Framework of Research and Development

Title of the study:

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).

Brief description of the Project & Framework of Research/Development:

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.

ATSN-201 will be released in Belgium as part of the phase 3 of ATSN-201-1 clinical trial, a 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. A total of 6 subjects is expected to be treated with ATSN-201 in Belgium. 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.

Study results are expected to provide pivotal evidence of ATSN-201 safety and efficacy for its use as a treatment of XLRS.

Description of the GMO

ATSN-201 is a GMO being developed by Atsena Therapeutics, Inc. for the treatment of X linked retinoschisis (XLRS). 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, designed to drive transgene expression specifically in rod and cone photoreceptors.

- The therapeutic transgene, which 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.

Nature and goal of the foreseen deliberate release:

The deliberate release of ATSN-201 is associated with vector shedding from patients who were administered with it. Recombinant Adeno-Associated Virus (AAV) vector shedding is commonly observed in studies involving AAV based vectors. Shedding occurs at very low levels. 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.

ATSN-201 is being tested as a potential therapy for treatment of XLRS. It is expected that introducing the functional human retinoschisin (hRS1) gene to photoreceptors in the eye, will restore or attenuate the deterioration of vision in patients with XLRS.

The assessment of the potential risks for human health and the environment linked to the deliberate release

The potential risk for human health and the environment of the release of ATSN-201 as described in this application is considered negligible for the 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. 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. 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, 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.

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. 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.

Potential advantages of the deliberate release

Potential advantages of the deliberate release of ATSN-201 include:

1. Therapeutic benefit for patients:
   ATSN-201 is a GMO being 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.
2. Advancement of scientific knowledge: The planned deliberate release will facilitate the gathering of essential data on safety, tolerability and initial efficacy data in human subjects. This information will enhance the progress of novel gene therapies while increasing our comprehension of XLRS biology and vector-based therapeutic strategies.

Proposed measures to limit the potential risks, to control and to ensure follow-up of the deliberate release

1. *Methods and procedures for controlling the dissemination of the GMO(s) in case of unexpected spread*
   To handling spills, the institution's Hazardous Drug Spill Procedure) will be followed

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. 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. 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.

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.

4. *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.

Site name and location in Belgium: UZ Gent; Corneel Heymanslaan 10; 9000 Gent, Belgium

Estimated number of patients in Belgium: 6 patients

Start and end date of the study in Belgium: 25th October 2026 to 25th October 2033