PROJECT
« A phase I/II, Open Labeled, Monocentric Study of Direct Intracranial Administration of a Replication Deficient Adeno-associated Virus Gene Transfer Vector Serotype rh.10 Expressing the Human ARSA cDNA to Children with Metachromatic Leukodystrophy»

N° EudraCT 2011-004410-42
Protocol: C 11-09

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

A. GENERAL INFORMATION

1. Details of notification

(a) Member State of notification FRANCE
(b) Notification number B/FR/12/GT03
(c) Date of acknowledgement of notification 18/06/2012
(d) Title of the project
A phase I/II, Open Labeled, Monocentric Study of Direct Intracranial Administration of a Replication Deficient Adeno-associated Virus Gene Transfer Vector Serotype rh.10 Expressing the Human ARSA cDNA to Children with Metachromatic Leukodystrophy
(e) Proposed period of release from 01/04/2012 until 01/12/2017

2. Name of institution or company:

INSERM- Institut National de la Santé et de la Recherche Médicale
101 rue de Tolbiac, 75654 Paris cedex 13, France

3. GMO characterization

(a) Indicate whether the GMO is a:

viroid	(.)
RNA virus	(.)
DNA virus	(x)
bacterium	(.)
fungus	(.)
animal	
mammal	**(-)**
insect	(.)
fish	(.)
other animal	(.)

specify phylum, class **Associated -Adenovirus**, Parvovirus- Dependovirus

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

The GMO, a gene therapy vector, is a non replicative AAVrh.10cuARSA vector. It is a modified single strand AAV2- DNA encapsulated in an AAVrh.10 capsid. The AAVrh10 capsid is derived from an AAV virus isolated in simians.

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

During the production of the AAVrh.10cuARSA clinical batch several tests have been conducted to determine the genetic stability of AAVrh.10cuARSA: 1) genomic structural identity test (Southern-blot and PCR-sequence) ; 2) Test of ARSA enzyme activity aiming to measure ARSA protein activity in cells transduced by AAVrh.10cuARSA vector; 3) Infectious Center Assay to detect and quantify potential replication of AAVrh.10cuARSA. All test results have confirmed the genetic stability of the non replicative AAVrh.10cuARSA vector.

In addition, genetic stability of the GMO in its container closure system before intracerebral administration is being monitored using the ARSA activity assay every 3 months from vialing of the vector during the first year and every 6 months during the second year and then annually thereafter if needed.
Stability has been demonstrated on the clinical lot up to present time of the IND writing at months 3, 6, 9 and 12.

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

Yes (.) **No (x)**

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

Yes (.) **No (x)**

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)**

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

The release of AAVrh.10cuARSA GMO consists in intracerebral injection performed in human patients with a rare disease called metachromatic leukodystrophy. This release in the brain should not lead to any incidence nor potential risk for the environment for the following reasons:

- The GMO (AAVrh.10cuARSA) is non pathogenic and unable to replicate under natural conditions. The mobilization of the AAVrh.10cuARSA genome, which does not encode any vital proteins for replication, would require co-infection of patient transduced cells by the GMO 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 the AAVrh.10cuARSA is delivered, and herpes brain infections are rare, the possibility that the vector could be mobilized after initial administration is very remote.

- The DNA insert of the GMO is packaged in the capsid of AAVrh10, an AAV serotype isolated in simians. The wild-type AAVrh.10 virus is known to infect simians but not humans. The presence of wild-type AAVrh.10 virus in humans has not been reported up to now.

- If there is a passage of the GMO in the blood of treated patient during the intracerebral delivery of the GMO, it is expected that that GMO may be detectable in the blood and urine of the treated patient few days after the intracerebral delivery of the GMO. The detection of the GMO in the urine of treated patients will be monitored after surgery (see below).

- Moreover, AAVrh.10cuARSA will be administered to the patient in a single administration by means of a neurosurgical procedure that uses a delivery system provided by the sponsor that consists of non-reusable microcatheters.

In spite of these precautions and characteristics of this vector, even an accidental exposure of the GMO to the environment (ex. spill of the vector) would very unlikely lead to an accidental introduction in human. Indeed:

- All healthcare professionals involved in the preparation and administration of AAVrh.10cuARSA to each patient will wear suitable gowns and gloves and will wear the appropriate mask 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 AAVrh.10cuARSA, the healthcare professionals involved in the clean-up procedure will also wear gowns and gloves, according to HCB recommendations.

- The clinical trial is monocentric and implies a Class 1 confinement. The neurosurgery operating room and the hospitalization rooms will be prepared according to HCB recommendations for gene therapy clinical trials. AAVrh10cuARSA (GMO) designed for this clinical trial has been classified as class I by the HCB (TG 5169, 2011).

- After surgery, waste containing the GMO, disposable and non-disposable surgical instruments or materials will be decontaminated according to HCB recommendations.

- The quantity of GMO viral particles released is low: one unique injection (a maximum of $4x10^{12}$ viral genome copies per patient administered in a total volume of 720µl/patient) to a limited number of patients (n= 5).

- The GMO, AAVrh.10cuARSA, can be specifically detected and quantified (see p 10). The GMO will be quantified in the urine of treated patient after the intracerebral administration of the GMO (see below).

Therefore, 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 environment to AAVrh.10cuARSA would not be harmful to humans, animals, or plants.

B. INFORMATION RELATING TO THE RECIPIENT OR DONOR ORGANISMS FROM WHICH THE GMO IS DERIVED

1 & 2 Recipient or donor organism characterization/ Name

The GMO is made of:

- Donor DNA sequences constituting the DNA insert of the non-replicative AAVrh.10cuARSA viral vector that originate from Human, Chicken, Rabbit, HerpesVirus and AAV2 (see table below).
- The viral capsid of AAV virus of serotype 10 which is the recipient of the DNA insert.

The GMO is therefore an AAV2-rh10 vector because its DNA insert contains the ITR of AAV2 virus and because the DNA insert is packaged in the capsid of AAVrh10.

Nature of Organism or element	Scientific name	Taxonomy	Reference	Phenotypic and Genetic characteristics
Insert donors				
Donor 1- Gene of interest	Homo sapiens	Homo- Primate	ARSA, NG_009260	cDNA from human ARSA gene (Arylsulfatase A). 1,52 kb. Type A
Donor 2- Enhancer	human Herpes virus 5 (CMV)	Cytomegalovirus, Herpesviridae	K03104.1	IE1 enhancer from human cytomegalovirus
Donor 3- promoter	Gallus gallus domesticus (chicken)	Galliform	E02199	promoter, splice donor and intron sequence from chicken ß-actin
Donor 4- Regulator	Oryctolagus cuniculus (rabbit)	Mammals Rodent	HBB2, V00882.1	Intron and splice acceptor of the rabbit β-globin gene
Donor 5- Regulator	Oryctolagus cuniculus (rabbit)	Mammals Rodent	HBB2, V00882.1	poly A sequence from β -globin gene
Donor 6-ITR (encapsulating sequence)	AAV2 wt	Parvovirus- Dépendovirus	K01624.1 (ITR 5') K01625.1 (ITR 3')	5' and 3' ITR from AAV2, 145 bp
Recipient- capsid	AAVrh.10	Parvovirus- Dependovirus	AY631965.1	Capsid of AAVrh10

3. Geographical distribution of the organism

(a) Indigenous to, or otherwise established in, the country where the notification is made:
Not known for AAV2 or AAVrh10.

(b) Indigenous to, or otherwise established in, other EC countries:
Not known for AAV2 or AAVrh10.

(c) Is it frequently used in the country where the notification is made?
No for the full GMO; yes for the different elements from which the GMO is derived

(d) Is it frequently kept in the country where the notification is made?
No for the full GMO; yes for the different elements from which the GMO is derived

4. Natural habitat of the organism

Wild-type (Wt) AAV, and specifically wt AAV2, are common in human. However, AAVrh.10 is a virus of simian origin and is not known to naturally infect humans.

5. Detection and Identification techniques

The identification and detection of donor DNA sequences are essentially based on PCR techniques. Restriction and sequencing allow distinguishing between the donor organisms from the vector.

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

AAV viruses are classified as Group / Class 1.

AAVrh10cuARSA (GMO) designed for this clinical trial has been classified as class I by the HCB (TG 5169, 2011).

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 (.)

In natural conditions, wild-type AAV2 virus is transmitted to human only. No data show the transmission to other species in these conditions. AAVrh.10 virus is not known to be transmitted to any other organism than monkeys.

AAVs are essentially transmitted through respiratory, digestive and sexual tractus.

The GMO is however incapable of replication

No AAV pathogenicity has been notified for the moment.

In human patients in whom they are administered, recombinant AAV are however potentially associated with risks of:

- Integration in the genome and therefore of oncogenesis
- Immune response against the capsid
- Transmission to germinal cells

However, in regards with preclinical data on AAV2-rh10 vectors (Sondhi et al., 2008; Sondhi et al. 2007; Piguet et al., 2012; Sevin and et al, in preparation), where no serious adverse event was reported, these risks are essentially theoretical.

8. Information concerning reproduction

Wild-type AAV2 replicates in cells that are co-infected by a virus presenting AAV helper functions (essentially adenovirus).

The AAVrh.10cuARSA is not replicable in the absence of wild-type AAV2 and AAV helper functions.

9. Survivability

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

Not applicable.

(b) relevant factors affecting survivability:

AAV viruses are destroyed with hydrochloride sodium 1%, phenol 5%, virocides (ex. Virkon®), heat, UV irradiations and high acidity or basicity (pH<2 and >12).

10. Ways of dissemination and factors affecting dissemination

Wild type AAV infections are common in human and probably occur from childhood. The ways of dissemination of wt AAV are poorly understood (Chen et al., 2005; Clark et al.; Schnepp et al., 2005).

The non-replicative AAVrh.10cuARSA (GMO) will be administered into the brain of patients with metachromatic leukodystrophy. There is very little risk that the GMO disseminates outside from the brain of treated patients during the neurosurgical procedure. As mentioned before, if there is a passage of the GMO in the blood of treated patient during the intracerebral delivery of the GMO, it is expected that the GMO may be detectable in the blood and urine of the treated patient few days after the intracerebral delivery of the GMO. The detection of the GMO in the urine of treated patients will be monitored after surgery (see below).

All measures will be taken to avoid the dissemination of the GMO during the filling of microcathethers and serynges and the neurosurgical procedure of intracerebral administration of the GMO according to HCB recommendations. Thus it is unlikely that AAVrh.10cuARSA will be disseminated into any ecosystem.

Previous genetic modifications of the recipient or donor organism already notified for release in the country where the notification is made (give notification numbers)
The same AAVrh.10cuARSA vector (GMO) was used for preclinical studies in France including toxicological and biodistribution in non-human primates and obtained necessary authorization from the CGG (numbers 5168 and 5169).

C. INFORMATION RELATING TO THE GENETIC MODIFICATION

1. Type of the genetic modification
Addition of enhancer/promoter sequences and human ARSA cDNA between the 5' and 3' ITR of AAV2
Packaging of the above DNA insert in the capsid of AAVrh10

2. Intended outcome of the genetic modification
Metachromatic Leukodystrophy (MLD) is a genetic disease that causes cerebral demyelination and neuronal loss leading to death within a few years.
The intracerebral injection of the GMO, AAVrh.10cuARSA vector, has a therapeutic objective: it aims to deliver the functional human ARSA enzyme to brain neurons and glial cells of MLD patients to arrest the course of their lethal disease.

3. (a) Has a vector been used in the process of modification?
Yes (-) No (X)
No viral vector has been used in the process of modification; only plasmids (see below)

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

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

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 : The production of the GMO (AAVrh.10cuARSA vector) relies on the transient transfection of HEK293T cells by two transfected plasmids: pPAK-MArh.10 and pAAV2-cu-ARSA. The constructions of these 2 plasmids have been made by molecular cloning.
The pPAK-MArh.10 plasmid provides the auxiliary adenoviral functions (genes VA, E2 and E4), as well as the rep gene from AAV2 and cap gene from AAVrh.10. It contains the kanamycin resistance gene. None of these sequences are present in the genome of the GMO once it has been produced.

The pAAV2-cu-ARSA plasmid contains the expression cassette necessary for the production of human ARSA enzyme located between the 5' and 3' ITRs of AAV2 virus. It also contains the kanamycin resistance gene. Only the expression cassette necessary for the production of human ARSA enzyme located between the 5' and 3' ITRs of AAV2 virus is present in the genome of the GMO once it has been produced.

6. Composition of the insert
The insert is the expression cassette of AAVrh.10cuARSA which codes for the human ARSA gene
(a) Composition of the expression cassette
- A "CU" promoter comprising :

- IE1 Enhancer from human cytomegalovirus (CMV) from human herpesvirus-5 : nt 152-533 of AAVrh.10cuARSA vector construct
- Promoter of chicken β-actin gene : nt 534-810
- Splice donor and intron of chicken β-actin gene: nt 811-1789
- Portion of intron and splice acceptor of the rabbit β -globin gene nt 1789-1880

- cDNA of the human ARSA gene: nt 1881-3433
- polyA from rabbit β -globin gene: nt 3434-3963

(b) Source of each constituent part of the insert
See above

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

- The IE1 enhancer from CMV derived from herpesvirus-5 + the chicken β-actin gene promoter, splice donor and portions of the intron from chicken β-actin gene + portion of intron and splice acceptor from ß-globin gene of rabbit constitute the CU promoter that allows expressing the cDNA of human ARSA gene.
- The cDNA from human ARSA protein allows producing a functional ARSA enzyme.
- The polyA from the rabbit β -globin gene allows increasing the stability of the RNA form the cDNA of human ARSA gene produced under the control of the CU promoter.

(a) Location of the insert in the host organism
-on a free plasmid (.)
- integrated in the chromosome (.)
- other, specify: The insert (expression cassette of AAVrh.10cuARSA) is located between the 5' and 3' ITRs of AAV2 and corresponds to the viral genome of the GMO (AAVrh.10cuARSA). The viral genome of GMO lacks the cap and rep genes of AAV2. In this way, the resulting GMO is incapable of replication. The schematic representation of the viral genome of the GMO is shown in the figure below.

Figure 1 : Schematic representation of the genome of AAVrh.10cuARSA vector (GMO). The insert is the expression cassette that spans from the CMV enhancer to the rabbit poly-A.

(b) Does the insert contain parts whose product or functions are not known?
Yes (.) **No (x)**

D. INFORMATION ON THE ORGANISM(S) FROM WHICH THE INSERT IS DERIVED

1.2. Nature and complete name

Manufacturing of the AAVrh.10cuARSA vector (GMO) relies on the transient transfection of HEK293T cells by two plasmids: pPAK-MArh.10 and pAAV2-cu-ARSA that are amplified before in E.Coli bacteria and are characterized by Aldevron (NO, USA).
pPAK-MArh.10 plasmid is used as a helper plasmid. It provides the auxiliary adenoviral functions (genes VA, E2 and E4), as well as the rep gene from AAV2 and cap gene from AAVrh.10. It contains the kanamycin resistance gene.

pAAV2cuARSA plasmid contains the genome of the AAVrh.10cuARSA vector, i.e, the cDNA of human ARSA enzyme, a constitutive CU enhancer/ promoter/intron sequence, a poly A sequence and the 5' and 3' ITRs of AAV2 . It has been generated using a modified pUC 19 backbone confering kanamycin resistance and harboring the elements for replication in E.Coli.

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 (.)

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)**

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: The GMO is non replicative; ie, it cannot enter an infectious cycle event in the presence of helper function. This is not the case for wt AAV2.

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

Yes: The GMO cannot replicate in contrast to the wild-type AAVrh10 with its AAVrh10 capsid and genome **(x)** No (.) Unknown (.)

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

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

But the GMO is incapable of replicating, even in the presence of helper functions, as rep and cap genes have been deleted from the AAV2 genome.

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

Yes (.) No (x) Not known (.)

2. Genetic stability of the genetically modified organism

During the production of the AAVrh.10cuARSA clinical batch several tests have been conducted to determine the genetic stability of AAVrh.10cuARSA:1) genomic structural identity test (Southern-blot and PCR-sequence); 2) Test of ARSA enzyme activity aiming to measure ARSA protein activity in cells transduced by AAVrh.10cuARSA vector; 3) Infectious Center Assay to detect and quantify potential replication of AAVrh.10cuARSA. All test results have confirmed the genetic stability of the non replicative AAVrh.10cuARSA vector.

In addition, genetic stability of the GMO in its container closure system before intracerebral administration is being monitored using the ARSA activity assay every 3 months from vialing of the vector during the first year and every 6 months during the second year and then annually thereafter if needed.
Stability has been demonstrated on the clinical lot up to present time of the IND writing at months 3, 6, 9 and 12.

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

Yes (.) No (x) Unknown (.)

4. Description of identification and detection methods

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

- Detection and quantification of viral DNA copies using qPCR. Primers span the expression cassette of AAVrh.10cuARSA genome.
- Detection of AAVrh.10 viral capsid (VP1, VP2 and VP3 proteins) with SDS electrophoresis followed by densitometry.

(b) Techniques used to identify the GMO in the environment

- Identity of the expression cassette: with enzyme restriction or DNA sequencing.
- Identity of viral capsid with SDS electrophoresis: identity is confirmed if the three bands corresponding to VP1, VP2 and VP3 are in stoechiometric proportions.

F. INFORMATION RELATING TO THE RELEASE

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

Metachromatic Leukodystrophy (MLD) is a rare genetic disease that causes cerebral demyelination and neuronal loss leading to death within a few years. The intracerebral injection of AAVrh.10cuARSA vector has a therapeutic objective: it aims to deliver the functional ARSA enzyme to brain neurons and glial cells of patients with MLD. In MLD patients where both alleles from the ARSA gene have a pathogen mutation, the ARSA enzyme latter is deficient.

The primary goal of the trial is the assessment of tolerance (safety) of the proposed treatment in 5 patients with early onsets forms of MLD. The secondary goal of the trial is the assessment of clinical benefits in the 5 enrolled patients.

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) for AAVrh10 No (x.) for AAV2

3. Information concerning the release and the surrounding area

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

The intracerebral injection of AAVrh.10cuARSA will take place in the neurosurgery operating room of Necker Hospital (149, rue de Sèvres, 75743 Paris Cedex 15, Ile de France, France).

Patients will be kept in the neurosurgery room until the end of the surgery. They will then be transferred in a single room in the same hospital service until the number of AAVrh.10cuARSA vector genome copy is in their urine below $5x10^4$ cp/ml. Thereafter, they will be transferred to a single room of the neuropediatric department of Bicêtre hospital (78 rue du général Leclerc, 94275 Le Kremlin Bicêtre, Ile de France, France).

(b) Size of the site (m^2): 100.04 m^2

(i) actual release site (m^2): 0.04 m^2 (brain of MLD patients)

(ii) wider release site (m^2): ~100 m^2 (pharmacy of Necker hospital, neurosurgery room and confinement room in Necker hospital)

(c) Proximity to internationally recognized 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:

5 patients will receive a maximum of 4.10^{12} copies of the AAVrh.10cuARSA genome in a total volume of 720 µl that will be injected in the brain of each MLD child.

(b) Duration of the operation:

The neurosurgery should last 3 hours approximately for each patient. Patients will only be treated once. The whole trial will last for 5 years.

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

After the neurosurgery, all treated patients will be transferred in a single room in the same hospital service until the number of AAVrh.10cuARSA vector genome copy is in their urine below $5x10^4$ cp/ml, at which point they will be transported to Bicêtre hospital.

Biological samples possibly containing AAVrh.10cuARSA genome will be collected, and decontaminated as recommended by the "Haut Conseil des Biotechnologies".

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

Not applicable

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.

As this is the first administration of AAVrh.10cuARSA to human, its potential impacts on human health are not known. However, recombinant AAV of different serotypes have been used in approximately 20 clinical trials. They had no environmental impact, nor did they have an impact on human health.

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 …Human

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

The intracerebral injection of AAVrh.10cuARSA vector has a therapeutic objective: it aims to deliver the functional ARSA enzyme to brain neurons and glial cells of patients with MLD. In MLD patients where both alleles from the ARSA gene have a pathogen mutation, the ARSA enzyme latter is deficient.

The GMO viral vector is anticipated to interact in human with the following mechanism, upon intracerebral injection:

Viral particles will transduce mostly neurons but also oligodendrocytes. Each of these cell types are deficient for the ARSA enzyme in MLD patients. Upon entry into the cells, viral particles go to the endosomes, are

uncoated and then the recombinant genome is transported into the nucleus where it undergoes a series of molecular transformations that result in its establishment as double stranded deoxyribonucleic acid (DNA) molecule that forms episomal concatemers. In non-dividing cells, these concatemers remain intact for the life of the cell.
The cellular machinery then produces the ARSA protein from this stable, non integrated double stranded DNA. The produced ARSA protein will complement the deficicency by two mechanisms:

1. The ARSA enzyme will reach the lysosomes of brain transduced cells which contain and express the AAV-borne transgene and will degrade the accumulated catabolites (sulfatides),
2. The ARSA enzyme will be released from brain transduced cells, recaptured by distant cells, routed towards their lysosomes and then degrade the accumulated sulfatides (cross-correction)

Hence, a relatively limited group of genetically modified cells may allow for the correction of extended brain territories. Transduced cells will express and deliver the enzyme continuously, thus constituting an intracerebral permanent source of ARSA enzyme production.

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 (.)

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

AAVrh.10cuARSA is incapable of replicating. It can only do so if it infects a cell that is co-infected by both a 1) wild-type AAV2 and 2) an auxiliary virus (adeno or herpesvirus). There is therefore a theoretical risk that the GMO vector could infect a human cell that is already infected by wild-type AAV2 and an auxiliary virus, which would result in the replication of the GMO. 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

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 (brain) expression of human ARSA enzyme

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.):

Potential benefit of brain gene therapy using AAVrh.10cuARSA vector for MLD patients

INSERM U745 has demonstrated in MLD mice the proof of efficacy of intracerebral gene therapy (Piguet et al., 2012). The results indicate that AAVrh.10cuARSA brain delivery has marked and rapid beneficial effects on sulfatide storage and pathological abnormalities in the brain of MLD mouse model. There was a clear evidence of ARSA expression in oligodendrocytes and correction of oligodendrocyte specific sulfatide species in these cells, a crucial point to target a demyelinating disease.
Using the same neurosurgical procedure and same 1X dose ($4x10^9$ vg/cm3), INSERM U745 has demonstrated in NHPs that the intracerebral injection of AAVrh.10cuARSA vector led to a significant overexpression (over 14% increase in activity) of recombinant ARSA in the whole brain without significant neuropathological abnormalities related to an immune reaction against the transgene into the brain (Sevin et al, in preparation).

Studies of asymptomatic heterozygous individuals that have one allele with a deleterious mutation of ARSA gene and on the other allele with a pseudodeficient variant of the ARSA gene suggest that 10% of normal ARSA activity level is sufficient to prevent the apparition of MLD symptoms (von Figura, 2001). It is however not known if this percentage of residual activity would be sufficient to arrest or better to reverse brain demyelination in affected MLD patients.
Therefore, results obtained in non-human primates indicate that the increase of ARSA activity achieved through the injection of a total of 4.10^{12} viral particles in MLD patients could lead to a therapeutic level of ARSA enzyme activity with a good safety profile.

Diffusion of AAVrh.10cu ARSA vector in off target tissues is limited and decreases with time
In NHPs injected with a 1X dose of AAVrh.10cuARSA and studied 3 months after vector administration, AAVrh.10 vector genome was mostly found in the CNS where it diffused largely. In accordance with previous studies on AAV vectors, very low level of vector genome was observed in peripheral tissues of some animals (liver, spleen, kidney, lymph node), but not in the testis (Sevin et al, in preparation).
Formal biodistribution study performed in NHPs with a 5X dose of AAVrh.10cuARSA vector showed that high levels of AAVrh.10cuARSA were found in different parts of the central nervous system. Vector genome was also quantified (limit of quantification = 50 cp/µg DNA) in peripheral organs such as the liver, spleen, lymph nodes and blood, but not in gonads. A decrease in vector genome copy was observed overtime (from D7 to D90) in the peripheral organs of most NHPs, likely reflecting the loss of episomal forms of AAVrh.10cuARSA in dividing cells in peripheral organs. Importantly, the AAVrh.10cuARSA vector was no longer detectable in urine at day 7, even using this 5X dose.

Tolerance of AAVrh.10cuARSA vector intracerebral administration
NHPs injected with the 1X dose planned for MLD patients (with respect to brain volume) of AAVrh.10cuARSA showed normal clinical, behavioral and standard biological parameters during the 3-months study, attesting for the perfect tolerance of the procedure. Transient abnormal hypersignals restricted to the injection site observed at brain MRI and traumatic inflammatory lesions observed at brain pathology were similar in PBS and AAVrh.10-injected hemispheres. However, perivascular cuffs were more prominent in the AAVrh.10cuARSA injected hemispheres, particularly in one NHP, suggesting a mild and limited inflammatory reaction. Nevertheless, no sign of demyelination or neuronal degeneration was observed.
Using the same procedure, toxicology grade AAVrh.10cuARSA was then administered at the 5X dose. There was no evidence for toxicity based on the clinical, neurobehavioral and standard blood parameters, or microscopic examination of peripheral tissues. Traumatic lesions due to neurosurgical procedure were similarly observed in control (vehicle) and AAVrh.10cuARSA-treated animals at early time-point (Day 7) and decreased significantly over time. The main adverse side effect, observed at the neuropathological level but without clinical symptoms, was a moderate to severe multifocal lymphoplasmacytic infiltration associated with spongiosis of myelin, without neuronal cell loss observed at Day 90 in the brain of 5/6 NHPs that received AAVrh.10cuARSA, likely the consequence of host immune response of NHPs against the human ARSA transgene.
The fact that the 1X primate group showed little or no immune response-related adverse effects suggests a dose-dependent immune response to the human ARSA transgene, which was to some extent predictable, because human and simian ARSA polypeptides differ by 17 amino-acids. We can anticipate that this is unlikely to occur in MLD patients who will have a mutated ARSA enzyme that differs from normal ARSA enzyme by one or two amino acid, and therefore conclude that AAVrh.10cuARSA has a good safety profile at the dose we propose to administer to MLD patients.

9. Possible environmentally significant interactions with biogeochemical processes (if different from the recipient or parental organism)
No

H. INFORMATION RELATING TO MONITORING

1. Methods for monitoring the GMOs

-Determination of AAVrh.10cuARSA concentrations in urine, blood and CSF of treated patients by quantitative polymerase chain reaction (QPCR) analysis
- Changes from baseline in anti-ARSA and anti-AAVrh.10 antibodies in serum and CSF of treated patients.

2. Methods for monitoring ecosystem effects

Not applicable

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

Transfer of donated genetic material from the patient to other organisms is impossible. The batch that is going to be used in the trial is indeed tested and manufactured in order to rule out the presence of replication-competent viruses. In the very improbable case of either transfection by the AAVrh.10cuARSA vector particle or of a direct transfer of vector DNA to other organisms, qPCR would allow to detect the transferred genetic material.

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

This clinical trial is a monocentric trial that takes place in the neurosurgery service of Necker hospital, and the neuropediatric service of Bicêtre hospital, Paris.

5. Duration of the monitoring:

The clinical trial will last 24 months following neurosurgery. Thereafter, patients will be monitored for the rest of their lives to assess for the tolerance of the treatment.

6. Frequency of the monitoring

Every 3 months for 2 years, then every 6 months for 3 years then every year for the whole life.

I. INFORMATION ON POST-RELEASE AND WASTE TREATMENT

1. Post-release treatment of the site

The pharmacy, the neurosurgery room, the hospitalization rooms in Necker and Bicêtre hospitals, as well as waste, will be treated for decontamination according to recommendations of the Haut Comité des Biotechnologies.

2. Post-release treatment of the GMOs

Not applicable

3. Waste

(a) Type and amount of waste generated

-Waste generated during the neurosurgery (catheters, polyethylene tubes, syringes, tubes, remaining vector).
-Waste generated during post operation follow up (compresses, gloves,)
-Urines from treated patients

(b) Treatment of waste

All of these generated wastes are disposable. After use, they are decontaminated as recommended by the "Haut Comité des Biotechnologies" and burnt.
The total volume of uninjected AAVrh.10cuARSA vector that remains will be of 164 µl per patient.
The total volume of waste will be sufficiently low to be contained in DASRI containers.

J. INFORMATION ON EMERGENCY RESPONSE PLANS

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

AAVrh.10cuARSA will be administered in the operating room by neurosurgical injections directly into the brain of MLD patients. Therefore, the spread outside 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

AAVrh.10cuARSA can be removed by decontamination according to recommendations of the Haut Comité des Biotechnologies.

Specifically, in the case of:

- Introduction of vector in the eyes: Wash the eyes during 15 minutes, and go to hospital emergency.
- Accidental injection of the vector: Wash the injected zone with soap and water, abundantly, and go to the hospital emergency.
- Aerosol emissions: 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 a serious undesirable effect associated to AAVrh.10cuARSA vector occurred, the inclusion of new patients in the clinical trial would be interrupted until the origins of this event would be determined and that measures of prevention of such an event would be taken.

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