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	Italy
(b)	Notification number	B/IT/16/02
(c)	Date of acknowledgement of notification	24/10/2016
(d)	Title of the project	

AAV8-mediated Low Density Lipoprotein Receptor (LDLR) Gene Replacement in Subjects with Homozygous Familial Hypercholesterolemia (HoFH).

Protocol Number FHGT002.

(e)	Proposed period of release	From 01/01/2017 until 12/12/2018

2. Notifier

Name of institution or company:

University of Pennsylvania (UPenn)
3400 Spruce St.
8046 Maloney Building
Philadelphia, PA 19104-4283
United States of America

3. GMO characterisation

(a) Indicate whether the GMO is a:

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

specify phylum, class …

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

Family: *Parvoviridae*
Genus: *Dependoparvovirus*
Species: Adeno-associated virus (AAV-derived-replication-deficient viral vector)

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

In general, DNA viruses have greater genetic stability than RNA viruses. The GMO is unable to replicate, even in the presence of a helper virus, since the genes essential for replication (*rep* and *cap*) are deleted.

Homologous genomic recombination may occur spontaneously in nature between the viral genomes of AAV strains only under circumstances where a cell of the host organism is infected simultaneously by two different strains of AAV and a helper virus (triple-infection).

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) NL

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

Yes (.) No (X)
If yes:
- Member State of notification …
- Notification number …

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

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

Yes (X) No (.)
If yes:
- Member State of notification United States of America
- Notification number IND 16710

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

The investigational agent is an AAV8 vector expressing the transgene human low density lipoprotein receptor, (hLDLR) under control of a liver-specific promoter (thyroxine-binding globulin, TBG) and is referred to in this document as AAV8.TBG.hLDLR. It is being developed for the treatment of Homozygous Familial Hypercholesterolemia (HoFH), a rare

genetic metabolic disorder characterized by absent or severely reduced capacity to catabolize circulating LDL particles by the hepatic LDL receptor.

AAV8.TBG.hLDLR is unable to replicate independently, even in the presence of a helper virus, since it lacks the *rep* and *cap* genes required for rescue/packaging. The genetic modifications do not affect its survival outside the host or probable mode of dissemination. Neither wild type AAV nor the experimental vector AAV8.TBG.hLDLR is known to be pathogenic to humans.

In the European Union (EU) according to Directive 2000/54/EC on the protection of workers from risks related to exposure to biological agents at work (Appendix III), wild type AAV is most appropriately designated a Risk Group 1 biological agent, defined in the EU as 'one that is unlikely to cause human disease'. Additionally AAVs are not assigned an Advisory Committee on Dangerous Pathogens (ACDP) category, so recombinant AAV viruses that contain no nucleic acid sequences of AAV except for the ITRs and no potentially hazardous nucleic acid fragment, are also classified biosafety Group/Class 1 or 2 depending on the European member state.

The intended application of AAV8.TBG.hLDLR is limited to a phase 1/2a clinical study enrolling a maximum of twelve consenting adult patients with HoFH. We estimate that three Italian patients will participate in the study (with a maximum of 10 Italian patients as the highest scale of release).

The administration of AAV8.TBG.hLDLR occurs only in the United States, at the University of Pennsylvania research inpatient unit. Following administration, the patients will be monitored for three days before returning to their home countries within one week from dosage. Blood draws occur in Italy at regular intervals according to clinical protocol. Trained nurse will perform the blood draws according to the well-established precautions routinely applied when obtaining clinical samples (which may potentially contain considerably more hazardous viruses than AAV8.TBG.hLDLR). Adherence to the laboratory manual ensures appropriate collection, processing and transportation of clinical samples are in place during blood draws in Italy. Adherence to established routine practices for the disposal of biohazardous materials in the healthcare setting covers accidental breakages during blood draws. The analysis of the samples will be done at three laboratories outside Italy: the vector concentration in plasma will be measured as vector genome.

In view of the low risk AAV8.TBG.hLDLR presents to people and the environment and considering that the intravenous administration in the United States as well as the analysis of blood samples outside Italy even further reduce the exposure to the vector, its overall risk for people and the environment can be evaluated as negligible.

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

1. Recipient or parental organism characterisation:

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

(select one only)

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

other, specify …

2. Name

(i)	order and/or higher taxon (for animals)	*Parvoviridae*
(ii)	genus	*Dependoparvovirus*
(iii)	species	Adeno-associated dependoparvovirus
(iv)	subspecies	…
(v)	strain	…
(vi)	pathovar (biotype, ecotype, race, etc.)	AAV2 (ITRs) /AAV8 (capsid)
(vii)	common name	Adeno-associated virus

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 Human and non-human primates.

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

Not applicable.

5. (a) Detection techniques

Optimised quantitative polymerase chain reaction (oqPCR).

(b) Identification techniques

Optimised quantitative polymerase chain reaction (oqPCR).

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

AAVs are not known to be a pathogenic virus in humans. AAVs are not assigned an Advisory Committee on Dangerous Pathogens (ACDP) category, so recombinant AAVs that contain no nucleic acid sequences of AAV except for the ITRs and no potentially hazardous nucleic acid fragment, are also classified biosafety Group/Class 1 or 2 depending on the European member state.

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

8. Information concerning reproduction

(a) Generation time in natural ecosystems:

Not applicable: the GMO is unable to replicate.

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

Not applicable: the GMO is unable to replicate.

(c) Way of reproduction: Sexual N/A Asexual N/A

(d) Factors affecting reproduction:

The GMO is unable to replicate. Reproduction of wild-type AAV is dependent on co-infection with helper virus (Adenovirus or Herpesvirus).

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	Not applicable.

(b) relevant factors affecting survivability:

The survival of AAV8.TBG.hLDLR outside of the host is expected to be the same as wild type AAV.

Wild-type AAV virus is inactivated outside the host by exposure to pH > 10 and by autoclaving for 30-45 minutes at 121°C and is susceptible to sodium hypochlorite (1-10% dilution of fresh bleach).

10. (a) Ways of dissemination

Dispersal (dissemination) of AAV is not documented definitively, but is likely through inhalation of aerosolized droplets, mucous membrane contact, parenteral injection, or ingestion.

(b) Factors affecting dissemination

AAV8.TBG.hLDLR is a replication-incompetent virus derived from AAV2 (ITRs) and AAV8 (capsid). The genetic modifications do not affect its survival outside the host or probable mode of dissemination. It is unable to replicate even in the presence of a helper virus.

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	(X)
(iii)	base substitution	(.)
(iv)	cell fusion	(.)
(v)	others, specify …	

2. Intended outcome of the genetic modification

AAV8.TBG.hLDLR is a recombinant adeno-associated virus (AAV) serotype 8 (AAV8) vector encoding the human low density lipoprotein receptor gene as a cDNA under the control of the liver-specific thyroxine-binding globulin (TBG) promoter. Other than the AAV serotype 2 inverted terminal repeat sequences (ITR) at each end of the single-stranded DNA virus genome, all other viral sequences have been removed and replaced with the human LDLR cDNA and control elements necessary to drive transgene expression. The viral genome is packaged in an AAV8 capsid, resulting in a recombinant viral vector that can drive expression of the LDLR gene in hepatocytes but that is unable to replicate further 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.

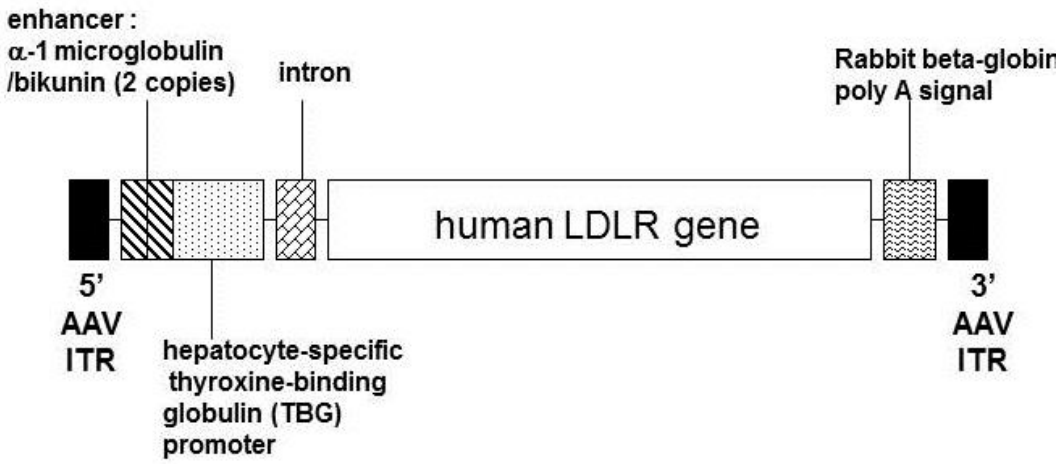
4. If the answer to 3(b) is yes, supply the following information

(a) Type of vector

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

(b) Identity of the vector

A schematic representation of the AAV8.TBG.hLDLR Vector is shown in the figure below:



(c) Host range of the vector

Bacterial cells.

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

Yes (X) No (.)

antibiotic resistance (X)
other, specify …

Indication of which antibiotic resistance gene is inserted:

The ampicillin resistance gene of the plasmids was replaced by the kanamycin resistance gene. This gene confers Kanamycin resistance to bacterial cells used for plasmid production.

(e) Constituent fragments of the vector

It is a vector genome expression construct that contains the human LDLR expression cassette inserted between two AAV2 inverted terminal repeats (ITRs). Expression of the human LDLR cDNA is driven from the hepatocyte specific-TBG promoter. A chimeric intron, a rabbit beta globin polyadenylation (polyA) signal and alpha 1 microglobulin /bikunin enhancer element complete the expression cassette. The plasmid backbone contains a kanamycin resistance (KanR) gene. The viral genome is packed in AAV8 capsid.

(f) Method for introducing the vector into the recipient organism

(i) transformation (.)
(ii) electroporation (.)
(iii) macroinjection (.)
(iv) microinjection (.)
(v) infection (.)
(vi) other, specify …

The final vector, AAV8.TBG.hLDLR is constructed from the plasmid stocks on a batch-by-batch basis by co-transfecting a Master Cell Bank (MCB) of Human Embryo Kidney (HEK) 293 cells with the three plasmid stocks:

- Cis plasmid (vector genome expression construct)
- Trans plasmid (packaging construct)
- Adenovirus helper plasmid

Note that the required helper functions are provided as a plasmid, NOT a viable adenovirus.

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	Not applicable.

6. Composition of the insert

(a) Composition of the insert

The expression cassette comprises:

- AAV Inverted terminal repeats (ITRs)
- Alpha 1 microglobulin/bikunin enhancer
- Human thyroxine-binding globulin (TBG) promoter
- Human LDLR cDNA
- Chimeric intron
- Polyadenylation signal

(b) Source of each constituent part of the insert

- AAV Inverted terminal repeats (ITRs): AAV2
- Alpha 1 microglobulin/bikunin enhancer: human
- Human thyroxine-binding globulin (TBG) promoter: human
- Human LDLR cDNA: human
- Chimeric intron: human
- Rabbit beta-globin Polyadenylation signal

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

- ITR sequences: cis acting sequences required for vector genome replication and packaging
- Human LDLR cDNA: active part of the vector
- Chimeric intron: facilitates the transport of mRNA. This is a common feature in gene vectors intended to mediate increased levels of gene expression
- Enhancer/promoter: enhance the expression of the transgene
- Polyadenylation signal: provides cis sequences for efficient polyadenylation of the antibody mRNA

(e) Location of the insert in the host organism

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

Though integration into the host cell chromosome is possible, the vector genome is predominately maintained as an episome in the nucleus of the host cell.

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

other, specify …

2. Complete name

(i)	order and/or higher taxon (for animals)	Primates
(ii)	family name for plants	…
(iii)	genus	Homo
(iv)	species	*sapiens*
(v)	subspecies	*sapiens*
(vi)	strain	…
(vii)	cultivar/breeding line	…
(viii)	pathovar	…
(ix)	common name	Human

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 (.) 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 (.) No (X)

If yes, specify

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

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

Following naturally acquired infection, wild type AAV DNA may persist mainly as circular double stranded episomes in human tissues. A very limited level of integration may occur in the host DNA: the genetic exchange is limited to human and wild type AAV.

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 (X) No (.) Unknown (.)

Specify …

AAV8.TBG.hLDLR is unable to replicate independently, even in the presence of a helper virus, since it lacks the *rep* and *cap* genes required for rescue/packaging.

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

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

Specify …

AAV8.TBG.hLDLR is unable to replicate independently, even in the presence of a helper virus, since it lacks the *rep* and *cap* genes required for rescue/packaging. Therefore, though it has the capacity to infect cells, the lack of replicative capacity will severely restrict dissemination.

(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

Based on the fact that long-term therapeutic activity of the investigational drug is not dependent on replication of the recombinant AAV and the known genetic stability of the parent wild type AAV, the genetic traits of the organism are expected to be stable.

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)

Neither wild type AAV nor the experimental vector AAV8.TBG.hLDLR is known to be pathogenic to humans.

4. Description of identification and detection methods

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

It will be determined by optimised quantitative polymerase chain reaction (oqPCR) using subject's blood samples.

(b) Techniques used to identify the GMO

Optimised quantitative polymerase chain reaction (oqPCR).

F. Information relating to the release

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

   A Phase 1/2a clinical study of AAV8-mediated Low Density Lipoprotein Receptor (LDLR) Gene Replacement in Subjects with Homozygous Familial Hypercholesterolemia.

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

   Yes (.) No (X)

   If yes, specify …

3. Information concerning the release and the surrounding area

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

   Not applicable for vector administration: the dosing of the subjects occurs in the United States at the University of Pennsylvania research inpatient unit. The patients will be monitored in the United States for three days, returning to the home countries within one week following vector administration.

   The monitoring of the subjects after their return to Italy occur at home (home-nursing blood sampling) and/or at a site close home. The monitoring sites are listed below:

   - Campus di Ematologia "Franco e Piera Cutino" c/o Ospedale Vincenzo Cervello
     Via Trabucco 118, 90146 Palermo
     Italy
   - Azienda Ospedaliera di Padova
     Via Giustiniani 2, 35128 Padova
     Italy

   The Italian region of residence of the enrolled patients is not known at this stage.

   (b) Size of the site (m^2):

   (i) actual release site (m^2): Not applicable.

   (ii) wider release site (m^2): Not applicable.

   The dosing of the subjects occurs in the United States at the University of Pennsylvania research inpatient unit.

(c) Proximity to internationally recognised biotopes or protected areas (including drinking water reservoirs), which could be affected:

Given the nature of the product administration (intravenous), the location (United States) and the scale of release (a maximum of 10 Italian patients as highest scale of release), the exposure to significant biotopes, protected areas and drinking water supplies is expected to be negligible.

(d) Flora and fauna including crops, livestock and migratory species which may potentially interact with the GMO

None, given the nature of the product administration (intravenous) and the location (United States).

4. Method and amount of release

(a) Quantities of GMOs to be released:

The dosing of the subjects occurs in the United States at the University of Pennsylvania research inpatient unit. The initial dose of AAV8.TBG.hLDLR will be 2.5×10^{12} GC/kg and if no DLTs are observed, then a total of 3 subjects will be administered that dose. According to pre-determined criteria, if safety is deemed adequate, the second dose group will be initiated at a dose of 7.5×10^{12} GC/kg. The study may enrol up to a maximum of 12 subjects: we estimate that three Italian patients will participate in the study (with a maximum of 10 Italian patients as the highest scale of release).

(b) Duration of the operation:

It is anticipated that the trial will start with Italian patients in Q1 2017 and the last patient (worldwide) is expected to be treated by Q4 2018.

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

Not applicable for vector administration: the dosing of the subjects occurs in the United States at the University of Pennsylvania research inpatient unit.

Following administration, the patients will be monitored in the US for three days before they travel back to Italy within one week. Blood samples will then be drawn in Italy and analysed outside Italy. During blood draws in Italy, established routine practices for dealing with potentially biohazardous materials are in place as well as protective equipment including laboratory coats and gloves. Instructions for collection, processing and transportation of the clinical samples are provided in the Laboratory Manual. Standard practices for the disposal of biohazardous materials in the healthcare setting cover accidental breakages during blood draws.

5. Short description of average environmental conditions (weather, temperature, etc.)

The administration of the vector will occur in the United States and follow-up sampling will be performed in Italy. Pennsylvania has a temperate climate and Italy a Mediterranean climate. The risk of release of AAV8.TBG.hLDLR into the environment is unrelated to climatic characteristics.

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.

No data available.

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

1. Name of target organism (if applicable)

(i)	order and/or higher taxon (for animals)	Primates
(ii)	family name for plants	…
(iii)	genus	Homo
(iv)	species	*sapiens*
(v)	subspecies	*sapiens*
(vi)	strain	…
(vii)	cultivar/breeding line	…
(viii)	pathovar	…
(ix)	common name	Human

2. Anticipated mechanism and result of interaction between the released GMOs and the target organism (if applicable)

Homozygous Familial Hypercholesterolemia (HoFH) is a rare genetic metabolic disorder characterized by absent or severely reduced capacity to catabolize circulating LDL particles by the hepatic LDL receptor. As a consequence, HoFH subjects present abnormal total plasma cholesterol (LDL-C) levels, resulting in severe atherosclerosis often leading to early onset of cardiovascular disease. Early initiation of aggressive treatment for these patients is therefore essential. Unfortunately, despite existing therapies, treated LDL-C levels could remain well above acceptable levels. Thus, the functional replacement of the defective LDLR via AAV-based liver-directed gene therapy (AAV8.TBG.hLDLR) may be a viable approach to treat this disease and decrease circulating LDL particles by increasing the hepatic LDL receptor expression.

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

No potentially significant interactions with other organisms in the environment are predicted.

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

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

Give details

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

AAV8.TBG.hLDLR is a replication-incompetent virus derived from AAV2 (ITRs) and AAV8 (capsid). The genetic modifications do not affect its survival outside the host or probable mode of dissemination. However, the lack of replicative ability prevents multiplication and therefore severely limits its ability to disseminate. Shedding has been monitored in both humans and animals following administration of similar vectors to AAV8.TBG.hLDLR, confirming the low level transient shedding of the vector.

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	…

Not applicable.

7. Likelihood of genetic exchange in vivo

(a) from the GMO to other organisms in the release ecosystem:

Negligible.

(b) from other organisms to the GMO:

Negligible.

(c) likely consequences of gene transfer:

The only mechanism by which the transgene could be mobilised is through a triple infection of the same cell by AAV8.TBG.hLDLR (containing the transgene), wild type AAV (providing the rep and cap functions) and a helper virus. This scenario is expected to be a rare event, especially since the vector target cells (liver) are not the natural target cells of helper viruses. If it did occur, it would only result in the production of more wild type AAV and more AAV8-hLDLR vector particles (which would still lack *rep* and *cap* genes and consequently could not be self-sustaining).

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.):

AAV8.TBG.hLDLR is a replication-incompetent virus derived from AAV2 (ITRs) and AAV8 (capsid). The genetic modifications do not affect its natural host and tissue tropism.

No specific studies have been conducted regarding transmission of AAV8.TBG.hLDLR between humans or animals and on the ecological impact of the vector in simulated natural environments.

9. Possible environmentally significant interactions with biogeochemical processes (if different from the recipient or parental organism)

None known or predicted.

H. Information relating to monitoring

1. Methods for monitoring the GMOs

   The vector concentration in plasma will be measured as vector genome by oqPCR post vector administration.

2. Methods for monitoring ecosystem effects

   The chance of ecosystem effects is negligible and monitoring is not planned.

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

   No plans for detecting transfer of genetic material to other organisms are considered necessary.

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

   Not applicable.

5. Duration of the monitoring

   In treated subjects only: patients will be monitored throughout treatment during the study period of 5 years. The vector concentration in plasma will be measured as vector genome up to 1 year post vector administration.

6. Frequency of the monitoring

   In treated subjects only: at regular intervals according to clinical protocol.

I. Information on post-release and waste treatment

1. Post-release treatment of the site

Not applicable for vector administration: the dosing of the subjects occurs in the United States at the University of Pennsylvania research inpatient unit.

The patients will be monitored in the United States for three days, returning to the home countries within one week following vector administration. Upon their return to home countries, the patients will resume their daily routine: normal hygiene practice will suffice.

Standard practices for the disposal of biohazardous materials in the healthcare setting cover accidental breakages during blood sampling in Italy.

2. Post-release treatment of the GMOs

3. (a) Type and amount of waste generated

Not applicable for vector administration: the dosing of the subjects occurs in the United States at the University of Pennsylvania research inpatient unit.

The patients will be monitored in the United States for three days, returning to the home countries within one week following vector administration. We estimate that three Italian patients will participate in the study (with a maximum of 10 Italian patients as the highest scale of release).

During the monitoring/blood sampling once the subjects have returned to Italy minimal waste is expected. Blood samples are drawn in Italy, but analysed outside Italy. During blood draws in Italy, established routine practices for the disposal of waste are in place, in accordance with treatment of potentially biohazardous materials in the healthcare setting.

3. (b) Treatment of waste

During blood draws in Italy, established routine practices for dealing with potentially biohazardous materials are in place: instructions for collection, processing and transportation of the clinical samples are provided in the Laboratory Manual. Standard practices for the disposal of biohazardous materials as common in the healthcare setting and according to local requirements ensure the appropriate treatment of waste.

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 and administration of the vector occurs in the United States only. There are no specific procedures planned for controlling the GMO in the case of unexpected spread in Italy, since the risk of spread is considered negligible.

In the theoretical event that wild type AAV, supplying the requisite replication gene products, were to co-infect a hepatocyte, along with a helper DNA virus such as adenovirus or herpes simplex virus and the AAV8.TBG.hLDLR vector (a triple co-infection), it is possible that vector replication could occur.

However, even if this rare event were to occur, the resulting virologic outcome would be increased synthesis of vector and wild type AAV, both intrinsically non-pathogenic viruses. It is therefore unlikely that such an event would present clinical symptoms and is therefore unlikely to become apparent.

If such spread were detected, the individual could be isolated pending further investigation, and consultation with AIFA and the Ministry of Environment in Italy.

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

There are no specific procedures planned for decontaminating areas in the case of unexpected spread, since the risk of spread is considered negligible.

In the unlikely event that transmission to an unintended human recipient occurred, this would likely be a local occurrence affecting a healthcare professional or close contact of a treated individual.

Decontamination of areas in which a recently treated patient had frequented (their home and or examination room at a medical facility) could be implemented by applying standard detergents to areas of likely contact (for example, frequent touch-points such as handles, door knobs, hard surfaces, railings and hand-holds, washing facilities and lavatories). Fomites could be autoclaved or incinerated.

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

The predicted habitat of AAV8.TBG.hLDLR is humans where it is expected to persist in a lysogenic state. AAV8.TBG.hLDLR is a disabled version of a non-pathogenic wild-type primate (human) AAV, modified by deletion of the *rep* and *cap* genes rendering it unable to replicate, even in the presence of a helper virus.

Decontamination of plants, (non-human) animals and soils will not be required.

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

AAV8.TBG.hLDLR will be regulated under medicines legislation in Italy, requiring stringent pharmacovigilance overseen by the Competent Authority (CA) AIFA. Information will be collected regarding all individual adverse events and submitted to the AIFA if they fulfil the criteria for a Serious Unexpected Suspected Adverse Reaction (SUSAR) as defined in the Clinical Trial Protocol. Development Safety Update Reports will be submitted to AIFA

on an annual basis while the trial is active. Procedures are in place at both AIFA and University of Pennsylvania to monitor, review and act on urgent safety information relating to medicinal products so that human health is protected.

In the extremely unlikely event that spread of the vector to an unintended human recipient was detected, the individual could be isolated pending further investigation, and consultation with AIFA and the Ministry of Environment in Italy.