PART 1 (COUNCIL DECISION 2002/813/EC)

SUMMARY NOTIFICATION INFORMATION FORMAT FOR THE RELEASE OF GENETICALLY MODIFIED ORGANISMS OTHER THAN HIGHER PLANTS IN ACCORDANCE WITH ARTICLE 11 OF DIRECTIVE 2001/18/EC

In order to tick one or several possibilities, please use crosses (meaning x or X) into the space provided as (.)

A. General information

1. Details of notification

(a)	Member State of notification	Germany
(b)	Notification number	B/DE/19/PEI3620
(c)	Date of acknowledgement of notification	26.11.2018
(d)	Title of the project	Clinical trial ALD-104 (EudraCT No. 2018-001145-14) titled: "A Phase 3 Study of Lenti-D Drug Product After Myeloablative Conditioning Using Busulfan and Fludarabine in Subjects ≤17 Years of Age With Cerebral Adrenoleukodystrophy (CALD)"
(e)	Proposed period of release	From 01.01.2020 to 30.09.2023 Lenti-D Drug Product and the recombinant Lenti-D LVV are not released in the environment.

2. Notifier

Name of institution or company: The Sponsor of ALD-104 is bluebird bio, Inc. with its wholly owned subsidiary, and legal representative in the EU, bluebird bio France.

3. GMO characterisation

(a) Indicate whether the GMO is a:

viroid		(.)	
RNA virus		(X)	*(replication incompetent Lenti-D lentiviral vector)*
DNA virus		(.)	
bacterium		(.)	
fungus		(.)	
animal		(.)	
-	mammals	(X)	*(autologous CD34+ cells transduced with Lenti-D lentiviral vector)*
-	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 LVV.

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.

Lineage: Totipotent stem cells

Differentiation: Pluripotent hematopoietic stem cells

Lenti-D lentiviral vector: The Lenti-D lentiviral vector (LVV) is a third generation, replication-defective, self-inactivating (SIN), human immunodeficiency virus-1 (HIV-1)-based LVV that is pseudotyped with the vesicular stomatitis virus envelope glycoprotein (VSV-G) and utilizes an internal MNDU3 promoter to control expression of the human *ABCD1* transgene that encodes the human adrenoleukodystrophy protein (ALDP).

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) GB, IT, FR and NL

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 GB
- Notification number RA010195/1
- Member State of notification DE
- Notification number: Clinical trial ALD-102, EudraCT number: 2011-001953-10
- Member State of notification FR
- Notification number DUO#TG6365

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 NIH RAC Protocol #1010-1073

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-104.

The 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. Transduced cells are not viable in the environment outside of the patient.

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

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

1. Recipient or parental organism characterisation:

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

(select one only)

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

other, specify …

2. Name

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

<u>For humans</u>:
Kingdom: Animalia
Phylum: Chordata
Chordata Class: Mammalia
Order: Primates
Family: Hominidae

<u>For HIV-1 and MoMuLV</u>:
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: Not applicable MoMuLV: Not applicable For VSV: Not applicable
(iv)	subspecies cells	For humans: Lineage: Totipotent stem For HIV-1: Not applicable MoMuLV: Not applicable For VSV: Not applicable
(v)	strain hematopoietic stem cells	For humans: Differentiation: Pluripotent 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
Mediteranean	X
Boreal	X
Alpine	X
Continental	X
Macaronesian	X

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

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

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

4. Natural habitat of the organism

(a) If the organism is a microorganism

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

(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 agents, as per Directive 2000/54/EC of the European Parliament and of the Council of 18 September 2000 on the protection of workers from risks related to exposure to biological agents at work. **Though HIV-1 is technically considered a parental organism of Lenti-D LVV**, **actual HIV-1 virus is not used in the manufacture of Lenti-D LVV**. Lenti-D LVV is produced by transient transfection of HEK293T cells with the plasmid transfer vector that encodes the RNA transcript packaged in the Lenti-D LVV and four packaging plasmids that encode the components required for production of Lenti-D LVV. Lenti-D LVV is budded from the production cells, harvested, and purified.

Lenti-D LVV is a SIN, replication defective LVV 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 LVV are modified to prevent translation, the provirus does not mobilize or transfer. Lenti-D LVV contains a functional copy of the human ABCD1 cDNA (the "therapeutic gene") and the minimum viral sequence necessary for efficient packaging into viral particles. As an added precautionary measure, all batches of LVV 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 LVV does not encode for any HIV proteins; the only HIV derived sequences in the transcript are the long terminal repeats (LTR) (made 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 SIN 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).

(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 (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 another Study, ALD-102, with the same genetic modified drug product in Europe, Lenti-D Drug Product (EudraCT #2011-001953-10). Study ALD-102 is a Phase 2/3 open-label, single-arm study to assess efficacy and safety of Lenti-D Drug Product after myeloablative conditioning with busulfan and cyclophosphamide. Study ALD-102 is currently ongoing in France, Germany, and the UK. As of 25 April 2018, 29 subjects have been treated with Lenti-D Drug Product in Study ALD-102.

Lenti-D Drug Product is the same in both Study ALD-102 and Study ALD-104. Lenti-D Drug Product consists of autologous CD34+ cell-enriched population that contains cells transduced with Lenti-D LVV encoding human ALDP protein, suspended in cryopreservative solution. Lenti-D Drug Product was granted an orphan status by the European Commission for treatment of adrenoleukodystrophy on 06 June 2012 (EU/3/12/1003).

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 the therapeutic gene to the autologous $CD34^+$ HSC 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 cerebral adrenoleukodystrophy (CALD).

For Lenti-D lentiviral vector:

The goal of the genetic modifications is 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 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) (*Lenti-D lentiviral vector*)
bacteriophage	(.)
virus	(X) (*autologous CD34+ cells transduced with Lenti-D lentiviral vector)*
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 LVV is a replication defective, SIN, third generation HIV-1 based LVV pseudotyped with the VSV-G envelope protein.

The Lenti-D LVV does not encode for any HIV proteins; the only HIV derived sequences in the transcript are the long terminal repeats (LTR) (made SIN by deleting promoter/enhancer sequences), attenuated regions of the 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 internal

MNDU3 promoter that consists of only the U3 enhancer/promoter region from the murine myeloproliferative sarcoma virus (MPSV) LTR, modified by deletion of the negative control region (NCR).

Identity of the plasmid vectors used in the production of Lenti-D lentiviral vector
Lenti-D LVV 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 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 LVV is not replication competent and does not encode any pathogenic genes.
Lenti-D LVV is a SIN, 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 LVV contains the therapeutic gene under the control of the internal MNDU3 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 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 LVV 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 adrenoleukodystrophy 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 LVV contain Ampicillin resistance cassettes to ensure the plasmids are maintained in bacterial stocks. Characterization studies have been conducted for each plasmid that demonstrated that carbenicillin was below the limit of quantitation.

(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 HSCs 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 cerebral macrophages and microglia should reduce levels of VLCFAs in the brain 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 LVV that is capable of modifying target $CD34^+$ HSCs, such that the proviral sequence containing the therapeutic cDNA is stably integrated into the genome of these cells

(e) Location of the insert in the host organism
- on a free plasmid (.)
- integrated in the chromosome (X)
- other, specify …

(f) 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

(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:

(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 (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 LVV 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. 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 LVV 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 LVV.

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 LVV is used to transduce the patient cells to add the human ABCD1 cDNA sequence into the autologous $CD34^+$ HSCs.

The Lenti-D Drug Product is manufactured at a GMP manufacturing site in the EU. Autologous HSCs are collected from the subject at the clinical site and transported to the manufacturing facility where they are transduced with the Lenti-D LVV to produce Lenti-D Drug Product. Each lot of Drug Product is tested to ensure identity and purity prior to release. The released Lenti-D 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-104 under highly controlled conditions in the transplant unit of a hospital. Patients have been myeloablated prior to treatment and will be followed for up to 15 years after Lenti-D Drug Product infusion. After infusion, the cells migrate to the bone marrow to reconstitute the bone marrow for HSCs 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:

The Lenti-D LVV 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+ HSCs *ex vivo*. The site of release of the Lenti-D LVV is not its natural habitat. Blood has been purified away from the autologous CD34+ HSCs.

3. Information concerning the release and the surrounding area

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

- Dr. med. Joern-Sven Kuehl
  Selbststaendige Abteilung fuer Paediatrische Onkologie,
  Haematologie und Haemostaseologie
  Department fuer Frauen- und Kindermedizin,
  Universitaetsklinikum Leipzig AoeR
  Liebigstr. 20a
  Leipzig, Germany D-0410
- Apceth Biopharma GmbH
  Haidgraben 5, Ottobrunn
  Germany D-85521

(b) Size of the site (m^2): "the patients will be treated in the above mention clinical site" m^2

(i) actual release site (m^2): … m^2

(ii) wider release site (m^2): … m^2

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

(d) Flora and fauna including crops, livestock and migratory species which may potentially interact with the GMO
Not applicable

4. Method and amount of release

(a) Quantities of GMOs to be released:

The Lenti-D Drug Product and the recombinant Lenti-D LVV 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+ HSCs per kg of body weight will be administered intravenously into each subject. Approximately 2-3 patients are estimated for treatment in the Member State.

(b) Duration of the operation:
The intravenous infusion of Lenti-D Drug Product is expected to last approximately 1 hour during the clinical trial.

(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 and the recombinant Lenti-D LVV are not released into the environment.

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

All waste is destroyed according to hospital bio-hazard disposal procedures.
The Lenti-D LVV is used to transduce *ex vivo* the autologous $CD34^+$ HSCs 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 LVV are modified to prevent translation, the provirus does not mobilize or transfer. Lenti-D LVV contains the therapeutic gene under the control of the internal MNDU3 promoter and the minimum viral sequence necessary for efficient packaging into viral particles. The integrated Lenti-D proviral DNA is flanked by identical human immunodeficiency virus-1 (HIV-1)-based 5' and 3' long terminal repeats (LTRs) each containing wildtype repeat (R) and unique 5 (U5) regions, but with modified unique 3 (U3) regions (ΔU3). The ΔU3 regions contain a deletion of the U3 enhancer/promoter that confers the self-inactivating (SIN) property that prevents LTR-driven transcription, reduces the possibility of replication-competent lentivirus (RCL) formation, and limits the potential for oncogenesis related to insertional mutagenesis. The extended packaging signal (Ψ+), central polypurine tract (cPPT) and Rev-response element (RRE) are incorporated to facilitate retroviral packaging, reverse transcription, and nuclear export of the viral RNA genome, respectively. Two STOP codons (not shown) are inserted in the gag region of Ψ+ to prevent Gag protein production. Expression of the ABCD1 transgene, encoding the adrenoleukodystrophy protein (ALDP), is under local control of the internal MNDU3 promoter. The internal MNDU3 promoter consists of only the U3 enhancer/promoter region from the murine myeloproliferative sarcoma virus

(MPSV) LTR, modified by deletion of the negative control region (NCR). The Lenti-D LVV production system uses a third-generation, 5-plasmid, split-packaging system to further reduce the possibility of RCL formation.

All manipulations of the Lenti-D Drug Product and Lenti-D LVV are carried out in GMP manufacturing facilities. 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.
As of 25 April 2018, 29 subjects have been treated with Lenti-D Drug Product in an ongoing Phase 2/3 trial, Study ALD-102 (EudraCT No: 2011-001953-10) and 15 subjects have rolled to a long-term efficacy and safety extension study of ALD-102, LTF-304 (EudraCT No: 2015-002805-13).

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

1. Name of target organism (if applicable)

(i)	order and/or higher taxon (for animals)	…
(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+ HSCs. 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 HSC 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. Transduced cells are not viable in the environment outside of the subject.

The recombinant Lenti D LVV is used to transduce ex vivo the autologous CD34+ HSCs and stably integrate the ABCD1 cDNA into the cell genome.

3. Any other potentially significant interactions with other organisms in the environment
Possible interaction with other foreign organisms, as HIV present in the patients, is extremely low as no HIV+ patients are exposed to the Lenti-D Drug Product. Subjects are screened prior to acceptance into the current ALD-104 clinical study.

No Lenti-D Drug Product is made from HIV positive subjects, therefore eliminating the possibility of recombination of the LVV with HIV. The transduced cells are not viable outside of the body of the treated subjects. Viral shedding is not possible due the use of a replication incompetent LVV. The administration of the GMO product to immunocompetent people leads to rejection of the GMO cells. In summary, no interactions are expected between Lenti-D Drug Product and other organisms in the environment.

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 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 LVV is replication incompetent and degrades rapidly in the environment as well; thus, it is not anticipated that Lenti-D LVV 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

(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+ HSCs. 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 LVV 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 HSCs 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 LVV does not encode any viral or pathogenic genes.

Lenti-D LVV 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 $CD34^+$ HSCs in a controlled and insulated manufacturing laboratory setting. Lenti-D Drug Product and Lenti-D LVV are not viable in the environment, and neither will be released in the environment.

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 LVV 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 Lenti-D LVV are not released into the environment.

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

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 study (Study LTF- 304, EudraCT number: 2015-002805-13), and will be monitored for an additional 13 years .

6. Frequency of the monitoring

Subjects are monitored for 24 months post-transplant as part of Study ALD-104. Subjects are then offered to participate in the long-term follow-up study, LTF-304, for an additional 13 years.

The Lenti-D LVV 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
Lenti-D Drug Product is not released in the environment. It is administered intravenously into the patient under standard controlled conditions for HSC transplant at a clinical site.

All waste remaining after the intravenous infusion of Lenti-D 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.
Lenti-D LVV is handled under highly controlled and isolated conditions (*in vitro*) in a GMP manufacturing facility outside of the EU.

2. Post-release treatment of the GMOs

The Lenti-D Drug Product is not released into the environment. Lenti-D 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 beyond the hospital bio-hazard disposal procedures noted in question I.1 above.

Lenti-D LVV 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.

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 LVV 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 LVV used to transduce *ex vivo* the autologous $CD34^+$ HSCs is a controlled and isolated GMP laboratory setting. Lenti-D Drug Product is not viable in the environment outside of the body of the treated patient.

Lenti-D LVV does not replicate, and the provirus is stably integrated into the genome of the patient $CD34^+$ cells. Lenti-D LVV 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, hospital decontamination and cleaning procedures are applied.
Lenti-D LVV is not released into the environment; it is released under highly controlled and isolated conditions to transduce autologous $CD34^+$ HSC *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 LVV 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.

Recombinant Lenti-D LVV 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 LVV in accordance with GMP. No undesirable effects are expected.