PART 1 (COUNCIL DECISION 2002/813/EC)

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

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

A. General information

1. Details of notification

(a)	Member State of notification	Germany
(b)	Notification number	B/DE/17/PEI3203
(c)	Date of acknowledgement of notification	22/09/2017
(d)	Title of the project	ASPIRO: A Phase 1/2, Randomized, Open-Label, Ascending-Dose, Delayed-Treatment Concurrent Control Clinical Study to Evaluate the Safety and Preliminary Efficacy of AT132, an AAV8-Delivered Gene Therapy in X-Linked Myotubular Myopathy (XLMTM) Patients
(e)	Proposed period of release	From 11/12/17 until 10/12/23

2. Notifier

Name of institution or company: Audentes Therapeutics Inc.

3. GMO characterisation

(a) Indicate whether the GMO is a:

viroid	(.)
RNA virus	(.)
DNA virus	(X)
bacterium	(.)
fungus	(.)
animal	
- mammals	(.)
- insect	(.)
- fish	(.)
- other animal	(.)

specify phylum, class …

(b) Identity of the GMO (genus and species) Genus: Dependoparvovirus; Species Adeno-associated depenoparvovirus A

…

(c) Genetic stability – according to Annex IIIa, II, A(10) stable as an episomal-nonintegrating viral vector

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) GB and FR

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 (X) No (.)

If yes:

- Member State of notification …
- Notification number B/../../…
  (released for clinical trial in the USA)

7. Summary of the potential environmental impact of the release of the GMOs.
None

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) Adeno-associated depenoparvovirus …

other, specify …

2. Name

(i)	order and/or higher taxon (for animals)	Parvoviridae
(ii)	genus	Dependoparvovirus
(iii)	species	Adeno-associated depenoparvovirus
(iv)	subspecies	
(v)	strain	Adeno-associated virus (AAV) – serotype 8
(vi)	pathovar (biotype, ecotype, race, etc.)	
(vii)	common name	Adeno-associated virus (AAV)

3. Geographical distribution of the organism

(a) Indigenous to, or otherwise established in, the country where the notification is made:
Yes (.) No (.) Not known (X)

(b) Indigenous to, or otherwise established in, other EC countries:
(i) Yes (.)

If yes, indicate the type of ecosystem in which it is found:

Atlantic	..
Mediteranean	..
Boreal	..
Alpine	..
Continental	..
Macaronesian	..

(ii) No (X)
(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 (X) No (.)

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 …	

No natural host known as AT132 is non-replicating rAAV8 expressing human MTM1. The AT132 lacks all of the viral protein coding seqeunces. AT132 is derived from AAV which is not currently know to cause disease. The virus causes a very mild immune response, lending further support to its appraent lack of pathogencity.

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

5. (a) Detection techniques
PCR

(b) Identification techniques
PCR…

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
related to Adeno-associated Virus

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:
…
(b) Generation time in the ecosystem where the release will take place:
…
(c) Way of reproduction: Sexual .. Asexual ..

(c) Factors affecting reproduction:
AAV vectors are nonpathogenic, replication-deficient viral vectors. AAVs do not have a propensity to integrate and, in the absence of evidence to the contrary, should present a low risk of gene transfer therapy-related delayed AEs. AAV are considered episomal vectors, although some studies have suggested that low frequency

integration events can have oncogenic consequences. Due to the absence of any precedence for oncogenicity from AAV vectors in humans, this risk is considered minimal. Viral vector, rAAV8-Des-hMTM1, is replication-deficient virus with Rep and Cap genes deleted.

9. Survivability

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

(i)	endospores	(.)
(ii)	cysts	(.)
(iii)	sclerotia	(.)
(iv)	asexual spores (fungi)	(.)
(v)	sexual spores (funghi)	(.)
(vi)	eggs	(.)
(vii)	pupae	(.)
(viii)	larvae	(.)
(ix)	other, specify	…

(b) relevant factors affecting survivability:
The survival and route of transmission of AT132 is not expected to be different from that of wild type AAV. However, AT132 is unable to replicate, even in the presence of a helper virus due to the deletions of the *rep* and *cap* genes.

10. (a) Ways of dissemination

Theoretically, it is possible that the AT132 could be release to the environment beyond the clinical trial patient receiving treatment with AT132. In this context, the accessible environment is considered to be non-patient humans or unintended human recipients: surgical and medical care staff, relatives and the environment compartment most likely to receive virus shed from the patient. The routine collection and disposal of the virus contaminated clinical waste is not considered to be an environmental release because of the measures routinely taken for biohazardous materials.

However, AT132is unable to replicate, even in the presence of a helper virus due to the deletions of the *rep* and *cap* genes. The likelihood of replication competent associated adenovirus (rcAAV) is considered low.

(b) Factors affecting dissemination
The routine collection and disposal of the virus contaminated clinical waste is not considered to be an environmental release because of the measures routinely taken for biohazardous materials. Moreover, AT132is unable to replicate, even in the presence of a helper virus due to the deletions of the *rep* and *cap* genes. The likelihood of replication competent associated adenovirus (rcAAV) is considered low.

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/../../…

C. **Information relating to the genetic modification**

1. Type of the genetic modification

(i)	insertion of genetic material	(X)
(ii)	deletion of genetic material	(.)
(iii)	base substitution	(.)
(iv)	cell fusion	(.)
(v)	others, specify …	

2. Intended outcome of the genetic modification
expression of human myotubularin

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

(b) Identity of the vector
Transgene Plasmid, pAAVAud-Des-hMTM1
…

(c) Host range of the vector
HEK 293

(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
The human MTM1 transgene sequence including the human promoter and polyadenylation (polyA) sequences are placed in a prokaryotic plasmid backbone providing kanamycin resistance. The transgene plasmid is designed such that the plasmid origin of replication (ori) and plasmid kanamycin resistance gene (kan) are in the counter direction to the direction of DNA transcription controlled by the p5

promoter in a human cell line. Therefore, neither of these genes is expressed in the HEK293 cell line.

(e) Constituent fragments of the vector

AAV Vector

rAAV8-Des-hMTM1 (AT132) is a non-replicating recombinant adeno-associated viral vector, serotype 8 (rAAV8), expressing the human MTM1 gene. The rAAV8 lacks all of the viral protein coding sequences. In this vector, transgene expression is controlled by the human desmin promoter, and RNA processing is mediated by the human β-globin gene second intron (HBB2) and polyadenylation (polyA) signal. This MTM1 expression cassette is flanked by AAV serotype 2 (AAV2) inverted terminal repeats (ITRs). Both the rAAV8-Des-hMTM1 serotype (AAV8) and promoter (Des) were selected to target skeletal muscle, the primary tissue affected in XLMTM. Please refer to Table 1 for details on the genetic elements of rAAV8-Des-hMTM1.

Table 1: Genetic Elements of the rAAV8-Des-hMTM1 Gene Therapy Vector

Range	Length	Name Tag	Name	Description
1-145	145	AAV2_ITR	AAV serotype 2 inverted terminal Repeat	Cis acting element allowing replication and packaging of vector genome in producer cells. Includes A, B, and C portions of dumbbell shaped hairpin, with expected 50:50 ration of flip and flop isoforms
146-157	12	-	Linker sequence	Short linker sequences joining two elements of vector
158-1217	1060	hDes	Human Desmin promoter	Segment of human genome encompassing the desmin gene promoter. Ends correspond to -984..+76 of transcript as defined in Reference human genome. Exact match to human reference genome but for SNP variation at bp 399, documented in dbSNP record rs2854885, and 790 bp documented in dbSNP record rs2854886
1218-1255	38	-	Linker sequence	Short linker sequences joining two elements of vector
1256-1361	106	-	hBglobin intron	*Bam*HI-*Rsa*I fragment of human beta-globin locus, encompassing splice donor of 2nd intron Exact match to NC_018922.2 reference genome (NCBI). Positions 5246759..5246654
1358-1802	445	-	hBglobin intron	Fragment of human beta globin intron, including splice acceptor. Extends from 392 bp upstream of splice acceptor, to 53 bp downstream. Exact match to positions 5246283..5245839 of reference genome NC_018922.2 (NCBI)
1274-1749	476	Beta_globin _intron	hBglobin intron	Intron sequences defined by two fragments of the human globin intron described above. This sequence extends precisely from splice donor to acceptor as per human RefSeq gene.
1818-3639	1822	hMTM1	Human MTM1 coding sequence	Human myotubularin (MTM1) cDNA sequence, from -10 relative to initiation codon to termination codon. Exact match to RefSeq record for MTM1, XM_011531173.2 (NCBI).
3640-3660	21	-	Linker sequence	Short linker sequences joining two elements of vector
3661-4420	760	Beta-globin_pA	Human beta globin poly-adenylation sequence	Fragment from human beta-globin gene locus, starting at *Bgl*II site approximately 212 bp upstream of actual polyadenylation site, and extending 548 bp downstream. Exact match to human reference genome NC_018922.2 (NCBI), positions 5245842..5245083.
4421-4433	13	-	Linker sequence	Short linker sequences joining two elements of vector

Range	Length	Name Tag	Name	Description
4434-4578	145	AAV2_ITR	AAV serotype 2 inverted terminal Repeat	Cis acting element allowing replication and packaging of vector genome in producer cells. Includes A, B, and C portions of dumbbell shaped hairpin, with expected 50:50 ration of flip and flop isoforms.

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

(i) transformation (.)
(ii) electroporation (.)
(iii) macroinjection (.)
(iv) microinjection (.)
(v) infection (.)
(vi) other, specify …
Transfection into HEK293 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 transgene cDNA for hMTM1 is under the control of the human desmin promoter (hDes), and RNA processing is mediated by the human β-globin gene second intron and polyadenylation (pA) signal.

…

(b) Source of each constituent part of the insert
Please refer to Section C.4.e.

(c) Intended function of each constituent part of the insert in the GMO
offers the potential for long-term expression of myotubularin

…

(d) Location of the insert in the host organism

- on a free plasmid (.)
- integrated in the chromosome (.)
- other, specify Inserted in the AAV8 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) human cDNA
- insect (.)
- fish (.)
- other animal (.)
(specify phylum, class) …

other, specify …

2. Complete name

(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	human myotubularin gene (hMTM1)

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 (.) No (X) Unknown (.)
Specify …

(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
AAV vectors are nonpathogenic, replication-deficient viral vectors. AAVs do not have a propensity to integrate and, in the absence of evidence to the contrary, should present a low risk of gene transfer therapy-related delayed adverse events. AT132 (rAAV8-Des-hMTM1) is a non-replicating rAAV8 expressing human MTM1. The rAAV8 lacks all of the viral protein coding sequences. The generation of a wild type AAV from the experimental product requires homologous recombination of AT132 with a co-infecting wild type AAV and a co-infecting helper virus (triple infection). It is not possible to generate a replication competent AAV containing the transgene.

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)
…

4. Description of identification and detection methods

(a) Techniques used to detect the GMO in the environment
A three passage assay has been developed consisting of cell-based amplification and passage followed by detection of rcAAV by real time qPCR (cap8 target).

(b) Techniques used to identify the GMO
PCR …

F. Information relating to the release

1. Purpose of the release (including any significant potential environmental benefits that may be expected)
Phase 1 clinical trial.
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 replication-defective AAV has no natural host

3. Information concerning the release and the surrounding area

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

(b) Size of the site (m^2): N/A … m^2
(i) actual release site (m^2): … m^2
(ii) wider release site (m^2): … m^2

(c) Proximity to internationally recognised biotopes or protected areas (including drinking water reservoirs), which could be affected:
none…

(d) Flora and fauna including crops, livestock and migratory species which may potentially interact with the GMO
none …

4. Method and amount of release

(a) Quantities of GMOs to be released:
Doses in the clinical study range from $1x10^{14}$ vg/kg to $51x10^{14}$ vg/kg

(b) Duration of the operation:
AT132 will be administered via an intravenous (iv) infusion over a period of up to 4 hours

(c) Methods and procedures to avoid and/or minimise the spread of the GMOs beyond the site of the release
Procedures are in place describing methods to store, transport and administer the viral vector. Additional procedures to adequately manage all materials that have been in contact with the GMO are available. All these procedures contain the appropriate measures to minimize spread of the GMO in the environment.

5. Short description of average environmental conditions (weather, temperature, etc.)
The weather in Germany is characterized by four well defined seasons, including at least one month with average temperatures below zero centigrade

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.
AAV vectors are nonpathogenic, replication-deficient viral vectors. AAVs do not have a propensity to integrate and, in the absence of evidence to the contrary, should present a low risk of gene transfer therapy-related delayed AEs. The genetically modified viral vector is not able to survive, disseminate in and/or displace other organisms and it not pathogenic to animals or plants.

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)	N/A
(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	…

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

3. Any other potentially significant interactions with other organisms in the environment
None

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
None

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

(i)	order and/or higher taxon (for animals)	…
(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:
None

(b) from other organisms to the GMO:
None

(c) likely consequences of gene transfer:
None

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.):
Although human infections are common, AAV is not known to be a pathogenic virus in humans (reviewed in Tenenbaum *et al*., 2003); AAV has never been implicated as an etiological agent for any disease (Blacklow *et al.,* 1968 a,b, 1971). Wild type AAV is not classified in Risk Groups 2, 3 or 4 in the European Union (EU) according to Directive 2000/54/EC on the protection of workers from risks related to exposure to biological agents at work. It is most appropriately designated a Risk Group 1 biological agent, defined in the EU as ‘one that is unlikely to cause human disease’.

There are no known natural predators, preys, parasites, competitors or symbionts associated with AAV. Primate (human) AAV serotypes are not known to actively transfer genetic material to organisms other than primates under natural conditions, although an absence of zoonosis is not documented.

However, for AAV viral vector, AT132, is unable to replicate independently, even in the presence of a helper virus, since it lacks the *rep* and *cap* genes required for rescue/packaging. The long-term safety of recombinant AAV vectors in humans is unknown; however, AAV vectors have been delivered to several hundred human subjects to date, in trials for cystic fibrosis, rheumatoid arthritis, Leber's congenital amaurosis, α1-antitrypsin deficiency, congestive heart failure, lipoprotein lipase deficiency, as well as haemophilia, and have been remarkably free of vector-related adverse events (Mingozzi and High, 2011).

Tenenbaum L, Lehtonen E, Monahan PE. Evaluation of Risks Related to the Use of Adeno-Associated Virus-Based Vectors. Current Gene Therapy. 2003; 3: 545-565.

Blacklow NR, Hoggan MD, Kapikian AZ, Austin JB, Rowe WP. Epidemiology of adenovirus-associated virus infection in a nursery population. Am. J. Epidemiol. 1968a; 88: 368-378.

Blacklow NR, Hoggan MD, Rowe WP. Serologic evidence for human infection with adenovirus-associated viruses. J. Natl. Cancer Inst. 1968b; 40: 319-327.

Blacklow NR, Hoggan MD, Sereno MS, Brandt CD, Kim HW, Parrott RH, Chanock RM. A seroepidemiologic study of adenovirus-associated virus infection in infants and children. Am. J. Epidemiol. 1971; 94: 359-366.

Mingozzi F, High KA. Therapeutic in vivo gene transfer for genetic disease using AAV: Progress and challenges. Nat Rev Genet. 2011; 12: 341-55.

9. Possible environmentally significant interactions with biogeochemical processes (if different from the recipient or parental organism)
…

H. Information relating to monitoring

1. Methods for monitoring the GMOs
Should it become indicated the viral vector can be detected by PCR.

2. Methods for monitoring ecosystem effects
AAV vectors are nonpathogenic, replication-deficient viral vectors. The genetically modified viral vector is not able to survive, disseminate in and/or displace other organisms and it not pathogenic to animals or plants.

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

4. Size of the monitoring area (m^2)
… m^2
N/A

5. Duration of the monitoring
N/A

6. Frequency of the monitoring
N/A

I. Information on post-release and waste treatment

1. Post-release treatment of the site
Surfaces that have been used during vaccination will be cleaned using 0.1% chloride-solution (or the equivalent). All waste products involved in all stages of the handling of AT132 must be disposed of per local hospital procedures and in compliance with biohazard standards of law.

2. Post-release treatment of the GMOs
…

3. (a) Type and amount of waste generated
Glass vials

3. (b) Treatment of waste
Used vials must be retained in a clear biohazard bag and kept until the site monitor has performed proper drug accountability. Pharmacist and the site may return the used and unused IMP vials and IMP infusion set to the Audentes Therapeutics designated depot

J. Information on emergency response plans

1. Methods and procedures for controlling the dissemination of the GMO(s) in case of unexpected spread
Allow aerosols to settle; contain spill and decontaminate with 10% chlorine bleach; allow sufficient contact time (30 min) before clean up.

2. Methods for removal of the GMO(s) of the areas potentially affected
Allow aerosols to settle; contain spill and decontaminate with 10% chlorine bleach; allow sufficient contact time (30 min) before clean up.

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

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