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/16/PEI2772

(c) Date of acknowledgement of notification: 19/05/2016

(d) Title of the project

Clinical trial HGB-207 titled: "A Phase 3 Single Arm Study Evaluating the Efficacy and Safety of Gene Therapy in Subjects with Transfusion-dependent β-Thalassemia, who do not have β^0/β^0 Genotype, by Transplantation of Autologous CD34+ Stem Cells Transduced Ex Vivo with a Lentiviral $\beta^{A\text{-}T87Q}$-Globin Vector in Subjects ≥12 and ≤50 Years of Age"

(e) Proposed period of release

It is anticipated that the recruitment period for clinical trial HGB-207 will be approximately 1 to 2 years (from 2016 to 2018).
The LentiGlobin BB305 Drug Product and the lentiviral vector LentiGlobin BB305 are not released in the environment. LentiGlobin BB305 is used in the ongoing clinical study HGB-205.

2. Notifier

Name of institution or company:

The Sponsor is bluebird bio, Inc. with its wholly owned subsidiary bluebird bio France.

3. GMO characterisation

(a) Indicate whether the GMO is a:

viroid	(.)
RNA virus	(X)
DNA virus	(.)
bacterium	(.)
fungus	(.)

animal

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

other, specify (kingdom, phylum and class)

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

The GMO consists of the 2 key elements briefly described below: a recombinant lentivirus which transduces autologous haematopoietic stem cells. LentiGlobin BB305 lentiviral vector: a recombinant HIV-1 RNA lentivirus (rLV) manufactured with 4 plasmids designed to express all the packaging components to generate a modified rLV. LentiGlobin BB305 lentiviral vector packaged RNA transcript encodes for the therapeutic $\beta^{A\ T87Q}$ globin gene. LentiGlobin BB305 Drug Product: autologous CD34+ haematopoietic stem cells transduced with the LentiGlobin BB305 lentiviral vector.

Lineage: Totipotent stem cells

Differentiation: Pluripotent haematopoietic stem cells

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

Sequences used to make the LentiGlobin BB305 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) *FR, GB, GR, IT*

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 *FR, GB, IT*
- Notification number

Please use the following country codes:
Austria AT; Belgium BE; Germany DE; Denmark DK; Spain ES; Finland FI; France FR; United Kingdom GB; Greece GR; Ireland IE; Iceland IS; Italy IT; Luxembourg LU; Netherlands NL; Norway NO; Portugal PT; Sweden SE

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

Yes (X) No (.)

If yes:

- Member State of notification *Australia, Thailand, USA*

- Notification number

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

No environmental impact is expected from the administration of LentiGlobin BB305 Drug Product to subjects in clinical trial HGB-207.

The LentiGlobin BB305 Drug Product consists of autologous CD34+ haematopoietic stem cells transduced with the lentiviral vector LentiGlobin BB305 encoding for the $\beta^{A\ T87Q}$ globin gene. Transduced cells are not viable in the environments outside of the patient. The LentiGlobin BB305 lentiviral vector is attenuated, replication incompetent and degrades rapidly in the environment as well.

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)
DNA virus	(.)
bacterium	(.)
fungus	(.)
animal	
- mammals	(X)
- insect	(.)
- fish	(.)
- other animal	(.)

(specify phylum, class)

other, specify

2. Name

(i) order and/or higher taxon (for animals)
Kingdom: Animalia Phylum: Chordata
Chordata Class: Mammalia
Order: Primates
Family: Hominidae
for HIV-1: Not applicable

(ii) genus
for humans: Homo;
for HIV-1: Lentivirus

(iii) species
for humans: Homo-sapiens;
for HIV-1: Human Immunodeficiency Virus 1

(iv) subspecies
for humans: Lineage: Totipotent stem cells
for HIV-1: Not applicable

(v) strain
for humans: Differentiation: Pluripotent haematopoietic stem cells
for HIV-1: HIV-1 NL4-3

(vi) pathovar (biotype, ecotype, race, etc.)
for humans : Not applicable
for HIV-1 : Not applicable

(vii) common name
for humans: human
for HIV-1: HIV-1

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 animals (for HIV-1)

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

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 (.) No (X)

If yes, specify
…

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

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

If yes:

(a) to which of the following organisms:

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

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

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

8. Information concerning reproduction

(a) Generation time in natural ecosystems:

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

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

(c) 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 contacts, 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)

None.

C. Information relating to the genetic modification

1. Type of the genetic modification

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

2. Intended outcome of the genetic modification

The goal of the genetic modification is to add a copy of the human $\beta^{A\text{-}T87Q}$-globin gene (the "therapeutic gene") to the autologous CD34+ haematopoietic stem cells by transduction. In vivo the transduced cells will differentiate and express the therapeutic gene improving the health of the target patients with transfusion-dependent β-thalassaemia.

3. (a) Has a vector been used in the process of modification?

Yes (X) No (.)

If no, go straight to question 5.

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

Yes (X) No (.)

If no, go straight to question 5.

4. If the answer to 3(b) is yes, supply the following information

(a) Type of vector

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

(b) Identity of the vector

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-glycoprotein (VSV-G) envelope protein.

The LentiGlobin BB305 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 β^{A}-globin gene.

(c) Host range of the vector

Lentiviral vectors pseudotyped with VSV-G are capable of transducing animal and insect cells. However, it is important to emphasize that LentiGlobin BB305 lentiviral vector is not replication competent and does not encode any pathogenic genes.

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

Yes (X) No (.)

antibiotic resistance (.)
other, specify
the $\beta^{A\text{-}T87Q}$-globin therapeutic gene product is identified by HPLC, and the lentiviral vector back-bone is identified and quantified by qPCR.

Indication of which antibiotic resistance gene is inserted..

(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 haematopoietic stem cells.

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.

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

This information has been provided to the appropriate EU national competent authorities. However, it is important to emphasize that no functional HIV genes are encoded in the LentiGlobin BB305 Drug Product insert. The insert encodes sequences necessary for the expression and production of the therapeutic human $\beta^{A\text{-}T87Q}$-globin therapeutic gene. 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.

(d) Location of the insert in the host organism

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

(e) Does the insert contain parts whose product or function are not known?
Yes (.) No (X)
If yes, specify

D. Information on the organism(s) from which the insert is derived

1. Indicate whether it is a:

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

other, specify

2. Complete name

This section is not applicable.

The donor, LentiGlobin BB305 lentiviral vector, is an artificial organism. 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-glycoprotein (VSV-G) envelope protein. The LentiGlobin BB305 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 β^A-globin gene.

(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:

(c) 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

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 LentiGlobin BB305 Drug Product insert is stably integrated into the genome of the cell, and does not have the capacity for mobilization. The RNA transcript packaged in the LentiGlobin BB305 lentiviral vector is stable and does not have capacity for replication.

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 LentiGlobin BB305 Drug Product is not released to the environment, and is not stable under uncontrolled environmental conditions. It is infused into the subject from whom the autologous cells were originally obtained, and is detected using qPCR. Detection of the LentiGlobin BB305 lentiviral vector is conducted with ELISA.

(b) Techniques used to identify the GMO

qPCR quantifies the amount of integrated vector in recipient cells.
HPLC is used to identify expression of therapeutic gene product.
ELISA is used to identify the LentiGlobin BB305 lentiviral vector.

F. Information relating to the release

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

The LentiGlobin BB305 Drug Product and the LentiGlobin BB305 lentiviral vector are not released into the environment. A subject is infused with LentiGlobin BB305 Drug Product with the goal of correcting disease symptoms of transfusion-dependent β-thalassaemia. The LentiGlobin BB305 lentiviral vector is used to transduce the subject cells to add the $\beta^{A\text{-}T87Q}$ gene sequence into the autologous CD34+ haematopoietic stem cells.

The LentiGlobin BB305 Drug Product is manufactured at a cGMP manufacturing site in the EU. Autologous haematopoietic stem cells are collected from the subject at the clinical site and transported to the manufacturing facility where they are transduced with the LentiGlobin BB305 lentiviral vector to produce the final LentiGlobin BB305 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 (.) No (X)
If yes, specify

The LentiGlobin BB305 Drug Product is not released in the environment; it is released under highly controlled conditions, in subjects enrolled in LentiGlobin BB305 studies for subjects with β-thalassaemia, who will be followed for 15 years after LentiGlobin BB305 Drug Product infusion. The LentiGlobin BB305 Drug Product (composed of modified haematopoietic progenitors) migrates to the bone marrow to reconstitute the bone marrow for haematopoietic cells production and is not viable outside the body of the specific recipient. It would be destroyed in any other recipient.

The LentiGlobin BB305 lentiviral vector is not released in the environment; it is released under highly controlled and insulated conditions (in vitro) at the EU GMP manufacturing site to transduce autologous CD34+ haematopoietic stem cells ex vivo. The site of release of the LentiGlobin BB305 lentiviral vector is not its natural habitat. Blood has been purified away from the autologous CD34+ haematopoietic 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 (m^2):
(i) actual release site (m^2): Not applicable.
(ii) wider release site (m^2): 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 LentiGlobin BB305 Drug Product and the LentiGlobin BB305 lentiviral vector are not released into the environment. It is planned that 3.0 million transduced autologous CD34+ haematopoietic stem cells per kg (subject weight) will be administered intravenously into the subjects.

Approximately 3.5 micro-grams of LentiGlobin BB305 lentiviral vector will be used for the ex vivo transduction of the autologous CD34+ haematopoietic stem cells in the controlled and insulated GMP manufacturing site located in the EU.

(b) Duration of the operation:

1 hour

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

The LentiGlobin BB305 Drug Product and lentiviral vector are not released in the environment. The LentiGlobin BB305 Drug Product (transduced cells) is not released in the environment. It is administered intravenously into the subject under standard controlled conditions for haematopoietic stem cell transplant at the clinical site. All waste is destroyed according to hospital bio-hazard disposal procedures.

All waste is destroyed according to the manufacturing facility bio-hazard disposal procedures after decontamination and use of disinfectant.

All manipulations of the LentiGlobin BB305 Drug Product and lentiviral vector are carried out in the licensed, controlled GMP manufacturing 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.

LentiGlobin BB305 Drug Product manufactured with LentiGlobin BB305 lentiviral vector is being studied in ongoing Study HGB-205 (Cavazzana-Calvo et al., 2014) in France and in Study HGB-204 (Thompson et al., 2014) in the US, Australia, and Thailand.

References:
Cavazzana *et al.,* 2014 - *Study HGB-205: Outcomes of Gene Therapy for Hemoglobinopathies Via Transplantation of Autologous Hematopoieitic Stem Cells Transduced Ex Vivo with a Lentiviral $\beta^{A\text{-}T87Q}$-Globin Vector (LentiGlobin BB305 Drug Product* - Blood. 2014 Dec 5;124:4797.

Thompson et al., 2014 - *Initial Results from the Northstar Study (HGB-204): A Phase 1/2 Study of Gene Therapy for β-thalassemia Major Via Transplantation of Autologous Hematopoietic Stem Cells Transduced Ex Vivo with a Lentiviral $\beta^{A\text{-}T87Q}$-Globin Vector (LentiGlobin BB305 Drug Product)* – Blood. 2014 Dec 5;124:549.

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

Target organism is the recipient. This section is not applicable. The transduced CD34+ autologous haematopoietic stem cells and the LentiGlobin BB305 lentiviral vector are not released in the environment.

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 LentiGlobin BB305 Drug Product is not released into the environment. The LentiGlobin BB305 Drug Product is composed of modified, autologous CD34+ haematopoietic stem cells. Upon infusion to the patient, the cells migrate to the bone marrow and reconstitute haematopoietic cell production. It is expected that the LentiGlobin BB305 Drug Product will have a therapeutic effect in infused patients suffering from transfusion-dependent β-

thalassaemia. The LentiGlobin BB305 lentiviral vector is used to transduce ex vivo the autologous CD34+ haematopoietic stem cells to insert the $\beta^{A\text{-}T87Q}$-globin gene.

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

The LentiGlobin BB305 Drug Product and LentiGlobin BB305 lentiviral vector are not released to the environment. The lentiviral vector is attenuated and degrades rapidly in the environment. The transduced cells are not viable in the environment outside of the subject.

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

The LentiGlobin BB305 Drug Product is not released into the environment.

The LentiGlobin BB305 Drug Product consists of autologous CD34+ haematopoietic stem cells transduced with the lentiviral vector LentiGlobin BB305 encoding for the $\beta^{A\text{-}T87Q}$-globin gene. Transduced cells are not viable in the environments outside of the subject. The LentiGlobin BB305 lentiviral vector is attenuated, replication incompetent and degrades rapidly in the environment as well.

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.

(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 LentiGlobin BB305 Drug Product is made with a replication defective vector that inserts the proviral DNA stably into the genome of the autologous CD34+

haematopoietic stem cells. Neither the insert nor the vector is capable of replication. Therefore, gene transfer to unintended organisms is not anticipated.

(b) from other organisms to the GMO:

The LentiGlobin BB305 Drug Product will exist as haematopoietic cells in the subject. While it is always possible that human subjects are infected with other organisms, there is no added risk to the subject as the LentiGlobin BB305 lentiviral vector does not encode any viral or pathogenic genes. LentiGlobin BB305 lentiviral vector is a self-inactivated lentiviral vector.

(c) likely consequences of gene transfer:

Once the LentiGlobin BB305 Drug Product is created, 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.):

The transduced cells are infused into the corresponding subject. The LentiGlobin BB305 lentiviral vector is used to transduce ex vivo the autologous CD34+ haematopoietic stem cells in the controlled and insulated manufacturing laboratory setting. Neither is viable in the environment. Neither will be released in the environment.
LentiGlobin BB305 Drug Product is being studied in ongoing Study HGB-205 (Cavazzana-Calvo et al., Blood. 2014 Dec 5;124:4797) in France and in Study HGB-204 (Thompson et al., 2014. Blood. 2014 Dec 5;124:549) in the US, Australia, and Thailand.

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 subject, the LentiGlobin BB305 Drug Product is monitored using qPCR for identification and quantification of the therapeutic insert. The therapeutic gene product, a modified form of the human β globin, is detected with HPLC.

Upon manufacturing, the LentiGlobin BB305 lentiviral vector is monitored by ELISA and classical cell culture techniques.

2. Methods for monitoring ecosystem effects

Not applicable. The LentiGlobin BB305 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 LentiGlobin BB305 Drug Product and lentiviral vector are not released into the environment.

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

Not applicable. The LentiGlobin BB305 Drug Product and lentiviral vector are not released into the environment.

5. Duration of the monitoring

Patients who receive LentiGlobin BB305 Drug Product are monitored for 15 years.

6. Frequency of the monitoring

Subjects are monitored for 24 months after drug product infusion according to the clinical protocols. After monitoring of a subject in the parent study has completed, subjects will be enrolled in the long-term follow-up protocol LTF-303 and will be monitored every 6 months through 5 years after drug product infusion and then annually through 15 years after drug product infusion. The LentiGlobin BB305 lentiviral vector is tested after manufacturing and at standard predefined stability time points.

I. Information on post-release and waste treatment

1. Post-release treatment of the site

The LentiGlobin BB305 Drug Product (transduced cells) is not released in the environment. It is administered intravenously into the subject under standard controlled conditions for haematopoietic stem cell transplant at the clinical site.

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

All waste is destroyed according to the manufacturing facility bio-hazard disposal procedures after decontamination and use of disinfectant (e.g., chlorine bleach, hydrogen peroxide, or detergent based disinfectant).

All manipulations of the LentiGlobin BB305 Drug Product and lentiviral vector are carried out under controlled conditions in a licensed GMP manufacturing facility located in the EU.

2. Post-release treatment of the GMOs

The LentiGlobin BB305 Drug Product and lentiviral vector are not released into the environment. The LentiGlobin BB305 drug product is infused into the patient as a one-time therapeutic treatment.

3. (a) Type and amount of waste generated

The waste generated following treatment of patients with the LentiGlobin BB305 Drug Product is minimal and consists mainly of residual cells remaining in the infusion bag.

The waste generated following manufacturing of the LentiGlobin BB305 Drug Product, i.e. following transduction of the autologous CD34+ haematopoietic stem cells with the LentiGlobin BB305 lentiviral vector is minimal and consists of residual cells or residual process solution. The waste is minimized as the efficacy of the product is highly dependent on the number of autologous cells that are transduced.

The waste generated following manufacturing of the LentiGlobin BB305 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 hospital or manufacturing facility bio-hazard disposal procedures 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 LentiGlobin BB305 Drug Product is not viable in the environment outside of the body of the treated patient. It is not possible for the Drug Product to spread into the environment. The LentiGlobin BB305 lentiviral vector is used to transduce ex vivo the autologous CD34+ haematopoietic stem cells in the controlled and insulated manufacturing laboratory setting. It degrades rapidly in the environment.

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

In case of accidental spill of the transduced cells or the LentiGlobin BB305 lentiviral vector, hospital or manufacturing facility decontamination and cleaning procedures are applied.

Waste is disinfected by appropriate products (e.g., paraformaldehyde, aqueous bleach, detergent based disinfectant, or hydrogen peroxide.

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

No plant, animal or soil will be in the manufacturing facility or the transplant unit where the LentiGlobin BB305 Drug Product is administered to the subject.

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

The LentiGlobin BB305 Drug Product (transduced cells) and the lentiviral vector LentiGlobin BB305 do not encode any pathogenic gene. The transduced cells are not viable outside of the body of the treated subjects. The lentiviral vector LentiGlobin BB305 degrades rapidly in the environment. Therefore no undesirable effects are expected.