMO.

Genetic stability has been assessed:

- on the starting materials of the clinical batches: the identity of all plasmids used for the transient tri-transfection has been controlled by full length sequencing (i.e. the plasmids encoding the provirus; the plasmid encoding structural and enzymatic proteins and the 2 plasmids encoding the VSV-G proteins– as two different serotypes are used for THV01-1 and THV01-2);
- on the final drugs products with identity of the GMOs demonstrated by transgene identity and integrity assessment. This is performed by sequencing the extracted DNA from transduced cells: cells have been transduced by the GMOs; then PCR on full vector length (from their 5' LTR to their 3' LTR) were performed followed by a sequencing of the amplified sequence;

Taken together, these tests guaranty genetic stability of the GMOs providing the specified storage conditions are met (i.e. -80°C +/- 10°C). Indeed, at this temperature, stability of the GMOs as detailed in the ICH Q1A (R2) "note for guidance on stability testing" and ICH Q5C "note for guidance on quality of biotechnological products" (i.e. the physical, chemical, biological and efficacy) is of at least 9 months (stability studies ongoing) whereas they are stable at 4°C for at least 30 hours.

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)

Absence of toxicity

Extensive single acute and successive injections of the GMOs during toxicity studies demonstrated complete absence of macro- and microscopic toxicity of the GMOs, event at the maximal injectable dose (ethic limitation due to maximal authorized volume in the specific animal). Control and vaccinated animals displayed similar behaviour, had similar weight gain and food consumption. Hepatic enzymes were monitored (ASAT; ALAT) as well as numerous inflammation markers (>10 cytokines quantified). Microscopic examination of organs listed in the relevant guidelines did not showed any toxicity at the microscopic level.

Formation of replication competent vectors

The risk of recombination leading to replication-competent vectors is almost inexistent as the GMOs are depleted from the promoter sequence of the 3'-LTR U3 region, rendering the expression of the HIV transgene dependant of an internal promoter. The promoter selected by THERAVECTYS is a human promoter devoid of any enhancer sequence. Moreover, the probability for the GMOs to encounter wild-type HIV particles *in vivo*, leading to formation of replication competent viruses, is low because the intended targeted population is patients that have an undetectable level of viraemia and thus very low amount of circulating viruses. Nevertheless, to assess whether replication competent vector has been generated during the manufacturing process, a specific test has been implemented during the manufacturing process and is part of the release tests.

Insertional mutagenesis

The risk of insertional mutagenesis leading possibly to activation of proto-oncogene or disruption of tumor suppressor gene leading to oncogenesis is very low as the lentiviruses have been showed not to integrate preferentially near transcription start sites. In addition, the GMOs will induce a cellular immune response that will lead to elimination of the transduced cells from the host.

Antibiotic resistance patterns

The GMOs do not confer antibiotic resistance properties.

Allerginicity

The GMOs have not been shown to trigger allergenic reaction during the nonclinical development studies performed to date.

4. Description of identification and detection methods

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

Detection of the GMOs is performed following DNA extraction using commercially available kit and using an optimized Taqman quantitative polymerase chain reaction (qPCR), based on the exonuclease activity of the 5'-3' Taq polymerase. The probe is an oligonucleotide specific to the backbone of our lentiviral vector' sequence flanked in both extremities by fluorophores. This specific assay is currently used to measure the titers of effective particles (able to transduce and integrate into the nucleus of cells).

Nonspecific detection of the GMOs can also be performed using commercially available kit to quantify the HIV capsid (p24 protein) by ELISA. The p24 can also be quantified by FACS following intracellular staining of the protein with specific commercially available antibody and by western blotting.

(b) Techniques used to identify the GMO

The identity of the GMOs can be demonstrated using direct and indirect methods as follows:

- Vector and transgene identity and integrity: cells are transduced by the GMOs; then PCR on full vector length (from their 5' LTR to their 3' LTR) is performed followed by sequencing;
- Pseudotyping envelope' identity: to assess the envelope identity, seroneutralisation assays is performed (using polyclonal antibodies specific to each envelope) followed by qPCR to quantify the remaining titer. It is therefore an indirect method to assess the neutralizing effect of the antibodies generated against each serotype and hence to control the envelope serotype.

F. Information relating to the release

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

The release is made in the context of the Phase I/II clinical trial THV01-11-01.

The objectives of this trial are comparison of the safety, tolerability and immunogenicity of the THV01 therapeutic treatment against HIV versus placebo in HIV-1 infected patients under HAART.

The expected benefit is to enable vaccinated patients to stop taking their antiretroviral therapies for a sustainable period of time, therefore increasing their quality of life, reducing the secondary effects related to antiretroviral treatment and strain resistance emergence as well as diminishing the treatment costs.

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 (.) No X

If yes, specify …

3. Information concerning the release and the surrounding area

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

The GMOs will be released at the following investigators sites:

- CIC Cochin-Pasteur (Dr LAUNAY)
  27 rue du Faubourg Saint Jacques
  75679 Paris cedex 14 – France ;
- CHU Saint-Etienne (Pr LUCHT)
  Hôpital Nord-CHU Saint Etienne
  42055 Saint Etienne Cedex 02- France ;
- CHU Purpan (Pr MASSIP)
  Place du dr Baylac
  31059 Toulouse cedex 9.

(b) Size of the site (m^2): … m^2
(i) actual release site (m^2): … m^2
(ii) wider release site (m^2): … m^2

Patients will be treated on an outpatient basis. Injections of the GMOs will be performed on a conventional hospital room.

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

None

(e) Flora and fauna including crops, livestock and migratory species which may potentially interact with the GMO
None

4. Method and amount of release

(a) Quantities of GMOs to be released:

This trial plans to enroll 36 patients: 27 patients will be vaccinated and 9 will receive the placebo.

Patients will be randomized in 4 groups (as three doses of the THV01 treatment will be assessed):

- 9 patients randomized in group 1 will receive intramuscular injections of the THV01-1 and THV01-2 at $5.0X10^{6}$TU, 8 weeks apart;
- 9 patients randomized in group 2 will receive intramuscular injections of the THV01-1 and THV01-2 at $5.0X10^{7}$TU, 8 weeks apart;
- 9 patients randomized in group 3 will receive intramuscular injections of the THV01-1 and THV01-2 at $5.0X10^{8}$TU, 8 weeks apart.

Taking into account the fact that there will be 3 centers in France and 2 in Belgium with similar recruitment envisaged, the estimated quantity of GMOs (THV01-1 and THV01-2) that will be released in France is: $6.0\ 10^{9}$ TU. This represents about 104 vials that have come into contact with the GMOs (either product vials or diluent vials used to perform extemporaneous dilutions before injection) and a total volume of GMO of approximately 28mL.

(b) Duration of the operation:

Duration of the study is 36 weeks (not taking into account the follow-up phase). However, patients will receive the THV01-1 and THV01-2, 8 weeks apart.

Duration of the operation lasts from the first investigational product injection (1st patient in group 1 injected) to the last investigational product injection (12th patient of the group 3 injected).

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

The GMOs are intended for release only at the investigational sites. They are supplied in vials closed with a bromobuthyl rubber stopper. Each vial is for single use and is supplied individually with individual secondary packaging.

The GMOs will be stored in a secure area with access limited to the investigator and authorized site staff, and must not be used for any other purpose other than the study. Administration of the study treatment will be carried out only by authorized site staff. This information will be specified appropriately on the labels.

Injection of the GMOs must be performed according to good clinical practice and the study protocol. The vials should remain hermetically closed at all times; to perform the product

sample, the seal should not been removed; only the top of the seal should be lift up. For extemporaneous dilutions, closed containers will also be provided. Therefore, the GMOs will remain at all times in closed containers except when in the syringe just prior injection.

Finally, as no shedding has been detected in blood or urine following injection of the maximal injectable dose in animals, no shedding is expected during the clinical trial.

5. Short description of average environmental conditions (weather, temperature, etc.)
Not relevant

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.
None

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	…
(iii)	genus	Hominidae
(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)

The expected effect of the THV01 treatment is the induction of a cellular immune response, mediated by cytotoxic T lymphocytes, against cells transduced by the lentiviral vectors and those infected by the HIV. This immune response should result in a long-lasting control of HIV replication allowing the patients to stop their antiretroviral therapies for a sustainable period of time: a "functional cure".

The mode of action of the GMOs can be summarized as follows:

i. The first step of the mode of action of the lentiviral vectors is formation of endocytosis vesicles of the plasmic targeted cell that will allow entry of the vectors into the host cell.

ii. After entry in the cell' cytoplasm, uncoating occurs followed by release of the vector' RNA. Reverse transcription takes place, then translocation of the DNA into the cell nucleus and integration of the provirus into the cell genome.

iii. This results in expression of the HIV antigen encoded by the vectors and its processing. The resulting epitopes, bound to MHC proteins (major histocompatibility complex), are exposed at the cell' surface where they will be recognized by cytotoxic T lymphocytes.

iv. Within a few days to weeks, all transduced cells presenting the epitopes at their surface are cleared by the host immune system.

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

The GMOs presented here are not expected to come into contact with other species than Human patients enrolled in the clinical trial, providing the specific procedures are followed.

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

Yes (.) No X Not known (.)

Give details

The GMOs are not competent for replication. Therefore no competitiveness or invasiveness is expected.

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

As specific procedures will be put in place during the trial and as the GMOs will remain at all times in closed containers except just prior injection, the GMOs are not predicted to be in contact with any ecosystems. Moreover, the GMOs are non-replicative vectors, it is anticipated that even if they come in contact with a non-targeted organism, no spread from this non-targeted organism will occur and hence no establishment.

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 (cf section above)

(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:

It is anticipated that the genetic exchange from the GMOs to other organisms in the release ecosystem is very unlikely.

Indeed and as already mentioned above (sections F4C; G5), the GMOs will be released during a clinical trial. They will be stored in secured access area and will remain in hermetically closed containers until injection into patients.

In addition, biodistribution studies demonstrated localization only at the injection site and draining lymph node, without shedding of the GMOs in blood or urine.

The only identified exchange that could occur would be in HIV infected patients, with wild-type virus. However, the patients eligible to this trial have undetectable viral load, thus minimizing the risk of recombination.

(b) from other organisms to the GMO:

No genetic exchange is expected between other organisms and the GMOs

(c) likely consequences of gene transfer:

The risk of recombination leading to replication-competent vectors is almost inexistent as the GMOs are depleted from the promoter sequence of the 3'-LTR U3 region, rendering the expression of the HIV transgene dependant of an internal promoter. The promoter selected

by THERAVECTYS is a human promoter devoid of any enhancer sequence. Moreover, the probability for the GMOs to encounter wild-type HIV particles *in vivo*, leading to formation of replication competent viruses, is low because the intended targeted population is patients that have an undetectable level of viraemia and thus very low amount of circulating viruses. Nevertheless, to assess whether replication competent vector has been generated during the manufacturing process, a specific test has been implemented during the manufacturing process and is part of the release tests.

The risk of insertional mutagenesis leading possibly to activation of proto-oncogene or disruption of tumor suppressor gene leading to oncogenesis is very low as the lentiviruses have been showed to integrate preferentially not near promoter region. In addition, the GMOs will induce a cellular immune response that will lead to elimination of the transduced cells from the host.

Taken together, the consequence of gene transfer is expected to be only the triggering of a cellular immune response which is the expected therapeutic effect of the GMOs.

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.):

No avalaible data

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

Monitoring could be performed to either detect the GMOs themselves, the integrated GMOs or their effect:

- Detection of the GMOs could be performed by RT-qPCR. However, survivability of the GMOs is of a few hours at room temperature (stability studies ongoing with the preclinical and clinical batches). Therefore, it does not seem relevant to perform monitoring of the GMOs themselves.
- Detection of the integrated GMOs will be performed during this Phase I/II trial by performing qPCR at several time points in blood sampled from vaccinated patients;
- Monitoring of the effects of the GMOs will be performed by physical examinations of patients as well as immunological and virological assessments (CD4+ T cell counts, cytokines, chemokines and integrins quantification, viral load and HIV DNA quantification);
- At all time, an inventory of the remaining vials, those used or destroyed and their respective location will be available.

Of note, no secondary transmission from Human to Human of the GMOs is expected.

2. Methods for monitoring ecosystem effects

As localization of integrated vectors' sequence was restricted to the injection site and draining lymph node, and because the release will be made in the context of a Human clinical trial, no specific method will be implemented to monitor the ecosystem effects of the GMOs.

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

As already mentioned, no genetic material is expected to be transferred from the GMO to other organisms. In case of suspected transmission, qPCR could be performed to detect any GMOs.

4. Size of the monitoring area (m^2)

Not relevant as the GMOs will be injected via intramuscular injection in conventional hospital room and as no transmission to ecosystem is expected.

5. Duration of the monitoring

Patients will receive the THV01-1 injection and the THV01-2 one, 8 weeks apart. Monitoring will be performed up to week 24 for all enrolled patients.

Patients will then stop their antiretroviral treatment and will be followed for an additional 12 weeks except in case of resuming therapies when they will be followed until date of HAART resumption.

Patients who have still not resumed therapies at week 36 will enter a follow-up phase which last 1 year except if patient has resumed earlier therapies.

6. Frequency of the monitoring

Treatment phase:
Monitoring visits are planned 1 and 6 weeks after the 1st injection; 1, 4 and 16 weeks after the second injection.

HAART interruption phase:
Monitoring visits are planned every 2 weeks for 2 months then every 4 weeks for 1 month or earlier in case of HAART resumption.

Follow-up phase
Patients who have not resumed therapy earlier will be followed every 4 weeks for 3 months then every 8 weeks for 9 months or earlier in case of HAART resumption.

I. Information on post-release and waste treatment

1. Post-release treatment of the site

Release of the GMOs will be performed in a clinical environment.

At all times, the GMOs will remain in closed containers except just prior injection where they will be sucked up in a syringe. Therefore, they should not come in contact with other material than the needle and the syringe to which it is connected.

If no accidental spilling has occurred, the site of the GMOs administration will be cleaned according to standards cleaning methods.

Patients will be followed on an outpatient basis. No particular precaution measures should be taken during the daily life of the patient once he/she has been discharged from the investigator' site.

2. Post-release treatment of the GMOs

The GMOs will be injected at three doses. For the lowest 2 doses (i.e. $5.0X10^6$ TU and $5.0X10^7$ TU), extemporaneous dilutions should be performed in a diluent provided in a closed vial.

The GMOs vials as well as the diluent ones should remain closed at all times (only the top of seal should be removed). Any remaining suspension, either in the GMOs originating vial or in the diluent vial will therefore remain inside the vials.

Return of all vials (those that contained the GMOs and the diluent ones) will be performed to the manufacturing site where they will be destroyed by incineration.

3. (a) Type and amount of waste generated

The GMOs will be injected at $5.0X10^6$ TU; $5.0X10^7$ TU and $5.0X10^8$ TU.

For the two lowest doses, 1 vial of GMO will be used per patient, per injection. However, extemporaneous dilutions will be performed using a diluent vial. Hence for the $5.0X10^6$ TU and $5.0X10^7$ TU doses, 2 vials will be contaminated with the GMOs plus 1 needle+syringe for the dilution and 1 needle+syringe to perform the injection.

For the highest dose, $5.0X10^8$ TU, 4 vials of the THV01-1 will be used and 7 vials of the THV01-2, per patient per injection. No dilution will have to be performed; therefore no additional waste except 1 needle and 1 syringe per injection.

Taking into account the fact that there will be 2 centers in Belgium and 3 in France; it is anticipated that the generated waste in France will be:

Dose (TU)	Waste per patient (2 injections) number of				Number of vaccinated patients	Estimated waste generated in France
	GMO vial	Diluent vial	Syringe	Needle		
$5.0X10^6$	2 (1+1)[1]	2	4	4	9	22 vials + 22 syringes + 22 needles
$5.0X10^7$	2 (1+1)[1]	2	4	4	9	22 vials + 22 syringes + 22 needles
$5.0X10^8$	11 (7+4)[1]	0	2	2	9	60 vials + 11 syringes + 11 needles

Figure 1: Estimated waste generated in France during the THV01-11-01 trial.
[1] Numbers in parenthesis represent the number of vial to be used for respectively the THV01-1 and the THV01-2 injections.

Hence, the total of the waste generated in France will be around: 104 vials that have come in contact with the GMOs, 55 syringes and 55 needles. Additional waste includes bandages and cottons.

3. (b) Treatment of waste

Vials that have come into contact with the GMOs will be shipped back to the manufacturing site where they will be stored in a restricted area until completion of the clinical trial. Upon order of the sponsor and after reconciliation, they will be destroyed according to a specific procedure, by incineration.

Needles and syringes as well as bandages and cottons used during the injection of the GMOs will be treated as biohazard waste at the investigational sites.

J. Information on emergency response plans

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

GMO projection on people
In case of people wearing protection clothes, spray disinfectant active on at least HIV over the concerned areas. Careful should be paid not to spray the disinfectant directly on the skin and the eyes. Protection clothes and hat should be thrown as biological waste to be incinerated.

Skin contamination
The skin area came in contact with the GMO should be immediately washed thoroughly with water for 15min then disinfected with bleach 2.6%.

GMO projection on the eyes
The eyes should be immediately washed thoroughly with water or physiological saline solution for 15min keeping the eyelids opened with eyes positioned face down. An ophthalmological examination should be performed as soon as possible.

Needle stick injury
Wash immediately and thoroughly with tap water and soap then rinse. Disinfect at least for 10min with bleach (1.3%). Rinse then abundantly with water.

Ingestion
In case of accidental ingestion of the GMO, vomiting should not be induced. A physician should be contacted immediately.

Close follow-up should be performed for at least 14 days.

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

Removal of the GMOs should be performed according to the procedure provided by THERAVECTYS at all investigators' sites which is briefly described below:

Workers should wear personal individual protection clothes. In case of accidental spilling of the GMOs (for instance in case of vial break or leak), the area should be recovered with paper towel. Hexanios G+R or any other strong disinfectant active on HIV should be added and left at least for 15min or as recommended by the manufacturer. The paper towels should then be removed using clamps and throw as a biological waste (to be incinerated). A second cleaning step should be performed using a disinfectant active on HIV.

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

Monitoring of the patients and of potential adverse events will be performed according to the study protocol.

In case of an undesirable effect recorded during the trial, the investigator and/or the safety review board should evaluate whether it is an adverse or a serious adverse event. If more than 2 dose limiting toxicities (defined as a grade 3 or higher adverse events) are monitored, trial will be stopped. In case of a life threatening (grade 4) adverse reaction, or in case of death, enrollment and treatment of ongoing patients will be immediately discontinued. Enrollment will only be resumed upon the decision of the sponsor if the safety review board can conclude that the causality of the event was unrelated or unlikely related to study treatment.