

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 Spain
- (b) Notification number B/ES/15/10
- (c) Date of acknowledgement of notification 10/11/2015
- (d) Title of the project
Phase I/II Gene Therapy Clinical Trial of scAAV9.U1a.SGSH for Mucopolysaccharidosis IIIA
- (e) Proposed period of release
The proposed period release for the complete clinical trial is from the first quarter of 2016 until the third quarter of 2018. The complete duration of the trial is 31 months or 2.6 years, from first patient recruited to last patient last visit. Any changes in this period of release will be notified to CIOMG/CNB.

2. Notifier

Name of institution or company: Abeona Therapeutics
10000 Cedar Avenue
Cleveland, OH 44106, USA

3. GMO characterisation

scAAV9.U1a.SGSH is an innovative gene therapy product with a proposed indication for treatment of mucopolysaccharidosis type IIIA (MPSIIIA – Sanfilippo type A syndrome).

This recombinant vector is a genetically modified organism (GMO) based on an adeno-associated virus serotype 9 (AAV9) without replication capacity, since the modified recombinant vector has a complete deletion of the wild type viral genes, except the ITRs. The deleted region is replaced by a recombinant expression cassette, so scAAV9.U1a.SGSH contains the code DNA (cDNA) for human N-sulfoglucosamine sulfohydrolase (SGSH) under the control of U1a promoter derived from the promoter of the gene encoding the murine spliceosome small nuclear RNAs.

(a) Indicate whether the GMO is a:

- viroid (.)
- RNA virus (.)
- DNA virus (X) rAAV- recombinant gene therapy product replication-deficient
- bacterium (.)

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

other, specify (kingdom, phylum and class)

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

Family: *Parvoviridae*

Genus: *Dependovirus*

Species: This is a recombinant AAV vector (replication deficient) derived from serotype 9 also named rh14.

scAAV9.U1a.SGSH is a genetically modified organism derived from wild type adeno-associated virus serotype 9 (rh14). This adeno-associated virus (AAV) is a member of the genus *Dependovirus*, which lies within the *Parvoviridae* family. Serotype 9 (also named rh14) was isolated from mammals, specifically humans and was serologically distinct from the known serotypes (for more information about AAV serotypes origin see Gao et. al. 2004 reference).

The identity of the genetically modified organism is scAAV9.U1a.SGSH, as unique name for this recombinant vector.

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

The GMO has been prepared by deletion of all viral genes (except inverted terminal regions or ITRs) and incorporating a gene expression cassette by recombinant DNA technology. Based on the absence of viral genes, this vector is replication incompetent and is therefore genetically stable by default, considering that it does not present any internal mechanism for genetic variation/transference.

The consistency of the genome sequence is confirmed by DNA sequencing of the three plasmids (vectors) used for the manufacturing of the GMO that is performed by restriction analysis and DNA sequencing. These tests are included in the release manufacturing certification of the plasmids. As the GMO is no able to replicate its genome, alterations in the genome are not expected.

scAAV9.U1a.SGSH is based on an AAV9 and they genetic stability are considered the same; the genome is maintained stable. It is important to remark that the GMO genome is maintained in the host cells without integration, as concatamers and as episomal forms.

4. Is the same GMO release planned elsewhere in the Community (in conformity with Article 6(1)), by the same notifier?

Yes (.) No (X)

If yes, insert the country code(s)

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

(*) At the time of the notification date, the presented GMO (scAAV9.U1a.SGSH) is the first time that will be used in humans as a treatment. No other releases are planned in the European Community.

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 (.) No (X)*

If yes:

- Member State of notification
- Notification number

(*) For the clinical development of this gene therapy vector is planned carrying out two simultaneous dose escalation clinical trials, sponsored by Nationwide Children's Hospital (NCH- Columbus, Ohio, EEUU) and Abeona Therapeutics. The initial patient enrolment of scAAV9.U1a.SGSH in MPS IIIA subjects is planned to be carried out at the Nationwide Children's Hospital in a unicenter clinical trial sponsored by this institution not yet authorized. The second international and multicenter clinical study is also a dose escalation study, consisting of a two cohorts of MPSIIIA patients totaling up to eighteen patients. This second patient enrolment of scAAV9.U1a.SGSH in MPS IIIA subjects will be carried out at two sites: Cruces University Hospital (Spain) and Adelaide Women's & Children's Hospital, Adelaide (Australia).

According to USA legislation there is no need to notify the GMO release. The Australian submission will be move forward in the following months.

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

The genetically modified organism of this voluntary release notification is a GMO based on adeno-associated virus serotype 9 or rh14 named scAAV9.U1a.SGSH. This GMO has a complete deletion of all viral genes (except inverted terminal regions or ITRs) and incorporates an expression cassette by recombinant DNA technology. This GMO is a replication incompetent virus with an expression cassette based on cDNA for human N-sulfoglucosamine sulfohydrolase (SGSH) under the control of U1a promoter derived from the promoter of the gene encoding the murine spliceosome small nuclear RNAs.

The scAAV9.U1a.SGSH is planned to be administered in a multicenter and international phase I/II gene therapy trial in Spain and Australia, treating a total of 18 patients in a dose escalation study. Since this is a competitive trial, it is not possible to determine in advance the number of patients to be treated in each country. The dose escalation will consist in the treatment of 2 Sanfilippo A patients with a low dose of 0.5×10^{13} recombinant viral genomes per kilogram (vg/kg) (dose adapted to patient weight) and a maximum of 16 Sanfilippo A patients with a high dose of 2×10^{13} vg/kg. It is expected that maximum weight would be around 50 kg, since this is a paediatric study and so the estimated maximum dose to be administered in a patient would be 10×10^{14} recombinant viral genomes or GMO. Patients will be administered one by one in a total period of 26 weeks, with safety periods of 4 to 1 week according to the dosing plan presented in the clinical protocol (attached).

The trial in Spain is going to be unicentric and conducted in University Hospital of Cruces (Bilbao) under the coordination of Dr. Aldamiz as Principal Investigator and Head of Metabolic Unit in this site. The GMO will be receipt in the pharmacy department and stored

at -80°C with limited access to the clinical team. The administration will be intravenous in an individual room of the hospital and patients will be at the hospital for a minimum of 48 h for a close monitoring. Then patient will be followed up for a total of 24 months within the clinical trial with a total of 11 site visits. GMO shedding samples (serum, urine, saliva, faeces) will be collected from basal time point until day 90 after vector administration. Samples at day 60 and 90 after administration are not expected to be positive, so they will be collected and only analysed in case that two consecutive negatives were not obtained in the previous samples (4, 8, 24, 48 hours, day 7, day 14 and day 30).

The highest GMO shedding is expected in the serum, due to the intravenous administration. Published preclinical data has demonstrated that rAAV9 present a half-life in circulation of approximately 4 hours over the first 10 hours post-injection, and approximately 10 hours over the next 3 days post-infection. If this is similar in humans, it should be expected to go through approximately 9-10 half-lives of GMO in circulation over 3 days, during which time the patients are mostly maintained in the hospital (from day 0 to day 2) under strict control.

In natural conditions, wild type AAV requires the presence of a helper virus (adenovirus or herpes virus) to replicate its genome in the respiratory tract of its host, that are exclusively primates human and non human. The scAAV9.U1a.SGSH is a non-replicative and has a limited capacity to infect mammalian cells, but not plant cells.

Preclinical mice data has demonstrated a very low shedding into the environment by urine, saliva or faeces. Samples were positive for a total of 35 days post-injections. Therefore, this rAAV has minimal chance to spread to other humans, animals or environment. Nevertheless, the patient will be isolated in an individual room for minimum of 48 hours and the professional health team involved in the study will be required to be trained in the safe handling of this GMO and biosafety practices. This will include instructions (also for the patient) for any accidental exposure and minimize any spread with other persons or environment.

Horizontal gene transfer is unlikely and due to scAAV9.U1a.SGSH sequence characteristics there is no possibility to confer a selective advantage to bacteria or other microorganisms, because it does not contain any prokaryotic promoters, any antibiotic or other types of resistance genes which would enhance or constrain their growth.

In regard to humans, the infection risk with this GMO is almost null due to its release by patients is limited on time and at very low doses, and, in the unlikely case that it is transmitted from a patient to a potential host, it contains a expression cassette that codify for a human protein that do not generate any disease or pathology. In fact, the human protein codified is still present in healthy subjects.

The likelihood that any negative effects could occur is considered negligible, there is no risk for people, animals, microorganisms and the environment.

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

1. Recipient or parental organism characterization:

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

(select one only)

viroid (.)
RNA virus (.)

DNA virus (X) Double stranded DNA. Serotype 2 (AAV2) for genome (ITRs) and serotype 9/rh14 (AAV9) for capsid
 bacterium (.)
 fungus (.)
 animal
 - mammals (.)
 - insect (.)
 - fish (.)
 - other animal (.)
 (specify phylum, class)

other, specify ...

Adeno-associated virus (AAV) is a small virus which infects humans and some other primate species. The virus is a small (20 nm) replication-defective, non-enveloped virus. Productive AAV infection requires co-infection with helper viruses that provide functions that aid in AAV replication.

The AAV genome is built of single-stranded deoxyribonucleic acid (ssDNA), either positive- or negative-sensed, which is about 4.7 kb. The genome comprises inverted terminal repeats (ITRs) at both ends of the DNA strand, and two open reading frames (ORFs): rep and cap. The first is composed of four overlapping genes encoding Rep proteins required for the AAV life cycle, and the second contains overlapping nucleotide sequences of capsid proteins: VP1, VP2 and VP3, which interact together to form a capsid of an icosahedral symmetry. The virus causes a very mild immune response, lending further support to its apparent lack of pathogenicity. AAV is not considered to have any known role in disease. It has been suggested to have a role in male infertility, as AAV DNA is more commonly found in semen samples from men with abnormal semen. However, no causal link has been found between AAV infection and male infertility.

As of 2008 there have been 12 AAV serotypes described. All of the known serotypes can infect cells from multiple diverse tissue types. Tissue specificity is determined by the capsid serotype and pseudotyping of AAV vectors to alter their tropism range will likely be important to their use in therapy.

Adeno-associated viruses (AAVs) have been widely used as a vector for gene therapy because of its safety profile, its ability to transduce both dividing and non-dividing cells, and its low immunogenicity. To date, AAV vectors have been used in over 117 clinical trials worldwide. Recently, promising results have been obtained from Phase 1 and Phase 2 trials for a number of diseases, including Leber's Congenital Amaurosis, Hemophilia, congestive heart failure, lipoprotein lipase deficiency, and Parkinson's disease.

2. Name
 - (i) order and/or higher taxon (for animals) ...
 - (ii) genus *Parvoviridae*
 - (iii) species *Dependovirus*
 - (iv) subspecies *Dependovirus*
 - (v) strain Serotypes 2 and 9
 - (vi) pathovar (biotype, ecotype, race, etc.) ...
 - (vii) common name Serotype 2 (AAV2) for genome (ITRs) and serotype 9/rh14 (AAV9) for capsid.

The description of AAV viruses is in point number 1 of this section.

scAAV9.U1a.SGSH is a genetically modified organism (GMO) based on a adeno-associated virus. This GMO is constructed based on AAV2 viral genome, as all the recombinant AAV gene therapy vectors nowadays. scAAV9.U1a.SGSH has a complete deletion of the AAV2 wild type viral genes, except the ITRs. It is worthy of mention that this recombinant virus is modified in one of inverted terminal sequences (ITRs) in order to encapsidate dimeric DNA molecules. The modification of one of its ITRs allows its self-complementarity. Thus the recombinant virus contains a double-stranded DNA produces a more rapid and efficient transcription. It incorporates a recombinant gene expression cassette that contains the cDNA for human N-sulfoglucosamine sulfohydrolase (SGSH) under the control of U1a promoter derived from the promoter of the gene encoding the murine spliceosome small nuclear RNAs. This GMO is constructed based on AAV9 viral capsid, as all the viral proteins (VP) are derived from this serotype to ensure a broad tropism that is the target to have a therapeutic approach in Sanfilippo A disease.

3. Geographical distribution of the organism

(a) Indigenous to, or otherwise established in, the country where the notification is made:
 Yes No Not known

(b) Indigenous to, or otherwise established in, other EC countries:

(i) Yes

If yes, indicate the type of ecosystem in which it is found:

Atlantic	<input checked="" type="checkbox"/>
Mediterranean	<input checked="" type="checkbox"/>
Boreal	<input checked="" type="checkbox"/>
Alpine	<input checked="" type="checkbox"/>
Continental	<input checked="" type="checkbox"/>
Macaronesian	<input checked="" type="checkbox"/>

(ii) No

(iii) Not known

Despite the fact that AAVs have been studied for the past 50 years, little is known about the natural infection by these viruses. However, it is worth of mention that as a result of natural infections, antibodies to AAV can be found in many animals including humans. Natural exposure to AAV can result in the production of antibodies from all four IgG subclasses, with a predominant IgG1 response and low IgG2, IgG3, and IgG4 responses. The characterization of the presence of these antibodies is the best way with update and available information that reviews the potential geographical distribution of these viruses. Surprisingly, approximately 30 - 80% of the population is seropositive for anti-AAV (all serotypes) and it has become apparent that approximately 60% of the population has neutralizing antibodies at age 10, which generally will persist into adulthood. In all human populations studied, which included samples from 10 countries and four continents (America, Europe, Africa, and Australia), prevalence of NAb to AAV7, AAV8, and AAV9 serotypes ranged from 30 to 15%. It is worth noting that significantly higher frequencies of NAb to all AAV serotypes were observed in Africa.

(c) Is it frequently used in the country where the notification is made?

Yes No

There are different research programs developing recombinant AAVs based on serotype 9 for genetic diseases. Bosch et al. 2013 is an example of a recent publication. All the preclinical work for the scAAV9.U1a.SGSH program was developed in USA. There is already a voluntary release notification of a recombinant AAV based on serotype 9 with notification code B/ES/14/09. The GMO presented in this voluntary release is based on the same parental organism, AAV9; and is proposed for a similar indication, treatment of mucopolysaccharidosis type III on a clinical trial with a unique center in Spain.

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

4. Natural habitat of the organism

- (a) If the organism is a microorganism

water
soil, free-living
soil in association with plant-root systems
in association with plant leaf/stem systems
other, specify :

Despite the fact that AAVs have been studied for the past 50 years, little is known about the natural infection by these viruses. However, it is worth of mention that as a result of natural infections, antibodies to AAV can be found in many animals including humans. Serotype 9 (also named rh14) was derived from isolates from three humans and was serologically distinct from the other known serotypes (for more information about AAV serotypes origin see Gao et. al. 2004 reference).

Wild type AAV survives in the environment as a persistent infection in the host vertebrate species or as a latent infection in the nucleus of some infected cells, where it may remain inactive indefinitely, or be reactivated giving rise to virus secretion. Due to its reliance on a helper virus for replication (typically adenovirus) AAV may be considered to exhibit seasonality. Temperate regions experience seasonal vagaries in the occurrence of adenoviral infections, with the highest incidences occurring in the autumn, winter, and early spring.

AAV is spread in nature via inhalation of aerosolized droplets, mucous membrane contact or ingestion, but does not cause any human disease. Specifically serotype 9 was derived from human isolates.

DNA replication occurs in the cell nucleus during lytic cycle. In its latent state, DNA is maintained either as a stable episome (circular or linear).

- (b) If the organism is an animal: natural habitat or usual agroecosystem:
No applicable.

5. (a) Detection techniques

Wild type adeno-associated viruses are generally detected in tissues by specific PCR according to general AAV sequences or specific for the serotype of analysis. It is also described that *in situ* hybridization (ISH) technique with a digoxigenin-labeled AAV probe in paraffin embedded tissues from the AAV positive cases has been developed in some cases. Additionally, AAV vector particles may also be detected using Enzyme-Linked Immunosorbent Assay (ELISA) methods.

(b) Identification techniques

Wild type adeno-associated viruses are generally detected in tissues by specific PCR according to general AAV sequences or specific for the serotype of analysis. It is also described that *in situ* hybridization (ISH) technique with a digoxigenin-labeled AAV probe in paraffin embedded tissues from the AAV positive cases has been developed in some cases. Additionally, AAV vector particles may also be detected using Enzyme-Linked Immunosorbent Assay (ELISA) methods. Finally the identification of a specific serotype sequences can be performed ultimately by DNA sequencing.

6. Is the recipient organism classified under existing Community rules relating to the protection of human health and/or the environment?

Yes No

If yes, specify

Wild type AAV is not classified in Risk Groups 2, 3 or 4 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. Therefore, it is most appropriately designated as a Risk Group 1 biological agent, defined in the EU as 'one that is unlikely to cause human disease'.

Additionally AAV's are not assigned an Advisory Committee on Dangerous Pathogens (ACDP) category, so recombinant AAV viruses are also classified biosafety Group/Class 1. Similar classifications of hazard have been assigned to AAV according to the definitions of the World Health Organisation (WHO), and in the USA, Canada and Australia. This all means that the risk is very low.

In fact, previous notifications for voluntary release of recombinant AAV in the context of human clinical trials such as B/ES/12/53 or B/ES/14/09 have been authorized by CIOMG with this biosafety classification.

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

Yes No Not known

AAVs are frequently found in humans and animals, but are not pathogenic, toxic, virulent, allergenic, or a carrier (vector) of a pathogen. The general known host range includes humans and nonhuman primates. It is worth of mention that in natural conditions, adeno-associated viruses require a helper virus for replication and so transmission. AAVs do not activate latent virus and is not able to colonise other organisms.

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:

In natural conditions, adeno-associated viruses require a helper virus for replication. The wild type AAV infection cycle, from infecting a cell to producing new infectious particles consists on the following steps: (1) attachment to the cell membrane; (2) receptor-mediated endocytosis; (3) endosomal trafficking; (4) escape from the late endosome or lysosome; (5) translocation to the nucleus; (6) uncoating; (7) formation of double-stranded DNA replicative form of the AAV genome; (8) expression of rep genes; (9) genome replication; (10) expression of cap genes, synthesis of progeny ssDNA particles; (11) assembly of complete virions, and (12) release from the infected cell.

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

The GMO will remain in the transduced cell and only the protein produced will have an effect in the surrounding cells. The genetically modified organism, rAAV9.CMV.SGSH, is a recombinant vector incapable of replication due to lack of all its viral genes.

(c) Way of reproduction: Sexual .. Asexual X

(c) Factors affecting reproduction:

Wild type AAV requires a helper virus (Adenovirus or Herpesvirus) for effective replication.

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 | None |

(b) relevant factors affecting survivability:

AAV does not form survival structures, but can remain infectious for at least a month at room temperature following simple desiccation or lyophilization.

As all GMOs based on adeno-associated viruses, scAAV9.U1a.SGSH is susceptible to appropriate virucidal disinfectants with activity for non-enveloped viruses such as 1-10% sodium hypochlorite (for at least 20 minutes), alkaline solutions at pH >9, 5% phenol, heat (>80°C for 60 minutes), UV radiation and extreme pHs (<2 and >12). Effective disinfectants require a minimum of 20 minutes contact time to be effective.

10. (a) Ways of dissemination

Wild-type AAV is disseminated by respiratory via, fecal-oral transmission, direct conjunctival and sexual transmission. Wild type AAV requires a helper virus (Adenovirus or Herpesvirus) for effective replication.

In natural conditions, for the typical primates serotypes (humans and non humans) it is not known any active transfer of genetic material to organisms other than primates, although an absence of zoonosis is not documented.

(b) Factors affecting dissemination
None.

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)

There are different research programs developing recombinant AAV GMO based on serotype 9 for genetic diseases,. Bosch et al. 2013 is an example of a recent publication.

All the preclinical work for the program with scAAV9.U1a.SGSH was developed in USA.

Additionally there is already a voluntary release notification of a recombinant AAV GMO based on serotype 9 with notification code B/ES/14/09. The GMO presented in this voluntary release is based on the same parental organism, AAV9; and is proposed for a similar indication, treatment of mucopolysaccharidosis type III on a clinical trial with a unique center in Spain. Another notification for voluntary release of a recombinant AAV virus based on a different serotype, also in the context of human clinical trial was the one with notification number B/ES/12/53.

C. Information relating to the genetic modification

1. Type of the genetic modification

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

2. Intended outcome of the genetic modification

scAAV9.U1a.SGSH is a genetically engineered recombinant virus from adeno-associated virus serotype 9 (AAV9/rh14). For GMO preparation, all codifying sequences for viral proteins except the Inverted Terminal Regions or ITRs (therefore the deletion of genetic material is marked) are replaced by a complete expression cassette containing a human N-sulfoglucosamine sulfohydrolase (SGSH) sequence and additional regulatory sequences needed for the protein expression such as promoter and poly A (therefore the insertion of genetic material is marked).

These genetic modifications allow complete loss of replication ability and makes possible the expression or synthesis of the protein SGSH by transduced cells. The purpose of this genetic modification is the preparation of an efficient vehicle to deliver the correct human N-sulfoglucosamine sulfohydrolase (SGSH) enzyme activity in the CNS and periphery of MPS IIIA patients to correct the course of their disease.

3. (a) Has a vector been used in the process of modification?
Yes No

If no, go straight to question 5.

- (b) If yes, is the vector wholly or partially present in the modified organism?
Yes No

If no, go straight to question 5.

Only the recombinant expression cassette will be included in the final GMO as genetic sequence. The viral proteins (VP) will constitute the capsid of the final GMO. The auxiliary genes will only be used for the correct packing of the GMO.

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

- (a) Type of vector

plasmid	<input checked="" type="checkbox"/>
bacteriophage	<input type="checkbox"/>
virus	<input type="checkbox"/>
cosmid	<input type="checkbox"/>
transposable element	<input type="checkbox"/>
other, specify	

- (b) Identity of the vector

To obtain the GMO a total of three plasmids (vectors) were used:

- p-trs-U1a-SGSH: provides the complete expression cassette that contains human N-sulfoglucosamine sulfohydrolase and a wild-type and a trs-deleted AAV2 inverted terminal repeat sequence (ITR). The modification of one of its ITRs allows its self-complementarity. Thus the recombinant virus contains a double-stranded DNA produces a more rapid and efficient transcription
- AAV2-9-helper: It is an AAV helper plasmid that encodes the 4 wild-type AAV2 rep proteins and the 3 wild-type AAV VP capsid proteins from serotype 9.
- Adenovirus helper pHelp: helper adenovirus plasmid that contains the required adenovirus genome regions for AAV replication (E2A, E4ORF6 and VA RNA).

- (c) Host range of the vector

There are no host identified for any of the three plasmids, since they are commercial products that are not available in the natural environment and do not have infectivity capacity. The genetic content of p-trs-U1a-SGSH is the human gene for the expression of the SGSH enzyme, therefore the hypothetic host would be humans. The AAV 2-9-helper plasmid encloses the genes that codify for the AAV capsid, and so hypothetic hosts would be the same than the wild type AAV, so humans and primates no humans. Finally, the pHELP plasmid contains approximately a 40% of adenovirus genome and do not contain any critical cis elements for the replication such as adenoviral inverted terminals. This final plasmid is only used for the manufacture (correct packing) of the GMO, and it is not present on the GMO.

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

antibiotic resistance
other, specify

Indication of which antibiotic resistance gene is inserted

The three vectors contain antibiotic resistance gene amp^r , which allows a selective amplification of them.

(e) Constituent fragments of the vector

The fragments that constitute each vector are presented in figures and tables of Annex I.

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

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

The vector components used to prepare the GMO, scAAV9.U1a.SGSH, are produced by a simultaneously transfection into human 293 cells with three plasmids containing the sequences for the generation of the recombinant AAV: recombinant gene expression cassette between ITRs, capsid genes and helper genes.

Only the recombinant expression cassette will be contained into the final GMO as genetic sequence. The viral proteins (VP) will constitute the capsid of the final GMO. The helper genes are only used for the correct packing of the GMO.

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

The vector components used to prepare the GMO, scAAV9.U1a.SGSH, are produced by a simultaneously transfection into human 293 cells with three plasmids containing the sequences for the generation of the recombinant AAV: recombinant gene expression cassette between ITRs, capsid genes and helper genes.

Only the recombinant expression cassette will be contained into the final GMO as genetic sequence. The VP proteins will constitute the capsid of the final GMO. The helper genes will only be used for the correct assembling of the GMO. Therefore, here is indicated a complete description of the recombinant gene expression cassette, contained in the p-trs-U1a-SGSH plasmid is provided (complete sequence in Annex 1).

The recombinant gene expression cassette is constructed by recombinant molecular techniques in the plasmid p-trs-U1a-SGSH. It contains the human SGSH gene expression cassette flanked by a wild-type and a trs-deleted AAV2 inverted terminal repeat sequence (ITR). The expression cassette contains the human SGSH cDNA sequence and the compact murine U1a small nuclear RNA promoter (an RNA Pol II promoter) to drive gene

expression. It also contains an SV40 polyadenylation signal that is used for efficient transcription termination.

The schematic of the vector genome and expression cassette is shown below:



(b) Source of each constituent part of the insert

1. ITR (inverted terminal repeat): one wildtype (wt) AAV2 inverted terminal repeat (ITR) sequence (wtTR), from AAV serotype 2.
2. dITR: AAV2 terminal repeat with deletion of terminal resolution site to force generation of self-complementary dimeric genomes.
3. U1a promoter: It is a compact RNA transcription element, derived from the mouse spliceosomal U1a small nuclear RNA gene. This promoter contains specialized transcription elements including a short bi-directional enhancer that does not interact with conventional mRNA promoters.
4. Human hSGSH gene: The transgene, hSGSH coding sequence cDNA, includes all eight exons of SGSH and the N-terminal signal peptide sequence.
5. SV40 polyadenylation signal from SV40 virus genome.

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

1. ITR: the inverted terminal repeat sequence function as both origin of vector DNA replication and packaging signal of vector genome. ITR sequences represent the only cis element required from the original vector to package AAV particles.
2. dITR: AAV2 terminal repeat with deletion of terminal resolution site to force generation of self-complementary dimeric genomes.
3. U1a promoter: the promoter/enhancer to drive a constitutive and high-level of human N-sulfoglucosamine sulfohydrolase expression.
4. Human hSGSH gene: to allow a correct production of human N-sulfoglucosamine sulfohydrolase and so obtain functional correction of lysosomal storage pathology.
5. Polyadenylation Signal: cis sequences for efficient polyadenylation of the hSGSH mRNA transcript. Its functions as a signal for transcriptional termination, a specific cleavage event at the 3' end of the nascent transcript and addition of a long polyadenyl tail.

(d) Location of the insert in the host organism

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

The described insert is recombinant and completely replaces the genome of the host or parental organism (AAV9 and 2).

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

Yes (.) No (X)

If yes, specify

The complete sequence of the insert is provided in the Annex 1.

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) Humans

(insert consist on the N-sulfoglucosamine sulfohydrolase (SGSH) cDNA)

- 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:

(b) to which of the following organisms:

humans (.)

animals (.)

plants (.)

other ..

- (b) are the donated sequences involved in any way to the pathogenic or harmful properties of the organism

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

If yes, give the relevant information under Annex III A, point II(A)(11)(d):

In fact the gene that is included in the vector is the correct human gene of SGSH, which defect is causing the disease in Sanfilippo A patients.

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

Considering the donor the human donating the SGSH gene for the insert and the recipient organism the AAV9 vector, it can be stated that there is no natural exchange of genetic material between them. Adeno-associated vector can infect humans but infections are completely asymptomatic.

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

The GMO secreted by body fluids will have survival or stability similar to the wild type virus. The genetically modified organism is complete deleted of any viral genes, so there is a complete block of any potential replication (even in presence of helper virus). The GMO will enter a cell and permanent within the nucleus as concatamers (linear or circular) with stable expression of the SGSH protein. The GMO cannot replicate, is only the protein which can pass from one cell to another due to a bystander effect.

- (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

The final GMO is an AAV recombinant virus, completely deficient for replication due to complete replacement of adeno-associated viral genes by the cassette of expression for human SGSH. See point E1a for further information.

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

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

Specify

As the GMO cannot replicate, the dissemination of the organism is limited to the administration of the GMO to the patients in the clinical study. Patients will be hospitalized for at least 48 hours in the University Hospital of Cruces under a close monitoring to control initial GMO shedding in body fluids. See point E1a for further information.

- (d) is the GMO in any way different from the recipient as far as pathogenicity is concerned?
Yes (.) No (X) Not known (.)
Specify

Wild type AAV is non pathogenic. Therefore, scAAV9.U1a.SGSH vector is non pathogenic as well as incompetent for the replication.

2. Genetic stability of the genetically modified organism

Specifically for this GMO, its genome has been constructed by recombinant DNA in the lab through a DNA plasmid. The gene expression cassette content in the plasmid is analyzed during the manufacturing process by several tests to assess its identity (Q-PCR, DNA sequencing and restriction analysis).

Considering that the GMO is containing the genome of the plasmid used for its production and cannot replicate further, the GMO is considered as genetically 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)

Point II(A)(11)(d) of Annex III: the recombinant AAV viruses are able to infect cells from humans and non human primates, but they are not pathogenic, toxic, virulent, allergenic, or a carrier (vector) of a pathogen. The most common known host range includes humans and nonhuman primates. They do not replicate or activate other latent virus and so they are not able to colonize other organisms.

Point II(C)(2)(i) of Annex III: With respect to human, animal and plant health, there are no toxic or allergenic effects of the recombinant AAV virus and its products do not represent toxic or allergenic effects. The recombinant AAV virus is not pathogenic and does not have colonization capacity, although it may persist within infected cells as episomal form.

In fact, recombinant adeno-associated virus (rAAVs) have been widely used for gene delivery in animal models, and are currently evaluated for human gene therapy after successful clinical trials in the treatment of inherited, degenerative or acquired diseases such as Leber congenital amaurosis, Parkinson disease, heart failure, etc. Recombinant adeno-associated virus carrying capsids from this serotype 9 transduces with different efficient different tissues, due to dissimilar tropism. In terms of the treatment of neurological diseases, the main reason for the emerging popularity of AAV9 is its remarkable ability to cross the BBB (Blood Brain Barrier).

4. Description of identification and detection methods

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

There are several techniques to detect the GMO in the environment, but the most common one is the quantitative PCR with primers for specific sequences. The correct primer design ensures the specific detection of the GMO. The shedding samples that will be obtained within the clinical trial (from serum, saliva, faeces and urine) will be analyzed with this technique that will allow the detection (with a low limit of detection of around 10 copies) and quantification of shedding profiles.

AAV vector particles can also easily be detected using Enzyme-Linked Immunosorbent Assay (ELISA) method. This is an indirect method able to detect and quantify the total antibodies developed by the body against the capsid proteins. The samples that will be obtained within the clinical trial will be analyzed with this technique that will allow the total antibodies quantification. The detection of antibodies by ELISA is a measure required as exclusion criteria in the study.

It is also described that in some cases it has been used an in situ hybridization (ISH) technique with a digoxigenin-labeled AAV probe in paraffin embedded tissues from the AAV positive cases. This technique is not use for this GMO.

Finally the identification of a specific GMO sequences can be performed in last instance by DNA sequencing.

(b) Techniques used to identify the GMO

All the techniques that have been described in point (a) to detect the genetically modified organism are also able to identify it, since they are specific.

F. Information relating to the release

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

The purpose of the voluntary release notification of the presented GMO, is a Phase I/II gene therapy clinical trial of scAAV9.U1a.SGSH for Mucopolysaccharidosis (MPS) IIIA. This study will include pediatric patients of 2 years old or greater, in order to assess the safety and clinical response to the therapy with the proposed GMO. The main purpose of this clinical development is to be able to restore the N-sulfoglucosamine sulfohydrolase (SGSH) enzyme activity in the central and peripheral nervous system to stop the progression of the disease caused by a deficiency of this enzyme.

This is an international clinical trial performed in two countries, Spain and Australia, with a total of 18 patients fulfilling the eligibility criteria. The study in Spain will be carried out in Cruces University Hospital, Bilbao, Spain with Luis Aldamiz as Principal Investigator. The study in Australia will be carried out in Adelaide Women's & Children's Hospital with Nick Smith as Principal Investigator. It is worth of mention that the initial development of this gene therapy virus scAAV9.U1a.SGSH will be carried out simultaneously with other dose escalation clinical trial sponsored by Nationwide Children's Hospital (NCH).

Both studies will be a dose escalation testing first a low dose of $0,5 \times 10^{13}$ viral genomes per kilogram (vg/kg), followed by a high dose of 2×10^{13} vg/kg. Patients will be administered one by one, according to a dose escalation plan with safety periods. Patients will be hospitalized for at least 48 hours after the drug administration for a close safety monitoring. Then, a follow up within the study will be performed by period visits to the hospital during a

period of 2 years. Additionally, considering that it is an advanced therapy, patients will be followed for a total of 5 years after study end for safety reasons.

No environmental benefit is expected. It is expected to detect GMO in serum, urine, saliva and faeces for a period of 30 days after vector administration. However, if necessary, the content of GMO in body fluids will be monitored in serum, urine, saliva and faeces until 90 days after treatment.

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

As previously indicated, in general the adeno-associated viruses hosts are humans and nonhuman primates. For this voluntary release, the genetically modified organism, scAAV9.U1a.SGSH will be administrated intravenously to a total of 18 patients in two different hospitals: University Hospital of Cruces Hospital, Baracaldo, Vizcaya, Spain and Adelaide Women's & Children's Hospital, Australia.

The hospital staff will receipt, storage, manipulate and destroy the gene therapy product and all biological samples (potentially containing also GMO) under biosafety rules type I (BSL-II). University Hospital of Cruces will follow internal procedures for biological product management.

3. Information concerning the release and the surrounding area

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

For Spain, country that applies for this voluntary release notification, the GMO will be administrated intravenously to pediatric patients recruited at University Hospital of Cruces. Hospital is located at the following address: Plaza de Cruces, 12, 48903 Baracaldo, Vizcaya – Spain.

(d) Size of the site (m²):

The recombinant adeno-associated vector will be receipt by the pharmacy service according to the sponsor instructions in the Pharmacy Manual. The storage will be performed in a -80°C freezer located at Biocruces Research Unit with a limited access. The final product preparation (defrosting, potential dilution and syringe preparation) will be performed in a biosafety cabinet type 2 (vertical flow for complete protection of environment and hospital staff). The syringe prepared for administration will be transported to the individual room where the patient will be hospitalized in a proper box to avoid spills and labeled as "containing GMO". All material use for GMO manipulation will be destroyed following internal procedures for biological material management and BSL-I.

Patient will be admitted at University Hospital of Cruces one by one, for a single intravenous administration. Patients will stay in an individual hospital room for at least 48 hours after gene transfer under a close monitoring and then they will came back to the hospital for period visits (day 7, day 14, day 30, etc).

- (i) actual release site (m²): Intravenous administration in an individual room at University Hospital of Cruces
- (ii) wider release site (m²): Not applicable.

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

The Cruces Hospital is located at approximately 40 km far from three protected areas: (1) Biosphere reserve: Urdaibai; (2) Protected biotype: San Juan de Gaztelugatxe and Itxina. It is important to highlight that GMO, as the parental organism from which is derived, are not pathogenic.

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

The wild type and recombinant AAV9 can infect mammal cells but not plant cells. Moreover, a limited release of the GMO to waste water is expected after the administration to the patients. Preclinical data is showing a positive shedding in faeces and urine samples for a period of 35 days with a very low amount of GMO genomes. Additionally the pH and biological conditions in urine and faeces diminish the potential survival of the genetically modified organism. The waste water is not an ecosystem where GMO can survive for a long period of time. Finally, the body fluids of treated patient will be neutralized with 10% bleach before its elimination by the regular ways until 90 days post-treatment (see patient instructions for further details). Taking into account these characteristics and the GMO replication incapacity, no interaction of the GMO with the flora, fauna, livestock or migratory species is expected.

4. Method and amount of release

- (a) **Quantities of GMOs to be released:**

The study design (international, multicenter and dose escalation) is described in point 1 of section F. A total of 18 patients will be treated in two sites (Spain and Australia) as a competitive recruitment, so we can not estimate the number of patients to be treated in each site beforehand.

Two patients (in total for both sites) will receive a low dose of $0,5 \times 10^{13}$ vg/kg, and 16 patients will receive a high dose of 2×10^{13} vg/kg. The administration will be only one for each patient and will be performed as a single intravenous injection of via peripheral limb vein.

It is expected that the GMO will be excreted to biological fluids in very small quantities several days after GMO administration. Preclinical data for animals administered with a dose 10 times higher than the maximum dose for the clinical trial showed shedding profiles of around 300 GMO genomes in 40 microliters of urine. The clinical trial design include the collection of shedding samples (serum, saliva, faeces and urine) for a total of 90 days after drug administration. It is expected that samples will be negative at day 30, but further samples (until day 90) will be analyzed in case no consecutive negative results are obtained. All the shedding results will be reported to CNB at the end of the study.

- (b) **Duration of the operation:**

The genetic modified organism will be stored frozen at -80°C in the Biocruces Research Unit. At the time that the patient administration will be performed, the frozen vials will be defrost inside a biological safety cabinet type 2 (vertical flow for safety reasons) and the syringe for infusion will be prepared. This may take around 30-45 minutes.

The syringe prepared for administration will be transported to the individual room where the patient is hospitalized in a proper box to avoid spills and labeled as "containing GMO". Patient will be admitted at University Hospital of Cruces one by one, for a single intravenous administration. The infusion will be given over approximately 10 to 20 minutes. The infusion tubing will be flushed using normal saline at the end of the infusion.

The material use for GMO manipulation will be destroyed following internal procedures for biological management and BSL-I.

Shedding analysis in body fluids of patient administered with the GMO will be performed for a total of 90 days after infusion.

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

The GMO will be stored in a limited-access area. All health personnel involved in this trial will use biosafety practices level 1 during transport, prior to and after administration and at final disposal. Patients will be hospitalized for at least 48 hours after GMO infusion for close monitoring. The potential presence of GMO in urine and faeces of treated patient will be neutralized with 10% bleach before regular elimination until day 90 post-treatment. In case of spillover, the perimeter of the spill will be limited with paper towels and appropriate virocidal agent will be use to clean the area. As all GMOs based on adeno-associated viruses, scAAV9.U1a.SGSH is susceptible to appropriate virucidal disinfectants with activity for non-enveloped viruses such as 1-10% sodium hypochlorite (for at least 20 minutes), alkaline solutions at pH >9, 5% phenol, heat (>80°C for 60 minutes), UV radiation and extreme pHs (<2 and >12). Effective disinfectants require a minimum of 20 minutes contact time.

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

The genetically modified organism is stored at Biocruces Research Unit at -80°C. The GMO will be used for treatment at University Cruces Hospital (Spain) in an independent room under hospital environmental conditions.

Potential shed GMO particles most likely may be at waste water at ambient temperature.

- 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.

All the previous release based on AAV9 parental organism has not provided yet any data on potential effects. Specifically, scAAV9.U1a.SGSH has demonstrated a shedding profile in preclinical studies with a dose 10 times higher than the maximum dose to be administered in the presented clinical trials have showed positive signal in urine and faeces with a very low number of vector genome copies for a period of 35 days. There is not shedding data in human clinical trials at the time of the present notification for shedding profile of scAAV9.U1a.SGSH, although the clinical trial at Nationwide Children's Hospital, Columbus, Ohio, USA will be started in the following months.

Nevertheless, it is important to consider that the GMO is not pathogenic, and no side-effects have been reported for the environment or human health after the release of similar GMOs (adeno-associated virus from serotypes 2, 5, 8 and 9).

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)

The gene transfer for mucopolysaccharidosis IIIA (MPS IIIA) will be performed in two Phase I/II studies (USA, Spain and Australia) in the following three years (six months of recruitment and two years of follow up). The proposed studies are designed to assess safety and clinical response to the restoration of N-sulfoglucosamine sulfohydrolase (SGSH) enzyme activity in the CNS and periphery following intravenous injection of rAAV9 vector carrying the human SGSH transgene under the control of the U1a promoter. Based on pre-clinical studies, rAAV9 and the U1a promoter will allow the efficient long-term transgene expression and secretion of functional SGSH, with great potential for achieving clinical benefits in treating neurological, as well as somatic disorders, in MPS IIIA patients. The GMO genome will not integrate in the host genome, since it is a non integrative viral vector, and will keep as extra-chromosomal concatamers (linear or circular).

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

Not expected. Considering that GMO genome is stable and shedding in waste water will come from urine shedding under extreme pH conditions, horizontal gene transfer to bacteria or other organisms most likely is minimal but cannot be excluded.

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

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

Give details

scAAV9.U1a.SGSH is replication incompetent by definition so unable to propagate and is therefore not subject to selective pressure.

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

The area where scAAV9.U1a.SGSH will be disseminated is the University Hospital of Cruces (Spain) and the product will be handled under BSL-I and appropriate internal procedures for biological waste. Even in the event of shedding in waste water no establishment in such system can be expected, because it is replication incompetent.

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 ...

None expected.

7. Likelihood of genetic exchange in vivo

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

Horizontal gene transfer to bacteria or other microorganisms is most likely minimal, but cannot be excluded.

The horizontal genetic transfer is unlikely and based on the scAAV9.U1a.SGSH sequence characteristics there is no possibility to provide a selective advantage to bacterias, since does not contain any prokaryotic promoters, any antibiotic- or other types of resistance genes or any genes, which would enhance or constrain their growth. Therefore, it is unlikely that scAAV9.U1a.SGSH would interfere with the control of pathogenic microorganisms or that it would have an effect on the natural dynamics of microbial populations or the biogeochemical cycles at any given site in the environment.

It is not expected any genetic exchange in vivo with other organism on the ecosystem. The GMO can be released to the environment but in infinitesimal amounts. On the other hand, the virus has a very low probability to be infective because of the physic-chemical characteristics of the biological fluids in which is released. Again, it is important to remind that the GMO has not replication capacity. It is highly unlikely that a release to the environment which will be infective will occur, based on the number of particles released, the need of direct contact between receptor mucosa and shedding products of the patient treated and the restriction of the virus or GMO hosts. In the extreme situation that GMO particles will be released, and they could reach a host (human or non human primate), there are not toxic effects described as derived from the virus or GMO itself, or the biological activity of the SGSH enzyme in the host species.

(b) from other organisms to the GMO:

Not expected.

(c) likely consequences of gene transfer:

None (GMO brings no selective advantages or disadvantages).

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.):

All voluntary releases previous notified with AAV9 as parental organism have not provided so far any data about the potential effects. Specifically, scAAV9.U1a.SGSH preclinical data in animals treated with a dose ten times higher than the maximum clinical dose planned have showed a positive shedding with a very low amount of GMO release in urine and faeces for a period of 35 days.

There are no references for scAAV9.U1a.SGSH behaviour in natural environment. It is expected that GMO will be degraded after administration to humans by endogenous protein and DNA catabolic pathways. Shed GMO DNA is not expected to be stable in waste water.

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

The potential GMO release to the environment will be analyzed in serum and biological fluids samples obtained from all treated patient according to the protocol of the clinical trial. Serum, saliva, urine and feces will be collected at Day 0, 1, 2, 7, 14, 30, 60 and 90 after GMO administration. The samples will be analyzed for GMO detection and quantification based on a specific qPCR. It is worth of mention that ELISA and PBLs samples will also be obtained to monitor the humoral and T cellular response that will also be assays to monitor the indirect effect of the GMO presence by means of antibodies and T reactive cells.

2. Methods for monitoring ecosystem effects

Not applicable.

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

Not applicable.

4. Size of the monitoring area (m²)

In Spain, the treatment will be prepared in a biosafety cabinet type 2 (vertical flow) of the Biocruces Research Unit. The patient treatment, by a single intravenous administration will be performed in an individual room at University Hospital of Cruces for each patient.

5. Duration of the monitoring

Based on preclinical data, GMO shedding will be analyzed for a total of 90 days after administration.

6. Frequency of the monitoring

GMO shedding monitoring will be performed in serum and biological fluids (saliva, urine and faeces) from all treated patients with the following schedule: screening (one month before treatment), day 0 or treatment day (4 and 8 hours after treatment), day 1, day 2, day 7, day 14, day 30, day 60 and day 90 after treatment.

I. Information on post-release and waste treatment

1. Post-release treatment of the site

In general terms, decontamination or site management will be performed according to local biosafety guidelines or procedures and BSL-I.

In case of spillover, the perimeter of the spill will be limited with paper towels and appropriate virocidal agent will be use to clean the area. As all GMOs based on adeno-associated viruses, scAAV9.U1a.SGSH is susceptible to appropriate virucidal disinfectants with activity for non-enveloped viruses such as 1-10% sodium hypochlorite (for at least 20 minutes), alkaline solutions at pH >9, 5% phenol, heat (>80°C for 60 minutes), UV radiation and extreme pHs (<2 and >12). Effective disinfectants require a minimum of 20 minutes contact time with GMO.

The destruction of all material used for GMO manipulation will be performed following internal procedures for management of biological agents and BSL-I.

2. Post-release treatment of the GMOs

In general terms, all equipments used during the procedure will either be disposed of in line with current biological hazard procedures or decontaminated with virucidal agents as dictated by the local biological hazard waste management plan.

3. (a) **Type and amount of waste generated**
Empty vials and used r scAAV9.U1a.SGSH vials as well as used delivery system components (guide tube, cannula, stylet, injection needle and syringe), gauzes and personal protective equipment and components used for collecting body fluids samples after administration.
The final volume of waste cannot be estimated but due to the competitive recruitment nature of the study design, but the final volume will not be high considering that the number of patient is limited due to the rareness of the condition and the phase I/II study design.
In any case, all waste will have a controlled elimination.
3. (b) **Treatment of waste**
Following vector administration, used scAAV9.U1a.SGSH vials as well as the used delivery system components (guide tube, cannula, stylet, injection needle and syringe) will be disposed of in a manner consistent with the standard practice of the institution for biohazardous materials and BLS-I rules. In addition any disposable surgical instruments or other materials used during the administration procedure or collection of body fluids will be disposed according to standard biosafety practice of the institution. All non-disposable surgical equipment will be cleaned using a chemical disinfectant with proven virucidal activity (e.g. hypochlorite 1% solution) and then sterilized by autoclaving according to standard practice of the institution.

J. Information on emergency response plans

1. **Methods and procedures for controlling the dissemination of the GMO(s) in case of unexpected spread**
In case of unexpected spread (e.g. spills) the affected area, perimeter will be lined with absorbing material, and then decontaminated using appropriate disinfectants as described before.
In case of injury, the injured site will be disinfected appropriately according to the best biosafety practice standards and internal procedures.
2. **Methods for removal of the GMO(s) of the areas potentially affected**
According to BSL-I health personnel involved in the trial will wear protective clothing.
In case of spillover, the area will be gently covered with paper towels and appropriate chemical disinfectant such as hypochlorite 1% solutions will be used to clean the area. The disinfectant will be applied for a minimum of 20 minutes as contact time before clean up.
3. **Methods for disposal or sanitation of plants, animals, soils, etc. that could be exposed during or after the spread**
Not applicable, since the GMO is not able to transduce plant cells.
4. **Plans for protecting human health and the environment in the event of an undesirable effect**
A safety follow up of 5 years after the end of the study will be performed for all patients according to the clinical trial protocol (attached). Adverse events will be registered and evaluated by clinical trial investigators and will be notified to competent authorities when they are relevant. All areas and facilities that will be used for the GMO administration will be cleaned and decontaminated using virocidal disinfectants as the ones described in this SNIF. There are no specific plans for the environment. Due to the preventive measures, such as the patient hospitalization for at least 48 hours after treatment, shedding control by disinfection

of potential contaminated areas, and patient body fluid neutralization; it is expected a residual GMO release. Additionally, considering that the GMO is non replicative there is also no further release expected from this point. In case undesirable effects for the environment would be observed, the study and GMO administration would be put on hold until appropriate measures are put in place to mitigate further risks.