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/19/17**
(c) Date of acknowledgement of notification: **1/07/2019**
(d) Title of the project: **A Phase II Clinical Trial to Evaluate the Efficacy of the Infusion of Autologous CD34+ Cells Transduced with a Lentiviral Vector Carrying the FANCA Gene (Orphan Drug) in Patients with Fanconi Anemia Subtype A**
(e) Proposed period of release **July 2019 - July 2023**

2. Notifier

Name of institution or company: Rocket Pharmaceuticals, Inc.

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 Fanconi Anemia subtype A patients (*Homo sapiens sapiens*) transduced with self-inactivating (SIN) lentiviral vector PGK-FANCA.Wpre*.

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

As previously detailed in application B/ES/12/37, the self-inactivating (SIN) lentiviral vector PGK-FANCA.Wpre* is used to transduce Fanconi Anemia subtype A (FA-A) patients' CD34+ cells. The PGK-FANCA.Wpre* is produced through a third-generation manufacturing system in which accessory viral genes are removed from the transfer vector and its 3'UTR has been modified to generate the SIN lentiviral vector.

The plasmid manufacturer 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, also carries out all the appropriate quality controls of each lentiviral supernatant production, including replication-competent lentivirus (RCL) analysis.

Genetic stability is very high, once the SIN-lentiviral vector is integrated in the genome of the CD34 cell. Thanks to the internal promoter (human PGK), transduced cells will express the FANCA protein and correct the genetic defect in patients' cells. Silencing or reduced transcription may happen when the vector integration occurs in areas of the genome with little transcription, silencing phenomena or low expression.

Additionally, the genetic stability of the integration of PGK-FANCA.Wpre* in the CD34+ genome is studied by quantitative PCR (qPCR) after the transduction of CD34+ cells.

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

   Yes (X) No (X)

   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 GMO (patients CD34+ cells transduced with the lentiviral vector) is very low. The GMO is safely manufactured, CD34+ cells are

transduced *ex vivo* in a facility compliant with Good Manufacturing Practices. Then, the cellular product is transported and handled until infusion in the patient in hospital facilities. Therefore, the risks of release to the environment are very limited and controlled throughout the process.

Additionally, both therapeutic lentiviral vector and the transduced CD34+ cells have biological characteristics that prevent their multiplication and/or spread outside the patient infused with the transduced cells. The lentiviral vector and the transduced cells cannot survive outside of the individual. The proliferation of transduced CD34+ cells only occurs during the own's patients' hematopoietic reconstitution and the cells cannot multiply outside the body (autologous cells).

There are no ecosystems which the GMO can spread. There is no genetic modification of germ cells in the patient, therefore no genetic modification can be transmitted.

The proposed use of the final product is the single intravenous administration thereof.

There cannot be interactions of the GMO with other foreign organisms. The lentiviral vector utilized is derived from HIV. It is known that only in the case of HIV infection present in the patient, there is a possibility of residual recombination between lentiviral vectors with the wild virus sequences. Therefore, HIV positive patients are not considered eligible to participate in the clinical trial. The patients receiving the GMO in the clinical trial must be specifically tested and they must be free of HIV. In this way, the possibility to allow recombination with wild type viruses is avoided. In none of the multiple clinical trials with this type of vector has been described the generation of recombination of therapeutic vectors to generate replication competent lentiviruses (RCLs)

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
Mediterranean	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 own hematopoietic microenvironment is the natural habitat of the GMOs (autologous transduced CD34+ cells).

5. (a) Detection techniques

The detection of the genetic modification is performed by molecular biology techniques (Western blot, real-time PCR (Q-PCR) and quantitative retrotranscriptase PCR (Q-RT-PCR)). Furthermore, flow cytometry and CD34 markers will be used to identify CD34+ cells (monoclonal antibody bound to a fluorochrome).

(b) Identification techniques

The same techniques are used: Q-PCR, RT-Q-PCR, Western blot and flow cytometry.

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

According to Royal Decree 664/1997, of May 12, on worker protection against biological hazards, lentiviral vectors are classified as biosafety level 2 agents. On the other hand, the transduced CD34+ cells have integrated the lentiviral vector and there is no possibility to produce replicative competent lentivirus. Therefore, biosafety confinement is not applicable for the manipulation of the product, administration and analyses of the patient's samples.

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 Fanconi Anemia subtype A (FA-A) patients. The transduced cells are infused autologously back in the patients. Detection of bacterial and viral agents, such as Human Immunodeficiency Virus (HIV), are performed routinely in patients. If the patient is positive for this pathogen agent, the patient will be excluded from participation in the clinical study to avoid residual recombination as explained above.

8. Information concerning reproduction

(a) Generation time in natural ecosystems:

The transduced CD34+ cells cannot spread in any natural ecosystem, since they can grow exclusively in special culture conditions or once transplanted into the patient (autologous cells). These transduced cells with the integrated vector will divide only to reconstitute

the hematopoiesis of the patient. In no case germ cells from the patient will be transduced because this protocol is an ex vivo gene therapy.

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

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

(c) Factors affecting reproduction:
Non-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 clinical trial only intends to infuse the transduced autologous cells back into the patient. The transduced CD34+ cells engraft in the patient to reconstitute his/her hematopoietic system.

(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)

A previous notification B/ES/12/37 was authorized for the conduct of the clinical trial Phase I/II clinical trial to evaluate the safety and efficacy of the infusion of autologous CD34+ cells transduced with a lentiviral vector carrying the *FANCA* gene (orphan drug) in patients with Fanconi Anemia Subtype A.

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 CD34+ progenitors from Fanconi Anemia subtype A (FA-A) patients transduced with the lentiviral vector PGK-FANCA.Wpre*.

CD34+ progenitors from Fanconi Anemia subtype A (FA-A) patients will be collected and transduced with the lentiviral vector. The therapeutic vector is integrated in the cellular genome and the gene will be constitutively expressed to generate the therapeutic protein FANCA. 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.

Fanconi anemia (FA) is a rare genetic disease. The impairment of DNA repair has been considered the principal and hallmark defect in FA cells. CD34+ cells transduced from the patient will with the lentiviral vector then be genetically corrected and therefore capable of activating the Fanconi anemia pathway by the mono-ubiquitination of FANCD2 and FANCI. These proteins may then migrate to areas of DNA damage, and in cooperation with other DNA repair proteins, promote DNA repair in these cells, as in healthy cells.

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

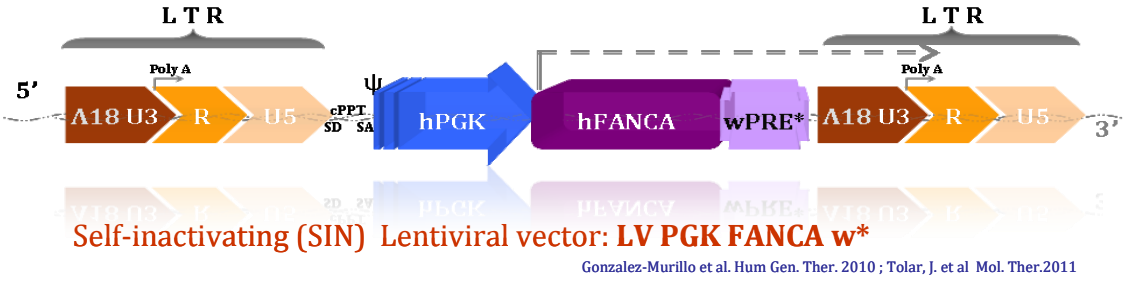
plasmid	(.)
bacteriophage	(.)
virus	(X)
cosmid	(.)
transposable element	(.)
other, specify	

(b) Identity of the vector

It is a lentiviral vector carrying the *FANCA* gene that has received orphan drug designation by EMA (EU 3/10/822) and FDA (16/5193) for the treatment of patients with Fanconi anemia subtype A. Lentiviral vectors used are third generation (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 regulator and envelope genes have been mutated. They are replication-defective viruses and the formation of wild virus or replication-competent virus has been discarded.

These vectors are produced produced by transient transfection of HEK293T cells in cell-factory stacks with 3 packaging plasmids (encoding HIV-1 rev, gag pol genes and the vesicular stomatitis G glycoprotein) and the pCCL PGK FANCA wpre* transfer plasmid.

This figure shows the construction already integrated into the genome of the target cell.



Self-inactivating (SIN) Lentiviral vector: **LV PGK FANCA w***

Gonzalez-Murillo et al. Hum Gen. Ther. 2010 ; Tolar, J. et al Mol. Ther.2011

(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. As vector manipulation is performed in a biosafety level 2 cabinet and the cells will be transduced *ex vivo*, the transduction of cells from other organisms cannot occur.

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

Yes (X) No (.)

antibiotic resistance (.)
other, specify

A regulatory agency compliant sucrose selection sequence is used. This sequence is designed to improve the lentiviral production, but it does not integrate in the target cells, therefore transduced cells will not express this sequence.

Indication of which antibiotic resistance gene is inserted

(e) Constituent fragments of the vector

The vector is constituted by a series of fragments, the most important one is the insert flanked by the LTRs and whose parts are detailed in point 6. The rest contains viral sequences of the plasmid pCCL-PGK-FANCAW-82-RO necessary for its amplification and selection. In particular, a PolyA origin of replication sequence, a SV40ori sequence, a pUCorigin sequence, a sucrose selection sequence and a CMV (cytomegalovirus) promoter that drives the expression of the insert in the producer cells.

(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 an LTR to the other (see figure, 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.
• PGK: internal promoter directs ubiquitous expression of genes of interest.
• *FANCA*: cDNA encoding the protein of Fanconi anemia subtype A.
• 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.
• PGK: Human.
• FANCA: 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 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.
- PGK: internal promoter that directs ubiquitous expression of genes of interest. It's the promoter sequence of the human gene PGK (Phosphoglycerate Kinase) that encodes the ubiquitous and moderate expression of PGK protein. This promoter element is located at hg 38 chrX:78,103,818-78,104,327 of the human phosphoglycerate kinase 1 (PGK1) gene (RefSeq NM_000291). The protein encoded by this gene is a glycolytic enzyme that catalyzes the conversion of 1,3-diphosphoglycerate to 3-phosphoglycerate. The glycolytic enzyme, PGK1, is central to nearly every eukaryotic cell metabolism so hPGK is considered a robust and constitutive promoter element ideal for gene therapy applications.
- *FANCA*: cDNA encoding the protein of Fanconi anemia subtype A. cDNA sequence of the human gene FANCA located on chromosome 16 at 16q24.3 long arm, encoding the protein complementation group A Fanconi anemia (FANCA).

- <u>WPRE, mutated</u> (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.
- <u>LTR 5'</u>: 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 worker protection against biological hazards, 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. Therefore, the lentiviral vectors are classified as requiring containment level 2 for handling.

On the other hand, the genetically modified CD34+ cell with the lentiviral vector, once integrated into the genome and because of the absence of RCLs is not considered to require a special level of containment.

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 (X) No (.) Not known (.)
Specify
FA-A patient cells are characterized by a DNA repair defect. A competitive advantage has been demonstrated for corrected cells survival compared to disease cells.

The competitive advantage of transduced/corrected CD34+ cells compared to the patient's disease cells can be a great advantage for therapeutic effect because even a few cells could reconstitute the patient's hematopoiesis with corrected cells expressing the *FANCA* gene. This is considered an advantageous possibility because patients with a spontaneous reversal of mutation in a hematopoietic stem cell have shown a high level of hematopoietic reconstitution by those reversed cells. This unique feature to Fanconi patients is so-called somatic mosaicism and mosaic patients are reported to recover normal blood counts.

On the other hand, the probability that the lentiviral vector acquires selective advantage or disadvantage is very low in relation to the integration site (discarding the expression of the therapeutic gene *FANCA*). In the case of integrative vectors there is a possibility of insertional mutagenesis, being in the case of lentiviral vectors a very remote option. Clinical data obtained in X1-SCID patients, CGD patients, and more recently in Wiscott-Aldrich patients have shown that transduction of hematopoietic cells with gamma-retroviral vectors have unquestionable clinical benefits, although in some cases lymphoma or myeloproliferative syndromes were generated due to transactivation of oncogenes by strong viral promoters used to express the genes. In order to minimize leukemogenic risks associated with gamma-retroviral vectors, a new generation of self-inactivating lentiviral vectors (LVs) was generated, which has already entered the clinic, and have enabled the development of new orphan drugs, such as current or as the so-called "lentiviral vectors carrying the Wiscott Aldrich protein (WASP-LV)" (Ref141/2000).

Compared to gamma-retroviral vectors, the lentiviral vectors have lower preference for integration in the regions near the transcription start site of genes. This fact, together with the advanced design of self-inactivating LVs implies a reduction of the risk of insertional oncogenesis. This was particularly the case for the LVs with the promoter present in the medicinal product of this trial, the promoter of the gene for phosphoglycerate kinase (hPGK).

(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 FANCA 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 vector propagation of SIN-lentiviral vectors. Because of the envelope, the lentiviral vector can transduce multiple cell types. Since the transduction of the $CD34^+$ cells will be *ex vivo* in the clinical trial and the transduced cells will be infused immediately into the patient, 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 all cases, 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, and in Fanconi anemia patients treated by our own group (FANCOLEN-I). 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 is done by molecular biology techniques (Western blot, real-time PCR (Q-PCR) and RT-Q-PCR). In addition, to identify CD34+ cells we will use flow cytometry and CD34 marker (monoclonal antibodies bound to a fluorochrome).

(b) Techniques used to identify the GMO

Western blot, real-time PCR (Q-PCR), RT-Q-PCR and flow cytometry.

F. Information relating to the release

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

The GMOs will not be released to the environment. The GMO defined as the hematopoietic progenitors CD34+ from Fanconi Anemia subtype A patients transduced with the lentiviral vector PGK-FANCA.Wpre* will be infused directly into the Fanconi Anemia subtype A patients (autologous cells) aiming to correct hematologic abnormalities in patients with this disease.

The lentiviral vector will facilitate the expression of FANCA protein in transduced CD34+ cells. After infusion, the modified CD34+ cells will engraft in the patient bone marrow and, those cells that were previously defective in proliferation and differentiation, will now behave with standard division capacity and should restore the patient's hematopoiesis.

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 release will occur in the context of a clinical study conducted at the Transfusion Center – Pediatric Oncohematology Departament, Hospital Infantil Universitario Niño Jesús in Madrid (Avenida Menendez Pelayo, nº 65, 28009, Madrid, Spain).

(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 planned to release CD34+ autologous cells for 5 patients in the clinical trial.

The dose of cells reinfused into the patient is that resulting from the transduction of the starting cell population of ≥ 5x10^5 CD34+ cells/kg patient weight.

(c) Duration of the operation:

4 years: approximately one year to complete patient enrollment with a total 3-year follow up post-infusion of patients in the study.

(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 GMO spreading, since the genetic modification of hematopoietic progenitors is performed *ex vivo* and these cells cannot survive outside the patient's 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.
A previous study still under development, with notification B/ES/12/37, has demonstrated absence of viral particles in the final drug product and absence of RCLs in the final product released for infusion of the patients, and in patients' follow up samples with some patients completing the 3 year follow-up.

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 one that happens to the infusion of hematopoietic progenitors corrected for the genetic defect, in the patient himself without the need for it to be treated by any previous conditioning, so no special requirements are needed in the hospital. The transduced hematopoietic progenitors will nest in the patient's bone marrow, and there, they will proliferate until reconstitute the patient's bone marrow and hematopoietic system. In none of the preclinical biodistribution or clinical studies in patients with Fanconi anemia treated in our study or in another ex vivo gene therapy studies has the generation of competent replication viruses been observed.

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

No interactions with other foreign organisms are expected, because patients receiving the GMO must be free of HIV to eliminate the possibility of recombination between the lentiviral vector and wild type HIV. Because of this, participation in the trial of HIV positive patients is disregarded.

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

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

Give details

As explained above, FA-A patient cells are characterized by a DNA repair defect. A competitive advantage has been demonstrated for corrected cells survival compared to disease cells.

This phenomenon is not described in any of the more than 200 patients treated by ex vivo gene therapy with lentiviral vectors.

As explained above, the cells of patients with Fanconi anemia type A are characterized by defects in DNA repair. It has been shown that corrected cells have a competitive advantage over diseased cells to survive, but in no case has it been observed nor is it reasonable to think that lentiviral particles can develop such an advantage, or that long-term RCL particles can be generated.

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 extend the GMO to other ecosystems. The clinical trial will be carried out in the Hospital Infantil Universitario Niño Jesús (Av. de Menéndez Pelayo, 65, 28009 Madrid, Spain).

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:

It is not reasonable that there may be interactions of the GMO with other foreign organisms. The lentiviral vector used derives from HIV, so theoretically recombination processes could occur between sequences of the lentiviral vector with sequences of the wild virus in patients infected with the HIV virus. Therefore, it is ruled out to perform therapy in HIV-positive patients.

(b) from other organisms to the GMO:

No such possibility.

(d) likely consequences of gene transfer:

The only consequence that could take place and that it would be very unlikely is that lentiviral vector sequences could be incorporated in the wild virus. But as stated above, it is highly unlikely since patients are studied if they are infected with the HIV virus.

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.):
Not applicable.

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

Patients will be followed for an initial period of 3 years and then annually up to 15 years after infusion with the genetically modified hematopoietic stem cells (GMO). Different samples, both blood and bone marrow, from the patients will be collected beginning in the first month 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. In bone marrow, samples instability cytogenetic studies will be carried out.

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
Quantitative PCR (Q-PCR) will allow to study/discard the transfer of genetic material to other organisms.

4. Size of the monitoring area (m^2)
Not applicable.

5. Duration of the monitoring
The follow-up period is 3 years from the infusion of transduced cells. Patients will be monitored in a companion long-term follow-up program for a total period of 15 years post-infusion (including the clinical trial period).

6. Frequency of the monitoring

After the infusion, blood samples will be taken at months 1, 2, 4, 6, 9, 12, 15, 18, 21, 24, 28, 32 and 36. Bone marrow samples will be taken at months 6, 12, 24 and 36 post-infusion.

I. Information on post-release and waste treatment

1. Post-release treatment of the site

Patients will be treated in standard rooms of the Pediatric Oncohematology Service of the Hospital Infantil Universitario Niño Jesús in Madrid, where they will remain at least 72 hours after the infusion simply to control their constants, as patients will not be treated with pre-infusion chemotherapy. They will be hospitalized in an area of restricted access, duly signposted, to which only the health personnel in charge of the patient and the authorized visits will have access.

The release of the final product (transduced CD34+ cells) will be carried out by direct infusion of the cells present in the infusion bag to the patient according to established norms. All staff will be informed that the CD34+ cells have been transduced with the lentiviral vector under a level 2 biosecurity and the staff will be trained to handle it, without the need for special waste management. Any residue generated during the handling activity of transduced CD34+ cells or that may have been in contact with the product should be deposited in special biosecurity and incinerated containers.

<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 desinfectant.**
- **Needles and syringes should be discarded in biosafety containers.**
- **After removing gloves, staff should wash their hands.**

<u>Administration of transduced CD34+ cells to the patient:</u>

- **Patients will be infused according to standard protocols of the Pediatric Onco-hematology Department of the Hospital Infantil Universitario Niño Jesús.**

<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 the investigators.**
- **Visits of immunocompromised, transplanted, undergoing chemotherapy or corticosteroids treatment persons, children and pregnant women are not allowed.**

• Only a maximum of two visitors at a time is allowed.

In case of spilled cellular 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 cleaning the spill up should follow cleaning operating standard procedures for biological materials, use a standard disinfectant like bleach and dispose of waste in a biosafety container.
• As a preventive measure, all surfaces that have been in contact with the transduced cells must be cleaned and disinfected.

Treatment of samples:

• Personnel handling samples of the patient should wear gowns and gloves.
• All surfaces that come in contact with the product should be disinfected as a preventive measure.
• 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 disinfected with bleach or disinfectants as a preventive measure.

2. Post-release treatment of the GMOs

No special procedures are needed to prevent the spread of GMOs outside the release site because transduced hematopoietic progenitors cannot spread beyond the patient's body.

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).
- The infusion bag to the patient of the final GMO.
- 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 Royal Decree 83/1999 for regulating the activities of production and management of medical waste and cytotoxic agents in the Community of Madrid (Comunidad de Madrid, BOCM 163), residues are classified into:
- Class I and II. The materials are inactivated (liquids with disinfectants and solids by autoclaving) and disposed following the guidelines.
- Class III. The waste is managed by the company CONSENUR, registered and qualified to do so, according to the provisions of the aforementioned Royal Decree.

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, etc..) 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 Consejo Interministerial de Organismos Modificados Genéticamente y a la Comisión Nacional de Bioseguridad (Interministerial Council of Genetically Modified Organisms and the National Biosafety Commission).

J. Information on emergency response plans

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

Patients will remain in the hospital for at least 72 hours after the infusion.

In the case of accidental release, the following preventive 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 periodically during the 3 years after the infusion of the transduced cells, and then annually for a period of 15 years to control the efficacy and safety of the procedure. Due to the reasons explained above, and in reference to the risk assessment, the development of specific plans to protect the environment will not be considered necessary.