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/15/PEI 2337
(c) Date of acknowledgement of notification 16/02/2015
(d) Title of the project
Safety and efficacy of a single subretinal injection of rAAV.hCNGA3 in patients with CNGA3-linked achromatopsia investigated in an exploratory, dose-escalation trial

(e) Proposed period of release From 01/03/2015 until 31/12/2015

2. Notifier
Name of institution or company: University of Tübingen, Department for Ophthalmology, Schleichstraße 12-16, 72076 Tübingen, Germany

3. GMO characterization

(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)
rAAV.hCNGA3 is a recombinant Adeno-associated Virus (AAV) pseudotyped with AAV8 capsid proteins. It contains about 3140 nucleotide sequence of the hCNGA3 expression cassette flanked by the nucleotides of inverted repeat sequences derived from AAV serotype 2. All native cap and rep sequence of the viral genome have been removed and replaced by the hCNGA3 expression cassette.

(c) Genetic stability – according to Annex IIIa, II, A(10):
Homologous recombination between the GMO and other AAV may only occur under circumstances in which a cell is infected simultaneously by the GMO, another AAV strain and a helper virus (triple infection). The GMO is unable to replicate in even in the presence of a helper virus and recombined AAVs that contain parts of the transgene and the native AAV coding sequence will be replicative deficient due to the packaging limit of the virion.

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 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 (.) No (X)
If yes:
- Member State of notification ...
- Notification number B/../../...

7. Summary of the potential environmental impact of the release of the GMOs:
rAAV.hCNGA3 is a recombinant, replication-deficient, adeno-associated virus-based vector that will be administered by a single dose subretinal injection to achromatopsia patients. The intended application of rAAV.hCNGA3 is limited to the Eyehospital Tübingen and the number of patients to be treated is restricted.
In addition, results obtained in non-human primates, a limited number of particles are expected to be shed from the patients. Due to the extremely low numbers of

rAAV.hCNGA3 particles potentially released into the environment during the study, either by accident or through shedding, horizontal gene transfer is unlikely.
Due to the lack of viral Rep and Cap genes, the vector will persist as episome and will not replicate or produce viral particles. The expression cassette will be transcribed and translated by host cell enzymes leading to expression of the human CNGA3 Protein.
Although human infections are common, wild type AAV is not known to be a pathogenic virus in humans and can be classified as a Risk Group 1 biological agent, defined in the EU as 'one that is unlikely to cause human disease' according to Directive 2000/54/EC on the protection of workers from risks related to exposure to biological agents at work.
Wild type AAV is not known to be involved in environmental processes and none of the genetic modifications made to wild type AAV during construction of rAAV.hCNGA3 is expected to have any impact on this property. As such, there is no expected impact to the environment following the release of rAAV.hCNGA3.
Nonetheless, the Eyehospital is expected to have adequately trained the health care professionals involved in the study in the safe handling of GMOs and to have best biosafety practices implemented in order to minimize any accidental exposure to the product. In view of the low risk rAAV.hCNGA3 presents to people and the environment and in view of the biorisk management measures applied to even further reduce the exposure to the vector, its overall risk for people and the environment can be evaluated as negligible.

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

1. Recipient or parental organism characterization:

(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/AAV8
(vii)	common name	Adeno-associated virus or AAV

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)

Typical cell line for scientific laboratories as model organism

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)

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	Specific hosts are humans and non-human primates

AAV survives in the environment as a persistent infection in the host species or as a latent infection in the nucleus of 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:
N.A.

5. (a) Detection techniques
Quantitative Polymerase Chain Reaction (QPCR), Enzyme-linked Immunosorbent Assay (ELISA)

(b) Identification techniques
QPCR, ELISA

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 are not known to be associated with any pathogenic effect and thus are not assigned an Advisory Committee on Dangerous Pathogens (ACDP) category. Recombinant AAV-based vectors are usually classified as Biosafety Class 1 or 2 (depending on the Member State).

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

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

If yes:

(a) to which of the following organisms:

humans (.)
animals (.)
plants (.)
other (.)

(b) Give the relevant information specified under Annex III A, point II. (A)(11)(d) of Directive 2001/18/EC
AAVs are frequently found in humans and animals, but they are not pathogenic, virulent, allergenic, or a carrier (vector) of a pathogen. The known host range includes humans and non-human primates. In natural conditions, wild type AAV is found to transmit to humans in the presence of a helper virus. It does not activate latent virus and is not able to colonise other organisms.

8. Information concerning reproduction

(a) Generation time in natural ecosystems:
Not applicable since the vector is not capable of replication.
(b) Generation time in the ecosystem where the release will take place:
(c) Not applicable since the vector is not capable of replication.

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

Factors affecting reproduction:
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	AAVs have the ability to form extrachromosomal concatemers that remain episomal for extended periods of time.

(b) relevant factors affecting survivability:
Replication of wild-type AAV is dependent on co-infection of helper viruses such as adenovirus or herpes-simplex virus. In presence of helper virus, AAV undergoes productive infection characterized by genome replication, viral gene expression and virion production. In absence of a helper virus co-infection, the virus DNA mainly remains as an extrachromosomal episome or may integrate into the host cell genome and in both cases the virus appears to remain latent.
AAV survives in the environment as a persistent infection in the host species or as a latent infection in the nucleus of infected cells, where it may remain inactive indefinitely, or be reactivated giving rise to secretion of virus.
Outside of the host, non-lipid enveloped viruses such as AAV are resistant to low level disinfectants, survive well outside of the laboratory environment and can be easily transmitted via fomites. AAV particles are resistant to a wide pH range (pH 3-9) and can resist heating at 56°C for 1 hour (Berns and Bohenzky, 1987).

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

(b) Factors affecting dissemination
Co-infection with a helper virus.
rAAV.hCNGA3 is a replication-deficient virus packaged into serotype 8 virions. The genetic modifications are not expected to affect its survival outside the host or probable mode of dissemination. However, the lack of replicative ability prevents multiplication and therefore limits its ability to disseminate.

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):

AAV-encoded expression of TRAIL in experimental human colorectal cancer leads to tumor regression (Mohr et al. 2004)

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:
rAAV-hCNGA3 has been designed for its delivery into retinal photoreceptor and expression of the CNGA3 transgene in cones. Its intended use is to produce CNGA3 protein in cone photoreceptors of subjects with a hereditary deficiency in CNGA3. The transgene comprises: See ERA

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

The proposed rAAV.hCNGA3 Drug Substance is produced using a transient double-transfection protocol of the HEK293 MCB. The phArr3.hCNGA3.WPREm vector plasmid encodes the human CNGA3 expression cassette flanked by the ITRs of AAV2. The pDP8-KanR plasmid encodes the helper Adenoviral sequences (VA RNA, E2A, E4) and the replication (rep) and capsid (cap) genes of AAV2 and AAV8 respectively. For both plasmids, a Master Cell Bank of 81 vials was produced and fully characterized by Aldevron (Fargo, ND, USA). High quality GMP-Source plasmids lots were then produced from these banks and subsequently characterized at Aldevron.

Map of phArr3.hCNGA3.WPREm

(c) Host range of the vector
All the plasmids contain bacterial origins of replication, and are therefore able to replicate in bacterial cells.

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

Yes (X) No (.)

antibiotic resistance (X)

Indication of which antibiotic resistance gene is inserted: Kanamycin

(e) Constituent fragments of the vector
The approximately 3700 bp genome of the proposed IMP rAAV.hCNGA3 vector consists of the AAV2 ITRs flanking the cDNA of the human CNGA3 protein driven by the cone-specific human Arrestin 3 promoter. The hCNGA3 expression is enhanced using a mutated WPRE sequence inserted in the 3' end of the transgene. The recombinant genome is packaged in the AAV8 capsid. The expression cassette comprises the following elements:

- Promoter of the human arrestin 3 : 0.4 Kb
- cDNA of the human Alpha subunit of the cone photoreceptor Cyclic Nucleotide-Gated cation channel (hCNGA3) : 2 Kb
- Woodchuck hepatitis virus (WHV) Post-translational Regulatory Element (WPRE) with a point mutation in the ATG codon of the WHV-X open reading frame: 0.54 Kb
- Polyadenylation signal of the Bovine Growth Hormone (BGH): 0.2 Kb

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

(i) Transformation (.)
(ii) electroporation (.)
(iii) macroinjection (.)
(iv) microinjection (.)
(v) infection (.)
(vi) other, specifyTransfection

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 rAAV.hCNGA3 is a hybrid adeno-associated virus–based vector that lacks all the viral protein-coding sequences and instead carries the **cDNA of the human Alpha subunit of the cone photoreceptor Cyclic Nucleotide-Gated (CNG) cation channel**. The hCNGA3 cDNA expression is under the control of the cone-specific human Arrestin 3 promoter and translation is enhanced using a mutated WPRE sequence inserted in the 3' end of the transgene. The expression cassette is flanked by the AAV serotype 2 Inverted Terminal Repeats (ITRs) and the 3700 bp recombinant genome is packaged in the AAV serotype 8 capsid.

(b) Source of each constituent part of the insert: see Table 1
See also Appendix IMPD

Table 1: Sources of constituents

Product	Manufacturer	Quality Control Laboratory
• phArr3.hCNGA.WPREm plasmid • pDP8-Kana plasmid	Aldevron, Fargo, ND 58104, USA	Aldevron, Fargo, ND 58104, USA
rAAV.hCNGA3	Etablissement Français du Sang – Atlantic Bio GMP, Saint-Herblain, France	• Etablissement Français du Sang – Atlantic Bio GMP • Gene Therapy Laboratory UMR1089, Atlantic Gene Therapies, Nantes, FRANCE • SGS-Vitrology ltd., Glasgow, United Kingdom • Quality Assistance SA., Donstiennes, Belgium

(c) Intended function of each constituent part of the insert in the GMO:
Promoter of the human arrestin 3 → initiates transcription
cDNA of the human Alpha subunit of the cone photoreceptor Cyclic Nucleotide-Gated cation channel (hCNGA3) → replaces mutated hCNGA3 gene
Woodchuck hepatitis virus (WHV) Post-translational Regulatory Element (WPRE) with a point mutation in the ATG codon of the WHV-X open reading frame → enhances expression by creating a tertiary structure when transcribed
Polyadenylation signal of the Bovine Growth Hormone (BGH) → allows the qualification of the vector genome titration assay with the BGH polyA sequence.

(d) Location of the insert in the host organism

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

(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 (CNGA3 cDNA)

2. Complete name

(i)	order and/or higher taxon (for animals)	Primates
(ii)	family name for plants	N/A
(iii)	genus	Homo
(iv)	species	sapiens
(v)	subspecies	sapiens
(vi)	strain	N/A
(vii)	cultivar/breeding line	N/A
(viii)	pathovar	N/A
(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 (.)
Although following naturally acquired infection, AAV DNA mainly persists as circular double stranded episomes in human tissues (Schnepp et al., 2005) it has been shown that some level of integration may occur in the host DNA (Kaeppel et al, 2013).

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: Due to the removal of the Rep and Cap genes, rAAV.hCNGA3 is replication incompetent even in the presence of wild-type AAV.

(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:

(c) is the GMO in any way different from the recipient as far as dissemination is concerned?
Yes (X) No (.) Not known (.)
Specify: The GMO cannot enter an infectious cycle even in the presence of helper function

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

Yes (.) No (X) Not known (.)
Specify: Neither wild type AAV nor rAAV5.CNGA3 are pathogenic to humans

2. Genetic stability of the genetically modified organism:
rAAV.hCNGA3 is replication incompetent. In absence of an intrinsic mechanism for genetic vari-ation or instability and based on the known genetic stability of wild type AAV, the genetic traits of the organism are expected to be stable.

3. Is the GMO significantly pathogenic or harmful in any way (including its extracellular products), either living or dead?
Yes (.) No (X) Unknown (.)

(a) to which of the following organisms?

humans (.)
animals (.)
plants (.)
other ...

(b) give the relevant information specified under Annex III A, point II(A)(11)(d) and II(C)(2)(i)
Humans are likely infected by wild type AAV through the respiratory tract, sexual and gastrointestinal route. AAV is capable of infecting either non-dividing or dividing cells.
In the presence of helper virus (adenovirus or herpes virus), AAV undergoes productive infection characterized by genome replication, viral gene expression and virion production.
In the absence of a helper virus co-infection, AAV DNA remains extrachromosomal or may integrate in the host DNA. In both situations the virus remains latent.
Wild type AAV is weakly immunogenic. AAV-induced immune reaction is seemingly restricted to the generation of neutralizing antibodies.
AAV has never been associated with any disease or pathological conditions in humans. AAV is not known to be associated to plants. rAAV.hCNGA3 is not expected to be pathogenic and does not interfere with any prophylactic or therapeutic treatments since it does not contain any sequences (no antibiotic-resistance genes) that could affect prophylaxis or treatment of pathogenic microorganism infection.
The CNGA3 cDNA present in the vector is a naturally occurring sequence in healthy humans. Expression of this protein by infected cells does not induce cytopathic effects.

4. Description of identification and detection methods

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

The number of vector genomes can be determined by quantitative PCR with primers specific for vector sequences. This technique however is only applicable where sufficient DNA can be recovered for analysis.

(b) Techniques used to identify the GMO
The vector is identified by quantitative PCR with primers specific for vector sequences.

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 to investigate the safety and efficacy of the subretinal injection of rAAV.hCNGA3 to Achromatopsia patients. 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 injected subretinal to Achromatopsia patients in the University Eyehospital Tübingen. Shedding of vector DNA in urine, saliva or faeces can be expected afterwards for a few days, however shed AAV-based vectors have been shown to be non-infectious.

3. Information concerning the release and the surrounding area

(a) Geographical location (administrative region and where appropriate grid reference):
University Eyehospital, Tübingen, Germany

(b) Size of the site (m^2):
(i) actual release site (m^2): 25 m^2
(ii) wider release site (m^2): ca. 100 m^2

(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:

The applied doses will vary between the
low dose $1x10^{10}$ vgp, the
Intermediate dose ≤ 5x10e10 vgp and the
high dose group $1x10^{11}$ vgp
Each group will consist of 3 patients (9 patients overall)

(b) Duration of the operation:
30 min

(b) Methods and procedures to avoid and/or minimise the spread of the GMOs beyond the site of the release
As a minimum, institutional risk assessments are required to determine appropriate precautions for the work tasks and processes. Standard biosafety practices are similar amongst the guidelines and are typically followed by medical facilities when handling injectable medicinal products and medical waste, such as:
• Restricted access
• Secure storage
• Training of personnel
• Availability of Personal Protective Equipment (PPE; laboratory coats, gowns, gloves and safety glasses)
• Established routine practices for dealing with potentially biohazardous materials such as patient samples/fluids and medical waste (autoclaves, sharps bins, incinerators, disinfectants and appropriate cleanable surfaces).
Vector will be prepared for subretinal injection in the surgery hall by the investigator according to the procedure manual. Due to the minimal manipulations involved in dose administration it is not considered necessary to require further precautions at the point of administration, beyond the use of disposable gloves.
The worker protection measures proposed for the preparation and administration of rAAV.hCNGA3 are therefore in line with those recommended globally for the handling of BSL-1 organisms in a research setting.

5. Short description of average environmental conditions (weather, temperature, etc.):
In Germany we have middle-European continental climate. The vector will be shipped on dry ice and immediately after arrival stored in a -80°C freezer. The surgery hall, where the vector is thawed and injected, is air conditioned.

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 available

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

1. Name of target organism (if applicable)

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

2. Anticipated mechanism and result of interaction between the released GMOs and the target organism (if applicable)
CNGA3 encodes the alpha subunit of the cone photoreceptor cyclic nucleotide-gated cation channel and mutations in this gene have been associated with complete and incomplete achromatopsia (Kohl et al., 1998; Wissinger et al., 2001; Burgueno-Montanes et al., 2014; Yang et al., 2014). In treated subjects, rAAV.hCNGA3 is expected to replace the defect gene to restore cone-mediated vision and delay cone degeneration.

3. Any other potentially significant interactions with other organisms in the environment
None expected for this product

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
Even in the event of shedding of DNA in waste water no establishment in such a system can be 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: 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 in direct proximity to the cones. The promotor is specific for cone cells, so no other cells could be affected.
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 rAAV.hCNGA3 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 rAAV.hCNGA3 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 would result in the expression of hCNGA3 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.):
Not available

9. Possible environmentally significant interactions with biogeochemical processes (if different from the recipient or parental organism):
None known or predictable since wild type AAV is not known to be involved in any biogeochemical process.

H. Information relating to monitoring

1. Methods for monitoring the GMOs

Collection of body fluids according to clinical protocol and risk management plan and quantification using a specific DNA QPCR method.

2. Methods for monitoring ecosystem effects
No monitoring is considered necessary

3. Methods for detecting transfer of the donated genetic material from the GMO to other organisms
The method for detecting transfer of the donated genetic material to other organisms will be QPCR. The presence of vector DNA sequences will be determined in serum, urine and saliva from the treated patients.

4. Size of the monitoring area (m^2)
Not applicable. Only subject's body fluids will be monitored after administration.

5. Duration of the monitoring
The body fluids of the treated subjects will be monitored until found negative (two consecutive negative samples) for the presence of vector DNA.

6. Frequency of the monitoring
At regular intervals according to clinical protocol, for five years after administration.

I. Information on post-release and waste treatment

1. Post-release treatment of the site
Decontamination of the IMP administration room by standard procedures will be used after administration. Any material or surface in contact with the product will be decontaminated with 10% bleach solution (allowing contact for at least 10 minutes) or autoclaved.
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.

2. Post-release treatment of the GMOs
Used and unused vials will be stored in a -80°C freezer for re-analyses up to five years after the end of the study. Then all vials will be autoclaved and disposed according to general biosafety guidelines.

3. (a) Type and amount of waste generated
Empty vials and used vials and the used delivery system components (guide tube, cannula, injection needles and syringes), gauzes, personal protective equipment (e.g. gloves etc) and components used for collecting body fluids samples after administration.

3. (b) Treatment of waste
Sharps such as needles will be disposed of in adequate sharp containers and incinerated. Disposables such as syringes, tubing and catheters will be

decontaminated by immersion in a chemical disinfectant with virucidal activity before incineration.
All the surgical materials (surgery tools, linens) and surgery waste (gloves, compresses) will be collected and autoclaved before washing and sterilization or incineration. All non-disposable surgical equipment will be cleaned using a chemical disinfectant with proven virucidal activity (e.g. hypoclorite solution) and then sterilized by autoclaving according to standard practice of the institution.

J. Information on emergency response plans

1. Methods and procedures for controlling the dissemination of the GMO(s) in case of unexpected spread
The solution of rAAV.hCNGA3 for subretinal injection will be prepared by the injector in the surgery hall in the Eyehospital Tübingen. In case of spillage the affected area, lineated with absorbing material, will be decontaminated using appropriate disinfectants.

2. Methods for removal of the GMO(s) of the areas potentially affected
For splashes to the eye of the GMO, rinse eye with eyewash for 15 minutes then report to hospital emergency room for evaluation. In case of accidental injection of material containing the GMO, wash area well with soap and water and report to hospital emergency room for evaluation.
In case of spills: allow aerosols to settle; wear protective clothes and goggles and gently cover the spill with adsorbent paper towel and apply freshly prepared 10% bleach starting at the perimeter and working towards the centre; allow at least 10 minutes contact time before clean up.

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

4. Plans for protecting human health and the environment in the event of an undesirable effect
No undesirable effects are expected.