PART 1 (COUNCIL DECISION 2002/813/EC)

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

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

A. General information

1. Details of notification

(a) Member State of notification: Spain
(b) Notification number: B/ES/08/47
(c) Date of acknowledgement of notification: 29/Jul/2008
(d) Title of the project: A Phase II, Multicenter, Randomized, Controlled, Open-Label Trial to Compare the Safety and Efficacy of Bilateral Intraputaminal (IPu) Administration of CERE-120 (Adeno-Associated Virus Serotype 2 [AAV2]-Neurturin [NTN]) Combined with Best Medical Therapy (BMT) versus BMT-alone in Subjects With Idiopathic Parkinson's Disease.
(e) Proposed period of release: From November 2008 until December 2010 approximately.

2. Notifier

Name of institution or company: Universidad de Navarra, Neurología-Neurociencias Clínica Universitaria, Avenida de Pío XII, 36, Facultad de Medicina (Pamplona, España)

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)

GMO: CERE-120
Genus: Dependovirus
Species: Adeno-associated Virus

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

During the CERE-120 development program (from the initial nonclinical stages to the most recent clinical batch manufactured) several tests have been conducted to determine the genetic stability of CERE-120: 1) molecular identity test; 2) *in vitro* transduction/neurturin ELISA to assess transgene expression; 3) *in vitro* potency assay on target cells to assess the biological activity of neurturin; and 4) replication-competent AAV2 detection test with activation of auxiliary virus to detect the presence of wild-type AAV2 in clinical lots. To date, all test results have confirmed the genetic stability of CERE-120.

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) SP

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.

CERE-120 comes from an AAV2 virus unable to replicate under natural conditions. AAV2 virus is not pathogenic and requires the presence of an auxiliary virus (adenovirus or herpes) to replicate. The presence of the wild-type AAV2 in CERE-120, which could be produced by non-homologous recombinations between plasmids during manufacturing, is assessed by a test that can detect replication-competent AAV2 after co-infection with adenovirus. To date, no replication-competent entities have been detected in clinical lots of CERE-120.

Since AAV2 is unable to replicate under natural conditions and requires an auxiliary virus to do so, the mobilization of the CERE-120 genome, which does not encode any vital proteins, would require co-infection of transduced cells with a wild-type AAV and an auxiliary virus (adenovirus or herpes). Because AAV and adenoviruses do not usually produce infections in the central nervous system (CNS) where CERE-120 is delivered, and herpes brain infections

are rare, the possibility that the vector could be mobilized after initial administration is very remote.

CERE-120 has been studied in a series of pre-clinical animal toxicology and biodistribution studies and in 2 clinical studies in which it was administered to humans. For the time being, there is no indication of environmental emission of viral products after the administration of CERE-120 to patients.

CERE-120 will be given to the patient in a single administration by means of a stereotactic neurosurgical procedure that uses a delivery system provided by the sponsor that consists of a guide tube, cannula, trocar needle, injection needle, and syringe. The delivery system is provided to the clinical site as a sterile, non-reusable system. All healthcare professionals involved in the preparation and administration of CERE-120 to each patient will wear suitable gowns and gloves and will wear the appropriate mask and eye protection when participating in any type of procedure that involves the product, and they will be given training in handling the product. In addition, if any accidental spills or breakages occur with CERE-120, the healthcare professionals involved in the clean-up procedure will also wear gowns, gloves, and will wear the appropriate eye protection according to the institution's guidelines for handling biohazardous material. All clinical sites will be given a Material Safety Data Sheet for CERE-120, on which the product and the appropriate precautions and handling instructions will be described. If the instructions are followed, the risk that adverse effects may occur is negligible. However, even if they are not followed, it is unlikely that CERE-120 is going to cause any problems, given that AAV2 has not been linked to any pathology or any human illnesses. After surgery, the CERE-120 vials and the components of the delivery system that have been used (guide tube, cannula, trocar needle, injection needle, and syringe) will be disposed of according to the usual practice of the institution for biohazardous sharps. All disposable surgical instruments or other disposable materials used during the intervention will also be disposed of, following the usual practice of the institution for biohazardous objects. All non-disposable surgical equipment and other materials used during the intervention will be cleaned with a chemical disinfectant with virocidal activity, (e.g., Virkon®), and will then be sterilized by autoclaving, following the usual practice of the institution.

Together, based on the characteristics of this vector, the administration route and negative results of viral emission, and the environmental protection measurements, we understand that unwanted exposure of the medium to CERE-120 would not be harmful to humans, animals, or plants.

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

1. Recipient or parental organism characterisation:

(a) Indicate whether the recipient or parental organism is a:

(select one only)

viroid (.)
RNA virus (.)
DNA virus (X)

bacterium (.)
fungus (.)
animal
- mammals (.)
- insect (.)
- fish (.)
- other animal (.)
  (specify phylum, class) …

other, specify …

2. Name
   (i) order and/or higher taxon (for animals) Parvoviridae
   (ii) genus Dependovirus
   (iii) species Adeno-associated Virus
   (iv) subspecies …
   (v) strain serotype 2
   (vi) pathovar (biotype, ecotype, race, etc.) …
   (vii) common name 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 (.)

   (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 human

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

5. (a) Detection techniques
- The quantitative PCR detecting DNase-resistant CERE-120 vector genomes by the amplification of a 110-bp region of the vector genome spanning the 3'-end of the CAG promoter and the 5'-end of the NGF pre-pro coding regions.
- AAV2 capsid ELISA technique quantifying intact AAV2 capsids from the conformational point of view.

(b) Identification techniques
- multiple primer quantitative PCR method to identify CAG promoter and NGF pre-pro domain of CERE-120
- polyacrylamide gel electrophoresis with a silver nitrate stain to identify AAV2 capsid proteins (VP1, VP2, and VP3)

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
Los AAV2 están clasificados en el Grupo/Clase 1.

7. Is the recipient organism significantly pathogenic or harmful in any other way (including its extracellular products), either living or dead?
Yes (.) No (X) Not known (.)

If yes:

(a) to which of the following organisms:

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

(b) give the relevant information specified under Annex III A, point II. (A)(11)(d) of Directive 2001/18/EC
No pathological, ecological, or physiological traits are present. In natural conditions, wild-type AAV2 in the presence of an auxiliary virus (adenovirus) is transmitted to humans only and is not known to colonize other species.

8. Information concerning reproduction

(a) Generation time in natural ecosystems:
Not relevant, since CERE-120 lacks viral protein-coding sequences.

(b) Generation time in the ecosystem where the release will take place: Not relevant

(c) Way of reproduction: Sexual .. Asexual ..
Not relevant

(c) Factors affecting reproduction:
Not relevant

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 co-infection of transduced cells with wild-type AAV2 and an auxiliary virus (adenovirus or herpes)

(b) relevant factors affecting survivability:
CERE-120 is sensitive to 1% sodium hypochlorite, 5% phenol, or common virocidals e.g., Virkon®. CERE-120 is sensitive to heat (>80°C for 60 minutes), and sensitive to UV radiation and extreme pHs (<2 and >12).

10. (a) Ways of dissemination
Once injected into the patients, there is no indication that the CERE-120 gene vector is emitted into either their serum or urine. Therefore, it is unlikely that CERE-120 will be disseminated into any ecosystems.

(b) Factors affecting dissemination
Not relevant

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/../../… Not relevant

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

CERE-120 is a genetically engineered viral vector (of the adeno-associated virus serotype-2, AAV2) that lacks the wild-type viral protein-coding sequences and instead, encodes a human neurturin (NTN) complementary deoxyribonucleic acid (cDNA). The deletion of native AAV2-coding sequences incapacitates the CERE-120 for replication. In the CERE-120 vector, transgene expression is controlled by the constitutive CAG promoter (a fusion of the minimal cytomegalovirus enhancer with the chicken β-actin gene promoter and splice donor and a rabbit β-globin gene splice acceptor intron) and the human growth hormone gene polyadenylation signal. This NTN expression cassette is flanked by the AAV2 inverted terminal repeats (ITRs). To promote efficient secretion of mature NTN from transduced cells, the natural pre/pro domain of the human NTN cDNA was replaced with that of the human β-nerve growth factor (NGF). The resulting hybrid precursor protein (pre-pro-hNGFnNTN cDNA hybrid) generates the mature secreted NTN protein after passing through the proteolytic processing of the cellular secretary path.

NTN, a functional and structural analog of glial cell line-derived neurotrophic factor (GDNF), has demonstrated neuroprotective and neuroregenerative properties in rodent and non-human primate models of Parkinson's disease (PD). CERE-120 will be administered by bilateral intraputaminal (IPu) injection to achieve the administration of NTN to the desired targets, thereby providing continuous NTN to the neurons affected by PD, which are in the process of degeneration, while minimizing the presence of NTN in regions outside the nigrostriatal system.

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

Yes (X): No (.)

Cere-p521 (CERE-120 genome)
Cere-p509 (AAV auxiliary)
Cere-p511 (Ad auxiliary)

If no, go straight to question 5.

(b) If yes, is the vector wholly or partially present in the modified organism?

Cere-p521: Yes	(X)	No	(.)
Cere-p509: Yes	(.)	No	(X)
Cere-p511: Yes	(.)	No	(X)

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
Cere-p521 a.k.a. pK-AAV-CAG-NGF/NTN

(c) Host range of the vector
Escherichia coli

(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
Note that the final CERE-120 vector contains no Kanamycin-resistant gene.

(e) Constituent fragments of the vector
- CAG promoter
- human NGF pre-pro domain
- human mature NTN cDNA
- human growth hormone gene polyadenylation signal
- 5’ and 3’ AAV2 ITRs
- pUC plasmid backbone modified to replace ampicillin resistance gene by that of kanamycin

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

(i) transformation (.)
(ii) electroporation (.)
(iii) macroinjection (.)
(iv) microinjection (.)
(v) infection (.)
(vi) other, specify CERE-120 is manufactured observing current correct Good Manufacturing Practices (cGMP) in HEK 293 cells by a triple plasmid transfection technique exempt from an auxiliary virus. The vector is obtained from clarified cell lysates that are purified through multiple-step chromatography and filtration processes.

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 molecular cloning

6. Composition of the insert

(a) Composition of the insert

The cDNA hybrid contains human neurturin (NTN) cDNA, in which the natural pre-pro domain has been substituted by that of human-nerve growth factor (NGF) in order to promote the efficient secretion of mature NTN by transduced cells.

(b) Source of each constituent part of the insert

The whole human NTN was obtained by PCR amplification from a universal cDNA repository (Clontech 7108-1).

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

The pre-pro-hNGFnNTN cDNA hybrid will be translated into a precursor protein that generates the mature secreted NTN protein after going through proteolytic processing of the cellular secretory path. NTN could prevent the degeneration and death of existing nigrostriatal neurons, as well as re-establish dopaminergic function in existing dysfunctional neurons.

(d) Location of the insert in the host organism

- on a free plasmid (.)
- integrated in the chromosome (.)
- other, specify The insert is cloned into the viral vector genome.

(e) Does the insert contain parts whose product or function are not known?

Yes (.) No (X)

If yes, specify …

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

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

2. Complete name

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

(vi) strain …
(vii) cultivar/breeding line …
(viii) pathovar …
(ix) common name …

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

E. Information relating to the genetically modified organism

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

(a) is the GMO different from the recipient as far as survivability is concerned?
Yes (.) No (X) Not known (.)
Specify …

(b) is the GMO in any way different from the recipient as far as mode and/or rate of reproduction is concerned?
Yes (X) No (.) Unknown (.)
Specify The GMO is incapable of replicating.

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

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

2. Genetic stability of the genetically modified organism
During the CERE-120 development program (from the initial non-clinical phases to the most recent clinical batch manufactured), several tests have been conducted to determine the genetic stability of del CERE-120: 1) molecular identification assay; 2) in vitro transduction/neurturin ELISA to assess transgene expression; 3) in vitro potency assay on target cells to assess biological activity of neurturin; and 4) detection assay of AAV2 capable of multiplying with activation of the auxiliary virus, to detect the presence of wild-type AAV2 in clinical batches. For the time being, all test results have confirmed the genetic stability of CERE-120.

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)
No pathological, ecological, or physiological traits are present. In natural conditions, and in the presence of an auxiliary virus (adenovirus), wild-type AAV2 is transmitted to humans only and is not known to colonize other species.

4. Description of identification and detection methods

(a) Techniques used to detect the GMO in the environment
The detection and determination of CERE-120 concentration is performed by a quantitative PCR method, as well as an AAV2 capsid ELISA technique. The dose-defining detection technique for CERE-120 is the quantitative PCR method. This method detects DNase-resistant CERE-120 vector genomes by the amplification of a 110bp region of the vector genome that spans the 3’ end of the CAG promoter and the 5’ end of the nerve growth factor pre-pro coding regions. The vector genome concentration of each problem lot is

determined by extrapolation of the number of copies of the vector genome per reaction from a purified, qualified vector genome DNA standard; for the calculation, a semi-logarithmic curve is used [$\log_{10}$ copies entered/reaction versus threshold cycle (CT)] and appropriate correction for sample dilution factors. The AAV2 capsid ELISA is a commercially available assay that allows for the quantification of intact capsids from the conformational point of view that are captured by a specific antibody and are detected by standard sandwich ELISA methods.

(b) Techniques used to identify the GMO

Two identification tests are performed on CERE-120 that allow for its specific identification. One of them is a molecular identification test based on a multiple primer quantitative PCR method, which identifies two independent sequences in the CERE-120 vector genome. Combined, the two primer/probe groups confirm the presence of the CAG promoter contiguous to the NGF pre-pro domain, the NGF pre-pro contiguous to the mature NTN cDNA domain, and specifically identify CERE-120. The research product meets the specification if it is determined that it is amplified by the two primer probe groups and if the quantitative result obtained by both primer probe groups does not differ by more than five-fold.

The other identification test is based on the separation of polypeptides by polyacrylamide gel electrophoresis and staining with silver nitrate; it is used to confirm the protein identification of CERE-120. The AAV2 viral capsid proteins (VP1, VP2, and VP3) are identified based on their size by comparison to molecular weight markers of known size. The problem sample meets the specifications when the critical bands (VP1, VP2, and VP3) have molecular weights similar to those of the reference standard.

F. Information relating to the release

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

Parkinson's disease (PD) is a neurodegenerative disorder of slow progression. Its pathogenesis involves the progressive functional loss of dopaminergic nigrostriatal neurons and ends up causing their death. NTN is a neurotrophic factor that can promote neuronal growth, reestablish function, and prevent neuronal death. The purpose of this study is to compare the safety and efficacy of gene therapy with CERE-120 versus an optimized and stable regimen of antiparkinsonian medication ("Best Medical Therapy", BMT), in subjects with idiopathic PD. Patients in this study will receive a single bilateral intraputaminal injection of CERE-120, a replication-defective AVV2 vector that expresses human NTN. It is assumed that in the targeted brain tissue, functional NTN will be synthesized and secreted, generating a neurotrophic effect.

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 administered by bilateral intraputaminal injection.

3. Information concerning the release and the surrounding area

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

CERE-120 will be only loaded to the delivery system in the operating room of Universidad de Navarra, Neurologia-Neurociencias Clinica Universitaria.

(b) Size of the site (m^2): … m^2

(i) actual release site (m^2): … m^2

(ii) wider release site (m^2): … m^2

The patients will be kept in the operating room (hospital room) until the operation is finished.

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

Not applicable

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

Not applicable

4. Method and amount of release

(a) Quantities of GMOs to be released:

The total dose of CERE-120 that will be given to each patient will be 5.4×10^{11} viral genome (vg) as a single administration. It is anticipated that each of the 11 sites will recruit 4-5 subjects. Two-thirds of them (approx. 3-4 patients per site) will receive CERE-120 (which will be compared to best symptomatic treatment); therefore the maximum release of CERE-120 per site would be 2.16×10^{12} vg (4 patients x 5.4×10^{11} vg).

(b) Duration of the operation:

CERE-120 is administered bilaterally into the putamen in the course of a single surgical intervention. The expression of CERE-120 is anticipated to be consistent.

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

All healthcare professionals involved in the preparation and administration of CERE-120 to each patient will wear suitable gowns, gloves, and will be covered with the appropriate mask and eye protection when participating in any type of procedure that involves the product, and they will be given training in handling the product.

In addition, if any accidental spills or breakages occur with CERE-120, healthcare professionals involved in the clean-up procedure will also wear gowns and gloves and will wear the appropriate eye protection according to the institution's guidelines for handling biohazardous material. All clinic sites will receive a Material Safety Data Sheet for CERE-120, which describes the product and the appropriate precautions and instructions for handling.

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

Hospital operating room

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

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)	Hominidae
(ii)	family name for plants	…
(iii)	genus	Homo
(iv)	species	Homo sapiens
(v)	subspecies	…
(vi)	strain	…
(vii)	cultivar/breeding line	…
(viii)	pathovar	…
(ix)	common name	…

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

   The objective of therapy with CERE-120 is to enhance the function of nigrostriatal dopaminergic neurons, thereby providing symptomatic improvement of motor function and reduction of "off" time in patients with Parkinson's disease (PD). Other important objectives of CERE-120 are to prevent the nigrostriatal neurons from continuing to degenerate and in this way slow or avoid progression of the disease. CERE-120 encodes the gene for neurturin (a growth factor known to reestablish and protect nigrostriatal dopamine neurons) within a viral vector (AAV2) that selectively transduces neurons and does so with minimal proinflammatory side effects or other undesirable side effects. CERE-120 is being developed as a medical treatment with the potential to improve the symptoms of PD and at the same time slow progression of the disease.

   CERE-120 is a viral vector (adeno-associated virus serotype-2, AAV2) made by genetic engineering that lacks the sequences that encode wild viral proteins and instead, encodes the human neurturin (NTN) complimentary deoxyribonucleic acid (cDNA). It has been shown that NTN, a functional and structural analog of glial cell line-derived neurotrophic factor (GDNF), has neuroprotective and neuroregenerative properties in rodent and non-human primate models of Parkinson's disease (PD). By incorporating the NTN gene into an AAV2 vector, it is possible to administer NTN to the nigrostriatal system in the brain in a controlled, sustained, and directed manner, thus providing NTN continuously to the degenerating neurons affected by PD, at the same time that the presence of NTN is minimized in regions outside the nigrostriatal system.

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

   Not known.

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

   ERE-120 is incapable of replicating. It can only do so if it infects a cell that it co-infected by wild-type AAV2 and an auxiliary virus (adeno or herpesvirus). There is the theoretical risk

that OMG will infect a human cell that is already infected by wild-type AAV and auxiliary virus, which would originate replication of the vector. This risk is extremely small.

6. Complete name of non-target organisms which (taking into account the nature of the receiving environment) may be unintentionally significantly harmed by the release of the GMO

Not applicable

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

7. Likelihood of genetic exchange in vivo

(a) from the GMO to other organisms in the release ecosystem:
Not anticipated

(b) from other organisms to the GMO:
Not anticipated

(c) likely consequences of gene transfer:
Local expression of human NTN

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.):

The CERE-120 genome is made up of the following genetic elements flanked by the AAV2 inverted terminal repeats (ITRs):

- 1.7-kb CAG promoter that is comprised of the human CMV enhancer, the chicken β-actin promoter and splice donor, and the rabbit β-globin gene splice acceptor
- 0.36-kb human nerve growth factor (NGF) pre-pro domain cDNA
- 0.3-kb human NTN mature protein cDNA
- 0.48-kb human growth hormone gene polyadenylation signal

Since the CERE-120 genome does not encode any viral proteins, to replicate would require co-infection of transduced cells with wild-type AAV2 and an auxiliary virus (adenovirus or herpes). Because AAV2 and adenoviruses do not usually produce infections in the central nervous system (CNS), which is where CERE-120 is delivered, and herpes brain infections are rare, the likelihood that the vector could be mobilized after initial administration is very remote.

In the pre-clinical pharmacodynamic studies, NTN expression after CERE-120 administration into the striatum is stable for for at least one year as observed so far. The volume of distribution of NTN can be controlled by manipulating the dose of CERE-120. These data allow the selection of pre-clinical and clinical doses that provide reasonable coverage of the striatum with NTN without producing significant distribution to structures

that lack a neuroanatomical relationship. In further experiments it has been established that the NTN protein that us expressed after CERE-120 administration is robust and bioactive, capable of protecting and reestablishing nigral neurons function and improving clinical motor function in PD animal models. In pre-clinical safety and toxicology studies and Phase 1 and US Phase 2 studies done with CERE-120 in human subjects with PD, no clinically important adverse events related to CERE-120 were seen.

CERE-120 and its product NTN are not able to cross the hematoencephalic barrier. In the two studies with human subjects with PD, with quantitative PCR testing of the urine of the 12 subjects enrolled in the Phase I study in the U.S., no indication of viral emission has been detected. Nor has the presence of CERE-120 been detected in any blood samples of subjects participating in Phase I and Phase II studies.

No environmental impact has been observed in any of the studies mentioned above.

9. Possible environmentally significant interactions with biogeochemical processes (if different from the recipient or parental organism)
Not applicable

H. Information relating to monitoring

1. Methods for monitoring the GMOs
   - Determination of CERE-120 concentrations in serum, urine, feces, and buccal swab by quantitative polymerase chain reaction (QPCR) analysis
   - Changes from baseline in NTN serum antibodies and AAV2

2. Methods for monitoring ecosystem effects
Not applicable

3. Methods for detecting transfer of the donated genetic material from the GMO to other organisms
Not applicable, since there is no risk of transfer of donated genetic material form the patient to other organisms. The batch that is going to be used in the trial is tested to rule out the presence of replication-competent viruses.

4. Size of the monitoring area (m^2)
Monitoring of treated patients

5. Duration of the monitoring
According to the protocol, i.e., 12 months after surgery

6. Frequency of the monitoring
At the baseline visit, the day after Surgery, at Month 1 and Month 12, the CERE-120 vector will be tested (QPCR) in serum, feces, urine, and buccal swab. In addition, if positive titers are detected at Month 1, the test will be repeated at the Month 3 Visit. At the baseline visit, at Month 1, Month 3, and Month 12, serum AAV2 antibodies will be tested (ELISA). At the baseline visit and at Months 3 and 12, serum NTN Antibodies will be tested (ELISA).

I. Information on post-release and waste treatment

1. Post-release treatment of the site
   To disinfect the work area, a virucide such as Virkon® will be used.

2. Post-release treatment of the GMOs
   All equipment that is used during the procedure will be disposed of according to current procedures for biohazardous materials or decontaminated with virucides according to the institution's plan for managing biohazardous waste. This will prevent any possible spread to other patients who are operated on subsequently in the operating room.

3. (a) Type and amount of waste generated
   - Used CERE-120 vials with less than 0.05 mL of product and the used components of the delivery system (guide tube, cannula, trocar needle, injection needle, and syringe).
   - All non-disposable surgical equipment and other materials that are used during the intervention.

   The volume of waste cited above will be very small.

3. (b) Treatment of waste
   After surgery, the used CERE-120 vials and the used components of the delivery system (guide tube, cannula, trocar needle, injection needle, and syringe) will be disposed of following the usual practice of the institution for biohazardous sharps. All disposable surgical instruments or other materials that are used during the intervention will also be disposed of following the usual practice of the institution for biohazardous objects.
   All non-disposable surgical equipment and other non-disposable materials that are used during the intervention will be cleaned with a chemical disinfectant with virucidal activity e.g., Virkon®, and will then be sterilized by autoclaving following the usual 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
   CERE-120 will be administered in the operating room by neurosurgical stereotactic injections directly into the brain of patients with Parkinson's disease. Once injected into the patients, there is no indication that the CERE-120 gene vector is emitted into the serum or the urine of these people. Therefore, the spread outside of the area (aside from the site of the injection into the brain of the patient) will be negligible.

2. Methods for removal of the GMO(s) of the areas potentially affected
   Allow aerosols to settle. Wear protective clothing and carefully cover spills with paper towels. Apply chemical disinfectant with virucidal activity, such as Virkon®). Start at the outer part of the spill and move towards the center. Let the disinfectant work for at least 30 minutes before cleaning it up.

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

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

If an undesirable effect occurs, the use of CERE-120 would stop until a complete assessment of the effect is performed and measures are put into practice to mitigate later risks. All areas and facilities that had been used to administer the product would be cleaned and decontaminated with virucides.