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 | Belgium |
| (b) | Notification number | …/../../…. |
| (c) | Date of acknowledgement of notification | ../../…. |
| (d) | Title of the project | A Phase 1/2 Ascending Dose Study to Evaluate the Safety and Effects on Progranulin Levels of PR006A in Patients with Fronto-Temporal Dementia with Progranulin Mutations (FTD-GRN). |
| | Proposed period of release | Q4_2021 to end of treatment period Q1_2028 |

2. Notifier

Name of institution or company:
Prevail Therapeutics, A wholly owned subsidiary of Eli Lilly and Company
430 East 29th Street, Suite 1520
New York, NY 10016
USA

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)
Genus: Dependoparvovirus
Species: Recombinant AAV9

(c) Genetic stability – according to Annex IIIa, II, A(10)
Adeno-associated virus (AAV) is a single stranded DNA virus that demonstrates a high degree of genetic stability as evidenced by the high degree of sequence conservation of the rep and cap genes from multiple AAV serotypes and genomovars. Sequence homologies often are >90% and >80% for the rep and cap genes, respectively. Furthermore, AAV uses host DNA polymerases for viral replication, which are characterised by high fidelity DNA polymerization and additional proofreading exonuclease activity leading to very low error rate of DNA replication, when compared, for example, to RNA polymerases used by RNA viruses. In support of genetic stability is the observation that AAV proviral DNA episomes, isolated from multiple human tissue samples, consistently have the expected canonical AAV2 rep and cap sequences. Homologous recombination is thought to have occurred between serotypes AAV2 and AAV3 based on phylogenic analysis of the AAV2/3 hybrid virus. It has not been observed for other serotypes, supporting that only under the presumably rare circumstance where a cell is infected simultaneously by two different serotypes of AAV and a helper virus (triple infection) would conditions be appropriate for such recombination to occur.

PR006A is expected to be highly genetically stable. Production of the vector in the manufacturing process rely on the host DNA polymerase which is has a low error rate. The PR006A vector genome will be assayed by sequencing for every batch to assure the sequence remains identical.

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) ES; FR; BE

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 Not applicable
- Notification number Not applicable

**Please use the following country codes:**
*Austria AT; Belgium BE; Germany DE; Denmark DK; Spain ES; Finland FI; France FR; Greece GR; Ireland IE; Iceland IS; Italy IT; Luxembourg LU; Netherlands NL; Norway NO; Portugal PT; Sweden SE*

6. Has the same GMO been notified for release or placing on the market outside the Community by the same or other notifier?
Yes () No (X)
If yes:
- Member State of notification
- Notification number

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

PR006A is a non-replicating recombinant vector derived from AAV containing a gene encoding human *GRN*, that may be effective for the treatment of patients with Fronto-Temporal Dementia with Progranulin (PGRN) Mutations.

The release of PR006A as described in this application is not expected to result in adverse environmental impact, for the following reasons:

- Lack of pathogenicity of the parental virus: Despite an estimated seroprevalence of up to 90% for some common human serotypes, no pathogenic effects of AAV have been identified.

- Replication-incompetent GMO: PR006A is a non-infectious recombinant AAV vector that lacks all AAV viral genes and cannot replicate without AAV-specific helper functions and helper virus activities. PR006A replication could only occur in the extremely unlikely event of a host cell being infected additionally by wild-type AAV and a helper virus such as human adenovirus or herpes simplex virus. If replication occurred, the only expected product would be increased synthesis of vector and WT AAV, both intrinsically non-pathogenic viruses.

- Minimal risk of transmission by vector shedding: Viral shedding was assessed in feces and urine in the GLP NHP study PRV-2018-028. There was no detectable PR006A DNA in any of the treatment groups at either timepoint. The results of no-trace of PR006A DNA in any of the treatment groups at different time points was obtained in the limits and the sensitivity of detection of the qPCR assay.

  Viral shedding, i.e., excretion/secretion of viral particles that could be transmitted to other individuals, will be assessed in saliva, urine, and stool samples and will be assessed on Days -1 (baseline), 7, and 14, and at Months 1, 2, 3, and 6.
  PR006A shed vector particles are unable to replicate and are quickly diluted in the environment, and thus their spread is thus inherently limited. Furthermore, potential hazards of exposure to PR006A to humans are predicated upon systemic administration of PR006A. Minimal exposure, such as environmental exposure, to persons other than the subjects receiving PR006A as part of the study would not be of sufficient dose to represent significant gene expression nor safety concerns in humans. If replication did occur, the only expected by-product would be the generation of more PR006A. The likelihood of such an occurrence is extremely low.
  Other than potential human hosts, exposure to PR006A is not expected to affect any non-target organisms, either directly or indirectly. The risk to humans and the environment associated with vector shedding of PR006A is thus low to negligible.

- Minimal risk of insertional mutagenesis: The risks of insertional mutagenesis are considered to be low to negligible, as the vast majority of recombinant AAV vector DNA persists as episomal rather than as integrated DNA. No clinical trials to date with AAV have reported incidences of insertional mutagenesis, with more than 2000 patients treated by 2019.

- Tissue-specific tropism of the vector and transgene expression: PR006A will be administered as a single dose via suboccipital injection into the cisterna magna by an interventional radiologist or neurosurgeon. Additionally, AAV9 displays a strong tropism for neural tissue. The vector is expected to remain concentrated in the brain, followed by

brain and muscle tissues. The expressed protein is identical to the endogenous protein. Even in the case of expression in non-target tissues, this would not result in any toxic effect.

- Minimal risk associated with the transgene: Exposure to PR006A is not expected to have any deleterious effects on health of humans, other species or the environment. If a person were to be inadvertently exposed, they may produce more PGRN, but this would not be expected to cause harm as PGRN is not toxic. No genes for toxins, potential oncogenes, growth factors or other genes that could be potentially harmful have been inserted into the GMO.

## 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) | ssDNA virus |
| (ii) | genus | Dependoparvovirus |
| (iii) | species | Adeno-associated virus |
| (iv) | subspecies | N/A |
| (v) | strain | AAV9 |
| (vi) | pathovar (biotype, ecotype, race, etc.) | N/A |
| (vii) | common name | N/A |

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:

| | |
|---|---|
| Atlantic | X |
| Mediterranean | X |
| Boreal | X |
| Alpine | X |
| Continental | X |
| Micronesian | 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 | In association with animals (primate hosts) |

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

5. (a) Detection techniques
AAV can be detected by quantitative polymerase chain reaction (qPCR) using primers specific for the viral genome.

(b) Identification techniques
AAV can be identified by qPCR using primers specific for the viral genome. It can as well be sequenced.

6. Is the recipient organism classified under existing Community rules relating to the protection of human health and/or the environment?
Yes (.) No (X)
If yes, specify

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 (.)

**Additional information:**

AAV is non-pathogenic and has not been classified under Directive 2000/54/EC of the European Parliament and of the Council of 18 September 2000 on the protection of workers from risks related to exposure to biological agents at work. AAV has no known pathogenic effects, even though the estimated seroprevalence of some common human serotypes is ~80% (European Parliament and of the Council 2000). Consequently, AAV fulfils the definition of a risk group 1 biological agent according to the Directive 2000/54/EC "a biological agent that is unlikely to cause human disease".

(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
Not applicable

8. Information concerning reproduction

(a) Generation time in natural ecosystems:
AAV is replication defective; thus, the generation time is variable depending on the presence or absence of a helper virus. PR006A is replication incompetent, even in the presence of a helper virus.

(b) Generation time in the ecosystem where the release will take place:
Not applicable, PR006A is a replication defective viral vector.

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

(d) Factors affecting reproduction:
The presence of a helper virus, such as adenovirus or herpes simplex virus, promotes AAV gene expression, genome replication and production of virions. In absence of a helper virus, wild-type AAV is replication-incompetent. Please note that the final GMO, PR006A, is replication-incompetent even in the presence of a helper virus due to the removal of the viral *rep* and *cap* genes.

9. Survivability

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

| | | |
|---|---|---|
| (i) | endospores | (.) |
| (ii) | cysts | (.) |
| (iii) | sclerotia | (.) |
| (iv) | asexual spores (fungi) | (.) |
| (v) | sexual spores (fungi) | (.) |
| (vi) | eggs | (.) |
| (vii) | pupae | (.) |
| (viii) | larvae | (.) |

(ix) other, specify AAV does not form survival structures

(b) relevant factors affecting survivability:
(b) Parvoviruses such as AAV are stable viruses that can persist in the environment for extended periods of time (thought to be on the order of several weeks). AAV particles are resistant to a wide range of pH (pH 3-9) and can resist elevated temperatures (55°C for 1 hour). AAV does not form survival structures. However, as with all viruses, replication of AAV cannot occur outside of a host cell.

10. (a) Ways of dissemination
AAV may be transmitted through direct or indirect contact. AAV may be transmitted through inhalation, ingestion and possibly sexual transmission.

(b) Factors affecting dissemination
Replication of the virus is only possible in host cells that have been co-infected with a helper virus (e.g. adenovirus, herpes simplex 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)

Recombinant AAV9 has been used previously worldwide in many gene therapy studies, however, PR006A has not previously been notified for release in Belgium.

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

The intended outcome of the genetic modification was to generate a recombinant AAV vector lacking viral genes and containing the human therapeutic gene, for the potential treatment of patients in patients with Fronto-Temporal Dementia with Progranulin Mutations.

PR006A contains a *GRN* expression cassette, where *GRN* expression is driven by a specific promoter.

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?
Partially: 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
A plasmid is the source of the entire AAV9 vector (GMO) genome insert. (A separate plasmid contains the viral rep and cap genes required for PR006A production)

(c) Host range of the vector
Bacteria, mammalian 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

(e) Constituent fragments of the vector

The plasmid vector DNA present in PR006A is limited to only the intended *GRN* expression cassette, the two small, viral inverted terminal repeats, and the promoter element to regulate expression.

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

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

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 cassette sequence codes for the human *GRN* gene, which is under the control of a ubiquitous promoter, and is flanked by inverted terminal repeats

(b) Source of each constituent part of the insert
The human *GRN* is human in origin. The other sequences in the genome and promoter are synthetic, viral and mammalian in origin.

(c) Intended function of each constituent part of the insert in the GMO
The expression cassette is limited to the required elements designed to optimize expression of functional human progranulin under control of a ubiquitous promoter. The inverted terminal repeats are necessary for the packaging of the vector genome into the capsid and the formation of the episomal concatemers in the transduced cells.

(e) Location of the insert in the host organism

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

(f) 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 (X)
- insect (.)
- fish (.)
- other animal (.)
(specify phylum, class) …

other, specify …

2. Complete name

(i) order and/or higher taxon (for animals) Primates

| | | |
|---|---|---|
| (ii) | family name for plants | 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:

(a) 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 (.) Not known (.)

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

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 (.)

## 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 PR006A viral genome has been significantly modified compared to the parental virus in order to render it replication incompetent. The AAV *rep* and *cap* genes have been removed, and only the viral ITR sequences, which are non-coding DNA sequences (<300 bp), have been retained. Thus, PR006A contains no native viral AAV coding genes.
Wild-type AAV requires the presence of a helper virus such as human adenovirus or herpes simplex virus to replicate. PR006A replication could only occur in the extremely unlikely event of a host cell being infected additionally by wild-type AAV and a helper virus such as human adenovirus or herpes simplex virus.

(b) is the GMO in any way different from the recipient as far as dissemination is concerned?
Yes (X) No (.) Not known (.)

Specify

As PR006A replication could only occur in the extremely unlikely event of a host cell being infected by two additional separate viruses, a wild type AAV and a helper virus such as human adenovirus or herpes simplex virus, the likelihood of dissemination is lower than that of wild-type AAV.

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

No pathogenic effects of wild-type AAV in humans are known. The introduction of the expression cassette, coding for PGRN, is not expected to result in development of pathogenicity. Thus, neither the wild-type AAV nor PR006A are known or expected to be pathogenic.

2. Genetic stability of the genetically modified organism

AAV is a single stranded DNA virus that demonstrates a high degree of genetic stability; based on this, PR006A is also expected to be genetically stable. The integrity of the vector genome is confirmed for every batch of PR006A drug substance that is released.

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)

AAV is non-pathogenic and has not been classified under Directive 2000/54/EC of the European Parliament and of the Council of 18 September 2000 on the protection of workers from risks related to exposure to biological agents at work. AAV has no known pathogenic effects, even though the estimated seroprevalence of some common human serotypes is ~80%. Consequently, AAV fulfils the definition of a risk group 1 biological agent according to the Directive 2000/54/EC "a biological agent that is unlikely to cause human disease".
A large body of data generated over the past ~20 years suggests that the safety risks associated with AAV gene transfer are low.

Safety results for PR006A evaluated in nonclinical efficacy and toxicology models have shown no adverse effects at doses up to $2.7 \times 10^{11}$ vg/ g brain in mice and $6.5 \times 10^{10}$ vg/g brain in NHPs (see efficacy studies PRV-2018-027, PRV-2019-002, and PRV-2019-004 and toxicology studies PRV-2018-021 and PRV-2018-028). There are no toxicities predicted from increases in PGRN limited to the CNS in *GRN* mutation carriers. In addition, the rAAV9 vector has had relatively wide human experience, as it has been administered intravenously and intrathecally (lumbar and ICM) at doses that are able to provide transduction of genes broadly in the CNS (Hudry and Vandenberghe 2019).
There has been no previous experience with administration of a vector in humans aimed at expression of *GRN*. Since FTD-GRN patients are heterozygous mutation carriers and express a certain amount of normal PGRN, the potential risk to develop an immune response to the "wild type" PGRN produced by the transduced cells may be considered as null.

4. Description of identification and detection methods

(a) Techniques used to detect the GMO in the environment
PR006A can be detected by polymerase chain reaction (PCR) based methods using vector genome specific primers.

(b) Techniques used to identify the GMO
PR006A can be detected by qPCR using vector genome specific primers and identity can be further confirmed by sequencing.

## F. Information relating to the release

1. Purpose of the release (including any significant potential environmental benefits that may be expected):
Release is planned during a Phase 1/2, gene therapy study with PR006A in subjects with Fronto-Temporal Dementia with Progranulin Mutations.

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 (.)

The replication incompetent GMO is administered by sub-occipital injection and transient/low levels of vector DNA shedding is possible. However, shed AAV-based vector 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):

| | |
|---|---|
| Site 1 | UZ Leuven, Herestraat 49, 3000 Leuven, Belgium |
| Site 2 | <<Name and Address>> |

(b) Size of the site ($m^2$):

(i) actual release site ($m^2$): Not applicable.

(ii) wider release site ($m^2$): Not applicable.

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

Not applicable. PR006A will be administered by a one-time single suboccipital injection in a hospital setting. Thus, it is not anticipated to come into contact with any recognised biotopes or protected areas.

(d) Flora and fauna including crops, livestock and migratory species which may potentially interact with the GMO:

Administration of PR006A will occur only within a controlled hospital setting; therefore, it is not anticipated that it will come into contact with plants, animals or soil.

4. Method and amount of release

(a) Quantities of GMOs to be released:

While dosing will be based on the patient's cohort, vector is only detectable after injection using sensitive qPCR analysis. Thus, the amount released is considered to be low to undetectable. Dosing of PR006A is anticipated to be between 1.0 and $2.0x10^{14}$ vg at maximum. The exact doses are confidential. Up to 15 patients overall and 2 patients in Belgium are foreseen.

(b) Duration of the operation:

The complete administration procedure, including PR006A preparation, is expected to take less than 8h.

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

PR006A will be shipped to study sites in line with standard recommendations for the transport of biohazardous materials. PR006A will be stored, prepared and administered by trained medical professionals, in a hospital setting only to patients that meet criteria

for inclusion into the clinical study PRV-FTD101. Staff will follow the waste and disposal policies as per local site requirement to dispose of consumables used in the preparation and administration of the GMO. The use of needles will be kept to a minimum.

PR006A is an investigational medicinal product (IMP) and will be released by a Qualified Person (QP) in Europe for EU countries, for clinical trial use after meeting defined specifications in terms of quality and safety of the product for administration to human subjects in accordance with the clinical study protocol. In addition, it will be used as per the clinical study protocol approved by both regulatory agencies and ethics committee in the country where the study is to be conducted. For this reason, the supply chain of the IMP and its management at site is governed in the context of clinical trial regulations, local law, and relevant guidelines for receiving, storing, handling, dispensing, accounting, and destruction of the IMP. The study Pharmacy/IMP (IP) Manual and training material located at investigational sites will provide pharmacy personnel and clinical medical staff with directions on use, storage and destruction of the IMP. It will also include directions for documenting the control of the IMP from the time of receipt at the trial site until final accountability and destruction or return. In addition, it describes the required processes for managing and documenting any issues, such as shipment or storage temperature excursions and reporting of technical product complaints. The risks related to the release into the environment of PR006A or risks to personnel, in the event there is a breach in container integrity and/or storage or accidental spillage at the site or during shipping/storage is considered to be negligible. The PR006A will only be handled by delegated, trained personnel and, in the event that a spillage did occur, as the product is non-pathogenic and non-replicative, spread of the PR006A and risks to the environment or personnel would be limited (instructions how to handle spills are provided in the Pharmacy manual).

Recombinant AAV vectors are non-replicative and are not expected to pose a risk of transmission.

Patients will receive PR006A as a single suboccipital injection into the cisterna magna. Following the ICM procedure, patients will be monitored for at least 24 hours in an inpatient setting until fully recovered, prior to being discharged from the clinic after completion of the visit assessments. Additional hospitalisation is possible based on the patient's clinical status and Investigator discretion. Additionally, viral vector shedding will be assessed in this study. This will indicate when vector shedding in bodily fluids has ceased. As PR006A is non-replicative, shed vector particles are unable to multiply and thus, the spread of the GMO is inherently limited.

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

Not applicable. Administration of PR006A will occur only within a controlled hospital 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.

Not applicable. No clinical studies have been carried out before with PR006A. PR006A has been well tolerated with no adverse effects at doses up to $2.7 \times 10^{11}$ vg/g brain in mice and

$6.5 \times 10^{10}$ vg/g brain in NHPs based on the results of efficacy studies PRV-2018-027, PRV-2019-002, and PRV-2019-004 and toxicology studies PRV-2018-021 and PRV-2018-028. The level of shedding in excreted fluids in humans is expected to be low.

## 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):

PR006A is a gene therapy medicinal product (GTMP) being developed for the treatment of frontotemporal dementia with progranulin mutations. It consists of a non-replicating recombinant AAV encoding a codon-optimised version of *GRN* that is under the regulatory control of a promoter element. It is expected that expression of functional *GRN* in the cells of the central nervous system (CNS) will restore PGRN levels, lysosomal function neuronal survival. Hence, the drug product is intended to serve as a treatment of FTD-GRN.

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

Persons other than the human subjects receiving the medicinal product will not be exposed to levels of PR006A that could represent potential hazard. Potential hazards of exposure to PR006A are predicated upon administration of PR006A. Minimal exposure, such as environmental exposure, to organisms other than the subjects receiving PR006A as part of the study would not be of sufficient dose to represent significant gene expression or potential safety risks in humans. As PR006A is also replication-incompetent, it is expected that the vector would be rapidly cleared from any non-target organisms without causing any harmful effects. Other than potential human hosts, exposure to PR006A is not expected to affect any non-target organisms, either directly or indirectly.

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

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

Give details

As PR006A is unable to replicate, post-release selection cannot occur.

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

As PR006A is unable to replicate and is not expected to spread to the environment to a significant degree and is not expected to become established in any ecosystems.

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:

N/A

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

7. Likelihood of genetic exchange in vivo

(a) from the GMO to other organisms in the release ecosystem:
It is expected that the PR006A vector genome will be transferred into neural cells within the brain of patients. The vast majority of PR006A vector genomes within subject cells are expected to be episomal, rather than integrated into the host cell DNA. As PR006A is non-replicative and is only expected to be shed in study subjects' bodily fluids to a limited extent, transmission and gene transfer to organisms other than the study subjects is considered unlikely.

(b) from other organisms to the GMO:
The probability of homologous recombination with related viruses that could lead to variants of the GMO is strongly reduced with the ITRs being the only viral sequences remaining in the vector, making up only 6% of the final vector sequence.

(c) likely consequences of gene transfer:
While recombination between PR006A and a wild-type AAV to generate a hybrid vector genome that contains both the transgene and the AAV *rep* and *cap* genes remains a theoretical possibility, such a molecule even if generated in a cell would not replicate unless a helper adenovirus/herpes virus was also present. Moreover, such a hybrid genome would be too large to package the hybrid DNA into an AAV particle. It is known that AAV possesses a packaging limit of approximately 5kb (Wu, Yang, and Colosi 2010), and a hybrid molecule of *rep-cap* genes plus the *GRN* expression cassette would be predicted to be in excess of this limit. The risks associated with gene transfer from wild-type AAV to PR006A are thus considered to be negligible.

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

No such studies have been conducted with PR006A.

9. Possible environmentally significant interactions with biogeochemical processes (if different from the recipient or parental organism):
PR006A is not known or predicted to have an impact on biogeochemical processes.

## H. Information relating to monitoring

1. Methods for monitoring the GMOs:
Vector shedding will be closely monitored. Other methods to monitor the effects of PR006A include both safety and efficacy assessments.

2. Methods for monitoring ecosystem effects:
The presence of PR006A in bodily fluids following administration of PR006A will be determined by qPCR. No other monitoring of the environment or unintended recipients is planned or considered necessary.

3. Methods for detecting transfer of the donated genetic material from the GMO to other organisms:
Transfer of vector genome to study subjects will be detected by qPCR, activity will be assessed using clinically relevant parameters and biochemical assays.

4. Size of the monitoring area ($m^2$):
Not applicable; monitoring techniques will only be used with regards to vector shedding in patients' bodily fluids.

5. Duration of the monitoring:
Viral shedding will be assessed on Days -1 (baseline), 7, and 14, and at Months 1, 2, 3, and 6. Safety and efficacy assessments will be conducted throughout the duration of the study.

6. Frequency of the monitoring:
Viral shedding will be assessed on Days -1 (baseline), 7, and 14, and at Months 1, 2, 3, and 6. Safety and efficacy assessments will be conducted throughout the duration of the study, as detailed in the clinical protocol.

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

1. Post-release treatment of the site:
Any surface area exposed to the GMO will be decontaminated using an appropriate viricidal agent, such as a fresh preparation of 1:10 dilution of bleach (0.07-0.09M sodium hypochlorite), for 10 minutes. Waste material should be decontaminated using chemical means in a similar manner to spills, autoclaving, irradiation, or incineration prior to disposal. All materials should be decontaminated according to the facility's recommended protocol.

This process should be discussed with the local environmental health and safety officer and/or biosafety committee before receipt of any PR006A product on site so that an appropriate plan and supplies are in place.

2. Post-release treatment of the GMOs:
Consumables used in the preparation and administration of the GMO that may have come into contact with PR006A will be decontaminated prior to disposal (either by autoclaving or by

treatment with an appropriate chemical disinfectant with effectiveness against AAV, and/or incinerated. Liquid waste will be decontaminated using an appropriate chemical disinfectant or autoclaved).

3. (a) Type and amount of waste generated:

- Used vials of the IMP containing PR006A
- Materials used for the preparation and administration of the study product, e.g. ICT administration set, syringes, needles
- Personal protective equipment, e.g. gloves

3. (b) Treatment of waste:

Refer to post-release treatment I.2.

## J. Information on emergency response plans

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

In the event that the contents of the PR006A containers are accidentally released and come in contact with shipping materials, pharmacy/hospital surfaces, the spillage will be decontaminated and removed according to institutional practice with disinfectants effective against AAV. At minimum this will entail the initial containment of any spillage, collection of the spill with absorbent material, and thorough cleaning of the contaminated surfaces with the appropriate compounds that deactivate the PR006A. Larger spills will require the removal of non-essential personnel from the affected area. Reporting of emergency situations immediately. Clean up operations should only be undertaken by trained personnel.

In case of accidental spills, the medical staff should alert people in the area of the spill, remove contaminated clothes and leave the area for 30 min. He/she should close the area and post “DO NOT ENTER”. After 30 min, he/she must wear a clean lab coat and wear gloves, glasses and a FFP2 mask. He/she must cover the spill with towels and other absorbent material starting from the edge toward the center. He/she must carefully pour the appropriate disinfectant over the absorbent material starting from the edge to the center. It must allow a sufficient contact time for the disinfectant to inactivate the GMO. After that, he/she must remove the paper towels and broken vials with tongs or forceps and discard in a biohazard waste bag. The PPE should be discarded in the biohazard bag.

Staff will be advised that care must be taken when manipulating tubes and that the use of needles should be kept to a minimum. In the event of injury, staff will follow local institutional procedures.

In case of accidental contact of PR006A with skin, eyes or clothing, staff will follow institutional procedures for the management of biohazardous material.

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

Any surface area exposed to the GMO will be decontaminated using an appropriate viricidal agent, such as a fresh preparation of 1:10 dilution of bleach (0.07-0.09M sodium hypochlorite), for 10 minutes. All materials should be decontaminated according to the facility’s recommended protocol.

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

Administration of PR006A will occur only within a controlled hospital setting; therefore, it is not anticipated that it will come into contact with plants, animals or soil and decontamination will not be required.

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

Staff will follow local law and institutional procedures for the handling and disposal of GMOs. Furthermore, safety recommendations and guidance on the management of incidents related to PR006A are provided in the safety instructions for investigators and staff included in this submission. All patients will be carefully monitored for any adverse reactions during this study. An independent data monitoring committee (iDMC) will be responsible for monitoring safety data from the study.

**References:**

European Parliament and of the Council. 2000. 'Directive 2000/54/EC of the European Parliament and of the Council of 18 September 2000 on the protection of workers from risks related to exposure to biological agents at work,'

Hudry E, Vandenberghe LH. Therapeutic AAV gene transfer to the nervous system: A clinical reality. Neuron. 2019;101(5):839-62