

PART 1 (COUNCIL DECISION 2002/813/EC)

SUMMARY NOTIFICATION INFORMATION FORMAT FOR THE RELEASE OF GENETICALLY MODIFIED ORGANISMS OTHER THAN HIGHER PLANTS IN ACCORDANCE WITH ARTICLE 11 OF DIRECTIVE 2001/18/EC

*In order to tick one or several possibilities, please use crosses (meaning x or X) into the space provided as (.)*

**A. General information**

1. Details of notification

- |   |                                    |
|---|------------------------------------|
| (a) Member State of notification  | Germany                            |
| (b) Notification number   | B/DE/16/PEI 2698                   |
| (c) Date of acknowledgement of notification   | 16/03/2016                         |
| (d) Title of the project: A Phase 3 Clinical Trial Of Retinal Gene Therapy For Choroideremia Using An Adeno-Associated Viral Vector (AAV2) Encoding Rab Escort Protein 1 (REP1) |                                    |
| (e) Proposed period of release  | From July 2016 until November 2017 |

2. Notifier

Name of institution or company: **NightstaRx Ltd**

3. GMO characterisation

(a) Indicate whether the GMO is a:

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

specify phylum, class ...

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

AAV2-REP1 is an adeno-associated virus-2 (AAV2) based gene therapy vector. The vector genome is comprised of an expression cassette with the human cDNA encoding REP1. The cDNA fragment was originally isolated from a human retinal cDNA library from unaffected individuals. AAV2-REP1 is a potential gene therapy medicinal product for the treatment of choroideremia.

The parental virus concerned in this summary is a primate (human) adeno-associated virus (AAV) with the following taxonomy:

Group: Group II (ss DNA)  
Family: *Parvoviridae*  
Genus: *Dependovirus*  
Species: Adeno-associated virus

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

AAV2-REP1 is expected to be genetically stable in isolation. In general, DNA viruses have greater genetic stability than RNA viruses. Firstly, DNA is more thermodynamically stable than RNA; secondly, replication of DNA is a much less error-prone process than the replication of RNA; and thirdly, more mechanisms exist in the host cell for repairing errors in DNA than in RNA. Further, AAV2-REP1 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. Similarly the mechanism for site specific integration into the genome of the host cell involves AAV Rep proteins which are absent in AAV2-REP1. Accordingly recombinant AAV (rAAV) do not integrate site specifically and exist in the latent form predominantly as episomal concatemers, although random integration may occur at low level. No transfer of genetic material between the GMO and other organisms is predicted. The transfer of genetic material is therefore limited to the theoretical genetic exchange of DNA by homologous recombination with wild type AAV, which could only occur if human cells were simultaneously infected with both wild type AAV and AAV2-REP1, in the presence of a helper virus. In the case of AAV2-REP1, such recombination could only result in the exchange of the REP1 expression cassette with the rep and cap genes of the wild type virus. 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 AAV2-REP1 (containing the transgene), wild type AAV (providing the rep and cap functions) and a helper virus. This scenario is expected to be a rare event, and would only result in the production of more wild type AAV and more AAV2-REP1 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 (X) No (.)

If yes, insert the country code(s) DK, NL, FI, GB

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 B/././...

**Please use the following country codes:**

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

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

Yes (X) No (.)

If yes:

- Member State of notification US, Canada
- Notification number B/././...

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

Wild type AAV is not known to be involved in environmental processes. It does not respire and does not contribute to primary production or decomposition processes. In its virion form, it does not display any metabolic activity. There are no known natural predators, preys, parasites, competitors or symbionts associated with wild type AAV (although it does require helper functions of co-infecting viruses for replication in nature). Primate (human) AAV serotypes are not known to actively transfer genetic material to organisms other than primates under natural conditions, although an absence of zoonosis is not documented. AAV2-REP1 is a disabled version of a non-pathogenic wild-type AAV, modified by deletion of the rep and cap genes rendering it unable to replicate, even in the presence of a helper virus. As a derivative of wild type AAV2, the primary indigenous vector of AAV2-REP1 is humans. AAV shows some species specificity, but can replicate in cells of a different species in vitro, provided it is in the presence of a helper virus to which that species is permissive. 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. Based on the nature of the GMO, the parental organism and the receiving environment, the deliberate release of AAV2-REP1 is not anticipated to have any direct effects on the environment (other than humans). The potential direct effects in humans are limited to the transmission of AAV2-REP1 to an unintended human recipient. Any potential adverse effects are expected to be the same as those which may be anticipated in patients receiving the treatment (immune response, potential for insertional mutagenesis and potential for germline transmission). Indirect effects of the release are limited to the consequences of the release of wild type AAV (through contamination of the medicinal product during manufacture or following recombination in the recipient's cells followed by shedding into the environment) and the possible fate of contaminating DNA sequences derived from the manufacturing process.

**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                                  | ...                                 |
| (vi)  | pathovar (biotype, ecotype, race, etc.) | AAV2                                |
| (vii) | common name                             | Adeno-associated virus type 2 /AAV2 |

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 (.)  
 Cell culture in scientific laboratories

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

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

Atlantic (X)  
 Mediteranean (X)  
 Boreal (X)  
 Alpine (X)  
 Continental (X)  
 Macaronesian (X)

(ii) No (.)  
 (iii) Not known (.)

(c) Is it frequently used in the country where the notification is made?  
 Yes (.)                      No (X)

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

4. Natural habitat of the organism

- (a) If the organism is a microorganism
- water (.)  
 soil, free-living (.)  
 soil in association with plant-root systems (.)  
 in association with plant leaf/stem systems (.)  
 other, specify Humans and other primates

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 secretion of virus.

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

5. (a) Detection techniques

- 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.
- Viral culture. Samples containing suspected infectious AAV particles may be cultured in vitro on a permissive cell line, in the presence of a helper virus.
- 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.

- (b) Identification techniques  
 Same as B 5 (a) above.

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 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 (Appendix III). It is most appropriately designated a Risk Group 1 biological agent, defined in the EU as 'one that is unlikely to cause human disease'.

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 host range includes humans and other primates. The wild type AAV transmits to humans in the presence of a helper virus. AAV is not known to be a pathogenic virus in humans nor other vertebrate species, and has never been implicated as an etiological agent for any disease. Despite the lack of evidence for pathogenicity, correlations have been made between the occurrence of male infertility and the presence of AAV viral DNA sequences in human semen, and the occurrence of miscarriage and the presence of infectious AAV in embryonic material as well as in the cervical epithelium. A clear association is hard to establish from the related studies, given that co-incident evidence of human papillomavirus infection is present in most subjects, and that AAV DNA can be detected in cervical samples in the majority of women. An additional, theoretical, risk of AAV infection is the risk of insertional mutagenesis caused by non-site specific integration of the AAV genome into the host-cell genome of infected cells. Such an event carries the risk of malignant transformation leading to cancer. There is no documented causal link between AAV infection and malignancies in humans, but it has been shown that wild type AAV may integrate at sites other than chromosome 19.

## 8. Information concerning reproduction

(a) Generation time in natural ecosystems:

AAV2-REP1 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.

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

AAV2-REP1 is unable to replicate independently, even in the presence of a helper virus, since it lacks the rep and cap genes required for rescue/packaging.

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

(c) Factors affecting reproduction:

The presence of a helper virus is a condition for replication of the wild type AAV.

## 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 can exist in the latent form as episomal concatemers or integrated into the host cell DNA.

- (b) relevant factors affecting survivability:

Productive (lytic) infection develops in cells co-infected with a helper virus such as adenovirus, Human papilloma virus (HPV), vaccinia virus or herpes simplex virus (HSV) to facilitate its replication.

10. (a) Ways of dissemination

AAV is thought to be spread in nature via inhalation of aerosolized droplets, mucous membrane contact or ingestion.

- (b) Factors affecting dissemination

For wild type AAV close contacts of an individual who may potentially be exposed to any shed vector via lacrimal fluid, blood, urine, saliva, semen, or faeces.

None for AAV2-REP1 as it is not capable of replication regardless of the presence of a helper virus.

11. Previous genetic modifications of the recipient or parental organism already notified for release in the country where the notification is made (give notification numbers)

..., B/././...

THOR - Tübingen Choroideremia gene therapy trial

open label Phase 2 clinical trial using an adeno-associated viral vector (AAV2) encoding Rab-escort protein 1 (REP1)

EudraCT number: 2014-005004-21

The notification number yet to be assigned.

### 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

AAV2-REP1 is a gene therapy vector for administration as a subretinal injection to prevent the deterioration of or restore the lost function of REP1 in ocular tissue of patients suffering from choroideremia.

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

Three DNA plasmids are used for the production of AAV2-REP1.

(c) Host range of the vector

The plasmids used in the manufacture of AAV2-REP1 are capable of replication in bacteria. They were constructed using standard molecular biological techniques for the precise excision and ligation of component elements using specific restriction enzymes followed by transduction and amplification in bacterial cells at each stage.

(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

(e) Constituent fragments of the vector

- Plasmid 1 encodes the AAV2-REP1 vector genome elements.
- Plasmid 2 encodes the AAV2 rep and cap genes.
- Plasmid 3 encodes the viral helper functions for vector production.

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

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



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

Human REP1 cDNA with promoter and enhancer elements.

(b) Source of each constituent part of the insert

The human REP1 cDNA is human in origin. The other sequences in the genome and promoter and enhancer elements are synthetic, viral and mammalian in origin.

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

The human REP1 cDNA replaces the faulty endogenous gene encoding REP1. The other elements of the vector ensure packaging of the genome and expression of the gene.

(d) Location of the insert in the host organism

- on a free plasmid (.)
- integrated in the chromosome (.)
- other, specify: Predominantly as episomal concatemers in the host cells.

(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

- mammals (.)

- insect (.)

- fish (.)

- other animal (.)

(specify phylum, class) ...

other, specify Human – REP1 cDNA

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):

...

4. Is the donor organism classified under existing Community rules relating to the protection of human health and the environment, such as Directive 90/679/EEC on the protection of workers from risks to exposure to biological agents at work?

Yes (.) No (X)

If yes, specify ...

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

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

AAV can integrate into the host DNA.

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

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

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

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

Specify ...

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

Yes  No  Unknown

Specify AAV2-REP1 as it is not capable of replication regardless of the presence of a helper virus, since it lacks the rep and cap genes required for rescue and packaging

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

Yes  No  Not known

Specify AAV2-REP1 is not capable of replication regardless of the presence of a helper virus, therefore dissemination is not possible.

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

Yes  No  Not known

Specify It is not known to be a pathogen.

2. Genetic stability of the genetically modified organism

AAV2-REP1 is expected to be genetically stable in isolation. AAV2-REP1 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. Similarly the mechanism for site specific integration into the genome of the host cell involves AAV Rep proteins which are absent in AAV2-REP1. Theoretically, genetic exchange of DNA by homologous recombination with wild type AAV could occur if human cells were simultaneously infected with both wild type AAV and AAV2-REP1, in the presence of a helper virus.

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

Yes  No  Unknown

- (a) to which of the following organisms?

humans

animals

plants

other ...

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

...

4. Description of identification and detection methods

(a) Techniques used to detect the GMO in the environment:  
Quantitative Polymerase Chain Reaction (qPCR)

(b) Techniques used to identify the GMO  
Same as E.4 (a) above.

## **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 is a clinical study of the safety and efficacy of AAV2-REP1. Neither positive nor negative impact on the environment 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 site of release is the sub-retinal space within study subjects, as specified in F.1 above.

3. Information concerning the release and the surrounding area

(a) Geographical location (administrative region and where appropriate grid reference):  
Hospitals in the EU territory authorized by national regulatory bodies to conduct the clinical trial.

(b) Size of the site (m<sup>2</sup>):  
(i) actual release site (m<sup>2</sup>): N/A  
(ii) wider release site (m<sup>2</sup>): N/A

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

(d) Flora and fauna including crops, livestock and migratory species which may potentially interact with the GMO  
None

4. Method and amount of release

(a) Quantities of GMOs to be released:  
It is anticipated that  $5 \times 10^{12}$  AAV2-REP1 genome particles will be released in the entire study.

(b) Duration of the operation:  
60 min

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

As this is a GMO of Biosafety Level 1 and will be involved in a clinical trial, its usage is limited to the hospital facilities already audited for dealing with biologic

hazardous and infectious material, including storage and waste management, and all participating medical staff will be trained in this sense. Moreover, study specific procedures will be implemented and the clinical staff trained in them in terms of the waste management, using protective equipment, administration and other procedures that will be fully detailed in study specific materials.

5. Short description of average environmental conditions (weather, temperature, etc.)  
This is not applicable because AAV2-REP1 will be used solely in a clinical setting.

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.  
None

**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)  
AAV2-REP1 vector will deliver the gene sequence encoding human Rab escort protein 1 into the target cells of the study subjects where it will persist episomally.

3. Any other potentially significant interactions with other organisms in the environment  
None

4. Is post-release selection such as increased competitiveness, increased invasiveness for the GMO likely to occur?  
Yes (.)                      No (X)                      Not known (.)  
Give details  
...

5. Types of ecosystems to which the GMO could be disseminated from the site of release and in which it could become established  
There is not any known ecosystem in which this GMO could be successfully established.

6. Complete name of non-target organisms which (taking into account the nature of the receiving environment) may be unintentionally significantly harmed by the release of the GMO: None

- (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:

Extremely unlikely. The vector will be injected into the subretinal space and is unable to replicate.

Due to the low numbers of vector DNA copies potentially released into the environment through shedding, horizontal gene transfer is highly unlikely.

- (b) from other organisms to the GMO:

Extremely unlikely. Since AAV2-REP1 contains the ITR-sequences of AAV2, there is a (remote) possibility of homologous recombination of the vector with wild type AAV2 in case of a co-infection in exposed persons. The result of such a recombination would be that AAV2-REP1 would gain functional genes of the AAV2 required for replication and encapsidation, but, in turn, would lose the transgene. Hence, recombination would lead to the formation of viruses that are identical to the starting material and replication incompetent.

- (c) likely consequences of gene transfer:

The genetic material from the rep and cap genes together with the transgene would be too large in size to be packed in an AAV capsid. Thus it is highly unlikely that the recombination would result in a replication-competent vector containing transgenes. Any recombination however, would result in the expression of REP1 by infected cells.

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

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

No available data.

**H. Information relating to monitoring**

1. Methods for monitoring the GMOs

The study subjects will be monitored throughout treatment by the Investigator at each site and delegates. The study will be conducted in compliance with GCP and appropriate study monitoring and data management activities will be employed. Any serious adverse event will be reported in the appropriate time-frame and according to the relevant regulatory

requirements. A comprehensive battery of laboratory evaluations, including blood tests, will be conducted at regular intervals to assess safety throughout the study following administration. Safety tests will include PCR testing for vector shedding at several timepoints after administration.

2. Methods for monitoring ecosystem effects

N/A

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

The vector genome contains unique sequences, which are not expected to be found in clinical samples not exposed to the vector, namely the REP1 expression cassette sequence in proximity with AAV2 inverted terminal repeats. Hence PCR based methods using vector genome specific primers can be used to detect GMO genetic elements with high specificity and sensitivity.

4. Size of the monitoring area (m<sup>2</sup>)

N/A

5. Duration of the monitoring

Throughout the study conduct.

6. Frequency of the monitoring

During the protocol defined Study Visits, as approved by local Regulatory Authorities.

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

1. Post-release treatment of the site

Except for those routinely applied at the particular hospital site, there are no specific procedures planned for decontaminating. In the unlikely event that transmission to an unintended human recipient occurred, this would likely be a local occurrence affecting a healthcare professional or close contact of a treated individual. Decontamination of areas in which a recently treated patient had frequented (their home and / or an examination room at a medical facility) could be implemented by applying standard detergents to areas of likely contact (for example, frequent touch-points such as handles, door knobs, hard surfaces, railings and handholds, washing facilities and lavatories). Fomites could be autoclaved or incinerated. Decontamination of plants, (non-human) animals and soils will not be required.

2. Post-release treatment of the GMOs

Following administration of AAV2-REP1 at a medical facility, used (or partially used) vials of vector, syringes used in dose preparation, injection kits and any other disposable instruments or other materials used during the dose preparation procedure will be disposed of in a manner consistent with the standard practice of the institution for potentially biohazardous materials and also in a manner consistent with the guidance given in the WHO Laboratory Biosafety Manual, 3rd Ed (2004) for BSL1/2.

3. (a) Type and amount of waste generated

Waste generated from the preparation and administration of AAV2-REP1 will be limited to:

- Used vials of the Investigational Medicinal Product.
- Used preparation equipment; syringes, needles, vials.
- Used injection kit.
- Containers used to transport potentially contaminated equipment to and from storage facility.
- Personal Protective Equipment used during dose preparation and administration.

It is anticipated that no more than 10 mL of waste will be generated in the whole study.

3. (b) Treatment of waste

AAV2-REP1 is a replication-deficient non-pathogenic virus which is considered to present a much lower hazard to human health than other human biological waste which is frequently disposed of in medical facilities. AAV2-REP1 is sensitive to inactivation by a variety of commonly available physical and chemical (for example sodium hypochlorite) methods. Following administration of AAV2-REP1 at a medical facility, used (or partially used) vials of vector, syringes used in dose preparation, injection kits will be disposed of in a manner consistent with the standard practice of the institution for potentially biohazardous materials and also in a manner consistent with the guidance given in the WHO Laboratory Biosafety Manual, 3rd Ed (2004) for BSL1/2.

**J. Information on emergency response plans**

1. Methods and procedures for controlling the dissemination of the GMO(s) in case of unexpected spread

There are no specific procedures planned for controlling the GMO in the case of unexpected spread. Wild type AAV is a non-pathogenic single-stranded DNA Dependovirus, requiring helper DNA virus for replication. AAV2-REP1 is derived from wild type AAV, but encodes no replication genes in the expression cassette and is incapable of independently replicating its genome. The potential for unexpected spread of AAV2-REP1 in the environment is extremely low, due to:

- Attenuation of the GMO rendering it even less replication competent than the parental virus (AAV2), by deletion of the replication genes.
- Subretinal administration to eligible patients by medical professionals in a medical facility.
- Limited host and tissue tropism (human/primate) of the parental virus (AAV2).
- None to low and transient incidence of shedding of infective virus from treated individuals.
- High levels of existing adaptive immunity in the human population.

Any spread of AAV2-REP1 to unintended human recipients is therefore highly unlikely, and would be isolated to single cases in discrete geographical locations. The risk of widespread infection is considered negligible. In the theoretical event that wild type AAV, supplying the requisite replication gene products, were to co-infect a retinal pigment epithelial or photoreceptor cell, along with a helper DNA virus such as adenovirus or herpes simplex



virus and the AAV2-REP1 vector (a triple co-infection), it is possible that vector replication could occur. However, even if this rare event were to occur, the resulting virologic outcome would be increased synthesis of vector and wild type AAV, both intrinsically non-pathogenic viruses. It is therefore unlikely that such an event would present clinical symptoms and is therefore unlikely to become apparent. If such spread were detected, the individual could be isolated pending further investigation, and consultation with the relevant authorities.

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

There are no specific procedures planned for decontaminating areas in the case of unexpected spread, since the risk of spread is considered negligible. In the unlikely event that transmission to an unintended human recipient occurred, this would likely be a local occurrence affecting a healthcare professional or close contact of a treated individual. Decontamination of areas in which a recently treated patient had frequented (their home and / or an examination room at a medical facility) could be implemented by applying standard detergents to areas of likely contact (for example, frequent touch-points such as handles, door knobs, hard surfaces, railings and handholds, washing facilities and lavatories). Fomites could be autoclaved or incinerated.

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

The predicted habitat of AAV2-REP1 is humans where it is expected to persist in a lysogenic state. AAV2-REP1 is a disabled version of a non-pathogenic wild-type primate (human) AAV2, modified by deletion of the rep and cap genes rendering it unable to replicate, even in the presence of a helper virus. Decontamination of plants, (non-human) animals and soils will not be required.

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

AAV2-REP1 will be regulated under medicines legislation, requiring stringent pharmacovigilance overseen by the regulatory authorities and ethics committees. Information will be collected regarding all individual adverse events and submitted to the regulatory authorities and ethics committees in line with the applicable regulatory requirements. Development Safety Update Reports will be submitted on an annual basis while the trial is active and interim reports will be submitted when required. Procedures are in place at the regulatory authorities and ethics committees to monitor, review and act on urgent safety information relating to medicinal products so that human health is protected.