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: Belgium
(b) Notification number: t.b.d.
(c) Date of acknowledgement of notification
(d) Title of the project: "Microdystrophin (GNT0004) Gene Therapy Clinical Trial in Duchenne Muscular Dystrophy: A phase I/II/III study with a dose determination part followed by an efficacy and safety evaluation, quadruple blind placebo-controlled part and then by a long-term safety follow up part, in ambulant boys".
(e)Proposed period of release: May 2026 – November 2031

2. Notifier
Name of institution or company:
GENETHON

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

other, specify (kingdom, phylum and class)

(b) Identity of the GMO (genus and species)
Family: Parvoviridae
Genus: Dependoparvovirus
Species: Adeno-associated parvovirus

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

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. GNT0004 is a recombinant AAV vector in which the wild-type AAV rep and cap genes are replaced by the hMD1 expression cassette. Thus, GNT0004 is unable to replicate independently, even in the presence of a helper virus, such as adenovirus, vaccinia virus or herpes simplex virus to achieve their productive cycle since it lacks the rep and cap genes required for replication and packaging, respectively. Therefore, GNT0004 is expected to be highly genetically stable. GNT0004 vector

genomes is assayed by specific qPCR before release. All batches are sequenced to confirm the absence of any changes.

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) France & Spain

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

Yes (X) No ()

If yes:

- Member State of notification FR & ESP
- Notification number

FR: Ref 21066895 – GNT-016
ESP: Ref B/ES/25/20

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

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

Yes (X) No ()

If yes:

- Member State of notification GB
- Notification number

GB Gosh: GMOSC #14 – GNT-016
GB New castle: GMSC – GNT-016

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

The release of GNT0004 as described in this application is not expected to result in adverse environmental impact, for the following reasons:

1. **Minimal risk of GNT0004 release in the environment:** GNT0004 will be given as a single peripheral intravenous (IV) infusion in study participants (6-10 years old males) with Duchenne muscular dystrophy (DMD), which is a lethal X-Linked neuromuscular disorder. GNT0004 will be administered to the patients in a controlled environment in a hospital setting by trained healthcare professionals.
2. **Lack of pathogenicity of the parental virus and the GMO:** Despite an estimated seroprevalence of up to 80% for some common human serotypes, no pathogenic effects of AAVs have been identified. The modifications which have led to the generation of the GMO, GNT0004, have not raised the pathogenicity.
3. **Replication-incompetent GMO:** GNT0004 is a non-pathogenic recombinant AAV vector that lacks all AAV viral genes and cannot replicate without AAV-specific helper functions and helper virus activities. GNT0004 replication could only occur in the extremely unlikely event of a host cell being infected by wild-type AAV and a helper virus such as human adenovirus, vaccinia virus or herpes simplex virus. If replication occurred, the only expected products would be GNT0004 and WT AAV, both intrinsically non-pathogenic viruses.
4. **Minimal risk of transmission by GNT0004 viral shedding**: GNT0004 is replication-incompetent and is not expected to survive, multiply or disperse if it were to be shed intact from the patients administered with GNT0004. AAV-based gene therapies are known to shed via bodily fluids. It has been shown consistently that vectors are shed for a short period of time, but then become undetectable in bodily fluids. The viral load shed in bodily fluids is expected to be low, compared to the necessary dose required to result in a significant gene expression in humans. Clearance of GNT0004 vector genomes in blood, urine, saliva and fece samples will

be assessed before and after GNT0004 administration. The frequency of samples post administration was every week until week 8, then monthly and every 3 months up to 1 year. This post administration vector shedding follow-up lasts until the clearance of vector (defined as indetectable results are obtained in two consecutive sample timepoints) in each type of biologic excretion/fluid for each individual patient who was enrolled in Part 1. From all doses administered in VOI-04, VOI-04-LT and 20180053TRP studies clearance of vector DNA has been observed in urine samples 8 weeks after administration in GRMD dogs and 93D after administration in WT rats, and because AAV vectors are non-replicating, the Sponsor considers the risk of transmission to non-treated individuals is low, with negligible environmental impact.

5. **Minimal risk of insertional mutagenesis:** No clinical trials to date with AAV have reported incidences of insertional mutagenesis. In addition, based on reviewed publications and on the results obtained in nonclinical studies the risk of adverse effects linked to vector integration, and increase tumourigenicity is considered very low.
6. **Tissue-specific transgene expression:** GNT0004 uses the SpC5.12 synthetic muscle and cardiac restricted promoter which restricts expression of the transgene hMD1.
7. **Minimal risk associated with the transgene:** Overall, results to date indicate no significant vector-related toxicity and demonstrate the feasibility of gene transfer with peripheral intravenous (IV) infusion administration. No genes for toxins, potential oncogenes, growth factors or other genes that could be potentially harmful have been inserted into the GMO. Overall, the risk to people (like other non-participant patients, site staff, etc), animals, microorganisms and the environment exposed to the GMO is negligible.

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	Dependoparvovirus
(iii)	species	Adeno-associated parvovirus
(iv)	subspecies	
(v)	strain	
(vi)	pathovar (biotype, ecotype, race, etc.)	rAAV8-hMD1
(vii)	common name	Recombinant Adeno associated virus

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) No (.)

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,	X hosts are humans

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

5. (a) Detection techniques
AAV can be detected by qPCR with primers specific to the virus genome.

(b) Identification techniques
AAV can be detected by qPCR with primers specific to the virus genome. It can also be identified by sequencing.

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
The AAV meets the definition of biological agent of Risk Group 1 according to Directive 2000/54/EC, defined as “biological agent that is unlikely to cause human disease”.

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

If yes:

(a) to which of the following organisms:

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

(b) give the relevant information specified under Annex III A, point II. (A)(11)(d) of Directive 2001/18/EC

8. Information concerning reproduction

(a) Generation time in natural ecosystems:
The generation time of wild-type AAV in a natural ecosystem will be very high, depending on the timing of coinfection with helper virus.

(b) Generation time in the ecosystem where the release will take place:
AAV replicates only in the presence of a helper virus. Replication competent AAV generation time of GNT0004 is not relevant since it lacks the rep and cap genes that are required for reproduction of rcAAV.

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

(d) Factors affecting reproduction: Wild type AAV which have humans as host are non-pathogenic viruses, and can only replicate in the presence of helper virus. Compared to the wild type virus, the safety profile of recombinant AAV particles, such as rAAV8-hMD1, was further increased by generating "gutted AAV vector" as they do not contain the Rep and Cap genes. As a result, rAAV8-hMD1 is replication-defective even in the presence of a helper virus. Genome replication of rAAV8-hMD1 can only occur in presence of both helper virus and wild type AAV virus. In case this occurs, the continued expression of the adeno helper genes and the wild type AAV genome are necessary, which is unlikely to occur. This makes the replication of rAAV8-hMD1 unlikely and self-limiting.

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
AAVs do not form survival structures

(b) relevant factors affecting survivability:

AAV can persist in the environment for extended periods of time (thought to be on the order of several weeks) as they are relatively resistant to dehydration. They could potentially survive for protracted periods in the environment and can remain infectious, thought to be in the order of several weeks. AAV, being a non-enveloped virus, can tolerate a wide range of pH (3-9) and elevated temperatures (55°C for 1 hour). However, as with all viruses, replication of AAVs cannot occur outside of a host cell. Treatment by autoclaving followed by incineration or immersion in chlorine bleach (Javel water) with 1% final active chlorine for 20 minutes, will destroy viral particles.

10. (a) Ways of dissemination

AAVs may be transmitted through direct or indirect contact with an affected individual patient via vector shedding. AAVs may be transmitted through inhalation, ingestion and possibly sexual transmission. However, AAV is a dependovirus and hence cannot replicate without co-infection with helper virus.

(b) Factors affecting dissemination

Replication of wildtype AAVs is only possible in cells that have been coinfected with a helper virus (e.g., Adenovirus, vaccinia virus or 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).

Not Applicable

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 modifications in GNT0004 was to generate a recombinant AAV lacking viral genes, with the exception of inverted terminal repeats (ITRs), so that the vector would be replication incompetent and serve only to introduce functional transgene encoding the hMD1 gene to patients with Duchenne Muscular Dystrophy (DMD) who have dystrophin gene mutation. This gene therapy is designed to deliver in DMD patient, whatever their genetic defect, an optimized microdystrophin protein, which is not the full dystrophin protein but would be able to significantly delay or markedly slow down the progression of the disease over time as already evidenced in AAV-microdystrophin injected rodent and canine dystrophic models of DMD. The microdystrophin protein is a non-toxic protein which is expected to be metabolised naturally and in the same manner as endogenous human dystrophin.

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

Yes (X) No (.)

If no, go straight to question 5.

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

Yes (X) No (.)

If no, go straight to question 5.

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

(a) Type of vector

plasmid	(X)
bacteriophage	(.)
virus	()
cosmid	(.)
transposable element	(.)
other, specify	

(b) Identity of the vector
Two helpers plasmids are used to produce GNT0004 drug substance (DS). Helper p5e18_VD2-8 plasmid contains the AAV rep and cap genes and pXX6 helper plasmid contains an expression cassette for a therapeutic gene of interest (GOI) flanked by the AAV2 inverted terminal repeats (ITR). Both are used in the rAAV vector production process by means of transient transfection in human HEK293T cells.

(c) Host range of the vector
Mammalian cells

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

Yes () No (X)

antibiotic resistance	(X)
other, specify	

Indication of which antibiotic resistance gene is inserted :
Kanamycin

(e) Constituent fragments of the vector
The fragments of the vector that end up in the GMO are the inverted terminal repeats (ITRs), Spc512 promoter, Codon optimized sequence from the human microdystrophin ORF hMD1co, Poly-A signal, and the AAV2 capsid protein.

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

(i)	transformation	(.)
(ii)	electroporation	(.)
(iii)	macroinjection	(.)
(iv)	microinjection	(.)
(v)	infection	(.)
(vi)	other, specify	transient transfection in human HEK293T cells.

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 insert contains a synthetic muscle and cardiac restricted promoter, codon optimized sequence from the human microdystrophin, and a polyadenylation signal. The cassette is flanked by the AAV2 inverted terminal repeats.

(b) Source of each constituent part of the insert.
Muscle and cardiac restricted promoter: synthetic
Codon optimized sequence from the human microdystrophin: Homo sapiens
Polyadenylation sequence: mammalian
AAV2 inverted terminal repeats: are viral DNA sequences in the vector.

(c) Intended function of each constituent part of the insert in the GMO
Muscle and cardiac restricted promoter: intended to drive gene expression
Codon optimized sequence from the human microdystrophin: gene transfer
Polyadenylation sequence: terminate transcription of the gene
ITRs: to facilitate replication and packaging of the cassette into the capsid.

(d) Location of the insert in the host organism

- on a free plasmid (.)
- integrated in the chromosome (.)
- other, specify In the recombinant AAV 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 (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 …
(iii) genus Homo
(iv) species Sapiens
(v) subspecies …
(vi) strain …

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

3. Is the organism significantly pathogenic or harmful in any other way (including its extracellular products), either living or dead?
Yes (.) No (X) Not known (.)
If yes, specify the following:

(b) to which of the following organisms:

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

(b) are the donated sequences involved in any way to the pathogenic or harmful properties of the organism
Yes (.) No (X) Not known (.)

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

4. Is the donor organism classified under existing Community rules relating to the protection of human health and the environment, such as Directive 90/679/EEC on the protection of workers from risks to exposure to biological agents at work?
Yes (.) No (X)
If yes, specify

5. Do the donor and recipient organism exchange genetic material naturally?
Yes (X) No (.) Not known (.)

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
GNT0004 viral genome has been significantly modified compared to the parental AAV by removing all viral genes and replacing them with an expression cassette. This modification has made GNT0004 unable to replicate independently, even in the presence of helper viruses, since it lacks the rep and cap genes required respectively for replication and packaging. The only remaining viral sequences are the ITRs which are noncoding sequences. Thus, GNT0004 contains no native viral coding genes. GNT0004 replication could only occur in the extremely unlikely event of a host cell being infected by wild-type AAV (providing the rep and cap functions) and a helper virus such as adenovirus, vaccinia virus or herpes simplex virus.

(c) is the GMO in any way different from the recipient as far as dissemination is concerned?
Yes (X) No (.) Not known (.)
Specify
As GNT0004 replication could only occur in the extremely unlikely event of a host cell being infected by two separate viruses, a wild type AAV and a helper virus such as human adenovirus, vaccinia virus or herpes simplex virus, the likelihood of dissemination is much lower than that of wild-type AAV.

(d) 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, encoding hMD1, is not expected to result in development of pathogenicity. Removal of viral genes in making the vector would be expected to further reduce any risk of pathogenesis. Thus, neither wild type AAVs or GNT0004 are known to have pathogenic effects.

2. Genetic stability of the genetically modified organism
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. Based on this, GNT0004 is also expected to be genetically stable. The integrity of the vector genome has been confirmed.

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. Consequently, AAV fulfils the definition of a group 1 biological agent according to the Directive 2000/54/EC (a biological agent that is unlikely to cause human disease).

4. Description of identification and detection methods

(a) Techniques used to detect the GMO in the environment
GNT0004 can be detected by quantitative Polymerase Chain Reaction (qPCR).

(b) Techniques used to identify the GMO
GNT0004 can be identified by qPCR and sequencing.

F. Information relating to the release

1. Purpose of the release (including any significant potential environmental benefits that may be expected)
GNT0004 is to be released for the purpose of a clinical trial (protocol GNT-016-MDYF) to determine the efficacy and safety, of a single peripheral intravenous (IV) infusion of GNT0004 in subjects with Duchenne Muscular Dystrophy.

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 release will be carried out in the context of a clinical trial at the site mentioned below.

3. Information concerning the release and the surrounding area

(a) Geographical location (administrative region and where appropriate grid reference):
Site 1: Brussels University Hospital - Queen Fabiola Children's University Hospital
Avenue Jean Joseph Crocq 15
1020 Brussels
Belgium

Site 2: UZ Leuven
Herestraat 49
3000 Leuven
Belgium

Site 3: HOPITAL DE LA CITADELLE
Boulevard du 12e de Ligne 1
4000 Liège
Belgium

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

(i) actual release site (m^2): N/A.

(ii) wider release site (m^2): N/A.

.

(c) Proximity to internationally recognised biotopes or protected areas (including drinking water reservoirs), which could be affected:
Not applicable as GNT0004 will be administered in a controlled 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.
Not applicable as GNT0004 will be administered in a controlled hospital setting. Thus, it is not anticipated to come into contact with plants, animals or soil.

4. Method and amount of release

(a) Quantities of GMOs to be released:
GNT0004 will be administered to patients as follow:
Part 2: 3.0×10^{13} vg/kg

(b) Duration of the operation:
The duration of the IMP administration is about 3 hours. The study duration is defined for each subject as the date signed written informed consent is provided over 5 to 6 years after IMP infusion.

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

As per applicable biosafety guidelines for Biosafety Class 1 genetically modified organisms (WHO Laboratory Biosafety Manual, 2004; Annex IV of Dir 2009/41/EC), the following standard biosafety practices are typically followed by medical facilities when handling potentially biohazardous materials: GNT0004 is an investigational drug shipped to sites in 4 mL type I glass vials (primary container) containing 2.5 mL of product stored in frozen form at a -80°C ± 10°C. Each IMP vial is packaged in an individual sealed box (secondary packaging). The required number of GNT0004 vials are shipped by a specialized courier from the manufacturing site to the unblinded Pharmacist or designee at clinical trial site in line with standard recommendations for the transport of biohazardous materials.

On site transportation occurs according to local sites procedures for the transportation of biohazardous material. The product will be diluted to the assigned concentration by mixing an appropriate amount of 0.9%NaCl IV bag containing 0.25% (w/v) of human serum albumin HSA. The total volume of injection is around 200 mL. Once diluted, GNT0004 IMP is administered by a unique intravenous infusion with an electric pump.

There is Pharmacy Manual available to be provided to the sites' pharmacy. Since GNT0004 is considered Biosafety Level 1 and is used in a clinical trial, it's usage will be restricted to hospital facilities which will have been audited for dealing with biologic hazardous and infectious material, including storage and waste management. All involved personnel at the site will be trained in best biosafety practices to be applied during thawing, transport to the administration room (closed infusion bag in a double packaging with the outer packaging consisting of a leakproof container) , precautions during administration and disposal of any biological waste. GNT0004 is used and approved as per the clinical study protocol by both regulatory agencies and Ethics Committees 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 returning IMP.

- **Training of hospital personnel:** The Sponsor will also provide the site with a pharmacy Manual that includes directions for documenting the control of the IMP from the time of receipt at the trial site until final accountability and destruction. 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 the GMO 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.

GNT0004 will only be handled by trained personnel and in the event that a spillage and/or accidental exposure did occur, the product is non-pathogenic and non-replicative, limiting spread and risks to the environment or personnel.

Personnel handling GNT0004 will wear personal protective equipment (PPE) (laboratory coats, gowns, gloves,safety glasses and overshoes) in line with standard local procedures for BSL-1 products.

An appropriate spill kit will be available in the areas where GNT0004 is prepared and administered in line with standard local procedures for BSL-1 products.

Established standard local procedures for handling potential biohazardous materials such as patient samples/fluids and medical waste (autoclaves, sharps bins, incinerators, disinfectants, and appropriate cleanable surfaces) will be followed.

As GNT0004 is non-replicative, shed viral 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 as administration of GNT0004 will occur in 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.

GNT0004 has been well tolerated and no significant safety signals have emerged.

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)	…
(ii)	family name for plants	…
(iii)	genus	Homo
(iv)	species	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)

GNT0004 contains a gene encoding for the human microdystrophin protein. After IV infusion to patients with Duchenne Muscular Dystrophy (DMD) who have dystrophin gene mutation, it's expected to significantly delay or markedly slow down the progression of the disease over time.

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 GNT0004 that could represent potential hazard. As GNT0004 is 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 GNT0004 is not expected to affect any non-target organisms, either directly or indirectly. Minimal exposure, such as environmental exposure, to organisms other than the subjects receiving GNT0004 as part of the study would not be of a sufficient dose to represent significant gene expression or potential safety risks.

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 GNT0004 is a replication incompetent, 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 GNT0004 is a replication incompetent, it 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

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:
GNT0004 is infused directly to the subject in the hospital setting. It is only expected to be shed in study subjects' bodily fluids to a limited extent. In addition, as GNT0004 vector is non-replicative, shed viral particles transmission and gene transfer to organisms other than the study subjects is considered unlikely. Therefore, due to the incapacity of replication, the non-infectious nature of the shed DNA and the negligible amounts shed, the risk to the environment can be considered negligible.

(b) from other organisms to the GMO:
The probability of homologous recombination of GNT0004 with related viruses that could lead to variants of the GMO is highly unlikely. Furthermore, the regions of homology between GNT0004 and wild type AAV are limited to the ITRs since the rep and cap genes are deleted from the recombinant vector.

(c) likely consequences of gene transfer:
While recombination between GNT0004 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 be packed into an AAV particle.

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.):
Other than viral shedding from the subject there have been no reports of ecological impact of recombinant AAV vectors.

9. Possible environmentally significant interactions with biogeochemical processes (if different from the recipient or parental organism)
GNT0004 is not known or predicted to have an impact on biogeochemical processes.

H. Information relating to monitoring

1. Methods for monitoring the GMOs
Viral shedding will be closely monitored during the clinical trial, including safety and efficacy assessments.

2. Methods for monitoring ecosystem effects
No monitoring for 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.
Quantitative PCR could be used to detect transfer, however, there have been no reports of such a transfer from the injected subject to other organisms.

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 in various body fluids (blood, urine, saliva and feces) until the end of the trial period.

6. Frequency of the monitoring
Viral shedding will be assessed prior GNT0004 administration and in samples taken several times throughout the study.

I. Information on post-release and waste treatment

1. Post-release treatment of the site

Decontamination of the GNT0004 preparation area and administration room after completing administration per standard local procedures with a disinfectant with documented efficacy against AAV. Effective disinfectants include chlorine bleach (Javel water) with 1% final active chlorine for 20 minutes (or locally approved equivalent viricidal agent).

2. Post-release treatment of the GMOs

All unused GNT0004 vials will be returned to the sponsor or designed at the end of the study. Final drug accountability will be verified by the sponsor or designee.

3. (a) Type and amount of waste generated

- Albunorm bottles.
- Number of used vials will depend on the patients assigned to any of the dose groups in the study site.
- Non-used thawed vials.
- Any other disposable consumables/instruments used during the handling, dose preparation and administration procedures.
- Non-disposable instruments such as the designated study pipettes, plastic trays that have been used during the dose preparation and administration procedures and have potentially come into contact with GNT0004.
- Paper towel or other solid waste such as biobins, sharps containers, PPE and injection connection tubin.

3. (b) Treatment of waste

All waste is to be disposed according to the local standard procedures in a manner consistent with the standard practice of the institution for waste with biological risk. In the medical facility, this will involve containment in sharps bins or clearly marked bags (e.g., biohazard, medical waste) prior to autoclaving and/or incineration either on or off site.

Dispose of Albunorm bottle in accordance with the procedure of the pharmacy.

Solid waste (used vials, non-used thawed vials, syringes, and other objects that may potentially come into contact with the IMP) must be disposed of as infectious clinical waste (i.e. DASRI in France) in specific containers, labelled GMO.

J. Information on emergency response plans

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

GNT0004 will be prepared/administered in a hospital environment.
Accidental spillage of the IMP must be contained using absorbent paper covered with a suitable sodium hypochlorite solution.
Handle with personal protective equipment (wear gloves, coats, and protective glasses. When outside the Class II BSC or equivalent, also wear an FFP2 mask).
Cover the area of the spillage and the broken pieces of vial (if applicable) with absorbent paper or other absorbent material.
Soak with chlorine bleach (Javel water) 1% final active chlorine concentration or Virkon 1% (volume identical to the spilt volume).
Leave in contact for 20 minutes.
Pick up all material, working from the outside in, destroy the contaminated objects by autoclaving followed by incineration or immersion in chlorine bleach (Javel water) with 1% final active chlorine for 20 minutes, then dispose of as infectious clinical waste.

Remove traces of disinfectant from the area of the spillage by thoroughly wiping the surface with water.

If a biological product is splashed on the skin, wash the affected area with aseptic soap and water, then rinse.Remove all contaminated clothing or soiled elements. Should local or systemic signs emerge, see a doctor.

If a product is splashed in the eyes (or mucosae), the eyes must be rinsed, using water, also the eyelids, for at least 15 minutes, holding the eye open. Obtain medical attention if soreness or redness persists.
If a product has been ingested do not induce vomiting. Obtain medical attention immediately. Never give anything by mouth to an unconscious or convulsing person
If a product has been inhaled, Remove from exposure. If there is difficulty in breathing, give fresh air. Obtain medical attention if symptoms occur.
In case of needle-stick injury do not bleed. Immediate cleaning of the injured skin area with soap and water and rinsing. Antisepsis with chlorinated derivative (Dakin® or chlorine bleach with 2.6% active chlorine diluted 1/5) or polyvidone iodine in dermal or defective solution, 70 ° alcohol (at least 5 min).

Report a workplace accident (contact with a biological vector). Schedule a medical visit (follow hospital procedure).

2. Methods for removal of the GMO(s) of the areas potentially affected.
Handle with personal protective equipment i.e., wear gloves, coats and protective glasses. When outside the Class II BSC, also wear an FFP2 mask.
Soak with chlorine bleach (Javel water) 1% final active chlorine concentration or Virkon® 1%.
Note that bleach is usually incompatible with stainless steel Class II BSC, is such case Virkon® 1% will be preferred.
Leave in contact for 20 minutes.
Pick up all material, working from the outside in, destroy the contaminated objects by autoclaving followed by incineration or immersion in chlorine bleach (Javel water) with 1% final active chlorine for 20 minutes, then dispose of as infectious clinical waste.
Remove traces of disinfectant from the area of the spillage by thoroughly wiping the surface with water.

3. Methods for disposal or sanitation of plants, animals, soils, etc. that could be exposed during or after the spread
Not applicable as the administration of GNT0004 will occur in a controlled hospital setting, therefore, it is not anticipated that it will come into contact with plants, animals or soil.
Furthermore, GNT0004 is not capable of infecting plants or microbes.

4. Plans for protecting human health and the environment in the event of an undesirable effect
Clinical staff will be trained and supported as well as follow local standard procedures for handling and disposal of genetically modified organisms and biological hazards. The material that has been in contact with the GMO must be disposed of as biohazardous waste. Furthermore, safety recommendations and guidance on the management of incidents related to GNT0004 are provided in the safety instructions for investigators and staff (gloves, masks, gowns, and eye protection will be used when taking patient samples).