

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/18/04*
- (c) Date of acknowledgement of notification *20 June 2018*
- (d) Title of the project *Efficacy and Safety of Bilateral Intravitreal Injection of GS010: A Randomized, Double-Masked, Placebo-Controlled Trial in Subjects Affected with G11778A ND4 Leber Hereditary Optic Neuropathy for Up to One Year.*
- (e) Proposed period of release From *01/01/2018* until *30/03/2021*

2. Notifier

Name of institution or company:  
*Sponsor*  
*GENSIGHT Biologics*  
*74 rue du Faubourg Saint Antoine*  
*75012 Paris*

3. GMO characterization

*GS010 consists of a recombinant AAV vector serotype 2 containing the human mitochondrial ND4 gene (rAAV2/2-ND4 vector) intended for the treatment of Leber Hereditary Optic Neuropathy (LHON).*

(a) Indicate whether the GMO is a:

- viroid (.)
- RNA virus (.)
- DNA virus (X), *AAV-derived replication-deficient vector*
- 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)

*Parvoviridae*

*Genus: Dependovirus*

*Species: AAV-derived replication-deficient vector*

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

*AAV2/2-ND4 is expected to be genetically stable. In general, DNA viruses have greater genetic stability than RNA viruses. Firstly, DNA is thermodynamically stable; secondly, replication of DNA is a much less error-prone process than the replication of RNA; and thirdly, mechanisms exist in the host cell for repairing errors in DNA. Further, Recombinant AAV2/2-ND4 is unable to replicate independently, even in the presence of a helper virus, since it lacks the rep and cap genes required for rescue/packaging. No transfer of genetic material between the GMO and other organisms is predicted. It is not possible for the AAV genome to contain both rep/cap genes and the transgene, as this is beyond the packaging limit of the virion. Therefore the only mechanism by which the transgene could be mobilised is through a triple infection of the same cell by Recombinant AAV2/2-ND4 (containing the transgene), wild type AAV (providing the rep and cap functions) and a helper virus. This scenario is expected to be an extremely rare event, and would only result in the production of more wild type AAV and more Recombinant AAV2/2-ND4 vector particles (which would still lack rep and cap genes and consequently could not be self-sustaining). Each batch of the experimental product is tested for the absence of replication competent AAV to assure low levels of contamination.*

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

Yes ☒ No ☐

If yes, insert the country code(s) *FR, UK, IT, BE, DE, NL*

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

Yes ☒ No ☐

If yes:

- Member State of notification *FR, UK, IT, BE*
- Notification number *Not yet provided.*

**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 ☐

If yes:

- Member State of notification *USA, Taiwan*
- Notification number *Not yet provided.*



7. Summary of the potential environmental impact of the release of the GMOs.  
*GS010 will be administered as a single intravitreal (IVT) injection to LHON patients. This route of administration should not lead to the release of the GMO in the environment. Indeed:*
- *Biodistribution data available so far have shown that IVT route of administration does not lead to the spread of the GMO to other organs or blood in animals, In the case this GMO would nevertheless be released in the environment via body fluids, this should not lead to any incidence for the as the GMO i) is non pathogenic and replication incompetent, and ii) will be destroyed by conventional water treatment.*
- The intended application of GS010 is limited to one hospital centre and the number of patients to be treated is very restricted (target of 22 patients enrolled for 18 treated patients). Nonetheless, the hospital centre is required to train the health care professionals involved in the study in the safe handling of GS010 and to have appropriate biosafety practices implemented in order to minimize any accidental exposure to the product, be it personnel, contact persons or the environment.*

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

1. Recipient or parental organism characterisation:
- (a) Indicate whether the recipient or parental organism is a:  
(select one only)
- |                |     |
|----------------|-----|
| viroid         | (.) |
| RNA virus      | (.) |
| 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) | <i>Parvoviridae</i>           |
| (ii)  | genus                                   | <i>Dependovirus</i>           |
| (iii) | species                                 | <i>Adeno-associated virus</i> |
| (iv)  | subspecies                              |                               |
| (v)   | strain                                  | <i>Serotype 2</i>             |
| (vi)  | pathovar (biotype, ecotype, race, etc.) | ...                           |
| (vii) | common name                             | <i>AAV2</i>                   |



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:  
*Approximately 50 to 80% of the European human population is seropositive to at least one AAV serotype.*
- (ii) No (.)
- (iii) Not known (.)
- (c) Is it frequently used in the country where the notification is made?  
 Yes (X) *for research, contained use.* No (.)
- (d) Is it frequently kept in the country where the notification is made?  
 Yes (X) *for research, contained use.* 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
- Mostly frequent human and non-human primate hosts but also other animals.*
- (b) If the organism is an animal: natural habitat or usual agroecosystem:  
*Not applicable*
5. (a) Detection techniques
- Specific q-PCR can be used to detect the vector DNA.*
- Viral proteins can be detected by Western Blot to detect VP1, VP2 and VP3. The presence of VP1, VP2, VP3 should be comparable to reference.*
- (b) Identification techniques
- Specific q-PCR can be used to detect the vector DNA.*
- Viral proteins can be detected by Western Blot to detect VP1, VP2 and VP3. The presence of VP1, VP2, VP3 should be comparable to reference.*
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
- Adeno-associated viruses (AAV) belong to the family Parvoviridae and there is no known link to any known human illness. AAV viruses are classified biosafety Group/ Class 1.*



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

*No pathological, ecological and physiological traits are present. In natural conditions, wild type AAV2 in the presence of a helper virus (adenovirus) is found to transmit to humans only and is not known to colonize other species.*

8. Information concerning reproduction

(a) Generation time in natural ecosystems:

*Not applicable. Wild-type AAV is a non-autonomous virus and is not capable of replication.*

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

*Not relevant.*

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

(d) Factors affecting reproduction:

*AAV is a replication-incompetent virus. Reproduction of wild-type AAV is dependent on co-infection with helper virus (Adenovirus or Herpesvirus).*

9. Survivability

(a) ability to form structures enhancing survival or dormancy:

(i) endospores (.)

(ii) cysts (.)

(iii) sclerotia (.)

(iv) asexual spores (fungi) (.)

(v) sexual spores (funghi) (.)

(vi) eggs (.)

(vii) pupae (.)

(viii) larvae (.)

(ix) other, specify AAV does not form structures to enhance its survival.



- (b) relevant factors affecting survivability:

*The stability of parvoviruses against physico-chemical stress is considered to be high. Parvoviruses are stable in the presence of lipophilic solvents, upon exposure to pH 3-9 or incubation at 56°C for 60 minutes. In dried condition, infectivity of parvovirus particles can be maintained over several weeks*

*Additional experiments performed with recombinant AAV vectors also demonstrates that rAAV retain infectivity and transduction capability at room temperature for at least a month after simple dessication or lyophilization (Tenenbaum, Lehtonen et al. 2003).*

10. (a) Ways of dissemination

*Wild-type (wt) AAV infections are common in human and probably occur from childhood. The ways of dissemination for wt AAV are poorly understood, but is likely to occur through inhalation of aerosolized droplets, mucous membrane contact, parenteral injection, or ingestion.*

- (b) Factors affecting dissemination

*wt AAV are not able to replicate unless a co-infection with an adenovirus occurs.*

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)

*The strain from which GS010 vector is derived is a wt AAV2/2 for both rep functions and cap proteins.*

### 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

*GS010 is a rAAV2/2in which the human ND4 optimized gene has been inserted and the sequences allowing replication have been deleted.*

*The objective of these genetic modifications is to obtain a replication-defective viral vector able to deliver the correct human ND4 sequence in target cells in LHON patients.*

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

Yes (X) No (.)

If no, go straight to question 5.



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

If no, go straight to question 5.

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

- (a) Type of vector  
plasmid (X)  
bacteriophage (.)  
virus (.)  
cosmid (.)  
transposable element (.)  
other, specify
- (b) Identity of the vector  
*Recombinant AAV2/2\_ND4 vector is obtained by triple transfection in HEK293 cells with plasmid that contains (1) the recombinant viral genome (ITR- transgene expression cassette) (2) the rep and cap orf sequences and (3) adenoviral helper sequences.*
- (c) Host range of the vector  
*Animal and bacterial cells.*
- (d) Presence in the vector of sequences giving a selectable or identifiable phenotype  
Yes (X) No (.)

antibiotic resistance (X)  
other, specify

Indication of which antibiotic resistance gene is inserted:

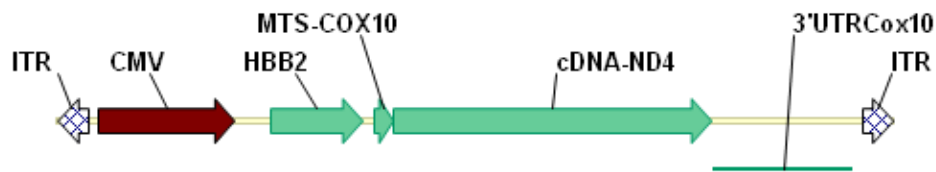
*Kanamycin resistance gene.*

*Note that the final GS010 product is controlled in order to ensure no kanamycin resistance gene is detected in drug product.*

- (e) Constituent fragments of the vector  
*The vector is a rAAV2 encoding an optimized gene of human NADH Dehydrogenase 4 (ND4) protein, under the control of the cytomegalovirus (CMV) immediate early promoter in an intron-containing expression cassette (beta globin intron, HBB2), flanked by the viral inverted terminal repeats (ITR) from AAV2. Each constituent is listed below:*
- AAV ITRS derived from serotype 2 (nt 6324-6453 and 3327-3454)*
  - CMV promoter (nt 2624-3277; 654 bp)*
  - HBB2 intron (nt 2129-2528; 399 bp)*
  - MTSCox10 (nt 1998-2081; 84 bp)*
  - Coding sequence of human ND4 codon-optimized for improved expression in human cells (nt 623-1996; 1373 bp).*
  - 3'UTR Cox10 (nt 11-605; 595 bp).*
  - Kanamycin resistance gene, aminoglycoside 3'-phosphotransferase (nt 4482-5273; 792 bp)*



*-Procaryotic origin of replication (nt 5488-6102; 615 bp) and phage f1 origin of replication (nt 3872-4327; 456 bp)*



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

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

*GS010 is manufactured in HEK 293 cells by a triple plasmid transfection technique exempt from an auxiliary virus.*

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 insert is composed of:*

- *a CMV promoter,*
- *the HBB2 intron,*
- *cis-acting elements of the Cox10 mRNA, and*

*The human wild-type mitochondrial NADPH Deshydrogenase (MT-ND4) optimized gene.*

(b) Source of each constituent part of the insert

<i>Part of the insert</i>	<i>Source</i>	<i>Intended function</i>
<i>CMV promoter</i>	<i>CMV</i>	<i>Induction of transgene expression in mammalian cells</i>
<i>HBB2 intron</i>	<i>human</i>	<i>Improvement of the transgene mRNA stability</i>
<i>Cis-acting elements of the Cox10 mRNA</i>	<i>human</i>	<i>Efficient mitochondrial delivery of the ND4 protein</i>
<i>ND4 transgene</i>	<i>human</i>	<i>Expression of a wt ND4 protein in the targeted cells. Use of nuclear codon bias.</i>



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

(d) Location of the insert in the host organism

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

*The insert is cloned into the viral vector genome.*

(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**

*The following information relates to the organism from which the inserted gene (MT-ND4) is derived.*

1. Indicate whether it is a:

viroid (.)

RNA virus (.)

DNA virus (.)

bacterium (.)

fungus (.)

animal

- mammals (X)

- insect (.)

- fish (.)

- other animal (.)

(specify phylum, class)

other, specify *human, with modification of codon bias.*

2. Complete name

(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*

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

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

If yes, specify the following:



(a) to which of the following organisms:

humans           (.)  
animals           (.)  
plants           (.)  
other           *Not applicable.*

(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):

*Not applicable.*

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 *Not applicable.*

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

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

## **E. Information relating to the genetically modified organism**

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

(a) is the GMO different from the recipient as far as survivability is concerned?

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

Specify

(b) is the GMO in any way different from the recipient as far as mode and/or rate of reproduction is concerned?

Yes   (*X*)                      No    (.)                      Unknown    (.)

Specify *The GMO has been designed to be defective for replication.*

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

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

Specify *GS010 is unable to replicate independently, even in the presence of a helper virus, since it lacks the rep and cap genes required for rescue/packaging. Therefore, though it has the capacity to infect cells, the lack of replicative capacity will severely restrict dissemination.*



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

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

Specify

2. Genetic stability of the genetically modified organism

*AAV2/2-ND4 is expected to be genetically stable. In general, DNA viruses have greater genetic stability than RNA viruses. Firstly, DNA is thermodynamically stable; secondly, replication of DNA is a much less error-prone process than the replication of RNA; and thirdly, mechanisms exist in the host cell for repairing errors in DNA. Further, Recombinant AAV2/2-ND4 is unable to replicate independently, even in the presence of a helper virus, since it lacks the rep and cap genes required for rescue/packaging. No transfer of genetic material between the GMO and other organisms is predicted. It is not possible for the AAV genome to contain both rep/cap genes and the transgene, as this is beyond the packaging limit of the virion. Therefore the only mechanism by which the transgene could be mobilised is through a triple infection of the same cell by Recombinant AAV2/2-ND4 (containing the transgene), wild type AAV (providing the rep and cap functions) and a helper virus. This scenario is expected to be a extremely rare event, and would only result in the production of more wild type AAV and more Recombinant AAV2/2-ND4 vector particles (which would still lack rep and cap genes and consequently could not be self-sustaining). Each batch of the experimental product is tested for the absence of replication competent AAV to assure low levels of contamination.*

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 *Not applicable.*

- (b) give the relevant information specified under Annex III A, point II(A)(11)(d) and II(C)(2)(i)

*Neither wild type AAV, nor the GS010 vector is known to be pathogenic to humans.*

4. Description of identification and detection methods

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

*Not applicable.*

- (b) Techniques used to identify the GMO

*• Polymerase Chain Reaction (PCR). PCR can be used to detect vector genome sequences associated with AAV in a qualitative or quantitative manner, using primers specific for the rep or cap genes. Detection of a specific serotype, or any AAV-like sequence, as well as distinction between wild type AAV and recombinant AAV is*



*possible, depending on the choice of primers. Note that the presence of vector genomes does not necessarily imply infectious virus particles.*

• *Enzyme-Linked Immunosorbent Assay (ELISA) methods. These methods may be used to detect AAV vector particles. They rely on the generation of specific antibodies to the vector capsid proteins, and can therefore be specific to an individual serotype, or cross-react with several AAV serotypes. Detection of vector capsid particles does not necessarily imply infectious virus particles.*

*Before batch release, the following identity controls are performed in order to characterize the GS010 viral vector:*

<i>Identity test by sequencing analysis of viral DNA</i>
<i>Viral protein identity</i>
<i>Protein purity profile</i>
<i>Viral Genome Titer by qPCR</i>
<i>Infectious Genome titer by qPCR</i>

## **F. Information relating to the release**

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

*Administration of the GS010 investigational medicinal product (IMP) to LHON patients in the frame of an authorized clinical trial in Spain.*

*The clinical study is entitled:*

***Efficacy and Safety of Bilateral Intravitreal Injection of GS010: A Randomized, Double-Masked, Placebo-Controlled Trial in Subjects Affected with G11778A ND4 Leber Hereditary Optic Neuropathy for Up to One Year.***

*The objective of this phase III is to assess the safety and efficacy of bilateral administration of intravitreal GS010 and to determine if a difference exists in the treated eyes versus placebo controlled eyes.*

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 (.) *Not applicable.*

If yes, specify

3. Information concerning the release and the surrounding area

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

*The clinical trial will be conducted at a single investigational site in Spain:*

*Hospital Universitario Ramón y Cajal  
Ctra. De Colmenar Viejo, km. 9,100,  
28034 Madrid, Spain*



- (b) Size of the site (m<sup>2</sup>): *Not applicable.*
  - (i) actual release site (m<sup>2</sup>):
  - (ii) wider release site (m<sup>2</sup>):
- (c) Proximity to internationally recognised biotopes or protected areas (including drinking water reservoirs), which could be affected:  
*Given the nature of the product administration, scale of contained use and procedures for waste treatment, the exposure to significant biotopes, protected areas and drinking water supplies is expected to be negligible.*
- (d) Flora and fauna including crops, livestock and migratory species which may potentially interact with the GMO  
*GS010 is a replication-incompetent virus derived from AAV2/2. The genetic modifications do not affect its natural host and tissue tropism. No transfer of genetic material between the GMO and other organisms is predicted.*  
*Given the nature of the product administration (intravitreal) and the low levels of shedding expected, based on previous studies done on former clinical trials, the risk of unintended exposure of flora and fauna to GS010 is minimal.*

#### 4. Method and amount of release

- (a) Quantities of GMOs to be released:  
*Doses to be administered for the proposed study 9<sup>E</sup>10 vg as a single intravitreal injection in one eye or two eyes.*  
  
*The proposed clinical trial aims to administer GS010 to 7 patients in Spain.*
- (b) Duration of the operation:  
*From Q1 2018 to Q3 2021.*
- (c) Methods and procedures to avoid and/or minimise the spread of the GMOs beyond the site of the release  
*All hospital staff handling the IMP must wear a gown, gloves, mask and goggles. Preparation of the IMP will be performed in a clean room at a campaign dedicated production and outside production schedules other preparations. Controlled atmosphere areas will be accessible only to authorized persons. Access is regulated by badge. The preparation is carried out in a microbiological safety cabinet type II by qualified personnel in the preparation and informed the nature of the IMP. The dressing procedures will meet the needs of individual protection from the IMP.*

#### 5. Short description of average environmental conditions (weather, temperature, etc.) *The clinical trial of GS010 will occur in Spain which has a temperate climate. The risk of release of GS010 in to the environment is unrelated to climatic characteristics.*

#### 6. Relevant data regarding previous releases carried out with the same GMO, if any, specially related to the potential environmental and human health impacts from the release. *No data available.*



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

1. Name of target organism (if applicable)

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

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

*The target cells for transduction are Retinal Ganglion Cells (RGC). This should result into transgene expression and synthesis of the wild-type ND4 protein inside RGC mitochondria. This is expected to improve respiratory chain function, which should prevent further RGC loss. Ultimately, further vision impairment and optic nerve damage are expected to be prevented.*

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

*No such interaction is anticipated.*

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

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

Give details

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

*GS010 is incapable of replicating.*

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:

*Genetic transfer between GS010 and cells of recipient human subjects in the clinical trial will be achieved through injection of GS010 into the eye of trial subjects. GS010 will only be used on trial subjects and is not intended to affect other organisms in the release ecosystem.*

- (b) from other organisms to the GMO:

*As rep and cap genes within the wild-type AAV genome has been replaced by the transgene expression cassette, homologous recombination between GS010 and other viruses is not anticipated.*

*In the event of a non-homologous recombination, the exchange of the transgene present in GS010 with the rep and cap genes of the wild type virus is possible. However, it is not possible to have both rep and cap genes plus the transgene in the same construct, due to the size of the construct which would prevent it being packaged into new viral structures. Thus, generation of replication competent GS010 is not possible.*

*As GS010 is replication deficient, co-infection with a helper virus would not pose any harm.*

*The presence of GS010, wild-type AAV and the helper virus in the same cell is not anticipated however it is a probable. This could render GS010 replication competent thus leading to an increase in the synthesis of transgene. However, the probability of this occurring is considered to be very low.*

- (c) likely consequences of gene transfer:

*No harm is anticipated.*

8. Give references to relevant results (if available) from studies of the behaviour and characteristics of the GMO and its ecological impact carried out in stimulated natural environments (e.g. microcosms, etc.):

*Studies to assess the impact of the GMO in stimulated natural environments have not been conducted.*

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**

*Based on biodistribution data available on GS010, the IMP will remain in the eye i.e. is very unlikely to disseminate outside the human body.*

*Presence of Raav2 vector in the blood will be tested at 2 weeks after administration a using a customized quantitative polymerase chain reaction (qPCR) assay.*

### **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<sup>2</sup>)**

*Not applicable.*

### **5. Duration of the monitoring**

*Presence of GS010 in blood samples will be monitored at 2 and 4 weeks after administration.*

### **6. Frequency of the monitoring**

*Presence of GS010 in blood samples will be monitored at Screening Visit (Visit 1), Week 2 (Visit 5) and Week 4 (Visit 6).*

## **I. Information on post-release and waste treatment**

### **1. Post-release treatment of the site**

*It is very unlikely that GS010 is released outside patient's eye.*

*In case of contamination, the site should be thoroughly cleaned with ethanol and the individuals should be placed under observation for any effect attributed to the IMP.*

### **2. Post-release treatment of the GMOs**

*All materials, including used vials and other items potentially contaminated by the IMP will be collected in specific containers for the disposal of GMO and destroyed by autoclaving and incineration.*



3. (a) Type and amount of waste generated

*The amount of waste generated is 2 vials containing a maximum of 250 µL of GS010 each and an administration kit for the IMP reconstitution required per treated eye. Administration kit includes: 2 syringes, 2 blunt fill needles, 2 needles, 2 syringe caps, 2 sterile non-woven dry gauzes, 2 sterile wetted wipes and 3 sample bags.*

3. (b) Treatment of waste

*At each visit, the responsible hospital staff will examine the drug dispensing form and compare the unused vials. At the end of the study or during the study when necessary all unused vials will be destroyed on site, in accordance with the waste disposal procedures for GMOs (autoclaving and incineration), and the destruction will be documented appropriately.*

*All materials, including used vials and other items potentially contaminated by the IMP will be collected in specific containers for the disposal of GMO and destroyed by autoclaving and incineration.*

*Certificates of destruction, or equivalent, must be completed for used and unused bottles and copies should be kept in the record of the trial.*

**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, the IMP spill should be contained with an appropriate solution of sodium hypochlorite on paper towel:*

- *Handle with individual protective equipment.*
- *Cover the spill area of paper towel.*
- *Soak with sodium hypochlorite at the appropriate concentration.*
- *After a while, clean the zone starting by the outside of the zone to the inside and destroy contaminated items by autoclaving and incineration.*
- *Remove traces of disinfectant from the spill by wiping the surface intensively with 70% alcohol.*

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

*Please see answer to J.1 above.*

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

*Please see answer to J.1 above.*

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

*Among the incidents where people could be accidentally exposed to a viral vector include: injury to the skin which is crossed or involving a splash in the eyes, nose, mouth or broken skin and also implies human exposure to fluids and / or to study drug.*



*In case such an incident occurs, the following procedure shall be implemented:*

- 1. Place injury under a flow of hot water with soap.*
- 2. Wounds should not be 'sucked', cleaned and pressed, as this may damage the tissues and encourage the spread of potential infection.*
- 3. Wounds should be covered with a dry medical bandage.*
- 4. If splashed in the eyes (after removal of contact lenses, if applicable), on broken skin or mouth should be rinsed immediately with intensive amount of water.*
- 5. Follow local safety procedures and immediately report the incident to authorities and departments or other key contacts as defined by the procedures of the hospital.*
- 6. The Sponsor will be contacted for further information or advice.*

*In case of contamination, the site should be thoroughly cleaned with ethanol and the individuals should be placed under observation for any effect attributed to the IMP.*