PART 1 (COUNCIL DECISION 2002/813/EC)

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

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

A. General information

1. Details of notification

(a)	Member State of notification	Belgium
(b)	Notification number	B/BE/26/BVW4
(c)	Date of acknowledgement of notification	Not available
(d)	Title of the project	

A Phase 1/2/3, Open-Label, Dose Escalation, Dose Expansion, and Randomized, Controlled Study to Evaluate the Safety and Efficacy of ATSN-201 Gene Therapy in Subjects with RS1-Associated X-linked Retinoschisis (LIGHTHOUSE)

(e) Proposed period of release

From 25th October 2026 to 18th September 2033

2. Notifier

Name of institution or company: Atsena 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

other, specify (kingdom, phylum and class)

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

Order: Piccovirales
Genus: Dependoparvovirus
Species: Adeno-associated virus (AAV)
Strain: the GMO is a pseudotyped AAV vector
Inverted terminal repeats (ITRs) present in the vector genome are derived from wild type AAV

The capsid was derived from bioengineering the capsid protein of wild-type AAV serotype. Specifically, AAV44.9, a naturally occurring AAV variant isolated from a laboratory stock of simian adenovirus SV15.

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

ATSN-201 is derived from bioengineering the capsid protein of wild-type AAV serotype. Specifically, AAV44.9, a naturally occurring AAV variant isolated from a laboratory stock of simian adenovirus SV15, was engineered to contain a single amino acid substitution from glutamic acid to aspartic acid at position 531 (E531D) (Boye *et al.*, 2020). The resulting AAV serotype was named AAV.SPR. The bioengineered capsid protein was rationally designed to reduce glycan interactions in the extracellular matrix with the aim to facilitate increased uptake of the AAV particle by photoreceptors which is the target tissue.

Wild-type AAV, is ubiquitous in the environment and naturally infect humans, but in the absence of a helper virus, the genome of the AAV remains latent in the cell nucleus, since it is replication-deficient by itself. In the literature, there is no clear association between AAV infection and human disease and there are no reports of pathogenicity as a result of the use of these vectors in numerous clinical studies. One report proposed a link between AAV infection and spontaneous abortions in a study of 81 clinical cases, however if this is a causal link or not has not been evaluated (Burguete *et al.*, 1999; Pereira *et al.*, 2010). As stated above, most humans (up to 60% or more) have antibodies to AAV suggesting infection (Li *et al.*, 2012; Fitzpatrick *et al.*, 2018). Consequently, AAVs are classified as Biosafety Level 1 (BSL 1) or Risk Group (RG) 1 (Baldo *et al.*, 2013).

Wild-type AAV persists in the cell nucleus mostly as extra-chromosomal episomes, however, it has been shown to integrate at low frequencies in a Rep protein-dependent manner primarily in the AAVS1 site, a region on the long arm of chromosome 19 (19q13-qter), as part of its life cycle (Deyle *et al.*, 2009).

Recent literature suggests that the wild-type AAV2 virus can clonally integrate in known cancer driver genes including CCNA2 (cyclin A2), TERT (telomerase reverse transcriptase), CCNE1 (cyclin E1), TNFSF10 (tumor necrosis factor superfamily member 1 10) and KMT2B (lysine-specific methyltransferase 2B), leading to overexpression of the target genes. This was observed in 11 of 193 studied case reports of HCC (Nault *et al.*, 2015), a rare form of liver tumor. However, the exact role of AAV in causing HCC in these patients is not clear. The data suggests that only 6 of these 11 cases were found to occur in the absence of other known risk factors (such as hepatitis virus B or C infection or alcohol consumption), whereas 4 of these 6 HCCs presented AAV-independent HCC-related mutations. Finally, the integrations detected were always partial genomes with no other distinct genetic patterns including small regions of homology. Thus, it remains to be determined whether AAV is simply a passenger mutation rather than a driver mutation in HCC formation (Büning *et al.*, 2015; Russell *et al.*,

2015). Importantly, these observations were made with wild-type AAV2, which is not the serotype used in ATSN-201.
While the etiology of this original finding is not entirely clear, a more recent publication (Logan *et al.*, 2017) suggests a possible link between the finding and sequences present in the wild-type AAV 3' untranslated regions (3'UTR) that can have enhancer-promoter activity. According to this model, integration of wild-type 3'UTR into the host genome would result in the transcriptional transactivation of neighboring genes. Of note, ATSN-201 genome is devoid of these sequences (wild-type AAV UTRs), thus, even in the event of a host genome integration, no transactivation activity on neighboring genes is expected. Importantly, no cases of HCC have been described to date in patients treated with AAV vectors in gene therapy CTs or in large animal models treated with AAV vectors and followed up long-term (Niemeyer *et al.*, 2009; Nathwani *et al.*, 2011; Nathwani *et al.*, 2014).
The genetic stability of ATSN-201 is expected to be equivalent to wtAAV. The GMO is mostly replication defective, lacking the *rep* and *cap* gene sequences from the genome in the vast majority of viral particles due to the design of the AAV production system. The manufacturing process also controls and quantifies the presence of replication-competent AAVs and other adventitious and extraneous agents that may also facilitate recombination events. All the plasmids used for the vector production have been verified by direct sequencing.

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) NL, IT, FR

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

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 DE
   - Notification number GMOB-2026-38974

   Member State of notification FR
   - Notification number 30957801

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 US, UK
   - Notification number UK: PID 19575
     US: Not applicable

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

ATSN-201 is a recombinant, mostly replication-deficient, AAV-based vector that will be administered subretinally by a single dose to patients with X-linked retinoschisis (XLRS).

The administration of ATSN-201 will be performed in Ghent University Hospital in Belgium, The environmental impact of ATSN-201 is considered negligible for the reasons summarised below:

1. The likelihood of the GMO to become persistent and invasive into natural habitants is considered extremely unlikely.
   Based on preclinical and clinical studies with similar vectors, doses and route of administration, vector genomes are known to be only shed in small quantities, through specific bodily fluids such as tears and nasal swabs, and cleared within a few days of vector administration (Weber et al., 2003; Maguire et al., 2008; Constable et al., 2016; Le Meur et al., 2018). Nevertheless, the amount released to the environment will overall be very small when using the routine procedures described and the vector is likely to be inactive or be deactivated by natural conditions. Wild-type AAV vectors are not known to cause any pathological effects or known sequelae. Moreover, AAVs are ubiquitously present, being detectable in many animal species and with most humans already showing previous exposure to AAVs (Gao et al., 2004; Li et al., 2012; Baldo et al., 2013; Zinn et al., 2014; Fitzpatrick et al., 2018). Therefore, there is considered to be no major risk to the environment. In addition, the GMO is mostly replication defective, lacking the rep and cap gene sequences from the genome due to the design of the AAV production system. Even if the formation of a maximal proportion of replication competent particles occurs (<0.000001%), these particles will still be helper-virus dependent for its replication. The amount of replication-competent viral particles is controlled during the manufacturing process.

2. No selective advantage has been conferred to the GMO.
   Viral particle replication capacity is minimal, and the GMO contains no elements for increased competitiveness or invasiveness. In addition, most of the viral genetic material has been removed reducing the capacity for recombination and/or the ability to provide competitive sequences to other organisms. Shed material is also infectious for a limited time, but the ability to cause significant infection or spread to other organisms is considered limited.

3. Spread of infectious ATSN-201 following release is limited.
   GMO shows poor potential for infection once shed via body fluids as shed material will predominantly contain only DNA fragments of ATSN-201 and is unlikely to contain infectious particles. In addition, due to the low numbers of vector DNA copies potentially released into the environment through shedding, horizontal gene transfer is highly unlikely. Even if horizontal gene transfer occurred, the sequences would not confer a selective advantage to other organisms such as bacteria since AAV does not contain any prokaryotic promoters, any antibiotic or other types of resistance genes or any genes, which would enhance or constrain their growth. As ATSN-201 contains the ITR-sequences of wild-type AAV, there is a (remote) possibility of homologous recombination of the vector with wild-type AAV of the same serotype in case of a co-infection in exposed persons. The result of such a recombination would be that ATSN-201 would gain functional genes of the wild-type AAV required for replication and encapsidation but, in turn, would lose the transgene. Hence, recombination would lead to the formation of viruses that are identical to the wild-type virus (non-pathogenic).

Finally, the possibility of gene transfer to species other than humans and (some) primates is low, given the host preference of AAV. In addition, the photoreceptor-specific promoter/enhancer element is a human-derived regulatory sequence that will limit transgene expression to this type of cells.

4. No immediate and/or delayed environmental impact of the interactions between the GMO and non-targeted organism is expected.
   No environmental impact is expected of the direct and indirect interactions of ATSN-201 as AAV vectors do not cause pathogenicity, are already present in the environment (including a high level of human exposure) and the GMO is expected to be released in relatively low amounts. In addition, the GMO has extremely limited replication capacity and thus, unlikely to propagate further.

5. Appropriate measures will be taken to avoid that personnel handling the GMO will come into direct or indirect contact with the GMO.
   Personnel are highly trained in the handling of infectious and/or GMO materials. Protocols for correct transport, storage, handling of the GMO and biologic samples, protection equipment to be used, handling and disposing of contaminated materials, and procedures to follow in case of spill are established and personnel will receive specific training. Thus, the possibility of accidental exposure will be very much reduced.
   Shed quantities will be minimal, through specific bodily fluids such as tears and nasal swabs, and cleared within a few days of vector administration (Weber et al., 2003; Maguire et al., 2008; Constable et al., 2016; Le Meur et al., 2018). Also, the vector is likely to be inactive or be deactivated by natural conditions. Finally, wild-type AAV vectors are not known to cause any pathological effects or known sequelae. Transgene expression is unlikely to cause any adverse effects, as it is constrained to photoreceptors, and the potential doses received are minimal. Therefore, exogenous gene expression will not be biologically significant or even detectable.

6. No effects on animal health are expected from consumption of the GMO and any product derived from it.

   The GMO is not intended as animal feed and is not expected to enter the food chain. Any accidental exposure of animals or plants is unlikely due to the hospital procedures and guidelines in terms of destruction of all contaminated material. Shed virus from dosed participants is expected to be minimal and not infectious.

7. No effects on biogeochemical processes are expected caused by possible direct or indirect interaction between the GMO and the target and non-target organisms in the vicinity of the GMO introduction.
   AAVs are not known to contribute to or be involved in any biogeochemical processes either directly or indirectly. AAV is not per se a food source (although their degradation products such as nucleic acid and protein may be recycled as an energy source) and they do not infect animals, microbes or plants known to participate in important biogeochemical processes such as carbon or nutrient availability.

Therefore, the environmental impact of a potential release of ATSN-201 is considered to be 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)	Piccovirales
(ii)	genus	Dependoparvovirus
(iii)	species	
(iv)	subspecies	Adeno-associated virus (AAV)
(v)	strain	The capsid is derived from bioengineering the capsid protein of wild-type AAV serotype. Specifically, AAV44.9, a naturally occurring AAV variant isolated from a laboratory stock of simian adenovirus SV15.
(vi)	pathovar (biotype, ecotype, race, etc.)	
(vii)	common name	

3. Geographical distribution of the organism

(a) Indigenous to, or otherwise established in, the country where the notification is made:

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

(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 (.) No (X)

4. Natural habitat of the organism

(a) If the organism is a microorganism

water (.)
soil, free-living (.)
soil in association with plant-root systems (.)
in association with plant leaf/stem systems (.)
other, specify
Specific hosts for AAV include primates, as well as avian, rat and mouse, caprine, porcine and bat species (Rapti *et al.*, 2021).

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

5. (a) Detection techniques
Droplet digital Polymerase Chain Reaction (ddPCR)

(b) Identification techniques
Droplet digital Polymerase Chain Reaction (ddPCR)

6. Is the recipient organism classified under existing Community rules relating to the protection of human health and/or the environment?
Yes (.) No (X)
If yes, specify
AAVs are not listed in Directive 2000/54 (protection of workers from risks related to exposure to biological agents at work).
AAVs are not known to be associated with any pathogenic effect. Nonetheless, recombinant AAV-based vectors are usually classified as Biosafety Class 1 or 2 depending on the Member State. It is concluded that ATSN-201 can be defined as a Risk Group 1 biological agent, defined in the EU as 'one 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

As mentioned before, wild-type AAVs, are ubiquitous in the environment and naturally infect humans, but in the absence of a helper virus, the genome of the AAV remains latent in the cell nucleus, since it is replication-deficient by itself. In the literature, there is no clear association between AAV infection and human disease and there are no reports of pathogenicity as a result of the use of these vectors in numerous clinical studies. One report proposed a link between AAV infection and spontaneous abortions in a study of 81 clinical cases, however if this is a causal link or not has not been evaluated (Burguete et al., 1999; Pereira et al., 2010). As stated above, most humans (up to 60% or more) have antibodies to AAV suggesting infection (Li et al., 2012; Fitzpatrick et al., 2018). Consequently, AAVs are classified as Biosafety Level 1 (BSL 1) or Risk Group (RG) 1 (Baldo et al., 2013).

Wild-type AAV persists in the cell nucleus mostly as extra-chromosomal episomes, however, it has been shown to integrate at low frequencies in a Rep protein-dependent manner primarily in the AAVS1 site, a region on the long arm of chromosome 19 (19q13-qter), as part of its life cycle (Deyle et al., 2009).

AAV does not contain any prokaryotic promoters, any antibiotic or other types of resistance genes or any genes, which would enhance or constrain their growth.

No environmental impact is expected of the direct and indirect interactions of ATSN-201 as AAV vectors do not cause pathogenicity, are already present in the environment (including a high level of human exposure) and the GMO is expected to be released in relatively low amounts. In addition, the GMO has extremely limited replication capacity and thus, unlikely to propagate further.

No effects on biogeochemical processes are expected caused by possible direct or indirect interaction between the GMO and the target and non-target organisms in the vicinity of the GMO introduction.

AAVs are not known to contribute to or be involved in any biogeochemical processes either directly or indirectly. AAV is not per se a food source (although their degradation products such as nucleic acid and protein may be recycled as an energy source) and they do not infect animals, microbes or plants known to participate in important biogeochemical processes such as carbon or nutrient availability.

8. Information concerning reproduction

(a) Generation time in natural ecosystems:

Replication of wtAAV depends on the co-infection with a helper virus (adeno or herpesvirus). In such conditions, generation time will depend on environmental conditions.

(b) Generation time in the ecosystem where the release will take place:

Not applicable as replication of wtAAV depends on a helper virus.

(c) Way of reproduction: Sexual N/A .. Asexual N/A ..
Reproduction of wtAAV is dependent on co-infection with a helper virus.
(c) Factors affecting reproduction:
The reproduction of AAV depends on the co-infection with a helper virus (adenovirus or herpesvirus).

9. Survivability

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

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

AAVs have the ability to form extrachromosomal concatemers that remain episomal for extended periods of time. AAVs themselves are naturally relatively stable particles, but will be degraded and deactivated by heat and chemical conditions such as acid or detergents.

(b) relevant factors affecting survivability:
Some viruses are relatively stable, resistant to dehydration and able to persist in the environment whereas other viruses are highly susceptible to dehydration and are rapidly inactivated outside the host. More stable viruses may be resistant to certain disinfectants and may therefore be able to persist in the environment. The persistence of the viral vector could increase the likelihood of exposure. AAV is generally considered to be highly stable (Baldo et al., 2013). However, stability studies with AAV1 have shown that exposure to multiple common disinfectants prevent AAV-mediated transgene expression and thus many detergents can be considered to inactivate AAV1 vectors and this is presumed to apply to other AAV serotypes (Howard et al., 2017). Overall, autoclaving, 0.25% peracetic acid, iodine, or 10% sodium hypochlorite were effective. Stability is also expected to decline with exposure to heat, UV radiation, or extreme pH.
Studies have shown that AAV may be detectable (shed) in some biological samples such as stools, but shows time-dependent decline in infectivity based on spiking controls as well as lack of infection of naïve animals housed in the same cage as treated animals (Reuter et al., 2012)

10. (a) Ways of dissemination

Wild-type AAV may be transmitted through direct contact with an infected individual (aerosols, blood) or through indirect contact with the contaminated environment (gastrointestinal transmission, shedding and/or contact with excreta). Sexual

transmission remains a theoretical risk of AAV transmission (Gao *et al.*, 2004) and is considered significantly reduced by the use of barrier protection methods.

(b) Factors affecting dissemination

AAV is dependent on a helper virus such as adenovirus or herpes virus for replication. The bioengineered capsid protein was rationally designed to reduce glycan interactions in the extracellular matrix with the aim to facilitate increased uptake of the AAV particle by photoreceptors, which is the target tissue. Therefore, the tropism of the GMO has been altered (compared to the wild-type virus), making it more specific and directed towards the targeted cells, and limiting its dissemination, as evidenced by preclinical biodistribution data.

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)

The Sponsor has not notified any previous modifications of the parental virus AAV44.9 for release in the EU.

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	(X)
(iv)	cell fusion	(.)
(v)	others, specify	

2. Intended outcome of the genetic modification

ATSN-201 is a GMO being developed by Atsena Therapeutics, Inc. for the treatment of X-linked retinoschisis (XLRS). ATSN-201 will be administered by subretinal administration. The expected physiological effects related to the therapeutic intervention is that ATSN-201 administration will enable transfer of functional human retinoschisin (*hRS1*) gene to photoreceptors in the eye and thereby restoring or attenuating the deterioration of vision in patients with XLRS.

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	

(c) Identity of the vector

ATSN-201 is not constructed or produced starting from the parental virus live virus stock. Each product batch is not derived from a viral seed stock or equivalent containing the wild-type and/or modified vector. Each batch is manufactured via a helper virus-free triple transfection process by which the genes necessary for viral protein expression and assembly are provided only in trans (Matsushita et al., 1998; Ayuso et al., 2010). The triple transfection process used for manufacture of the drug substance is based on cell culture and transient transfection of adherent human embryonic kidney epithelial cells (HEK 293) with three plasmid constructs:

1. Trangene Plasmid
2. Packaging plasmid
3. Helper plasmid

That is, three elements are required for the generation of functional AAV particles, with important AAV elements provided in trans, thus the system is designed to obtain infective but replication deficient viral particles. The genome of AAV particles formed will be predominantly constituted of the transgene expressing cassette. Therefore, the parental vector is not part of the manufacturing process.

(c) Host range of the vector

Bacteria. The plasmids have a bacteria replication origin and do not replicate in mammalian cells.

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

Yes (X) No (.)

antibiotic resistance	(X)
other, specify	

Indication of which antibiotic resistance gene is inserted

Kanamycin resistance

(d) Constituent fragments of the vector

Transfer vector sequence will be encapsidated in the AAV particles and will constitute the genome of ATSN-201, that will be transferred to transduced cells. The genome includes a synthetic hRS1 cDNA and a human rhodopsin kinase promoter (hGRK1), which drives transgene expression specifically in rod and cone photoreceptors.

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

(i)	transformation	(.)
(ii)	electroporation	(.)
(iii)	macroinjection	(.)
(iv)	microinjection	(.)
(v)	infection	(.)
(vi)	other, specify	Transfection

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	(.)

Not applicable

6. Composition of the insert

(a) Composition of the insert

For the recombinant vector ATSN-201, the wild-type AAV genome, containing the rep and cap genes, was replaced with a therapeutic transgene expression cassette. The cassette includes a promoter, regulatory elements for the transgene hRS1, a poly adenylation signal and stuffer sequence, flanked by AAV ITRs.

(b) Source of each constituent part of the insert

- AAV inverted terminal repeats (ITRs) are derived from wild-type AAV2.
- The promoter and the hRS1 transgene sequence derive from human sequences.
- The poly A signal element derives from bovine sequences.

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

- The promoter is designed to drive transgene expression specifically in rod and cone photoreceptors.
- The therapeutic transgene has been designed to introduce the functional human retinoschisin (hRS1) gene to photoreceptors in the eye, thereby restoring or attenuating the deterioration of vision in patients with XLRS.
- The polyadenylation sequence is included to drive a specific cleavage event to terminate transcription and the addition of a long polyadenylate tail to enhance transcript stability
- The expression cassette is flanked by the ITRs, which facilitate the packaging of the rAAV genome and DNA replication within target cells.

(d) Location of the insert in the host organism

- on a free plasmid (.)
- integrated in the chromosome (.)
- other, specify

The insert is located between the ITRs of the vector.

(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

The promoter and transgene sequence are derived from human sequences. The coding sequence has been codon optimised to increase protein expression. The polyA element derives from bovine sequences.

2. Complete name

(i)	order and/or higher taxon (for animals)	Primates; Hominidae
(ii)	family name for plants	Not applicable
(iii)	genus	Homo
(iv)	species	*Homo Sapiens*
(v)	subspecies	Sapiens
(vi)	strain	…
(vii)	cultivar/breeding line	Not applicable
(viii)	pathovar	Not applicable
(ix)	common name	Human

(i)	order and/or higher taxon (for animals)	Artiodactyla
(ii)	family name for plants	Not applicable
(iii)	genus	Bos
(iv)	species	*Bos taurus*
(v)	subspecies	
(vi)	strain	
(vii)	cultivar/breeding line	
(viii)	pathovar	

(ix) common name Cattle

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
No selective advantage has been conferred to the GMO. Viral particle replication capacity is minimal, and the GMO contains no elements for increased competitiveness or invasiveness. In addition, most of the viral genetic material has been removed, reducing the capacity for recombination and/or the ability to provide competitive sequences to other organisms. Shed material is also infectious for a limited time, but the ability to cause significant infection or spread to other organisms is considered limited.

(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

ATSN-201 has been modified to reduce the integration risk in several ways. Firstly, the sequence encoding Rep proteins has been deleted from recombinant AAV vectors, and hence integration in the host genome is significantly reduced (Penaud-Budloo et al., 2008). Secondly, in ATSN-201, a photoreceptor-specific enhancer/promoter is used, limiting the expression of the carried transgene to this particular cell type. Finally, ATSN-201 genome is devoid of wild-type UTR sequences, which were described to have enhancer-promoter activity (Logan *et al.*, 2017) causing transcriptional transactivation of neighboring genes.

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

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

Specify

The GMO cannot enter an infectious cycle even in the presence of a helper virus due to the removal of the viral rep and cap genes. ATSN-201 replication could only occur in the extremely unlikely event of a host cell being infected by three separate viruses: ATSN-201, wild-type AAV and a helper virus such as adenovirus or herpes simplex virus.

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

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

Specify

Neither wild type AAV nor ATSN-201 are considered pathogenic to humans or a risk to the environment.

2. Genetic stability of the genetically modified organism

The genetic stability of ATSN-201 is expected to be equivalent to wtAAV. The GMO is mostly replication defective, lacking the rep and cap gene sequences from the genome in the vast majority of viral particles due to the design of the AAV production system. The manufacturing process also controls and quantifies the presence of replication-competent AAVs and other adventitious and extraneous agents that may also facilitate recombination events.

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 (Not applicable)

(b) give the relevant information specified under Annex III A, point II(A)(11)(d) and II(C)(2)(i)

As mentioned before, AAVs are classified as Biosafety Level 1 (BSL 1) or Risk Group (RG) 1 (Baldo et al., 2013). AAV vectors do not cause pathogenicity, are already present in the environment (including a high level of human exposure) and the GMO is expected to be released in relatively low amounts. In addition, the GMO has extremely limited replication capacity and thus, unlikely to propagate further.

4. Description of identification and detection methods

(a) Techniques used to detect the GMO in the environment
Droplet digital PCR with primers specific for vector sequences.

(b) Techniques used to identify the GMO
Droplet digital PCR with primers specific for vector sequences.

F. Information relating to the release

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

ATSN-201 is an investigational medicinal product developed for the treatment of X-Linked retinoschisis (XLRS), a rare congenital disease of the retina caused by mutations in the *RS1* gene. The present GMO will be deployed in a multicentre phase 1/2/3 CT for the treatment of X-linked retinoschisis (XLRS) in paediatric and adult patients. The study will be comprised of a dose-escalation phase, a dose expansion phase and a randomized, controlled phase with the dose selected in previous phases. Phase 1/2 parts of the proposed CT, are currently ongoing in the USA, to evaluate safety and tolerability in the dose-escalation phase and dose expansion phase of the study (NCT05878860) or Atsena study number ATSN-201-1. The main study duration will be 52 weeks (12 months) with subjects followed for an additional 4 years.
The primary objective during phase 1/2 of the clinical trial (CT) is to evaluate safety and tolerability of the product, while efficacy will be evaluated during the phase 3 part of the study. Patients with a clinical diagnosis of XLRS caused by mutations in RS1 will be enrolled, with ≥18 years of age for phase 1 and the first cohort of phase 2, ≥6 and < 18 years for the second cohort of phase 2, and ≥6 years for phase 3. Male patients will be included in phase 1/2/3, and female patients may be included as part of phase 3.

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
ATSN-201 will not be released in the environment. The GMO will be administered subretinally exclusively to XLRS patients in controlled clinical sites, including 1 hospital in Belgium.

3. Information concerning the release and the surrounding area

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

Administration will be in a normal patient treatment room of a hospital. ATSN-201 will not be released in the environment.

The location of the hospitals participating in the trials is the following:

- Ghent University Hospital

(b) Size of the site (m^2):
Not applicable. A specific size for the site of release cannot be defined as ATSN-201 will be administered to patients as part of a clinical trial.
(i) actual release site (m^2):
(ii) wider release site (m^2):

(e) Proximity to internationally recognised biotopes or protected areas (including drinking water reservoirs), which could be affected:
Not applicable. Shed material is potentially non-infectious and poses no environmental threat.

(f) Flora and fauna including crops, livestock and migratory species which may potentially interact with the GMO
Not applicable. Administration of ATSN-201 will occur only within a controlled hospital setting and therefore, it is not anticipated that it will come into contact with plants, animals or soil.

4. Method and amount of release

(a) Quantities of GMOs to be released:

This is a phase 3, single-arm, open-label, randomised, multi-centre, study designed to evaluate the safety and efficacy of subretinally administered ATSN-201 gene therapy in subjects ≥ 6 years of age with RS1-associated XLRS. Subjects will be determined to be eligible for bilateral or unilateral administration and then randomized to one of the following groups: control or ATSN-201 (1.1×10^{10} vg/eye).
A total of 6 subjects is expected to be treated with ATSN-201 in Belgium. Therefore, the estimated quantity of ATSN-201 that will be released in Belgium is the following:

ATSN-201 dose	Bilateral/unilateral	Estimated vg/person	Number of patients	Total vg
1.1×10^{10} vg/eye	Unilateral	1.1×10^{10} vg/person	3	3.3×10^{10} vg
	Bilateral	2.2×10^{10} vg/person	3	6.6×10^{10} vg
Total vg released in Belgium				9.9×10^{10} vg

(b) Duration of the operation:

Participants will receive a single subretinal administration of ATSN-201 (1.1×10^{10} vg/eye). The procedure is expected to last 1 hour. The study will include a 12-month main study period, followed by a 4-year extension study period. Subjects may be included in a separate gene therapy long-term follow-up registry for a total of 15 years after ATSN-201 administration.

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

During the administration procedure, only standard hospital safety procedures will be performed to limit contamination as the risks from the GMO are considered low from a risk of spreading as well as safety/pathogenesis (no serious concerns or pathogenicity are considered likely).
Safety procedures will include:

- Training of pharmacy staff about the product and potential risks. This will reduce the risk of accidents by creating familiarity with procedures and processes.
- Use of protective equipment (gowns and/or lab coats) as well as gloves and goggles will also limit contamination and exposure to staff in case of spill.
- Preparation of the vector will take place in a isolator unit. This will limit aerosol and other exposure to staff working in the same facility.
- All disposables that come into contact with the GMO will be disposed of in bins for specific hospital waste (UN3291) within the patient room.
- Disinfection of contaminated surfaces will be performed as described with detergents and chemical agents commonly used to disinfect biohazard materials and surfaces.

Routine safety and hospital procedures are considered adequate to control the unlikely event of exposure to HCPs during the preparation. It is considered that there is no additional risk of exposure to ATSN-201 compared to, for example, blood borne human viruses during routine blood extraction from patients and volunteers in a clinical setting. Any meaningful exposure would be by accidental contact or spillage. The routine safety procedures and use of personal protective equipment and biological safety cabinets, for example, are designed to reduce these aspects and staff will be fully trained to address these situations and apply disinfection procedures as required. AAV vectors are replication defective, and therefore they do not spread through propagation means.

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

ATSN-201 and diluent vials must be stored at or below -60°C. vials will be thawed at room temperature. Preparation, transport, and administration will be performed at room temperature. Formulated ATSN-201 can be held for up to 6 hours at 5°C ± 3°C from the initial time of preparation (breach of the stopper) before being brought to room temperature in the surgical suite.

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.
None available

G. Interactions of the GMO with the environment and potential impact on the environment, if significantly different from the recipient or parent organism

1. Name of target organism (if applicable)

(i)	order and/or higher taxon (for animals)	Primates; Hominidae
(ii)	family name for plants	N/A
(iii)	genus	Homo
(iv)	species	*Homo Sapiens*
(v)	subspecies	Sapiens
(vi)	strain	N/A
(vii)	cultivar/breeding line	N/A
(viii)	pathovar	N/A
(ix)	common name	Human

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

X-Linked retinoschisis (XLRS) is a rare congenital disease of the retina caused by mutations in the *RS1* gene. XLRS is one of the most common causes of juvenile macular degeneration in males, with a world-wide prevalence ranging from 1:5,000 to 1:25,000. It is a symmetrical, bilateral disorder with onset in the first decade of life. Affected males exhibit reduced visual acuity (typically in the 20/60 to 20/120 range) that cannot be improved with glasses.
The expected physiological effects related to the therapeutic intention is that ATSN-201 administration will introduce the functional human retinoschisin (h*RS1*) gene to photoreceptors in the eye, thereby restoring or attenuating the deterioration of vision in patients with XLRS.

3. Any other potentially significant interactions with other organisms in the environment.
No interaction with other organisms in the environment is anticipated

4. Is post-release selection such as increased competitiveness, increased invasiveness for the GMO likely to occur?
Yes (.) No (X) Not known (.)
Give details
ATSN-201 is not only lacking all AAV genes except ITRs in the genome, but it is designed to be naturally replication defective, and even if some residual particles retain the competence to replicate, AAV propagation would still require a helper virus. Therefore, release to the environment is not expected to give rise to a serotype that will survive in the environment. Similarly, transfer of genes from ATSN-201 to other AAVs in the environment is unlikely as the homologous sequences for recombination (*i.e.*, the ITRs) are small and, even in the case that genetic material was transferred, this material is unlikely to have a survival advantage (*e.g.*, antibiotic resistance genes

are absent) or a pathogenic advantage (*i.e.*, there are no transgene or regulatory sequences that are likely to function efficiently in animals as they are all human sequences, except for the bovina poly(A) sequence, and they do not have pathogenic effects even when overexpressed).

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

No important dissemination of the GMO is expected as it is mostly non-replicative.

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:

Highly unlikely. Spread of infectious ATSN-201 following release is limited by the fact that the GMO shows poor potential for infection once shed via body fluids as shed material will predominantly contain only DNA fragments of ATSN-201 and is unlikely to contain infectious particles. In addition, due to the low numbers of vector DNA copies potentially released into the environment through shedding, horizontal gene transfer is highly unlikely. Even if horizontal gene transfer occurred, the sequences would not confer a selective advantage to other organisms such as bacteria since AAV does not contain any prokaryotic promoters, any antibiotic or other types of resistance genes or any genes, which would enhance or constrain their growth.

As ATSN-201 contains the ITR-sequences of wild-type AAV, there is a (remote) possibility of homologous recombination of the vector with wild-type AAV of the same serotype in case of a co-infection in exposed persons. The result of such a recombination would be that ATSN-201would gain functional genes of the wild-type AAV required for replication and encapsidation but, in turn, would lose the transgene. Hence, recombination would lead to the formation of viruses that are identical to the wild-type virus (non-pathogenic).

In addition, the possibility of gene transfer to species other than humans and (some) primates is low, given the host preference of AAV. In addition, the photoreceptor-specific promoter/enhancer element is a human-derived regulatory sequence that will limit transgene expression to this type of cells.

Any accidental exposure of animals or plants is unlikely due to the hospital procedures and guidelines in terms of destruction of all contaminated material. Shed virus from dosed participants is expected to be minimal and not infectious.

(b) from other organisms to the GMO:
Negligible. As ATSN-201 contains the ITR-sequences of wild-type AAV, there is a (remote) possibility of homologous recombination of the vector with wild-type AAV of the same serotype in case of a co-infection in exposed persons. The result of such a recombination would be that ATSN-201would gain functional genes of the wild-type AAV required for replication and encapsidation but, in turn, would lose the transgene. Hence, recombination would lead to the formation of viruses that are identical to the wild-type virus (non-pathogenic).

(c) likely consequences of gene transfer:
The risk of ATSN-201 gene integration is minimal/negligible.
Negative findings on integration have so far been made with wild-type AAV and not with recombinant clinical vectors despite a large number of CTs and patients treated, including at dose levels much higher than what would occur with wild-type AAV infection (Büning *et al.*, 2015; Russell *et al.*, 2015; Gil-Farina *et al.*, 2016). Despite the numerous patients treated with AAV gene therapy, including with similar serotypes and/or constructs to ATSN-201, no prohibitive safety signals or important integration events have been observed despite many years of follow up.

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.):
No such studies have been conducted.

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9. Possible environmentally significant interactions with biogeochemical processes (if different from the recipient or parental organism)
AAVs are not known to contribute to or be involved in any biogeochemical processes either directly or indirectly. AAV is not *per se* a food source (although their degradation products such as nucleic acid and protein may be recycled as an energy source) and they do not infect animals, microbes or plants known to participate in important biogeochemical processes such as carbon or nutrient availability.

H. Information relating to monitoring

1. Methods for monitoring the GMOs
Droplet digital PCR (ddPCR) with primers specific for vector sequences.

2. Methods for monitoring ecosystem effects

The presence of ATSN-201 in bodily fluids following administration will be determined by ddPCR.

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

The most sensitive method for detecting transfer of vector genetic material to other organisms (i.e. the host) will be Droplet digital PCR. The presence of vector DNA sequences will be determined in various biological matrices including serum, tears, saliva, urine and nasal swabs. Others have shown that AAV material found in excreta may not be infectious and thus transfer of donated genetic material from the patient to other organisms is not envisaged and will not be monitored.

4. Size of the monitoring area (m^2)
Not applicable.

5. Duration of the monitoring
Detection of the GMO in different excreta and/or biomaterials will be performed. Vector presence in blood will be performed. Additionally, vector shedding will be collected for tears, saliva, urine and nasal swabs. Samples will be collected at Baseline (day -7 to day -2) and at specified times after administration as indicated in the protocol. Analysis will continue until 3 consecutive samples are negative (classified as: at or below the limit of detection of the assay).

6. Frequency of the monitoring
Vector biodistribution and viral shedding will be performed at day-7 to -2, at day 1, day 7, day 14, day 28, week 8, month 6 and month 12 weekly until week 8; monthly thereafter until month from month 6 to month 12. Analysis will continue until 3 consecutive samples are negative.

I. Information on post-release and waste treatment

1. Post-release treatment of the site
Disinfection of contaminated surfaces will be performed using an appropriate validated disinfection detergent, for instance Descogen® 3% for 30 minutes, or any other method indicated as routine cleaning and disinfection virucidal agent by UZ Gent biosafety competent department.

2. Post-release treatment of the GMOs

All disposables that come into contact with the GMO will be disposed of in bins for specific hospital waste (UN3291) within the patient room.

3. (a) Type and amount of waste generated

All leftovers of the GMO and diluent and all products/disposables (vials, syringes, needles and related items, gloves, gowns etc) that were in contact with the GMO will be disposed of as hazardous medical waste (following hospital procedures).

4. (b) Treatment of waste
The hazardous medical waste (HMW) bins are closed within the contained use rooms and transported within 24 hours to the recycling park on site. There the HMW is

stored in a closed room until collection by the licenced waste collection service (IHM) followed by incineration at Indaver.
Non-disposable materials will be decontaminated by treatment with an appropriate disinfectant and/or autoclaving oxygen-releasing solution (i.e. Descogen® 3% for 30 minutes) will be used for regular disinfection on used surfaces. In case of a spill, staff will be instructed to wear gloves (if not already wearing) and treat the surface with oxygen-releasing solution (e.g. Descogen® 3% for 30 minutes). All additional material such as absorbent tissues used to absorb the material will be discarded and destroyed as described above. Any broken material (i.e. the container closure system, syringes, etc.) will also be similarly destroyed.

J. Information on emergency response plans

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

Surfaces will be disinfected with an appropriate validated disinfection detergent. In case of a spill, contaminated surfaces will be disinfected with a suitable validated disinfectant (e.g. Descogen® 3% for 30 minutes or 1000 ppm chlorine solutions).

Even if a person comes into contact with the GMO, while handling or after release, no immediate and/or delayed effects on his/her health are expected as AAV infection has been associated with no known pathology in any species. Immune responses will limit persistence in most cases, whereas the tissue-specific restriction of expression imposed by the promoter will also limit off-target expression.

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

AAV is generally considered to be highly stable (Baldo *et al.*, 2013). However, stability studies with AAV1 have shown that exposure to multiple common disinfectants prevent AAV-mediated transgene expression and thus many detergents can be considered to inactivate AAV1 vectors and this is presumed to apply to other AAV serotypes (Howard *et al.*, 2017). Overall, autoclaving, 0.25% peracetic acid, iodine, or 10% sodium hypochlorite were effective. Stability is also expected to decline with exposure to heat, UV radiation, or extreme pH.
Specific methods for removal of ATSN-201 from areas potentially affected will be followed as per clinical site-approved internal procedures such as decontamination of surfaces with a suitable validated disinfectant (e.g. Descogen® 3% for 30 minutes or 1000 ppm chlorine solutions).

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

Not applicable. Administration of ATSN-201 will occur only within a controlled hospital setting; therefore, it is not anticipated that it will come into contact with plants, animals or soil. Furthermore, ATSN-201 cannot infect plants or microbes.

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

Measures will be taken to avoid that personnel handling the GMO will come into direct or indirect contact with it. The GMO will be transported under validated and controlled conditions, in frozen form, to the hospital site under monitored and temperature-controlled conditions by a courier specialized in this type of transport. Once at the hospital site, the product will be stored until used in a monitored freezer at or below -60°C. The freezer will be inaccessible to unauthorized personnel. Personnel are highly trained in the handling of infectious and/or GMO materials. Protocols for correct transport, storage, handling of the GMO and biologic samples, protection equipment to be used, handling and disposing of contaminated materials, and procedures to follow in case of spill are established and personnel will receive specific training.

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