ALD-102 Clinical Trial Application
Section III.1.1.6 – Part 3: SNIF for GMO Notification (according to 2002/813/EC)

Introduction

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 the Phase II/III clinical trial ALD-102 in the United Kingdom (UK) and France. 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 on two GMOs: Lenti-D Drug Product utilized in clinical study ALD-102 and Lenti-D lentiviral vector which is used to manufacture Lenti-D Drug Product.

Lenti-D Drug Product consists of autologous $CD34^+$ hematopoietic stem cells transduced with the Lenti-D lentiviral vector and suspended in CryoStor® CS5 (BioLife Solutions) cryopreservative solution containing 5% dimethyl sulfoxide (DMSO) in the final immediate container for the intended medical use.

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 competent authority 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.

PART 1

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

A. GENERAL INFORMATION

1. Details of notification

a) Member State of notification

France and United Kingdom

b) Notification number: B/FR/15/GT04

EudraCT number: 2011-001953-10

c) Date of acknowledgement of notification

21/01/2015

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 childhood cerebral adrenoleukodystrophy (CCALD)".

e) Proposed period of release

It is anticipated that the recruitment period for clinical trial ALD-102 will be approximately 2 years (from September 2013 to September 2015).

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 bluebird bio France.

3. GMO characterization

a) Indicate whether the GMO is a:

Viroid	☐
RNA virus	■ *(replication incompetent Lenti-D lentiviral vector)*
DNA virus	☐
bacterium	☐
fungus	☐
animal	■
- mammals	■ *(autologous CD34+ cells transduced with Lenti-D lentiviral vector)*
- insect	☐
- fish	☐
- other animal	☐ specify phylum, class

other, please specify (kingdom, phylum and class)

b) Identity of the GMO

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

Lenti-D Drug Product: autologous $CD34^+$ hematopoietic stem cells transduced with the Lenti-D lentiviral vector.

Lineage: Totipotent stem cells

Differentiation: Pluripotent hematopoietic stem cells

Lenti-D lentiviral vector: The Lenti-D lentiviral vector is a 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)

Lenti-D Drug Product:

Genetic sequences are stably integrated into the DNA of the transduced autologous $CD34^+$ cells. Identity and stability of integration of an intact provirus in the Lenti-D Drug Product, devoid of rearrangements, is verified by proviral sequencing of transduced test cells.

Lenti-D lentiviral vector:

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 ☐	No ■
If yes, insert the country code(s):	

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

Yes ☐	No ■
If yes: – Member State of notification - Notification number	

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

Yes ☐	No ■
If yes: – Member State of notification - Notification number	

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

Lenti-D Drug Product:
No environmental impact is expected from the administration of Lenti-D Drug Product to patients in clinical trial 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 ex vivo transduction of patient autologous $CD34^+$ cells with Lenti-D lentiviral vector will be conducted outside of the EU.

Lenti-D lentiviral vector:
The Lenti-D lentiviral vector is replication incompetent, and degrades rapidly in the environment. Because genetic sequences 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 is not imported in the EU.

B. INFORMATION RELATING TO THE RECIPIENT OR PARENTAL ORGANISMS FROM WHICH THE GMO IS DERIVED

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 actually 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 characterization:

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

Viroid	☐
RNA virus	■ (*replication incompetent Lenti-D lentiviral vector and HIV-1*)
DNA virus	☐
bacterium	☐
fungus	☐
animal	■
- mammals	■ *(autologous CD34+ cells transduced with Lenti-D lentiviral vector)*
- insect	☐
- fish	☐
-other animal	☐ (please specify phylum, class)

other, please 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

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

a) Indigenous to, or otherwise established in, the country where the notification is made: Yes ■ No ☐ Not known ☐
b) Indigenous to, or otherwise established in, other EC countries: (i) Yes ■ If *yes*, indicate the type of ecosystem in which it is found: Atlantic ■ Mediterranean ■ Arctic ■ Alpine ■ Continental ■ (ii) No ☐ (iii) Not known ☐
c) Is it frequently used in the country where the notification is made? Yes ☐ No ■
d) Is it frequently kept in the country where the notification is made? Yes ☐ No ■

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	☐
in association with animals	■ (for HIV-1)
other (specify)	

(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.

5. 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 ■	No ☐

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 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 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 ■	No ☐	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

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 ■

d) 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 (fungi) ☐
(vi) eggs ☐
(vii) pupae ☐
(viii) larvae ☐

(ix) other, please 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.

10. 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 βA-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 $\beta^{A\text{-}T87Q}$-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 [$\beta^{A\text{-}T87Q}$]).

C. INFORMATION RELATING TO THE GENETIC MODIFICATION

1. Type of the genetic modification

(i) Insertion of genetic material	■(*autologous CD34+ cells transduced with Lenti-D lentiviral vector and Lenti-D lentiviral vector*)
(ii) Deletion of genetic material	■ *(Lenti-D lentiviral vector*)
(iii) Base substitution	■ (*autologous CD34+ cells transduced with Lenti-D lentiviral vector and Lenti-D lentiviral vector*)
(iv) Cell fusion	☐
(v) Other, please 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. In vivo the transduced cells will differentiate and express the therapeutic gene improving the health of patients with CCALD.

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 ■	No ☐
If no, go straight to question 5.	

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

Yes ■	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	■ (*Lenti-D lentiviral vector*)
bacteriophage	☐
virus	■ (*autologous CD34+ cells transduced with Lenti-D lentiviral vector)*
cosmid	☐
transposable element	☐

other, please specify

b)

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 Lenti-D lentiviral vector:

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.

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).

The ex vivo transduction of patient autologous $CD34^+$ cells with Lenti-D lentiviral vector will not be conducted in the EU.

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 ■ No☐

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.

Antibiotic resistance ■

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, please specify:

For Lenti-D Drug Product: ex vivo transduction into $CD34^+$ autologous hematopoietic stem cells are transduced ex vivo with the Lenti-D lentiviral vector.

For Lenti-D lentiviral vector: transient transfection of HEK293T cells with the four packaging plasmids and the transfer plasmid which encodes the RNA transcript packaged in the Lenti-D lentiviral vector.

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, please specify

6. Information on 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:
The intended function of the inserted human ABCD1 cDNA in the autologous genetically modified $CD34^+$ cells is 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 CCALD.

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.

d) Location of the insert in the host organism

- on a free plasmid	☐
- integrated in the chromosome	■

- other, please specify

e) Does the insert contain parts whose product or function are not known?

Yes ☐ No ■

If yes, please specify

D. INFORMATION ON THE ORGANISM(S) FROM WHICH THE INSERT IS DERIVED (DONOR)

1. Indicate whether it is a:

Viroid	☐
RNA virus	■
DNA virus	☐
bacterium	☐
fungus	☐
animal	■
- mammals	■
- insect	☐
- fish	☐
- other animal	☐ (please specify phylum, class)

other, please specify

2. Complete name

This section is not applicable.

The donor, 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 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 ■	Not known ☐

If *yes*, please specify the following

a) 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 ■ 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 related to exposure to biological agents at work?

Yes ■	No ☐

If yes, please specify:

Group 2

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

Yes ☐	No ■	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 ■ Not known ☐

Please 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 ■ Not known ☐

Please specify

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

Yes ☐ No ■ Not known ☐

Please specify

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

Yes ☐ No ■ Not known ☐

Please specify

2. Genetic stability of the genetically modified organism

Lenti-D Drug Product: The Lenti-D Drug Product proviral insert is stably integrated into the genome of the cell, and does not have the capacity for mobilization.

Lenti-D Lentiviral vector: 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 ■	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) and II (C) (2) (i)		

4. Description of identification and detection methods

a) Techniques used to detect the GMO in the environment

Lenti-D Drug Product:

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.

Lenti-D lentiviral vector:

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

For Lenti-D Drug Product:

A patient with CCALD is infused with Lenti-D Drug Product (transduced patient cells) with the goal of halting the progress of CCALD.

For Lenti-D lentiviral vector:

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.

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 ☐

If yes, please specify

For Lenti-D Drug Product:

The Lenti-D Drug Product (transduced patient cells) is not released in the environment; it will be intravenously infused into a limited number of patients in clinical trial ALD-102 under highly controlled conditions in the transplant unit of a hospital, who 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.

For Lenti-D lentiviral vector:

Currently, the manufacture of Lenti-D lentiviral vector and the ex vivo transduction of patient autologous $CD34^+$ cells with Lenti-D lentiviral vector is conducted outside of the EU.

The recombinant Lenti-D lentiviral vector is not released in the environment; it is released under highly controlled and isolated conditions (in vitro) in the manufacturing laboratory to transduce autologous $CD34^+$ hematopoietic stem cells ex-vivo. The site of release of the recombinant lentiviral vector Lenti-D lentiviral vector is not its natural habitat.

3. Information concerning the release and the surrounding area

This section is not applicable as neither GMO is released into the environment.

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

Not applicable.

b) Size of the site (m^2): Not applicable.

(i) actual release site (m^2):

(ii) wider release area (m^2):

c) Proximity to internationally recognized 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:

For Lenti-D Drug Product:

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.

For Lenti-D lentiviral vector:

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.

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 minimize 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 manipulations of the Lenti-D Drug Product and recombinant lentiviral vector are carried out under Biosafety Level 2 containment.

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 at the manufacturing site outside the EU. All waste is destroyed according to the manufacturing facility bio-hazard disposal procedures after decontamination and use of disinfectant (such as chlorine bleach 10%, Wescodyne, or detergent based 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 for the production of the viral particles.

5. Short description of average environmental conditions (weather, temp. 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

This section is not applicable.

Lenti-D Drug Product and the recombinant Lenti-D lentiviral vector are not released in the environment; it is not anticipated that either GMO will interact with the environment outside the body of the treated patient and thus no potential impact on the environment is anticipated from either GMO.

1. Name of target organisms (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)

For Lenti-D Drug Product:

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 CCALD. 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 CCALD. Expression of ALDP in the brain microglia should reduce levels of VLCFAs and, thereby, mitigate the cerebral demyelination that is the hallmark of CCALD.

For Lenti-D lentiviral vector:

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 ■	Not known ☐

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). To date, it is estimated that more than 20 subjects have been treated in gene therapy studies involving lentiviral vectors and HSCs, with no published cases of therapy-related leukemia or lymphoma in any patient (Cavazzana-Calvo et al, 2010; Cartier et al, 2009; Scaramuzza et al, 2012; Montini et al, 2012).

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

For Lenti-D Drug Product:

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.

For Lenti-D lentiviral vector:

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:

For Lenti-D Drug Product:

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. Therefore, gene transfer to unintended organisms is not anticipated. 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.

For Lenti-D lentiviral vector:

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:

For Lenti-D Drug Product:

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. Additionally, the risk of a theoretical recombination event is mitigated by excluding HIV-positive patients from the clinical trial.

For Lenti-D lentiviral vector:

Not applicable; Lenti-D lentiviral vector is a self-inactivated lentiviral vector.

c) likely consequences of gene transfer:

For Lenti-D Drug Product:

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

For Lenti-D lentiviral vector:

Not applicable; Lenti-D lentiviral vector is a self-inactivated lentiviral vector.

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 simulated 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

For Lenti-D Drug Product:

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.

For Lenti-D lentiviral vector:

Not applicable

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

For Lenti-D Drug Product:

Not applicable. The Lenti-D Drug Product is not released into the environment. Once Lenti-D Drug Product is intravenously administered to patients, the donated genetic material is not expected to transfer to other organisms.

Lenti-D lentiviral vector is a self-inactivating, replication defective lentiviral vector. The proviral sequence is stably integrated into the recipient cell genome. Genes derived from HIV in the Lenti-D lentiviral vector are modified to prevent translation and contain the minimum viral sequences necessary for efficient packaging of the RNA transcript into viral particles; thus the provirus does not mobilize or transfer. As an added precautionary measure, all batches of lentiviral vector are tested for the possibility of replication competent lentivirus (RCL).

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

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

The Lenti-D Drug Product is administered intravenously to patients in the transplant unit of a hospital; there are no plans to monitor the transplant unit for the presence of Lenti-D Drug Product.

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

For Lenti-D Drug Product:

Subjects are monitored 2-years post-transplant according to the ALD-102 clinical protocol. Subjects will be asked to participate in a long-term follow-up study for an additional 13 years.

For Lenti-D lentiviral vector:

All batches of Lenti-D lentiviral vector are tested for the possibility of replication competent lentivirus (RCL).

I. Information on post-release and waste treatment

1. Post-release treatment of the site

For Lenti-D Drug Product:

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.

For Lenti-D lentiviral vector:

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 GMO

For Lenti-D Drug Product:

The Lenti-D Drug Product is not released into the environment. 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.

For Lenti-D lentiviral vector:

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

For Lenti-D Drug Product:

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.

For Lenti-D lentiviral vector:

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

For Lenti-D Drug Product:

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.

For Lenti-D lentiviral vector:

This is not applicable. 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. 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 facility outside of the EU. Lenti-D lentiviral vector is unstable in the environment. Lenti-D lentiviral vector is not expected to disseminate and spread unexpectedly in the environment.

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

For Lenti-D Drug Product:

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 (Betadine™), paraformaldehyde, aqueous bleach solutions or detergent-based disinfectant.

For Lenti-D lentiviral vector:

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 outside of the EU 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

Not applicable - plants, animals, and soil are not expected to 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

For Lenti-D Drug Product:

The Lenti-D Drug Product (transduced patient cells) does not encode any pathogenic genes. 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.

For Lenti-D lentiviral vector:

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