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

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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/14/09

(c) Date of acknowledgement of notification: 04/11/14

(d) Title of the project: Phase I/II safety, tolerability and initial efficacy study of adeno-associated viral vector serotype 9 containing human Sulfamidase gene after intracerebroventricular administration to patients with Mucolopolysaccharidosis IIIA (Sanfilippo A syndrome).

(e) Proposed period of release: From September 2015 until May 2016.

2. Notifier

Name of institution or company: Laboratorios del Dr. Esteve, S.A.
Av. Mare de Déu de Montserrat, 221
08041 Barcelona

3. GMO characterisation

(a) Indicate whether the GMO is a:

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

other, specify (kingdom, phylum and class)
Human recombinant adeno-associated vector serotype 9 (AAV9) containing human Sulfamidase is a breakthrough product for the treatment of Mucolopolysaccharidosis IIIA (Sanfilippo A Syndrome).

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

Family: Parvoviridae
Genus: Dependovirus
Species: Recombinant adeno-associated virus (AVV) non-replicative

The complete name of the recombinant AAV is *AAV9-CAG-coh-SGSH (*or *AAV9-hSulfamidase*).

(c) Genetic stability – according to Annex IIIa, II, A(10)
The genetic stability is equivalent to the wild type AAV; however, as the vector is replication deficient, the stability after the replication will not be relevant.
The genetic stability of the batches of Baculovirus used to manufacture the GMO as well as the batches used for the clinical trial will be confirmed by qPCR and sequencing techniques.

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

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

7. Summary of the potential environmental impact of the release of the GMOs.
The AAV9 virus is an adeno-associated virus non-pathogenic, isolated by molecular biology techniques from human tissues. The GMO has been developed from wild type AAV9 modifying the genome and removing all genes codifying the viral proteins. During this process, the *AAV9-hSulfamidase* loses all replicative capacity.

AAV9, as well as the recombinant virus, can infect mammal cells but not vegetal cells. Consequently, no risk on the flora can be expected. Regarding the fauna, as the AAV9 is non-pathogenic, the virus would not imply any environmental impact.
On the other hand, a limited release in waste water during the first days after administration can be expected; however, *AAV9-hSulfamidase* will not survive and shed in the waste water.

Taking into account these characteristics, the probability that there is an exchange of genetic material with other organisms is negligible, thus the release of the GMOs will not pose an environmental risk.

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	Dependovirus
(iii)	species	Adeno-associated virus
(iv)	subspecies	…….
(v)	strain	Serotype 9
(vi)	pathovar (biotype, ecotype, race, etc.)	…..
(vii)	common name	AAV9

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:

An high percentage of the world population (between 30-50%) is seropositive for at least one of the AAV serotype. Some scientific studies show that approximately 50% of world population is seropositive for AAV9.

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	

The AAV have been isolated from human and non-human primates, although other animals can be hosts. Specifically, the AAV9 has been isolated from human tissues.

(b) If the organism is an animal: natural habitat or usual agroecosystem:
Not applicable

5. (a) Detection techniques: Specific PCR techniques to detect the vector DNA.
(b) Identification techniques : Specific PCR and sequencing techniques to detect the vector DNA.

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

Wild type AAVs are not related with any disease, and, therefore, these viruses are considered as non-pathogenic viruses. For this reason, wild type AAVs, as well as the recombinant one, are classified into the biosecurity group Group/Class 1 (insignificant or null risk).

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
The AAVs are not pathogenic, virulent, allergenic or vectors carrying a pathogen. The range of the most famous host includes human and non-human primates.

8. Information concerning reproduction

(a) Generation time in natural ecosystems:
Not relevant. *AAV9-hSulfamidase* lacks all viral replicative proteins.

(b) Generation time in the ecosystem where the release will take place:
Not relevant. *AAV9-hSulfamidase* lacks all viral replicative proteins.

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

(d) Factors affecting reproduction:
For the replication of the wild type AAVs, it is necessary to co-infect the host cells with helper viruses as *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 (fungi)	(.)
(vi)	eggs	(.)
(vii)	pupae	(.)
(viii)	larvae	(.)
(ix)	other, specify	None

(b) relevant factors affecting survivability:
Wild type AAVs are susceptible to commercial virucidals (Limoseptol 1.25%), 1% sodium hypochlorite (at least for 10 minutes), 5% phenol, 0.25% SDS and sensitive to UV radiation, to heat (>80ºC for 60 minutes) and to extreme pHs (<2 and >12).

10. (a) Ways of dissemination:
Wild type AAVs are disseminated by respiratory way, fecal-oral way, direct contact of the virus with the eye conjunctiva or mucosa and by seminal way. This is the first time the vector *AAV9-hSulfamidase* will be administered in humans. Based on the preclinical studies, it is not expected to find vector remains after 30 days from the administration.

(b) Factors affecting dissemination:
Viruses have been detected by PCR in urine, saliva, feces and semen; however, the detection of these particles does not imply an infective property of the 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)
Not applicable.

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
AAV9-hSulfamidase is a genetically engineered vector from adeno-associated virus serotype 9 (AAV9). During the construction, the codifying sequence for viral proteins is deleted and it has been replaced by the expression cassette containing the codon optimized sequence of human Sulfamidase enzyme (SGSH) and the regulatory sequences needed for the protein expression.
The deletion of the complete protein sequence of AAV9 gives replicative incapacity.

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

If no, go straight to question 5.

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

If no, go straight to question 5.

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

(a) Type of vector

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

(b) Identity of the vector:
Baculovirus.

(c) Host range of the vector: Insect cells (Sf9 cells) because Baculoviruses infect only invertebrates.

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

antibiotic resistance	(.)
other, specify	

Indication of which antibiotic resistance gene is inserted:

(e) Constituent fragments of the vector:
Two Baculovirus constructs are required for the generation and manufacturing of *AAV9-hSulfamidase*: Baculovirus Rep2Cap9 and Baculovirus SGSH.

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

(i)	transformation	(.)	
(ii)	electroporation	(.)	
(iii)	macroinjection	(.)	
(iv)	microinjection	(.)	
(v)	infection	(X)	Co-infection of Sf9 cells with 2 Baculoviruses containing the sequences needed to generate the GMO.
(vi)	other, specify		

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 genetic material (genome) that is introduced into the receptor organism (AAV9) is composed of different parts with different biological origins.
Firstly, the genome is flanked by DNA sequences from the AAV serotype 2 called Inverted Terminal Repeats (ITR). These sequences are needed to encapside the genome inside the viral capsid during the GMO generation process.
Secondly, the genome is formed of an expression cassette containing a promotor, the codifying sequence for the human Sulfamidase and the polyA signal, since it is intended to express the gene of Sulfamidase in the transducted cells.
The promotor is a hybrid and ubiquitous promotor (CAG) composed of the Citomegalovirus enhancer (CMV) and the chicken β-actine promotor. The codifying sequence is the human Sulfamidase, an important enzyme for the degradation of sulfate heparan in mammal cells. This sequence is codon optimized in order to get a major expression. And, finally, the insert contains the polyA signal from the rabbit β-globine.
The sequences ITRs, CAG and polyA have been largely tested in human.

The insert is represented in the following picture:

(b) Source of each constituent part of the insert

- ITRs: from AAV serotype 2.
- CAG promoter: enhancer from Cytomegalovirus (CMV) and the promotor sequence from the chicken β-actin.
- Codon-optimized human Sulfamidase: synthetic origin codifying for human Sulfamidase protein (SGSH).
- PolyA sequence from rabbit β-globin.

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

- ITRs from AAV2: these sequences flank the codifying sequence and are needed to encapside the genome of the GMO into viral capsids.
- CAG promoter: this sequence is needed to activate the protein transcription ubiquitously after the cell infection by the GMO. This promotor is a hybrid promotor, composed of the Citomegalovirus enhancer (CMV) and the chicken β-actin promotor.
- Codifying sequence for human Sulfamidase: this is needed to produce the Sulfamidase protein, the enzyme involved in the heparan sulfate metabolism in mammal cells.
- PolyA signal: this sequence is needed to control the end of the transcription.

The ITR, CAG promoter and PolyA sequences have been tested in animals and humans previously.

(d) Location of the insert in the host organism

- on a free plasmid (.)
- integrated in the chromosome (.)
- other, specify: the insert replace completely the genome of the receptor organism (AAV9).

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

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

1. Indicate whether it is a:

viroid	(.)	
RNA virus	(.)	
DNA virus	(.)	
bacterium	(.)	
fungus	(.)	
animal	(X)	
- mammals	(X)	Human Sulfamidase is considered as the main component for the insertion of the codifying sequence, because this is the only sequence that can be translated to a protein with biological function once the GMO is liberated.
- insect	(.)	
- fish	(.)	
- other animal	(.)	
(specify phylum, class)		
other, specify		

2. Complete name

(i)	order and/or higher taxon (for animals)	Primate
(ii)	family name for plants	…
(iii)	genus	Homo
(iv)	species	*Homo sapiens…*
(v)	subspecies	…
(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):
…

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 (.)
If yes, specify
Not applicable.

5. Do the donor and recipient organism exchange genetic material naturally?
Yes (X) No (.) Not known (.)

E. Information relating to the genetically modified organism

1. Genetic traits and phenotypic characteristics of the recipient or parental organism which have been changed as a result of the genetic modification

(a) is the GMO different from the recipient as far as survivability is concerned?
Yes (.) No (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
The GMO generated cannot replicate in the host cell even if helper viruses (*Adenovirus* and *Herpesvirus)* are present. During the generation of GMO, the AAV9 genome of the receptor organism (AAV9) needed has been completely replaced by the cassette of expression of human Sulfamidase.

(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 patient.

(d) is the GMO in any way different from the recipient as far as pathogenicity is concerned?
Yes (.) No (X) Not known (.)
Specify:
As in the human Sulfamidase expression cassette no pathogenic sequences are present and the receptor organism (wild type AAV) is not pathogenic, any difference of pathogenicity between the wild type and the GMO will be observed. In any case, it is important to highlight that the GMO is not an integrative or a replicative vector.

2. Genetic stability of the genetically modified organism
The GMO genome is analyzed during the manufacturing process by several tests to assess the identity of the vector (qPCR and sequencing techniques). As does wild type AAV9, the GMO keeps the genome stable. Moreover, due to the incapacity for replication of the GMO and the non-presence of the intrinsic mechanism for the genetic variation, the GMO will be 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)
Annex IIIA, point (A)(11)(d): The recombinant AAV vector infects mammal cells, but vectors are not pathogenic, toxigenic, virulent, allergenic or pathogen carriers. The GMO is incompetent for replication and, consequently, it cannot colonize other organisms.

Annex IIIA, Point II(C)(2)(i): Regarding the human and animal health or the phytosanitary field, no toxic or allergenic effects of the recombinant AAV vector or its products have been described. The GMO is not pathogenic and cannot colonize new habitats, although it will persist in the infected cells in episoma forming concatamers.

4. Description of identification and detection methods

(a) Techniques used to detect the GMO in the environment:
Specific PCR in order to detect the GMO genome.

(b) Techniques used to identify the GMO:
Specific PCR and sequencing techniques in order to detect the GMO genome.

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 release of the GMO is to perform a clinical trial to administrate the *AAV9-hSulfamidase* to 6 patients with Mucolopolysaccharidosis IIIA (Sanfilippo A Syndrome). No environmental benefit is expected.

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

   Yes (X) No (.)

   If yes, specify:
   The GMO will be administrated by intracerebroventricular via to 6 patients at Hospital Sant Joan de Déu of Barcelona.

3. Information concerning the release and the surrounding area

   (a) Geographical location (administrative region and where appropriate grid reference):
   The GMO will be administrated by intracerebroventricular via at Hospital Sant Joan de Déu de Barcelona (Spain).

   Hospital Sant Joan de Déu
   Passeig Sant Joan de Déu, 2
   08950 Esplugues de Llobregat
   Barcelona

   The GMO will be released by the urine, saliva, faeces or semen for some days after the administration (not longer than 30 days); however, it is expected that the GMP release will not be infectious.

   (b) Size of the site (m^2):
   The GMO will be administrated in the Operating Room of Neurosurgery (by a standard procedure of intaverebroventricular puncture). After the administration, the patient will stay at UCI for the first 24 hours after the administration. Finally, patients will stay at the Hospital for 7-10 days after administration.

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

   It is important to highlight that the recombinant vector, *AAV9-hSulfamidase*, is non-pathogenic.

   (c) Proximity to internationally recognised biotopes or protected areas (including drinking water reservoirs), which could be affected:
   The Hospital is located in the region where the natural reserve Delta del Llobregat is delimited.

   (d) Flora and fauna including crops, livestock and migratory species which may potentially interact with the GMO
   The wild type and recombinant AAV9 infect mammal cells but not plant cells. Moreover, only a limited release of the GMO to a waste water is expected after the administration to the patients. However, the waste water is not an ecosystem where the GMO can survive. Taking into account these characteristics and the replicative 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:
This is the first clinical trial where the *AAV9-hSulfamidase* is administered in humans. During the clinical trial, 2 dose levels of GMO will be administered to 2 cohorts of patients. One cohort of three subjects will receive *AAV9-hSulfamidase* by intracerebroventricular injection at the dosage level of 6.8E14 viral genomes. Depending on the results with this cohort, the dosage to be administered to the 3 subjects of the second cohort will be adapted halving or duplicating the dosage and the total dosage will be 3.4E13 or 1.4E14 viral genomes respectively.

It is expected that the GMO will be excreted to biological fluids several days after the administration of the vector, being in small quantities.

(b) Duration of the operation:
The GMO will be administered to the patients by surgery in the Operating Room of Neurosurgery of Hospital Sant Joan de Déu for no longer than 1 hour.

(c) Methods and procedures to avoid and/or minimise the spread of the GMOs beyond the site of the release
All persons involved in this clinical trial will work following Good Practice of biosecurity before and after the administration of the GMO, the transport of the GMO and the waste treatment.

5. Short description of average environmental conditions (weather, temperature, etc.)
The administration of the GMO will be done in the Hospital Sant Joan de Déu. The site is a smoke free healthcare environment with a climate control system that maintains temperatures of 23-25ºC at rooms and 21±2ºC at the surgical area.

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.
The GMO *AAV9-hSulfamidase* has not been released in human previously. However, it is important to highlight that the GMO is nonpathogenic and the human Sulfamidase is a protein without known toxic effects.
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 and 8).

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	*Homo sapiens*
(v)	subspecies	…
(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 *AAV9-hSulfamidase* will be administered as gene therapy to Sanfilippo A patients, which are subjects with a non-functional Sulfamidase protein. This enzyme is involved in the heparan sulfate catabolism and its inactivation produces a heparan sulfate accumulation.
After the administration of the vector, it is expected that the GMO genetic material will be transferred to the host cells and the protein Sulfamidase will be translated. If Sulfamidase protein levels are restored, the symptoms of the disease will improve. The GMO genome will not integrate in the host genome and will keep as extra-chromosomal concatamers.

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

4. Is post-release selection such as increased competitiveness, increased invasiveness for the GMO likely to occur?
Yes (.) No (X) Not known (.)
Give details
The GMO cannot replicate and, therefore, its dissemination or selection is not expected.

5. Types of ecosystems to which the GMO could be disseminated from the site of release and in which it could become established
The areas where *AAV9-hSulfamidase* will be administered are inside the hospital and they will be duly treated. The GMO can be released to waste water, but it is unlikely to hold the infective capacity and no consequence is expected.

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
Not applicable.

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

7. Likelihood of genetic exchange in vivo

(a) from the GMO to other organisms in the release ecosystem:
The GMO released to the environment is not expected to keep the infective properties and, due to the replicative incapacity, no dissemination pattern is expected.
If any GMO is released keeping the infective capacity, it could infect any mammal. However, no toxic effects derived from the enzyme Sulfamidase have been described in any 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.):
None available.

9. Possible environmentally significant interactions with biogeochemical processes (if different from the recipient or parental organism)
None.

H. Information relating to monitoring

1. Methods for monitoring the GMOs
The GMO release will be analyzed in biological fluids from the treated patient according to the protocol of the clinical trial: in blood, saliva, urine and feces. The analysis will be done regularly by qPCR until 2 consecutive samples of the biological fluids are negative.

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 (m^2)
Not applicable.

5. Duration of the monitoring
The biological fluids from the treated patients will be monitored regularly according to the protocol of the clinical trial until 2 consecutive samples are negative for the GMO.

6. Frequency of the monitoring
The monitoring of the detection of GMO will be done regularly until 2 consecutive samples will be negative for the GMO.

I. Information on post-release and waste treatment

1. Post-release treatment of the site
Post-release treatment will be done according to the disinfection protocol from the Committee of Infections of Hospital Sant Joan de Déu. (http://www.hsjdbcn.org/polymitaImages/public/comites/infeccions/2014_03_11_protocolo_antisepticos_desinfectantes.pdf)

2. Post-release treatment of the GMOs
The bio waste and the material in contact with the GMO will be treated following the center management plan for healthcare risk waste III. Any surface will be treated for disinfection following the disinfection protocol from the Committee of Infections of Hospital Sant Joan de Déu. (Limoseptol 1.25%).

3. (a) Type and amount of waste generated
Surgery material used for the administration of the virus, elements of protection for staff and carers. Syringes, needles used for the administration of the vector and sampling. Biological fluids and material from the patients.
The final volume of waste cannot be estimated but, taking into account the characteristics of the clinical trial with a low number of patients, the final volume will not be high and the waste will have a controlled elimination.

(b) Treatment of waste
The materials that have been in contact with the GMO (vials, needles, syringes, gloves, etc.) will be deposited in a biohazards container type III. Once hermetically sealed, containers will be transported to a waste room in the facilities of the Hospital. Every 48 hours, an authorized waste management company risk truck transports containers to a sterilizing plant. All surfaces that could have been in contact with the GMO will be disinfected with Limospetol (glutaraldehyde 1.25% with benzalkonium chloride).
Gauze, disposable towels and clinical waste generated in surgery are compiled in black bags and deposited in special containers and incinerated.

In the rooms disinfection will be performed with aldehydes and the waste will be eliminated in biohazards containers.
Disposable sheets and clothing will follow a specific disinfection protocol (washing for 15 minutes at 85ºC followed by a washing cycle at lower temperature and drying at 160ºC).
Biological samples processed at the laboratory will be eliminated in biohazard containers.
Careers will be informed about the specific disinfection measures and the treatment of biowaste: surface disinfection, toilets and clothes with aldehydes or diluted bleach.

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 all personnel shall be protected with goggles, surgical masks, disposable gloves and gowns following the criteria of good clinical practice (although these protective measures are not needed for the manipulation of the GMO type I). The zone will be isolated with closed doors and notification of the incidence. The protocol in case of accidental release of biological product of the Prevention and the Environment Service of the Hospital will be followed, including the use for absorbent material and disinfectants to restrain the spill.
Spilled material, as well as gloves, gowns and masks will be considered bio hazardous waste type III and, as such, will be removed. In case of injury, the wound will be disinfected with antiseptics.

2. Methods for removal of the GMO(s) of the areas potentially affected
The decontamination protocol includes absorbent material in order to restrain the spill, the disposal in biohazard container and disinfecting surfaces with aldehydes.

3. Methods for disposal or sanitation of plants, animals, soils, etc. that could be exposed during or after the spread
Not applicable.

4. Plans for protecting human health and the environment in the event of an undesirable effect
An exhaustive monitoring of the patients will be performed until 5 years after the administration according to the protocol of the clinical trial. The side effects will be registered and evaluated by the clinical researchers and, when relevant, will be notified to the Health Authorities.
No specific plans for the environment are contemplated as the release is expected to be residual and dissemination is not expected (because of the replicative incapacity). If any side effect on the environment is detected, the use of GMO will be stopped until good measures are adopted in order to eliminate the risk.