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/17/14**
(c) Date of acknowledgement of notification, **7/7/2017**
(d) Title of the project,

Clinical Trial Phase I/II study to evaluate the safety and efficacy of the infusion of autologous CD34^{+} cells ex vivo transduced with a lentiviral vector carrying the Integrin beta 2 (ITGB2) gene for patients with Leukocyte Adhesion Deficiency Type I (LAD-I)

(e) Proposed period of release **December 2017-December 2020**

2. Notifier

Name of institution or company:
Centro de Investigaciones Energéticas Medioambientales y Tecnológicas (CIEMAT)

3. GMO characterisation

(a) Indicate whether the GMO is a:

viroid		(.)	
RNA virus		(.)	
DNA virus		(.)	
bacterium		(.)	
fungus		(.)	
animal			
-	mammals	(**X**) Genetically modified autologous CD34+ cells	
-	insect	(.)	
-	fish	(.)	
-	other animal	(.)	specify phylum, class

other, specify (kingdom, phylum and class)

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

Human hematopoietic progenitors CD34$^+$ from Leukocyte Adhesion Deficiency Type I patients (*Homo sapiens sapiens*) transduced with self-inactivating (SIN) lentiviral vector Chim.hCD18.wpre*.

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

Self-inactivating (SIN) lentiviral vector Chim.hCD18.wpre* is used to transduce CD34$^+$ cells.

The Chim.hCD18.wpre* lentiviral vector will be produced through a third generation manufacturing system, in which viral genes were removed from the transfer vector and its 3'UTR was modified to generate the SIN lentiviral vector.

Plasmid Factory stores, amplifies and carries out quality control of transfer vector and accessory plasmids. The genetic stability of each plasmid will be tested by sequencing of each new plasmid production. The company producing the lentiviral vector supernatant also carries out several quality controls of each lentiviral supernatant production, including replication-competent lentivirus (RCL) analysis.

Additionally, the genetic stability of the integration of Chim.hCD18.wpre* in the CD34$^+$ genome is studied by quantitative PCR (qPCR) after the transduction of CD34$^+$ derived cells.

On the other hand, the internal promoter, Chim, drives the expression of CD18. In rare instances, the methylation of promoter regions might cause the silencing of the vector. Moreover silencing or reduce transcription of Chim will happen, when the vector integration occurs in low gene transcription areas. However, these silencing events will be irrelevant since millions of transduced cells, with stable transgene expression will be infused.

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) **GB**

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

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

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

The potential environmental impact of the release of the GMOs ($CD34^+$ cells transduced with Chim.hCD18.wpre* vector) is very low. Since the GMOs are $CD34^+$ cells *ex vivo* transduced in a facility which meets the Good Manufacturing Practice (GMP) principles, and then the GMOs are infused into the patient in hospital facilities, all of which limits its environmental risk.

On the other hand, both therapeutic lentiviral vector and transduced $CD34^+$ cells have biological characteristics that prevent their multiplication and/or spread outside the infused patient. They cannot survive outside of the individual and the proliferation of transduced $CD34^+$ cells only occurs during the own patient's hematopoietic reconstitution and they cannot multiply in another individual since they are autologous cells.

There are no ecosystems which the GMO can spread. In the patient there is no genetic modification of germ cells therefore cannot be transmitted.

There cannot be interactions of the GMO with other foreign organisms because patients receiving the GMO must be free of HIV. Only in the case of HIV infection exists in the patient may be residual recombination between lentiviral vectors with the wild virus sequences. That's why it is discarded to perform the therapy in HIV positive patients.

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

other, specify

2. Name
(i) order and/or higher taxon (for animals) **Primate, Hominidae**
(ii) genus ***Homo***
(iii) species ***Homo sapiens***
(iv) subspecies ***Homo sapiens sapiens***
(v) strain
(vi) pathovar (biotype, ecotype, race, etc.)
(vii) common name

3. Geographical distribution of the organism

(a) Indigenous to, or otherwise established in, the country where the notification is made:
Yes (**X**) No (.) Not known (.)

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

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

Atlantic	**X**
Mediteranean	**X**
Boreal	**X**
Alpine	**X**
Continental	**X**
Macaronesian	**X**

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

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

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

4. Natural habitat of the organism

(a) If the organism is a microorganism

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

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

The patient's hematopoietic microenvironnement is the natural habitat of the GMOs (transduced $CD34^+$ cells).

5. (a) Detection techniques

The detection of the genetic modification is performed by molecular biology techniques (Western blot, quantitative PCR (qPCR) and quantitative reverse transcription PCR (qRT-PCR). Besides, fluorescence-activated cell sorting (FACS) is used to identify $CD34^+$ cells or $CD18^+$ cells.

(b) Identification techniques

The same techniques are used: qPCR, qRT-PCR, Western blot and FACS.

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

According to RD 664/1997, of May 12, on the protection of workers from risks related to exposure to biological agents at work, lentiviral vectors are classified as level II of biosafety. On the other hand, the transduced $CD34^+$ cells do not require classification as they have integrated the lentiviral vector and there is no possibility to produce replicative competent retrovirus. Only at the time of the lentiviral transduction of $CD34^+$ cells, containment level 2 for handling is required.

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 genetically modified human hematopoietic progenitors CD34$^+$ are collected from Leukocyte Adhesion Deficiency Type I (LAD-I) patients. These transduced cells are infused autologously in the patients. Detection of bacterial and viral agents, such as Human Immunodeficiency Virus (HIV), Hepatitis C Virus (HCV) and Hepatitis B Virus (HBV), are performed routinely in both patients and hematopoietic progenitor CD34$^+$. And they will be excluded from the clinical study, if they are positive for these pathogen agents.

Moreover, lack of replicative-competent lentivirus (RCLs) is determined after the genetic modification of the hematopoietic progenitors and before their infusion in the patients.

8. Information concerning reproduction

(a) Generation time in natural ecosystems:

The transduced CD34$^+$ cells cannot spread in any natural ecosystem, since they can proliferate exclusively under specific culture conditions or transplanted in patients. These transduced cells with the vector integrated will divide to reconstitute the patient's hematopoietic system only. Under no circumstances, germ cells will be affected.

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

Not applicable.

(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	

(b) relevant factors affecting survivability:

The survival ability of the transduced CD34^{+} is entirely dependent on their homing and engraftment in the patient's hematopoietic microenvironment.

10. (a) Ways of dissemination

The transduced CD34^{+} cells engraft in the patient to reconstitute his/her hematopoietic system. There would be no possibility to be transmitted to another individual, at least that different person was under myeloablative conditioning and infused. Additionally, since the transduced CD34^{+} cells cannot survive outside of the patient, there is no way of dissemination.

(b) Factors affecting dissemination

In the case that the transduced hematopoietic progenitors were infused or injected in an individual different than the donor, the recipient's immune system would eliminate them since both transduced cells and recipient are not histocompatible.

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)

No previous genetic modifications have notified the release. Only the confined use for experimental studies has been reported (n º A/ES/05/06, A/ES/04/I-04 and A/ES/12/I-21).

C. **Information relating to the genetic modification**

1. Type of the genetic modification

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

2. Intended outcome of the genetic modification

The GMO are hematopoietic progenitors CD34$^+$ from Leukocyte Adhesion Deficiency Type I patients transduced with the lentiviral vector Chim.hCD18.wpre*. The therapeutic vector is integrated in the cellular genome and the therapeutic protein CD18 is expressed constitutively. These genetically modified hematopoietic progenitors will be infused in the patients autologously. After the infusion, the modified CD34$^+$ will reconstitute the patient's hematopoietic system and the therapeutic protein will be expressed in all the derived blood cells, in particular in cells from the myeloid lineage, where the CD18 expression will restore the adhesion deficiency defect allowing the corrected cells to migrate to the infection sites. And finally, the patient´s susceptibility to infections will be reduced and therefore their health status will improve. In the most severe cases of the disease, this treatment will allow them to survive above the age of two.

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

(b) Identity of the vector

The lentiviral vector Chim.hCD18.wpre* is based on the HIV-1 lentivirus. Lentiviral vectors used are third generation vectors (4 plasmids are used for

their production which increases its security), improved (containing sequences that enhance expression as cPPT, central polypurine tract, and WPRE*, mutated woodchuck hepatitis virus post-regulatory element) and self-inactivating (LTR with deletions so that once integrated are not active). These vectors are based on the lentivirus HIV-1 which accessory genes have been deleted and regulatory genes have been mutated. These vectors are pseudotyped with an envelope different than the wild type one, the VSV-G (vesicular stomatitis virus envelope). They are replication-defective viruses; it is not known the formation of wild virus or replication-competent virus.

These vectors are produced by co-transfection of 4 plasmids into 293T cells: transferring vector (pCCLsincPPT-Chim.hCD18.wpre*), packaging vectors (rev and gag-pol) and the VSV envelope.

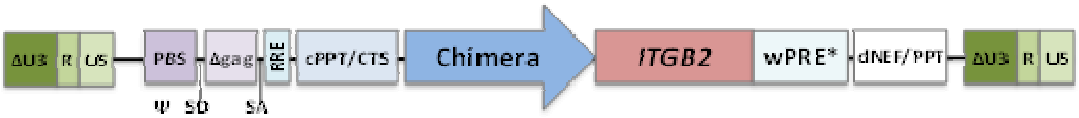

Figure 1: Scheme of the integrated provirus pCCLsincPPT-Chim.hCD18.wpre

(c) Host range of the vector

Because the lentiviral vector is pseudotyped with VSV-G envelope, the vector is able to transduce numerous cell types of different species. However, the transduction of cells from other organisms cannot occur, since vector manipulation is performed in a laboratory containment level II and the cells will be transduced *ex vivo*.

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

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

antibiotic resistance (.)
other, specify

The vector expresses the hCD18 gene that can be identified or detected by PCR, RT-PCR or flow cytometry (FACS).

Indication of which antibiotic resistance gene is inserted

There is no integration of an antibiotic resistance gene.

(e) Constituent fragments of the vector

The expression plasmid is constructed on pUC19 (plasmid from University of California) and has a size of 9,966 bp. From the plasmid in eukaryotic cells, 6,539 bp mRNA is transcribed. In each vector 2 identical molecules are encapsidated that are retro-transcribed during infection in a DNA containing the modules described in Section 6.

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

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

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 is composed of the following parts to be integrated from a LTR to the other (see figure 1, section 4. B).

- **LTR: Long Terminal Repeat (sequence derived from the lentivirus).**
- **SD: Splice Donor or donor splicing.**
- **Ψ: packaging signal.**
- **PBS: primer binding site.**
- **ga: deleted gag gene.**
- **RRE: Rev Response Element.**
- **SA: Splicing Acceptor.**
- **cPPT: central Polypurine Tract, regulates expression of the transgene.**
- **Chim: internal promoter that directs myeloid expression of genes of interest.**
- ***hCD18*: cDNA that codify for CD18 protein.**
- **Wpre, mutated: Woodchuck pre-regulatory element. Stabilizes and improves transgene expression. In this case is mutated to improve the safety and efficacy of the sequence.**

(b) Source of each constituent part of the insert

LTR: sequence derived from lentivirus and cytomegalovirus.

- **SD: Lentivirus.**
- **Ψ: Lentivirus.**
- **PBS: Lentivirus.**
- **ga: Lentivirus.**
- **RRE: Lentivirus.**
- **SA: Lentivirus.**
- **cPPT: Lentivirus.**
- **Chim: Human.**

- **hCD18: Human.**
- **Wpre, mutated: woodchuck hepatitis virus.**

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

- **LTR 3': Long Terminal Repeat (sequence derived from the lentivirus). The LTR is formed by the merge of regions U3-R-U5 that results from the retro-transcription of the vector and before integration. The wild type LTR of HIV-1 was mutated by removing the U3, so the resulting LTR is unable to stimulate gene expression in either the plasmid or in the integrated form following the retro-transcription. To synthesize the messenger RNA, the powerful promoter/enhancer of the Cytomegalovirus (CMV IE-I avg) was incorporated into 3'RU5 sequence, so that this promoter directs expression of the RNA to be packaged into infectious capsids. This sequence at any time will be part of the virus so that after integration will be unable to form new infectious particles: this is a self-inactivating vector or SIN.**
- **SD: Splicing Donor. The presence of post-processing signal/transcriptional improves the titles by reducing the degradation of RNA. There is an SD sequence within the Psi.**
- **Ψ: packaging signal. Sequence with a secondary structure characteristic which is 4 loops (SL1, SL2, SL3, SL4) that are necessary for the correct incorporation of viral RNA in the capsid.**
- **PBS: Primer Binding Site: Includes the sequence where it joins to the transfer RNA that serves as a primer for retro-transcription of the virus.**
- **ga: deleted gag gene. Sequences coding for viral proteins have been removed deliberately and form part of the genes provided in trans for the production of the vector. This residual non-coding sequence meets the need of maintaining structures involved in encapsidation of RNA as SL4).**
- **RRE: Rev Response Element.**
- **SA: Splice Acceptor or acceptor of splicing. The presence of post-transcriptional processing signals improves titles by reducing the degradation of RNA.**
- **cPPT: Central Polypurine Tract, regulates expression of the transgene.**
- **Chim: Internal promoter that directs the myeloid expression of the genes of interest. The Chimera promoter is the result of the fusion of the promoter regions of the genes CTSG and cFES. The CTSG gene codes for the cathepsin G protein which is a serine protease specific for neutrophils. The cFES gene codes for the Fes / Fps protein tyrosine kinase also expressed by neutrophils and macrophages. In this fusion of CTSG-cFES promoter regions the TATA box of the CTSG promoter region has been removed so that the expression is directed only by the promoter region of the cFES gene.**
- **hCD18: cDNA of CD18 protein encoded by ITGB2 that is located in 21q22.3.**
- **Wpre*: (Woodchuck hepatitis virus (WHV) post-transcriptional Regulatory Element) is an original sequence of the hepatitis viruses of the woodchuck with the capacity to stabilize mRNA by increasing the amount of protein produced. The wild version encodes the protein X related to hepatocarcinoma. The mutated version has eliminated the possible sites critical for expression of that protein.**
- **LTR 5': The LTR is formed by the merge of regions U3-R-U5 that results from the retro-transcription of the vector and before integration. The wild**

LTR of HIV-1 was mutated by removing 18 bases of the region promoter/enhancer U3 (Δ18U3) so that the resulting LTR is unable to stimulate gene expression in either the plasmid or in the integrated form following the retro-transcription, so after integration will be unable to form new infectious particles: a self-inactivating vector or SIN.

(d) Location of the insert in the host organism

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

(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 (**X**)
- insect (.)
- fish (.)
- other animal (.)
(specify phylum, class)

other, specify

2. Complete name

(i)	order and/or higher taxon (for animals)	**Primates**
(ii)	family name for plants	**Hominidae/Retroviridae**
(iii)	genus	***Homo*/Lentivirus**
(iv)	species	***Homo sapiens*/Lentivirus HIV-1**
(v)	subspecies	***Homo sapiens sapiens***
(vi)	strain	**VIH-1**
(vii)	cultivar/breeding line	…
(viii)	pathovar	…
(ix)	common name	**Human being and HIV-1 lentivirus**

3. Is the organism significantly pathogenic or harmful in any other way (including its extracellular products), either living or dead?
Yes (.) No (**X**) Not known (.)

If yes, specify the following:

(b) to which of the following organisms:

humans (.)
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):
…

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

According to the RD 664/1997 of 12 May on the protection of workers from risks related to exposure to biological agents at work, HIV is classified as group 3 biological agents. However, part of its genome has been changed by deleting the viral sequences necessary for propagation, thereby suppressing their infectivity, requiring containment level 2 for handling.

5. Do the donor and recipient organism exchange genetic material naturally?
Yes (.) No (**X**) Not known (.)

E. Information relating to the genetically modified organism

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

(a) is the GMO different from the recipient as far as survivability is concerned?
Yes (.) No (**X**) Not known (.)
Specify

(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

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

Specify

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

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

Specify

2. Genetic stability of the genetically modified organism

Genetic stability is very high once the vector is integrated into the genome of the CD34$^+$ cell, thanks to the internal promoter the hCD18 protein is expressed and corrects the genetic defect.

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)

There is neither infectivity nor propagation of sin lentiviral vectors. Since the transduction of the CD34$^+$ cells is done ex vivo and infused immediately, there will be no possibility to infect any other cell type during the clinical trial. The integration of the vector into the target cell may not activate latent viruses and could not colonize other organisms. In any case the GMO is not pathogenic or harmful.

As in other ex vivo transduction protocols performed with CD34$^+$ cells, the cell product under the transduction process is washed with infusion medium of the patient. Many reagents have already been included in the transduction medium used for the gene therapy of other diseases such as SCID-X1, ADA-SCID, beta-thalassemia or adrenoleukodystrophy. In none of these cases any adverse effect has been associated with cellular product infusion. As expected, residual doses of some reagents must not generate any therapeutic or toxic effect.

It is not considered that the infusion of lentiviral particles present in the infusion medium will transduce cells of the patient as previous studies have shown that complement inactivates VSV-G envelope used for the packaging of our therapeutic vector. So that, in the presence of complement, the vectors will infect human cells with efficiency 95 times lower.

4. Description of identification and detection methods

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

The detection of the genetic modification is performed by molecular biology techniques (Western blot, quantitative PCR (qPCR) and quantitative reverse transcription PCR (qRT-PCR). Besides, fluorescence-activated cell sorting (FACS) is used to identify $CD34^+$ cells and $CD18^+$ cells.

(b) Techniques used to identify the GMO

The same techniques are used: qPCR, qRT-PCR, Western blot and FACS.

F. Information relating to the release

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

The GMOs are not released to the environment, but they are infused in the LAD-I patients to correct the leukocyte adhesion deficiency characteristic of this disease.

The GMO are hematopoietic progenitors $CD34^+$ from Leukocyte Adhesion Deficiency Type I patients transduced with the lentiviral vector Chim.hCD18.wpre*. The therapeutic vector is integrated in the cellular genome and the therapeutic protein CD18 is expressed constitutively. These genetically modified hematopoietic progenitors will be autologously infused in the patients. After the infusion, the modified $CD34^+$ will reconstitute the patient's hematopoietic system and the therapeutic protein will be expressed in all the derived blood cells, in particular in cells from the myeloid lineage, where the CD18 expression will restore the adhesion deficiency defect allowing the corrected cells to migrate to the infection sites. And finally, the patient´s susceptibility to infections will be notably reduced.

The clinical trial is not expected to have any effect on the environment.

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 GMO release will occur in the context of a clinical trial performed in several medical centers.

(b) Size of the site (m^2): **Not applicable.**
(i) actual release site (m^2):
(ii) wider release site (m^2):

(c) Proximity to internationally recognised biotopes or protected areas (including drinking water reservoirs), which could be affected:
Not applicable.

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

Not applicable.

4. Method and amount of release

(a) Quantities of GMOs to be released:

It is expected the GMO release for 8 patients, with an infusion about 2-8x10^6 cells/kg.

(b) Duration of the operation:

3 years.

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

There is no possibility of spreading, since the genetic modification of hematopoietic progenitors is performed ex vivo and these cells cannot survive outside the hematopoietic niche. Then, no special methods are set up to prevent spreading.

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

Not applicable.

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.

No data from previous releases of the same GMO.

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	***Homo sapiens***
(v)	subspecies	***Homo sapiens sapiens***
(vi)	strain	…
(vii)	cultivar/breeding line	…
(viii)	pathovar	…
(ix)	common name	**Human being**

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

The interaction will be the standard in hematopoietic stem cell transplants that are performed routinely in hospitals. $CD34^+$ cells transduced will graft in the patient's bone marrow and will proliferate there to reconstitute the patient's hematopoietic system. It is not expected direct vector interaction with the patient's cells. Thus, biodistribution studies were performed to ensure that transduction of other cells is not happening.

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

It is expected no interaction with other foreign organisms, because patients receiving the GMO must be free on HIV to eliminate the possibility of recombination between our lentiviral vector and wild type HIV. Because of this, it is discarded to perform the gene therapy in HIV positive patients.

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

Because this is a clinical trial there is no possibility to disseminate the GMO to any other ecosystem.
The clinical trial will be carried out in the Hospital Infantil Universitario Niño Jesús (Av. de Menéndez Pelayo, 65, 28009 Madrid, España).

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	…

7. Likelihood of genetic exchange in vivo

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

Only in the event that there is HIV infection in the patient could be residual recombination between sequences of the vector with sequences of the wild virus. Because of this, it is discarded to perform the gene therapy in HIV positive patients.

(b) from other organisms to the GMO:

No such possibility.

(c) likely consequences of gene transfer:

The only consequence that would be very unlikely: lentiviral vector sequences could be included in the wild virus genome. However, this would not have relevant biological consequences.

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.):

Do not exit.

9. Possible environmentally significant interactions with biogeochemical processes (if different from the recipient or parental organism)

Not applicable.

H. Information relating to monitoring

1. Methods for monitoring the GMOs

The patients will be monitored after being infused with genetic modified hematopoietic progenitors to evaluate the safety for five years. Different samples, both blood and bone marrow, from the patients will be collected beginning in the third week after the infusion to measure hematological parameters (percentage of hematopoietic progenitors and several blood lineages) and quantify the GMO presence by PCR, which is a highly sensitive and reliable technique that allows the amplification and detection of sequence of interest. Moreover, clonality studies will be performed to identify the vector integration site in the cellular genome, in order to detect any clonal dominancy caused by the genetic modification.

Additionally, a long-term subject monitoring will be done. The patients will be checked routinely for ten years to confirm the absence of long-term side-effects.

2. Methods for monitoring ecosystem effects

Not relevant as there will be no impact on the ecosystem.

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

Not applicable.

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

Not applicable.

5. Duration of the monitoring

Monitoring of patients will be at least 5 years.

6. Frequency of the monitoring

After the infusion, blood samples will be taken weekly during the first 8 weeks, monthly until the 1st year, and at least every 12 months thereafter. Bone marrow samples will be taken at 1, 3, 6 and 12 months post-infusion, and at least annually thereafter.

I. Information on post-release and waste treatment

1. Post-release treatment of the site

Patients will be treated in standard rooms for hematopoietic transplants of the different clinical centers, where they will remain at least 72 hours after infusion.

The release of the final product (transduced $CD34^+$) will be performed by infusion to the patient in a hospital. The preparation of the site will be adapted to the rules of that center for this type of intervention. The place where the product is prepared for infusion should be decontaminated before and after handling, with a conventional disinfectant solution.

All staff will be informed that the $CD34^+$ cells transduced with the lentiviral vector are considered a product of biosafety level II and they will be trained for the handling following proper standards for that level of biosafety (product handling, equipment and materials used, correct waste disposal, etc..). Any waste generated during the handling of the transduced $CD34^+$ cells or have been in contact with the product must be deposited in special biosafety containers and incinerated.

<u>Staff should wear protective clothing, according to the following:</u>
• Gowns should be worn.
• Gloves should be worn for any procedure that may involve direct contact with skin.
• All equipment and work surfaces should be cleaned with bleach.
• Needles and syringes should be discarded in biosafety containers.
• After removing gloves, staff should wash their hands.

<u>Administration of CD34 cells transduced to the patient:</u>
• Patients will be infused according to standard protocols of the different clinical centers involved in the clinical trial.

<u>Patient management that is discharged after treatment:</u>
• No specific procedures are considered once the patient is discharged.

<u>Patient management having problems after treatment:</u>
• No specific procedures are considered different from common at the Hospital.

<u>Procedures to be followed by staff and visitors:</u>
• Staff who is pierced with needles that have had contact with the transduced cells must follow standard procedures for this type of accident. They should notify to the department of labor security and to the investigators of the clinical trial.
• Any staff member involved in the trial who is feeling sick should inform the department of labor security and to the investigators.
• Visits of immunocompromised, transplanted, undergoing chemotherapy or corticosteroids treatment persons, children and pregnant women are not allowed.
• Only allowed a maximum of two visitors at a time.

<u>In case of spilled product:</u>
• In areas where the product is handled, stored and transported, there must always be available disinfectant, such as bleach.

• If a spill of the product occurs, the staff cleaning up should follow the standards specified in the previous section.
• All surfaces that have been contaminated should be cleaned and disinfected.

<u>Treatment of samples:</u>
• Personnel handling samples of the patient should wear gowns and gloves.
• All surfaces that come in contact with the product should be disinfected with bleach.
• Devices such as needles and syringes should be deposited in a biosafety container.
• All samples must be clearly labeled with a biosafety label.
• All waste material should be decontaminated with bleach or disinfectants.

Transplanted patients will be hospitalized in a restricted area properly marked and the access will be restricted only to medical personnel in charge of the patient and authorized visitors.

2. Post-release treatment of the GMOs

No special procedures to prevent the spread of GMOs outside the release site because transduced hematopoietic progenitors cannot spread beyond the patient's body. So, the procedures are the usual ones for patients who have been infused with hematopoietic progenitors.

3. (a) Type and amount of waste generated

The types of waste are:

- **Waste generated during the preparation and handling of the final product ($CD34^+$ cells transduced with the lentiviral vector).**
- **Residues resulting from the final GMO infusion of the patient.**
- **The residues from the cleaning of work areas.**

The volume of waste generated will be the usual of this type of procedure and will not be large volumes. Most residues will be inactivated by autoclaving and will not be more than two autoclave bags, which then pass to incineration. Liquid waste will be treated with disinfectants and be no more than 1 L.

3. (b) Treatment of waste

The proposed treatment for different types of waste will be adapted to current regulations. As established in RD 83/1999 for regulating the activities of production and management of medical waste and cytotoxic agents in the "*Comunidad de Madrid*" (BOCM 163), residues are classified into:

- **Class I and II. The materials are inactivated (liquids by disinfectants and solids by autoclaving) and disposed following the rules.**
- **Class III. The waste is managed by the company CONSENUR, registered and qualified to do so, according to the provisions of the previous cited RD.**

In general, it is expected that solid waste (such as gowns, masks, etc) are deactivated by autoclaving, and then incinerated conventionally. Liquid waste and surfaces will be treated with a suitable disinfectant. All other waste

(bandages, swabs…) will be incinerated in the hospital in the same way that the usual clinical waste.

In the case of occurrence of any incident or accident, it must be reported immediately to the "*Ministerio de Agricultura y Pesca, Alimentación y Medio Ambiente*".

J. Information on emergency response plans

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

As a precaution, the containment level 2 (class II) procedures will be held at the site of release. Treated patients will be kept in the hospital as long as necessary according to the usual hospital service protocols related to hematopoietic transplantation with conditioning.

In the case of accidental release, the following procedures will take place:
Isolate the spill area; absorb spilled solution with paper towels or other absorbent material. The area will be treated with 5% bleach, 0.5% sodium hydroxide solution or a solution of disinfectant. Also other disposables should be used and a proper use of the dustpan. After cleaning up spills, put all the cleaning materials used in a contaminated site, in a sturdy disposable plastic bag. When all contaminated materials have left the room, rinse the area with clean water using additional towels.

After the cleaning, all contaminated materials should be placed properly, appropriately labeled, and discarded as biohazardous waste in specific containers. Finally, remove gloves and wash hands thoroughly with soap and clean water.

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

In case of spilled product:
- **In areas where the product is handled, stored and transported, there must always be available disinfectant, such as bleach.**
- **If a spill of the product occurs, the staff to clean up should follow the procedures specified in the previous section.**
- **All surfaces that have been contaminated should be cleaned and disinfected.**

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

See the previous section.

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

In the clinical trial, patients will be monitored for at least 5 years after the single dose treatment to control clinically significant adverse elements. For the reasons given previously and referring to the risk assessment is not considered necessary to write specific plans to protect the environment.