

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 (.)

This Summary Notification Information Format for deliberate release of a genetically modified organism (GMO) has been prepared for the purposes and according to the procedures envisaged by Article 11 of Directive 2001/18/EC and is submitted in support of the Clinical Trial Application for Study ALD-102 in Germany. Per the “*COUNCIL DECISION of 3 October 2002 establishing, pursuant to Directive 2001/18/EC of the European Parliament and of the Council, the summary notification information format for notifications concerning the deliberate release into the environment of genetically modified organisms for purposes other than for placing on the market (2002/813/EC)*” (Part 1), this document provides the information required for the GMO: Lenti-D Drug Product which consists of autologous CD34⁺ cell-enriched population that contains cells transduced with lentiviral vector that encodes an ABCD1 cDNA for human ALDP, suspended in cryopreservation solution in the final immediate container for the intended medical use. Thus, the GMO consists of these 2 key elements: the lentivirus vector and autologous CD34⁺ hematopoietic stem cells.

Lenti-D lentiviral vector is used to transduce the autologous CD34⁺ hematopoietic stem cells. The Lenti-D lentiviral vector is a replication defective, self-inactivating (SIN), human immunodeficiency virus-type 1 (HIV-1) based lentiviral vector that encodes the human ATP-binding cassette, sub-family D, member 1 (ABCD1) cDNA. Lenti-D lentiviral vector is not manufactured in the European Union (EU)

In Part 1 of this document, the information entered reflects (in a condensed form) the information submitted to the Paul Ehrlich Institute in accordance with Articles 6 and 7 of Directive 2001/18/EC.

Part 2 is not applicable as neither of the GMOs described in this document consist of or contain genetically modified higher plants.

A. General information

1. Details of notification

- | | | |
|-----|---|--|
| (a) | Member State of notification | Germany |
| (b) | Notification number | B/DE/17/PEI3281 |
| (c) | Date of acknowledgement of notification | 08.12.2017 |
| (d) | Title of the project | Clinical trial ALD-102 titled: "A phase 2/3 study of the efficacy and safety of hematopoietic stem cells transduced with Lenti-D lentiviral vector for the treatment of cerebral adrenoleukodystrophy (CALD)". |

Proposed period of release

It is anticipated that the recruitment period for clinical trial ALD-102 will be ongoing through December 2018.

The Lenti-D Drug Product and the recombinant Lenti-D lentiviral vector are not released in the environment.

2. Notifier

Name of institution or company:

The Sponsor of ALD-102 is bluebird bio, Inc. with its wholly owned subsidiary, and legal representative in the European Union, bluebird bio France.

3. GMO characterisation

(a) Indicate whether the GMO is a:

- | | |
|----------------|---|
| viroid | (.) |
| RNA virus | (x) (<i>replication incompetent Lenti-D lentiviral vector</i>) |
| DNA virus | (.) |
| bacterium | (.) |
| fungus | (.) |
| animal | (x) |
| - mammals | (x) (<i>autologous CD34+ cells transduced with Lenti-D lentiviral vector</i>) |
| - insect | (.) |
| - fish | (.) |
| - other animal | (.) |

specify phylum, class ...

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

Two GMOs are described below: Lenti-D Drug Product and Lenti-D lentiviral vector.

Lenti-D Drug Product: Lenti-D Drug Product consists of autologous CD34+ cell-enriched population that contains cells transduced with lentiviral vector that encodes an ABCD1 cDNA for human ALDP, suspended in cryopreservation solution in the final immediate container for the intended medical use.

Lineage: Totipotent stem cells

Differentiation: Pluripotent hematopoietic stem cells

Lenti-D lentiviral vector: replication defective, self-inactivating (SIN) recombinant HIV-1 RNA lentiviral vector (rLV) manufactured with 5 recombinant plasmids designed to express all the packaging components to generate a modified rLV. The Lenti-D lentiviral vector packaged RNA transcript encodes the therapeutic ABCD1 cDNA.

Lineage: HIV-1

Differentiation: Not Applicable

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

Sequences used to make the Lenti-D lentiviral vector are verified by complete sequencing of the plasmids prior to initiating manufacturing of the lentiviral vector. The identity of the RNA transcript packaged in the lentiviral vector is confirmed by sequencing the provirus.

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) UK and FR

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

Yes (x) No (.)

If yes:

- Member State of notification UK and FR
- Notification number Not applicable

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 (x) No (.)

If yes:

- Member State of notification USA
- Notification number Not applicable

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

No environmental impact is expected from the administration of Lenti-D Drug Product to patients in Study ALD-102.

The Lenti-D Drug Product consists of autologous CD34⁺ hematopoietic stem cells transduced with the Lenti-D lentiviral vector encoding the ABCD1 cDNA. Transduced cells are not viable in the environments outside of the patient.

The Lenti-D lentiviral vector is replication incompetent, and degrades rapidly in the environment.

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

The recipient is Homo sapiens.

Lenti-D lentiviral vector: The recipient organism is HIV-1, and the parental organisms include HIV-1, Moloney murine leukemia virus (MoMuLV), Vesicular Stomatitis Virus (VSV), and homo sapiens.

Lenti-D Drug Product: The recipient is homo-sapiens, and the parental organism is the Lenti-D lentiviral vector (see above).

It is important to emphasize that DNA sequences from parental organisms are derived from readily available DNA subclones, and that at no point is any work carried out with these organisms.

Please note that the answers for questions 3-10b in this section are given only for organism HIV-1.

1. Recipient or parental organism characterisation:

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

(select one only)

- | | | |
|----------------|-------------------------|--|
| viroid | (.) | |
| RNA virus | (x) | (replication incompetent Lenti-D lentiviral vector and HIV-1) |
| DNA virus | (.) | |
| bacterium | (.) | |
| fungus | (.) | |
| animal | (x) | |
| - mammals | (x) | (autologous CD34 ⁺ cells transduced with Lenti-D lentiviral vector) |
| - insect | (.) | |
| - fish | (.) | |
| - other animal | (.) | |
| | (specify phylum, class) | ... |
| other, specify | ... | |

2. Name

(i) order and/or higher taxon (for animals)

For humans:

Kingdom: Animalia
Phylum: Chordata
Chordata Class: Mammalia
Order: Primates
Family: Hominidae

For HIV-1 and MoMuLV:

Group IV: RNA reverse transcribing viruses
Family: Retroviridae
Subfamily: Orthoretrovirinae

For VSV:

Group V: Mononegavirales
Family: Rhabdoviridae
Genus: Vesiculovirus

(ii) genus

For humans: Homo
For HIV-1: Lentivirus
For MoMuLV: Gammaretroviral
For VSV: Vesiculovirus

(iii) species

For humans: Homo-sapiens
For HIV-1: Human Immunodeficiency Virus 1
For MoMuLV: Murine Leukemia Virus
For VSV: Vesicular Stomatitis Virus

(iv) subspecies

For humans: Lineage: Totipotent stem cells
For HIV-1: Not applicable
MoMuLV: Not applicable
For VSV: Not applicable

(v) strain

For humans: Differentiation: Pluripotent hematopoietic stem cells
For HIV-1: HIV-1 NL4-3
For MoMuLV: Moloney
For VSV: Vesicular stomatitis Indiana virus

(vi) pathovar (biotype, ecotype, race, etc.)

For humans: Not applicable
For HIV-1: Not applicable
For MoMuLV: Not applicable
For VSV: Not applicable

(vii) common name

For humans: human
For HIV-1: HIV-1
For MoMuLV: MoMuLV
For VSV: VSV

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 : in association with animals	(x) (for HIV-1)

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

5. (a) Detection techniques
For HIV-1, multiple detection techniques are currently applied including ELISA and quantitative PCR in humans assessed for infection.
- (b) Identification techniques
For HIV-1, multiple identification techniques are currently applied including ELISA and quantitative PCR in humans assessed for infection.

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

In terms of classification of hazard, HIV-1 is considered as a group 3 biological agent as per the European Economic Community classification for the protection of workers with biological agents (Directive 2000/54/EC).

Though HIV-1 is technically considered a parental organism of Lenti-D lentiviral vector, actual HIV-1 virus is not used in the manufacture of Lenti-D lentiviral vector.

Lenti-D lentiviral vector is produced by transient transfection of HEK293T cells with the plasmid transfer vector that encodes the RNA transcript packaged in the Lenti-D Lentiviral vector and four packaging plasmids that encode the components required for production of Lenti-D lentiviral vector. Lenti-D lentiviral vector is budded from the production cells, harvested, and purified.

Lenti-D lentiviral vector is a self-inactivating, replication defective lentiviral vector containing a wild-type U3 5' that requires the Tat protein to produce the viral particles. The proviral sequence is stably integrated into the recipient cell genome. Because genes derived from HIV in the Lenti-D lentiviral vector are modified to prevent translation, the provirus does not mobilize or transfer. Lenti-D lentiviral vector contains the therapeutic gene and the minimum viral sequence necessary for efficient packaging into viral particles. As an added precautionary measure, all batches of lentiviral vector are tested for the possibility of replication competent lentivirus (RCL).

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

Yes (x) No (.) Not known (.)

If yes:

- (a) to which of the following organisms:

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

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

HIV-1 is a human blood born pathogen that causes acquired immune-deficiency syndrome (AIDS). HIV can also persist as a latent provirus. Its primary host is *Homo sapiens*.

The recombinant HIV-1 based Lenti-D lentiviral vector does not encode any HIV genes and is self-inactivating and replication deficient. The Lenti-D lentiviral vector does not encode for any HIV proteins; the only HIV derived sequences in the transcript are the long terminal repeats (LTR) (made self-inactivating (SIN) by deleting promoter/enhancer sequences), attenuated regions of the group antigen (gag), central polypurine tract (cPPT), and rev-response element (RRE), all of which aid in the production, packaging, or transfer of the transcript containing the therapeutic gene.

8. Information concerning reproduction

- (a) Generation time in natural ecosystems:

The generation time of HIV replication in patient cells has been estimated at approximately 1.2 – 1.8 days. HIV infects and replicates in human immune cells.

Lenti-D lentiviral vector does not encode any HIV genes and is self-inactivating and replication deficient. Reference: Rodrigo *et al.*, 1999 - Proc Natl Acad Sci U S A. 1999 Mar 2;96(5):2187-91.) Coalescent estimates of HIV-1 generation time in vivo. Rodrigo AG, Shpaer EG, Delwart EL, Iversen AK, Gallo MV, Brojatsch J, Hirsch MS, Walker BD, Mullins JI.

- (b) Generation time in the ecosystem where the release will take place:
HIV-1 is not released.

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

- (a) Factors affecting reproduction:

HIV-1 is a blood borne retrovirus that reproduces by infecting human immune cells.

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 | HIV can persist as a latent provirus. |

- (b) relevant factors affecting survivability:
HIV-1 is highly unstable in an uncontrolled environment.
10. (a) Ways of dissemination
HIV-1 is a blood-born pathogen which primarily disseminates by sexual contact, blood contact and vertical transmission from mother to child.
- (b) Factors affecting dissemination
Any situation that increases ways of dissemination is explained in 10.a)
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)

bluebird bio is currently conducting a study in France (HGB-205, EudraCT #: 2012-000695-42) entitled “A Phase 1/2 Open Label Study Evaluating the Safety and Efficacy of Gene Therapy of the β -Hemoglobinopathies (Sickle Cell Anemia and β -Thalassemia Major) by Transplantation of Autologous CD34⁺ Stem Cells Transduced Ex Vivo with a Lentiviral β A-T87Q-Globin Vector (LentiGlobin® BB305 Drug Product).”

In this study, subjects are treated with LentiGlobin BB305 Drug Product, a gene therapy product consisting of autologous CD34⁺ hematopoietic stem cells transduced with LentiGlobin BB305 lentiviral vector encoding the β^{A-T87Q} -globin gene. Subjects with either severe sickle cell disease or β -thalassemia major can be enrolled in this trial.

LentiGlobin BB305 lentiviral vector is a replication defective, self-inactivating (SIN), third generation human immunodeficiency virus type 1 (HIV-1) based lentiviral vector pseudotyped with the vesicular stomatitis virus G (VSV-G) envelope protein, carrying the human β -globin gene with a single modification at codon 87 (β^{A87} Thr:Gln [β^{A-T87Q}]).

C. Information relating to the genetic modification

1. Type of the genetic modification
- | | | |
|-------|-------------------------------|--|
| (i) | insertion of genetic material | (X) (autologous CD34 ⁺ cells transduced with Lenti-D lentiviral vector and Lenti-D lentiviral vector) |
| (ii) | deletion of genetic material | (X) (Lenti-D lentiviral vector) |
| (iii) | base substitution | (X) (autologous CD34 ⁺ cells transduced with Lenti-D lentiviral vector and Lenti-D lentiviral vector) |
| (iv) | cell fusion | (.) |
| (v) | others, specify ... | |
2. Intended outcome of the genetic modification

For Lenti-D Drug Product:

The goal of the genetic modification is to add a functional copy of the human ABCD1 cDNA (the “therapeutic gene”) to the autologous CD34⁺ hematopoietic stem cells by transduction ex vivo. In vivo the transduced cells will differentiate and express the therapeutic gene in the aim to improve health of patients with CALD.

For Lenti-D lentiviral vector:

The goal of the genetic modifications are to create a recombinant lentiviral vector that is capable of modifying target CD34⁺ cells such that the proviral sequence containing the therapeutic cDNA is stably integrated into the genome of these cells. Additional genetic modifications include modifications that prevent replication competent lentivirus (RCL) in transduced 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	(x) (<i>Lenti-D lentiviral vector</i>)
bacteriophage	(.)
virus	(x) (<i>autologous CD34⁺ cells transduced with Lenti-D lentiviral vector</i>)
cosmid	(.)
transposable element	(.)
other, specify ...	

- (b) Identity of the vector

Identity of the vector used in the production of Lenti-D Drug Product:

Lenti-D lentiviral vector is a replication defective, self-inactivating (SIN), third generation human immunodeficiency virus-type 1 (HIV-1) based lentiviral vector pseudotyped with the vesicular stomatitis virus-glycoprotein (VSV-G) envelope protein.

The Lenti-D lentiviral vector does not encode for any HIV proteins; the only HIV derived sequences in the transcript are the long terminal repeats (LTR) (made self-inactivating (SIN) by deleting promoter/enhancer sequences), attenuated regions of the group antigen (gag), central polypurine tract (cPPT), and rev-response element (RRE), all of which aid in the production, packaging, or transfer of the transcript containing the therapeutic gene. Additional sequences are derived from the human ABCD1 cDNA sequence and the Moloney murine leukemia virus MND promoter.

Identity of the plasmid vectors used in the production of Lenti-D lentiviral vector:

Lenti-D lentiviral vector is produced by transient transfection of HEK293T cells with the transfer vector (that encodes the packaged RNA transcript) and four packaging plasmids (that encode the components necessary for making the viral envelope and

package the RNA transcript). This multi-plasmid system was designed to prevent recombination and emergence of a replication competent lentivirus (RCL). These five plasmids encode minimal elements (described above) from the HIV-1 genome essential for packaging of the vector RNA. All of the accessory viral genes that are dispensable were removed from the system, including the HIV envelope, vpr, vpu and nef. The ABCD1 cDNA is encoded in the transfer vector.

(c) Host range of the vector

Host range of the Lenti-D lentiviral vector:

Lentiviral vectors pseudotyped with VSV-G are capable of transducing animal and insect cells. However, Lenti-D lentiviral vector is not replication competent and does not encode any pathogenic genes.

Lenti-D lentiviral vector is a self-inactivating, replication defective lentiviral vector containing a wild-type U3 5' that requires the Tat protein for the production of the viral particles. The proviral sequence is stably integrated into the recipient cell genome. Because genes derived from HIV in the Lenti-D lentiviral vector are modified to prevent translation, the provirus does not mobilize or transfer. Lenti-D lentiviral vector contains the therapeutic gene under the control of the MND promoter and the minimum viral sequence necessary for efficient packaging into viral particles. As an added precautionary measure, all batches of lentiviral vector are tested for the possibility of replication competent lentivirus (RCL).

Host range of the packaging plasmids used in the production of Lenti-D lentiviral vector:

The packaging plasmids used in the production of Lenti-D lentiviral vector contain origins of replication sequences that allow them to propagate in bacterial cells. The plasmids are maintained in bacterial cells due to the presence of antibiotic selection; in the absence of antibiotic selection, these plasmids are not maintained in the bacterial cells and are eventually lost.

Mammalian cells are transiently transfected with the packaging plasmids. As there is no selection to maintain these plasmid vectors in the mammalian cell culture, the packaging plasmids are eventually lost from the transiently transfected mammalian cells.

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

antibiotic resistance (x)
 other, specify

Confirmation of presence of vector sequences in the recipient cells is conducted by qPCR and assessment of the expression of ALD protein (ALDP) is performed by immunohistochemistry in peripheral blood leukocytes.

Indication of which antibiotic resistance gene is inserted: The packaging plasmids used in the manufacture of Lenti-D lentiviral vector contain Ampicillin resistance cassettes to ensure the plasmids are maintained in bacterial stocks.

- (e) Constituent fragments of the vector
This information has been provided to the appropriate EU national competent authorities.
- (f) Method for introducing the vector into the recipient organism
 - (i) transformation (.)
 - (ii) electroporation (.)
 - (iii) macroinjection (.)
 - (iv) microinjection (.)
 - (v) infection (.)
 - (vi) other, specify ex vivo transduction into CD34+ autologous hematopoietic stem cells by chemical means.

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

This information has been provided to the appropriate EU national competent authorities.

(b) Source of each constituent part of the insert

This information has been provided to the appropriate EU national competent authorities.

It is important to emphasize that no functional HIV genes are encoded in the Lenti-D Drug Product insert. The constituent parts of the sequences are necessary for the expression and production of the therapeutic human ABCD1 cDNA. HIV sequences are necessary for the packaging and delivery of the insert. The insert has no replication function and does not encode any pathogenic genes.

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

For Lenti-D Drug Product: No functional HIV genes are encoded in the Lenti-D Drug Product insert (provirus). The insert encodes sequences necessary for the expression and production of the therapeutic human ABCD1 gene. to express functional ALDP. Expression of ALDP in the brain microglia should reduce levels of VLCFAs and, thereby, mitigate the cerebral demyelination that is the hallmark of CALD. HIV sequences are necessary for the packaging and delivery of the insert. The insert has no replication function and does not encode any pathogenic genes.

For Lenti-D lentiviral vector:

The intended function of the constituents of the genetic inserts of the plasmids are to create a recombinant lentiviral vector that is capable of modifying target CD34⁺ cells such that the proviral sequence containing the therapeutic cDNA is stably integrated into the genome of these cells.

- (b) Location of the insert in the host organism

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

- (c) 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 (x)
- mammals (x)
- insect (.)
- fish (.)
- other animal (.)
- (specify phylum, class) ...
- other, specify ...

2. Complete name

This section is not applicable.

The donor, Lenti-D lentiviral vector, is a replication defective, SIN, third generation HIV-1 based lentiviral vector pseudotyped with the VSV-G envelope protein.

The Lenti-D lentiviral vector does not encode any HIV genes; the only HIV derived sequences in the transcript are the 3' LTR (made SIN by deleting promoter/enhancer sequences), cPPT, and RRE, all of which aid in the production, packaging, or transfer of the transcript containing the therapeutic gene. Additional sequences are derived from the human ABCD1 cDNA and the MND promoter of the Muloney murine leukemia virus.

- (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 ...

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 Group 2

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

The Lenti-D Drug Product proviral insert is stably integrated into the genome of the cell, and does not have the capacity for mobilization. The RNA transcript packaged in the recombinant Lenti-D lentiviral vector is stable and does not have capacity for replication outside its host cell.

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)

...

4. Description of identification and detection methods

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

The Lenti-D Drug Product is not released to the environment, and is not stable under uncontrolled environmental conditions. It is infused into the patient from whom the autologous cells were originally obtained, and is detected using qPCR. Detection of the recombinant Lenti-D lentiviral vector is conducted with ELISA.

(b) Techniques used to identify the GMO

qPCR quantifies the amount of integrated vector in recipient cells.

Immunohistochemistry will be used to identify expression of therapeutic gene product.

ELISA is used to identify the recombinant Lenti-D lentiviral vector.

F. Information relating to the release

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

The Lenti-D Drug Product is not released into the environment. A patient with CALD is infused with Lenti-D Drug Product with the aim of halting the progress of CALD. The recombinant Lenti-D lentiviral vector is used to transduce the patient cells to add the human ABCD1 cDNA sequence into the autologous CD34⁺ hematopoietic stem cells.

The Lenti-D Drug Product is manufactured at GMP manufacturing sites in the EU and USA. Autologous hematopoietic stem cells are collected from the subject at the clinical site and transported to the manufacturing facility where they are transduced with the Lenti-D lentiviral vector to produce the final Lenti-D Drug Product. Each lot of Drug Product is tested to ensure identity and purity prior to release. The released Drug Product is then transported from the drug product manufacturing site back to the clinical site under controlled conditions, where it is stored prior to infusion.

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

Yes (x) No (.)

If yes, specify

For Lenti-D Drug Product:

The Lenti-D Drug Product is not released in the environment; it is intravenously infused into patients in clinical trial ALD-102 under highly controlled conditions in the transplant unit of a hospital, who have been myeloablated prior to treatment and will be followed for up to 15 years. After infusion, the cells migrate to the bone marrow to reconstitute the bone marrow for hematopoietic cells production. Lenti-D Drug Product is not viable outside the body of the specific recipient. In addition, HLA mismatched cells would be rapidly destroyed in a non-immunocompromised host.

The Lenti-D lentiviral vector is not released in the environment; it is released under highly controlled and isolated conditions (in vitro) at the GMP manufacturing site to transduce autologous CD34+ hematopoietic stem cells ex vivo. The site of release of the Lenti-D lentiviral vector is not its natural habitat. Blood has been purified away from the autologous CD34+ hematopoietic stem cells.

3. Information concerning the release and the surrounding area

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

Not applicable.

- (b) Size of the site (m²): Not applicable.

(i) actual release site (m²): Not applicable.

(ii) wider release site (m²): Not applicable.

- (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:

The Lenti-D Drug Product and the recombinant Lenti-D lentiviral vector are not released into the environment.

Lenti-D Drug Product is administered intravenously to patients in the transplantation unit of a hospital. At least 5.0 million transduced autologous CD34+ hematopoietic stem cells per kg (subject weight) will be administered intravenously into each subject.

Approximately 45 millilitres of Lenti-D lentiviral vector will be used for the ex vivo transduction of the autologous CD34+ hematopoietic stem cells in the controlled and insulated GMP manufacturing site located in the EU.

- (b) Duration of the operation:

The intravenous infusion of Lenti-D Drug Product is expected to last 1 hour.

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

The Lenti-D Drug Product (transduced cells) and the recombinant Lenti-D lentiviral vector are not released into the environment.

The Lenti-D Drug Product is administered intravenously into the patient under standard controlled conditions for hematopoietic stem cell transplant at a clinical site.

All waste is destroyed according to hospital bio-hazard disposal procedures.

The recombinant lentiviral vector Lenti-D is used to transduce ex-vivo the autologous CD34⁺ hematopoietic stem cells in the controlled and isolated manufacturing laboratory setting. All waste is destroyed according to the manufacturing facility bio-hazard disposal procedures after decontamination and use of disinfectant.

The proviral sequence is stably integrated into the recipient cell genome. Because genes derived from HIV in the Lenti-D lentiviral vector are modified to prevent translation, the provirus does not mobilize or transfer. Lenti-D lentiviral vector contains the therapeutic gene under the control of the MND promoter and the minimum viral sequence necessary for efficient packaging into viral particles. Lenti-D lentiviral vector is a self-inactivating, replication defective lentiviral vector containing a wild-type U3 5' that requires the Tat protein to produce the viral particles.

All manipulations of the Lenti-D Drug Product and lentiviral vector are carried out in the licensed, controlled GMP manufacturing facility. Appropriate cleaning procedures, including the use of disinfectants with viricidal activity, are in place at the facility.

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.

Not applicable.

ALD-102 is the first clinical trial in which Lenti-D Drug Product has been administered.

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

Given the autologous nature of Lenti-D DP, this section is not applicable.

1. Name of target organism (if applicable)
 - (i) order and/or higher taxon (for animals) ...
 - (ii) family name for plants ...
 - (iii) genus ...
 - (iv) species ...
 - (v) subspecies ...
 - (vi) strain ...
 - (vii) cultivar/breeding line ...
 - (viii) pathovar ...
 - (ix) common name ...
2. Anticipated mechanism and result of interaction between the released GMOs and the target organism (if applicable)

The Lenti-D Drug Product is composed of modified, autologous CD34⁺ hematopoietic stem cells. The Lenti-D Drug Product is intravenously infused into patients with CALD. Upon infusion into the patient, the cells migrate to the bone marrow and reconstitute hematopoietic cell production. It is expected that the Lenti-D Drug Product will have a therapeutic effect in treated patients with CALD. Expression of ALDP in the brain microglia should reduce levels of VLCFAs and, thereby, mitigate the cerebral demyelination that is the hallmark of CALD. The recombinant Lenti-D lentiviral vector is used to transduce ex-vivo the autologous CD34⁺ hematopoietic stem cells and stably integrate the ABCD1 cDNA into the cell genome.

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

None.

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

Yes (.) No (x) Not known (.)

Give details

SIN lentiviral vectors lack the strong enhancer/promoter long terminal repeat (LTR) sequences of γ -retroviral vectors, and, unlike γ -retroviral vectors, do not preferentially integrate near gene promoter regions. Therefore, lentiviral vectors are less likely to transactivate oncogenes, and have demonstrated a significantly curtailed probability of oncogenic transformation in vitro and in vivo (Biffi et al, 2011).

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

The Lenti-D Drug Product is not released into the environment. Transduced cells are not viable in the environment outside of the patient, and thus it is not anticipated that Lenti-D Drug Product could become established in any environment outside the patient.

The Lenti-D lentiviral vector is replication incompetent and degrades rapidly in the environment as well; thus, it is not anticipated that Lenti-D lentiviral vector could become established in any environment.

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

None. This section is not applicable. The Lenti-D Drug Product (transduced CD34⁺ autologous hematopoietic stem cells) and the recombinant Lenti-D lentiviral vector are not released in the environment.

(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:

The Lenti-D Drug Product is made with a replication defective vector that inserts the proviral DNA stably into the genome of the autologous CD34⁺ hematopoietic stem cells. Neither the insert nor the vector is capable of replication. In addition, Lenti-D Drug Product is unstable in an uncontrolled environment and is not viable outside the body of the recipient (treated patient) as HLA mismatched cells would be rapidly destroyed in a non-immunocompromised host.

Lentiviral vectors pseudotyped with VSV-G are capable of transducing animal and insect cells. However, it is important to emphasize that Lenti-D lentiviral vector is not replication competent and does not encode any pathogenic genes.

- (b) from other organisms to the GMO:

The Lenti-D Drug Product will exist as hematopoietic cells in the patient. While it is always possible that human patients are infected with other organisms, there is no added risk to the patient as the Lenti-D lentiviral vector does not encode any viral or pathogenic genes.

Lenti-D lentiviral vector is a self-inactivated lentiviral vector; vector that does not transduce autologous cells is removed from the drug product during the manufacturing process. Additionally, the risk of a theoretical recombination event is mitigated by excluding HIV-positive patients from the clinical trial.

- (c) likely consequences of gene transfer:

Once the Lenti-D Drug Product is manufactured, no further gene transfer is anticipated.

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.):

Lenti-D Drug Product is infused into the corresponding patient. The recombinant Lenti-D lentiviral vector is used to transduce ex-vivo the autologous CD34⁺ hematopoietic stem cells in a controlled and insulated manufacturing laboratory setting. Lenti-D Drug Product and Lenti-D lentiviral vector are not viable in the environment, and neither will be released in the environment.

A study with a similar approach using a previous lentiviral vector referred to as CG1711hALD was published in 2009 (Cartier *et. al.*, Science 2009 Nov 6;326(5954):818-23).

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

Upon infusion into the patient, the Lenti-D Drug Product is monitored using qPCR for identification and quantification of the therapeutic insert. The therapeutic gene product is detected via immunohistochemistry.

Upon manufacturing, the Lenti-D lentiviral vector is monitored by ELISA and classical cell culture techniques.

2. Methods for monitoring ecosystem effects

Not applicable. The Lenti-D Drug Product and lentiviral vector are not released into the environment.

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

Not applicable. The Lenti-D Drug Product and lentiviral vector are not released into the environment.

4. Size of the monitoring area (m²)

Not applicable. The GMOs are not released in the environment.

5. Duration of the monitoring

Patients who receive Lenti-D Drug Product will be asked to enrol in a long-term follow-up protocol and will be monitored for up to 15 years post-treatment.

6. Frequency of the monitoring

Subjects are monitored for 24 months post-transplant as part of Study ALD-102. Subjects are then offered to participate in a long-term follow-up study for an additional 13 years.

The Lenti-D lentiviral vector is tested after manufacturing and at standard predefined stability time points. All batches of Lenti-D lentiviral vector are tested for the possibility of RCL.

I. Information on post-release and waste treatment

1. Post-release treatment of the site

The Lenti-D Drug Product (transduced cells) is not released in the environment. It is administered intravenously into the patient under standard controlled conditions for hematopoietic stem cell transplant at a clinical site.

All waste remaining after the intravenous infusion of the Drug Product is destroyed according to hospital bio-hazard disposal procedures.

All manipulations of the Lenti-D Drug Product will be carried out in compliance with appropriate Biosafety Level containment in a licensed GMP manufacturing facility located in the EU and USA.

Lenti-D lentiviral vector is handled under highly controlled and isolated conditions (in vitro) in a GMP manufacturing facility outside of the EU. After manufacture of the Lenti-D Drug Product, the manufacturing facility is cleaned according to the biohazard procedures of the institution.

2. Post-release treatment of the GMOs

The Lenti-D Drug Product is not released into the environment. The drug product is infused into the patient as a one-time therapeutic treatment. There are no further post-release or waste treatments after infusion of the Lenti-D Drug Product (transduced patient cells) beyond the hospital bio-hazard disposal procedures noted in question 1 above.

Lenti-D lentiviral vector is not released into the environment; it is released under highly controlled and isolated conditions (in vitro) in a GMP manufacturing facility outside of the EU to transduce autologous CD34⁺ hematopoietic stem cells ex-vivo.

3. (a) Type and amount of waste generated

The waste generated following treatment of patients with the Lenti-D Drug Product is minimal and consists mainly of residual cells remaining in the infusion bag. All waste is destroyed according to hospital bio-hazard disposal procedures.

The waste generated following manufacturing of the recombinant lentiviral vector Lenti-D lentiviral vector is minimal and consists of residual process solutions that may have contacted the lentiviral vector and residual inactivated viral particles.

All waste is destroyed according to the bio-hazard disposal procedures of the GMP manufacturing facility after appropriate disinfection.

3. (b) Treatment of waste

All waste is destroyed according to hospital or manufacturing facility bio-hazard disposal procedures.

J. Information on emergency response plans

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

The manufacturing facility where the recombinant Lenti-D lentiviral vector used to transduce ex-vivo the autologous CD34⁺ hematopoietic stem cells is a controlled and isolated GMP laboratory setting. The Lenti-D Drug Product (transduced patient cells) is not viable in the environment outside of the body of the treated patient.

The Lenti-D lentiviral vector does not replicate, and the provirus is stably integrated into the genome of the patient CD34⁺ cells. Lenti-D lentiviral vector is unstable in the environment.

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

In case of accidental spill of the Lenti-D Drug Product (transduced patient cells), hospital decontamination and cleaning procedures are applied. Disinfection of waste is provided by products such as povidone-iodine, paraformaldehyde, aqueous bleach solutions or detergent-based disinfectant.

Lenti-D lentiviral vector is not released into the environment; it is released under highly controlled and isolated conditions to transduce autologous CD34⁺ hematopoietic stem cells ex-vivo in a GMP manufacturing facility that follows GMP procedures in working with and disposing of GMOs.

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

No plants, animals, and soil will be in the transplant unit where the Lenti-D Drug Product is administered to the patient or where the Lenti-D lentiviral vector is manufactured.

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

Lenti-D Drug Product is manufactured and shipped in accordance with GMP. The transduced cells are not viable outside of the body of the treated patients. Patients will be monitored for potential clonal skewing and leukemogenesis during the 2-year enrolment period for study ALD-102. After completion of ALD-102, subjects will be asked to participate in a 13-year long-term follow-up study and will be monitored for clonal skewing and leukemogenesis during this study as well.

Recombinant Lenti-D lentiviral vector does not encode any pathogenic genes and degrades rapidly in the environment. No plans are in place other than controlled manufacture and shipping of the Lenti-D lentiviral vector in accordance with GMP.