Adviesraad voor Bioveiligheid
Conseil consultatif de Biosécurité

Advice of the Belgian Biosafety Advisory Council on the notification B/BE/26/BVW4 of the company Atsena Therapeutics, Inc. for deliberate release in the environment of genetically modified organisms other than higher plants for research and development

FINAL version: 17/09/2026
Ref. SC/1510/BAC/2026_0790

Context

The notification B/BE/26/BVW4 has been submitted by Atsena Therapeutics, Inc. to the Belgian Competent Authority in May 2026 for a request of deliberate release in the environment of genetically modified organisms (GMOs) other than higher plants for research and development according to Chapter II of the Royal Decree of 21 February 2005.

The planned activity concerns a clinical trial with the title: *" 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)".*

Retinoschisin (RS1)-associated X-linked retinoschisis (XLRS) is a rare congenital disease of the retina caused by mutations in the RS1 gene, which encodes retinoschisin, a protein involved in intercellular adhesion and likely retinal cellular organization. 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 symmetric bilateral macular involvement 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. Visual acuity often deteriorates in the first and second decades, but then remains stable until the fourth or fifth decade. Thereafter, visual acuity may drop to blindness.

The study is designed in three parts: a dose escalation phase (Part A), a dose expansion phase (Part B) and a randomized, controlled phase (Part C). The main goals of this phase I/II/III study are to assess the safety, tolerability of ATSN-201 and to determine efficacy of ATSN-201 on visual acuity and function. The investigational medicinal product ATSN-201 will be administered subretinally into one eye (unilaterally) or both eyes (bilaterally) to children aged ≥ six years and adults with RS1-associated XLRS. The delivery of the functional human RS1 gene to the retained photoreceptors of patients with XLRS may restore vision in patients with XLRS or slow its decline.

Compared to the wild-type AAV virus, the AAV1 encapsidated rAAV vector lacks the *rep* and *cap* viral sequences rendering it unable to replicate, even in the presence of a helper virus, which was confirmed by very low amounts of Rep2 amplicon following qPCR, suggesting the presence of rcAAV in the analysis of batches.

Overall, up to 106 subjects will be included in this Phase I/II/III study, wherefore, six are expected in Belgium. ATSN-201 will be administered at three different dose levels (low, mid and high doses) as a single dose via subretinal injection in pediatric and adult participants with RS1-Associated X-linked Retinoschisis disease. This study will be conducted at one clinical site located in Flanders. The national territory is considered as the potential release area of ATSN-201.

The dossier has been officially acknowledged by the Competent Authority on 03 June 2026 and forwarded to the Biosafety Advisory Council (BAC) for advice.
Within the framework of the evaluation procedure, the BAC, under the supervision of a coordinator and with the assistance of its Secretariat, contacted experts to evaluate the dossier. Three experts from the common list of experts drawn up by the BAC and the Service Biosafety and Biotechnology (SBB) of Sciensano and one expert from the SBB answered positively to this request. The experts assessed whether the information provided in the notification was sufficient and accurate to state that the deliberate release of the genetically modified organism would not raise any problems for the environment, animal health or human health (people coming in contact with the treated patient and/or with the GMO) in the context of its intended use. See Annex I for an overview of all the comments from the experts.

The scientific evaluation has been performed considering following legislation:
- Annex II (principles for the risk assessment) and annex III (information required in notifications) of the Royal Decree of 21 February 2005.
- Commission Decision 2002/623/EC of 24 July 2002 establishing guidance notes supplementing Annex II to Directive 2001/18/EC.
The pure medical aspects concerning the efficacy of the medicinal product and its safety for the treated patients, as well as aspects related to social, economic or ethical considerations, are outside the scope of this evaluation.

On 09 July 2026, based on a list of questions prepared by the BAC, the Competent Authority requested the notifier to provide additional information about the notification. The answers from the notifier to these questions were received by the Competent Authority on 31 July 2026 and transmitted to the secretariat of the BAC on the same day. This complementary information was reviewed by the coordinator and the experts, and resulted in a second list of questions, which was transmitted to the notifier on 11 August 2026. The answers of the notifier were received on 31 August 2026 and reviewed by the coordinator, after which the BAC concluded with respect to the environmental aspects associated to the proposed clinical trial.

In parallel with the scientific evaluation of the notification, the Competent Authority also made the dossier available on its website for the one-month public consultation foreseen in the above-mentioned Royal Decree. The Competent Authority received one reaction from the public that didn't require any feedback from our part.

Summary of the scientific evaluation

1. The characteristics of the donor, the recipient or parental organism

The donor, recipient and parental organisms were found to be adequately described in the dossier.

As confirmed by the applicant, ATSN-201 drug substance (DS) is produced by triple transient transfection of HEK-293 cells

2. Information related to the characteristics of the GMO and the medication

Information related to the molecular characteristics of ATSN-201 were found to be adequately described in the dossier.

The likelihood of recombination of sequences between Rep2 and human genomic sequences in encapsidated AAV genomes is considered negligible due to the lack of homology with Rep2 and the absence of ITRs flanking Rep2 required for encapsidation. This is further supported by 100% sequence concordance with the reference sequence by NGS and stringent control of residual host-cell DNA levels.

3. The conditions of the release

This phase I/II/III study will consist of three parts: a dose escalation phase (Part A), a dose expansion phase (Part B) and a randomized, controlled phase (Part C). In Part A, 3 dose levels of ATSN-201 will be evaluated in adult subjects, to select the maximum recommended dose for Part B. In Part C of the study, eligible patients will be treated with ATSN-201 or will have no treatment; subjects assigned to ATSN-201 will receive the drug as a one-time subretinal injection of ATSN-201 in one eye or both eyes. Subjects who do not receive treatment can choose to receive ATSN-201 in one or both eyes after the 1-year Main Study Period. The GMO will be administered via a subretinal injection, in hospital centres. Subjects will be monitored for 12 months to assess treatment outcomes. Afterwards, all the subjects will continue the study for long-term follow-up for a total of five years after ATSN-201 administration.

Shedding data collected from the study will further contribute to a proper environmental risk evaluation. These shedding data will need to be evaluated considering the observed quantity of shed viral vector material, and the period during which shedding is observed. Preliminary results from the shedding analysis performed during the Part A and B of this first-in-human study have been provided. Shedding of ATSN-201 was evaluated in serum, tears from both eyes, saliva urine and nasal secretions. During Part C of the study, samples for shedding analysis will be collected at different timepoints up to 12 months post administration of the treatment until 3 consecutive samples are negative (ddPCR assessment). Should ddPCR analysis reveal a detectable presence of Rep2 sequences, it is key to determine whether the observed signal corresponds with shed rcAAV viral vector genome and thereby adapt the precautionary measures for patients and care-takers accordingly to prevent contamination via tears, saliva, sputum, or cough.

Based on the shedding results from Seitz et al. (2017)[1] (showing quantifiable shedding one week after subretinal injection of rAAV-8 in non-human primates) and on the recommendation provided in the EPAR document from EMA for Luxterna, an AAV vector-based gene sub-retinal therapy for the treatment of adult and paediatric patients with vision loss, the applicant was asked to ensure that the treated eye of the patient is adequately protected by an eye bandage for at least 7 days post-injection.

[1] I.P. Seitz et al. 2017. "Superior Retinal Gene Transfer and Biodistribution Profile of Subretinal Versus Intravitreal Delivery of AAV8 in Nonhuman Primates". Invest Ophthalmol Vis Sci. 2017 Nov 1;58(13):5792-5801

Following BAC's request, the notifier provided a patient instruction leaflet that explains and summarizes all critical information and instructions for patients and their families as a safeguard against potential rAAV vector transmission to other people or release into the environment once patients leave the hospital setting.

To ensure consistency across the ICF, CAF and SNIF documents, contraception requirements have been aligned as follows: patients of reproductive age treated with the IMP must agree to use a highly effective method of contraception for at least 6 months following administration of ATSN-201.

Taken together, the information related to the conditions of the release were found to be adequately described in the dossier.

4. The risks for the environment or human health

The GMO is a recombinant, replication-deficient adeno-associated virus-based (rAAV) vector not harbouring any antibiotic resistance genes. Like the wild-type AAV virus, a rAAV vector is not known to be pathogenic. The genetic modification introduced in the AAV-based vector does not confer on the GMO any known properties that could pose risks to the human population or the environment.

There is only a remote possibility of homologous recombination between the ITR-sequences of ATSN-201 and wild-type AAV in case a triple infection by ATSN-201, wild type AAV2 (providing the *rep2* and *cap2* functions) and a helper virus occurs in exposed persons. Such a recombination event would result in a gain of functional AAV genes required for replication and encapsidation but should in turn lead to the loss of the transgene (and in essence results again in a WT AAV2). It was also remarked that the genetic material from *rep* and *cap* genes together with the transgene size would be too large to be packaged in AAV capsid, making it impossible to form a replication competent viral particle that contains the transgene and the *rep* and *cap* genes necessary for multiplication.

AAV virus particles are stable viruses that can persist in the environment for extended periods of time (thought to be on the order of several weeks). AAV particles are resistant to a wide range of pH (pH 3-9) and can resist elevated temperatures (55°C for 1 hour).

In order to align with the instruction given in the product information document (EPAR) of EU registered medicinal products containing rAAV, a lifelong restriction on donating blood, organs tissues and cells for transplantation is recommended.

In the case of transfer of rAAV to an unintended immune-competent human recipient, the risks are expected to be considerably reduced compared to any potential risk for the participant, since the rAAV vector is unable to replicate and the transferred 'dose' (from e.g., aerosol, splashing or fomites) is orders of magnitude lower than that received by the receiving patients. It is expected that in the worst case, the exposed individual will develop immune responses to the AAV capsid proteins.

The BAC concludes that, based on the non-pathogenic and non-replicative nature of ATSN-201 and the assumed lower amounts of shed and intact viral particles of ATSN-201 compared to the therapeutic dose, the overall risk associated to exposure and transmission to other individuals can be considered low to negligible.

5. The monitoring, control, waste treatment and emergency plans proposed by the applicant

Following SBB's request, the composition of the mandatory personal protective equipment, consisting of a laboratory coat, mask, protective glasses, and gloves, has been aligned between the documents. The Applicant has been informed that the use of an apron alone is not sufficient; a laboratory coat is a minimum requirement, with an apron used as an additional protective measure if needed. FFP2 mask will be used during spill management activities and during IMP preparation procedures performed outside a biosafety cabinet.

Disinfectants, such as Dismozon® plus at a concentration of 3.6% for 15 minutes, based on magnesium monoperoxyphthalate hexahydrate (MMPP) or Descogen® at 3% for 30 minutes, a potassium peroxymonosulfate-based disinfectant solution, will be used for decontamination of the ATSN-201 preparation area, the administration room after completing administration and in the event of spill. Potassium peroxymonosulfate was shown to inactivate AAV in a serotype- and contact-time-dependent manner (Korte et al. 2021[2]). Concentrations of 5000-6000 ppm sodium hypochlorite are also reported in the literature for effective AAV inactivation (Korte et al. 2021[2]). The appropriate documents have been updated accordingly.

Since propagation of ATSN-201 is unlikely, the BAC supports the view that, in terms of risk for the environment or human health, the proposed measures that are described in the revised documents are proportionate and adequate in the context of the intended trial provided that the additional requests as outlined in the conditions here below are met.

Conclusion

Based on the scientific assessment of the notification made by the Belgian experts, the Biosafety Advisory Council concludes that it is unlikely that ATSN-201 developed to treat patients with RS1-Associated X-linked Retinoschisis, by means of endogenous production of a functional human RS1 protein to the retained photoreceptors of the patients, will have any adverse effects on human health or on the environment in the context of the intended clinical trial provided that all the foreseen safety measures are followed.

Therefore, the Biosafety Advisory Council issues a **positive advice with the following conditions**:

- The notifier and the investigators must strictly apply the clinical trial protocol, and all the safety instructions as described in the following documents:
    - Latest version of the ICF
    - Latest version of the Protocol
    - SNIF public and confidential v3.0
    - CAF_ATSN-201 v2.0
    - CAF_ ATSN-201 confidential annex v2.0
    - Patient instruction leaflet ATSN-201

[2] Korte, J, *et al.* (2021). "Inactivation of Adeno-Associated Viral Vectors by Oxidant-Based Disinfectants." Hum Gene Ther 32(13-14): 771–781.

- As committed by the applicant in its responses to the SBB requests dated 31-Jul-2026, and in accordance with the updates made to the CAF, the ICF will be revised to include a lifelong recommendation to refrain from donation blood, organs, tissues, and cells, in alignment with the product information (EPAR) for EU-authorised medicinal products containing recombinant AAV

- Any protocol amendment must be previously approved by the Competent Authority.

- The notifier is responsible to verify that each study centre has qualified personnel (experienced in handling infectious material) and that the investigator has the required authorizations to perform the clinical trial activities inside the hospital (laboratory, pharmacy, hospital room, consultation room...) according to the Regional Decrees transposing Directive 2009/41/EC on Contained use of genetically modified micro-organisms.

- The Biosafety Advisory Council should be informed within two weeks when the first patient starts the treatment and the last patient receives the last treatment.

- At the latest six months after the last visit of the last patient enrolled in the trial, the notifier must send a report to the competent authority at the attention of the Biosafety Advisory Council detailing the biosafety aspects of the project. This report will contain as a minimum:
    - The total number of patients enrolled in the trial and the number of patients from Belgium;
    - A summary of all adverse events documented by the investigators as likely or definitely related to the study medication;
    - A report on the accidental releases, if any, of ATSN-201.

Dr. ir. Geert Angenon
President of the Belgian Biosafety Advisory Council

Annex I: Compilations of comments of experts in charge of evaluating the dossier B/BE/26/BVW4 (ref. SC/1510/BAC/2026_0609, SC/1510/BAC/2025_0674 and SC/1510/BAC/2025_0749)

Adviesraad voor Bioveiligheid
Conseil consultatif de Biosécurité

Compilation of comments of experts in charge of evaluating the dossier B/BE/26/BVW4
And comments submitted to the notifier

09 July 2026
Ref. SC/1510/BAC/2026_0609

Mandate for the Group of Experts: Mandate of the Biosafety Advisory Council (BAC) of 28 May 2026.
Coordinator: Rik Gijsbers (KULeuven)
Experts: Expert 2 (KULeuven), Expert 1 (ULiège), Expert 3 (Lausanne University Hospital), Expert 4 (SBB)
SBB: Sheela Onnockx

INTRODUCTION

Dossier **B/BE/26/BVW4** concerns a notification from Atsena Therapeutics Inc. for the deliberate release in the environment of genetically modified organisms other than higher plants according to Chapter II of the Royal Decree of 21 February 2005.
The notification has been officially acknowledged on 03 June 2026 and concerns a clinical trial entitled "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)". The investigational medicinal product is a recombinant AAV vector carrying human retinoschisin 1 (hRS1) expression cassette.

♦ INSTRUCTIONS FOR EVALUATION

Depending on their expertise, the experts were invited to evaluate the genetically modified organism considered in the notification as regards its molecular characteristics and its potential impact on human health and the environment. The pure medical aspects concerning the efficacy of the medicinal product and its safety for the treated patient are outside the scope of this evaluation.
The comments of the experts are roughly structured as in
- Annex II (principles for the risk assessment) of the Royal Decree of 21 February 2005
- Annex III (information required in notifications) of the Royal Decree of 21 February 2005
- Commission Decision 2002/623/EC of 24 July 2002 establishing guidance notes supplementing Annex II to Directive 2001/18/EC.

List of comments received from the experts

Remark: The comments below have served as basis for a list of questions that the Competent authority forwarded on 09-07-2026 to the notifier with a request to provide additional information. The comments or remarks highlighted in grey correspond to the questions addressed to the notifier.

List of comments/questions received from the experts

2. INFORMATION RELATED TO THE INVESTIGATIONAL MEDICINAL PRODUCT

2.1. Description of the production system

(e.g. maps of the vectors used, characteristics of the cell lines used, possibility of complementation or recombination….)

Comment 1 (**Expert 1**)

Has evaluated this item and has no questions/comments.

Comment 2 (**Expert 2**)

During the manufacturing process HEK293 cells were used (see e.g. page 7 of 27 in the public CAF and page 8 of 28 in the CAF confidential annex). However, also on page 8 of 28 in the CAF confidential annex HEK293T cells are mentioned as the origin of the HEK293 MCB. Is the conclusion correct, that HEK293T cells (a derivative of HEK293) were actually used in the manufacturing process? Why not mention more accurately HEK293T consequently instead of HEK293 in the documents?

SBB Comment

The expert's observation could be sent to the applicant as follows:

According to the Public CAF document, section 2.1.2, HEK293 cells have been used in the manufacturing process. However, according to the Confidential CAF document, it is unclear whether HEK293T or HEK293 cells were used. The applicant is requested to clearly indicate in both documents which cells have been used in the manufacturing process.

Coordinator's comment

I agree with the expert and SBB. Consequent use of wording is key.

The sentence "Overall, it can be concluded that 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." (see page 7 of 27 in the public CAF) should be rephrased in order to avoid the use of "mostly". >99.999999 % compared to <0.000001 % is not accurately described by "mostly".

SBB Comment

The expert's observation could be sent to the applicant as follows:

It is stated in section 2.2 of the CAF Public document that: "Overall, it can be concluded that 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 sentence should be rephrased to avoid the use of "mostly," as it does not accurately reflect the magnitude of the difference. A comparison of >99.999999% versus <0.000001% indicates an overwhelming predominance rather than what would ordinarily be described as "mostly."

Coordinator's comment

I agree with the expert. I would rather suggest the use of a statistical test and a p value instead.

SBB Comment

The recommendation to use a statistical test instead of the p-value could be added in the question sent to the applicant.

Comment 3 (**Expert 3**)
It is documented in the confidential CAF page7 that Rep 2 has homology with a human gene. Was this reported before? It suggests that the encapsidation plasmid might be subjected to homologous recombination with the human gene during viral production and, although at a very low level after shedding. Did the applicant test the presence of contaminants gene fragments in encapsidated genomes?

SBB Comment
The expert's observation could be sent to the applicant as follows:
It is stated on page 7 of the confidential CAF that Rep 2 shares homology with a human gene. Has this homology been reported previously? This observation raises the theoretical possibility that the encapsidation plasmid could undergo homologous recombination with the human gene during viral production and, albeit at a very low frequency, potentially after shedding. Has the applicant assessed whether gene fragments or related recombinant sequences are present as contaminants in the encapsidated viral genomes?

Coordinator's comment
I agree with the expert. As far as I know there is no homology. Homology would imply that there is a common ancestral gene.

Comment 4 (**Expert 4**)
Has not evaluated this item.

2.2. Demonstration of absence of formation of replication-competent virus
(e.g. assessment of risk of generation of replication competent AAV, test methods and test data, ….)

Comment 1 (**Expert 1**)
Has evaluated this item and has no questions/comments.

Comment 2 (**Expert 2**)
Has evaluated this item and has no questions/comments.

Comment 3 (**Expert 3**)
Has evaluated this item and has no questions/comments.

Comment 4 (**Expert 4**)
Has not evaluated this item.

2.3. Diagram (map) of the clinical vector

Comment 1 (**Expert 1**)
Has evaluated this item and has no questions/comments.

Comment 2 (**Expert 2**)
Has evaluated this item and has no questions/comments.

Comment 3 (**Expert 3**)

Has evaluated this item and has no questions/comments.

Comment 4 (**Expert 4**)
Has not evaluated this item.

2.4. Molecular characterisation of the clinical vector

(e.g. annotated sequence of the genome, genetic stability, ….)

Comment 1 (**Expert 1**)
Has evaluated this item and has no questions/comments.

Comment 2 (**Expert 2**)
Has evaluated this item and has no questions/comments.

Comment 3 (**Expert 3**)
Has evaluated this item and has no questions/comments.

Comment 4 (**Expert 4**)
Has not evaluated this item.

2.5. Description of the insert

(e.g. description of the expression cassette, potential harmful properties of the transgene, ….)

Comment 1 (**Expert 1**)
Has evaluated this item and has no questions/comments.

Comment 2 (**Expert 2**)
Has evaluated this item and has no questions/comments.

Comment 3 (**Expert 3**)
Has evaluated this item and has no questions/comments.

Comment 4 (**Expert 4**)
Has not evaluated this item.

2.6. Biodistribution and shedding

(e.g. shedding data, administered dose, route of administration, biodistribution data, methods used for detection of viral shedding….)

Comment 1 (**Expert 1**)
Has evaluated this item and has no questions/comments.

Comment 2 (**Expert 2**)
Has evaluated this item and has no questions/comments.

Comment 3 (**Expert 3**)
In the confidential CAF page 14, it is mentioned (but not proven) that “the vector is likely to be inactive or be deactivated by natural conditions.”

AAV particles are very stable. For example, after drying 6 days on a metal surface and being harvested by dilution, rAAV1 viral were shown to transduce cells with only a slightly reduced efficiency (Assaying the Stability and Inactivation of AAV Serotype 1 Vectors. Douglas B. Howard and Brandon K. Harvey)
Although stability could be different for different serotypes, it is doubtful that "natural conditions" will inactivate the vector.
Other arguments: "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." are fully acceptable.
However, the sentence "the vector is likely to be inactive or be deactivated by natural conditions." should be deleted.

SBB Comment

The expert's comment is acknowledged and could be communicated to the applicant as follows:
In section 3.5 of the CAF confidential document (page 14/28), it is stated, without supporting evidence, that "the vector is likely to be inactive or be deactivated by natural conditions". However, AAV particles are known to be very stable. For example, after drying 6 days on a metal surface and being harvested by dilution, rAAV1 viral were shown to transduce cells with only a slightly reduced efficiency (Douglas *et al*. 2017). While the environmental stability may vary among AAV serotypes, the available evidence does not support the general assertion that "natural conditions" are likely to inactivate the vector. The applicant is therefore requested to delete this statement or, alternatively, to provide appropriate scientific evidence to substantiate it.

Coordinator's comment

I completely agree with the expert. Also, the applicant does not use wording correctly. Wild-type AAV vectors do not exist, instead these are to be referred to as wt AAV virus.

SBB comment:

The coordinator's comment will be communicated to the applicant as follows:
Additionally, in the same section, the applicant uses incorrect terminology. "Wild-type AAV vectors" is not an appropriate term, as wild-type AAV is a virus rather than a vector. The correct terminology is "wild-type AAV virus" (wt AAV).

Comment 4 (**Expert 4**)
Has evaluated this item and has no questions/comments.

Additional SBB Comment:

Timepoint for shedding samples collections is inconsistent across the SNIF (page 22), CAF (page 16) and table 1 "Schedule of activities" in the protocol (page 29):

- SNIF: 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
- CAF: Predose phase (Day -90 to -8), at Day1, Day 7, Day 14, Day 28, at Week 8, week 12 and at Month 6 and 12
- Protocol: D-7 to D-2, D1, D7, D28, W8, W12, W24, Month 12

Please check these data and ensure timepoints for shedding samples are correctly reported between the different documents.

Coordinator's comment

I agree with the SBB. Overall, there are a significant number of typos, misinterpretations and incomplete sections. The document could be improved by proof-reading and more coherent reporting.

3. INFORMATION RELATED TO THE CLINICAL TRIAL

3.3. Storage of the clinical vector at the clinical site

(e.g. storage location, conditions of storage, …)

Comment 1 (**Expert 1**)
Has evaluated this item and has no questions/comments.

Comment 2 (**Expert 2**)
Has evaluated this item and has no questions/comments.

Comment 3 (**Expert 3**)
Has not evaluated this item.

Comment 4 (**Expert 4**)
Has evaluated this item and has no questions/comments.

3.4. Logistics for on-site transportation of the clinical vector

(information on logistics of in-house transportation, characteristics of the container, disinfection procedures, labelling of the containers, ...)

Comment 1 (**Expert 1**)
Has evaluated this item and has no questions/comments.

Comment 2 (**Expert 2**)
Has evaluated this item and has no questions/comments.

Comment 3 (**Expert 3**)
Has evaluated this item and has no questions/comments.

Comment 4 (**Expert 4**)
Has evaluated this item and has no questions/comments.

3.5. Reconstitution, finished medicinal product and administration to the patients

(e.g. mode of administration, information on dosing and administration schedule, information on concomitant medication,…)

Comment 1 (**Expert 1**)
Please see the comment III) in the section 3.6 of this report

Comment 2 (**Expert 2**)
Has evaluated this item and has no questions/comments.

Comment 3 (**Expert 3**)
Has evaluated this item and has no questions/comments.

Comment 4 (**Expert 4**)
Has evaluated this item and has no questions/comments.

3.6. Measures to prevent dissemination into the environment (e.g. control measures, PPE, decontamination/cleaning measures after administration or in the case of accidental spilling, waste treatment, recommendation given to clinical trial subjects, …)

Comment 1 (**Expert 1**)

I) In the SNIF document, page 22, point 4 (b) Treatment of waste, it is written that:

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.

Please consider that this part is confused in which the applicant proposed a list of procedures of treatment of waste but also referred treatment in case of spill. This part is unclear:

1) “Non-disposable materials will be decontaminated by treatment with an appropriate disinfectant and/or autoclaving oxygen-releasing solution (i.e. Descogene 3% for 30 minutes) will be used for regular disinfection on used surfaces. “

Comment:

-This sentence mixed procedures for decontamination of non-disposable materials and for regular disinfection on used surfaces. The interpretation of the “used surfaces” seems to concern currently the surfaces of the laboratory furniture. If this understanding is valid, thus please remove the part “will be used for regular disinfection on used surfaces.” , which could not be considered as a procedure of “Treatment of waste”
-There is no description of “non-disposable materials.”
-“An appropriate disinfectant” : please explicit this one.
-“And/or” : this term “and” is not appropriate because non-disposable materials could be previously treated by disinfectant as 1000 ppm chlorine solution such as proposed by the applicant which is not appropriate to be followed by a treatment by oxygen-releasing solution (i.e. Descogene 3% for 30 minutes). Indeed, in water, this product released H_2O_2 and in contact of rests of bleach, it can cause an exothermic chemical reaction that generates heat, toxic gases (including chlorine gas) and oxygen in the lab, which can lead to an explosion or serious respiratory problems.
-Autoclaving oxygen-releasing solution (i.e. Descogene 3% for 30 minutes): The appropriate term of this treatment is not autoclaving: it must be replaced by “disinfection / decontamination by oxygen-releasing solution”.

2) ”All additional material such as absorbent tissues used to absorb the material will be discarded and destroyed as described above.” A reference to a section must clearly be added to this sentence because the expression "as described above" is too vague.

3) “Any broken material (i.e. the container closure system, syringes, etc.) will also be similarly destroyed.” A reference to a section must clearly be added to this sentence because the expression “similarly destroyed” is too vague.

SBB comment:

The expert's observation is well-founded and could be sent to the applicant as follows.

Section I.4.b of the SNIF (page 23/27) :

“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.”

Several points in this section are incomplete or inaccurate and should be updated as follows:

1- A description of the expected “non-disposable materials” should be included
2- Example(s) of “appropriate disinfectant” should be included
3- “and/or” : the term “and” is not appropriate because non-disposable materials could be previously treated by disinfectant as 1000 ppm chlorine solution such as proposed by the applicant. The latter cannot be followed by a treatment by oxygen-releasing solution (i.e. Descogene 3% for 30 minutes). Indeed, in water, this product releases H_2O_2 and in contact of rests of bleach, it can cause an exothermic chemical reaction that generates heat, toxic gases (including chlorine gas) and oxygen in the lab, which can lead to an explosion or serious respiratory problems.
4- The term "autoclaving" is not appropriate for treatment with an oxygen-releasing solution (e.g., Descogene 3% for 30 minutes). This procedure should be described as "disinfection or decontamination using an oxygen-releasing solution." Please correct the terminology.
5- The expression "as described above" is too vague. Please replace it with a specific reference to the relevant section or subsection to ensure clarity and facilitate navigation for the reader.
6- The expression “similarly destroyed” is too vague. Please replace it with a specific reference to the relevant section or subsection to ensure clarity and facilitate navigation for the reader.

Coordinator’s comment

I agree with the expert and suggestion by the SBB.

Proposition of a better wording:

Non-disposable materials may first be treated with a disinfectant such as a 1000 ppm chlorine solution, as proposed by the applicant. However, this procedure should not be followed by treatment with an oxygen-releasing disinfectant solution (e.g., Descogen® 3% for 30 minutes). In aqueous solution, this product releases hydrogen peroxide (H_2O_2). Residual chlorine-based disinfectants (bleach/hypochlorite) may react with hydrogen peroxide, resulting in a vigorous exothermic reaction with the release of oxygen gas and heat. Depending on the conditions and products involved, hazardous gases may also be generated. Such reactions represent a safety risk in the laboratory and may lead to pressure build-up, fire hazards, or serious respiratory exposure.

SBB comment:

The question to the applicant has been updated as suggested by the coordinator

II) In the document CAF confidential p13-14, in section 3.6.a:

Non disposable material will be autoclaved or disinfected with an appropriate validated disinfection detergents. Surfaces will be disinfected with an appropriate validated disinfection detergent, for instance 1000 ppm chlorine solution.

Please consider that chlorine solution could not be defined as a detergent: chlorine solution is commonly used as a disinfectant, oxidizing product, but it does not contain surfactants able to clean or degrease soiled surfaces. If you apply it to a surface that is too soiled, its disinfecting capacity could be neutralized.

SBB comment:
The expert's observation is well-founded and could be sent to the applicant as follows.
In both CAF documents page 14, section 3.6.a, it is stated that: “Surfaces will be disinfected with an appropriate validated disinfection detergent, for instance 1000 ppm chlorine solution”.
However, please note that chlorine solution cannot be considered as a detergent. Chlorine solutions are commonly used as disinfectants due to their oxidizing properties, but they do not contain surfactants that enable the cleaning and degreasing of soiled surfaces. Furthermore, the efficacy of chlorine-based disinfectants can be significantly reduced when applied to very soiled surfaces. Therefore, please specify a validated disinfection detergent that can be used for decontamination of surfaces.

Coordinator’s comment
I agree with the expert and suggestion by the SBB.

In the document CAF confidential p13-14, in section 3.6.b:

b) Personal protective equipment.

The personnel performing the dilution of the GMO will use universal precautions and appropriate PPE (as applicable): personal apron, gloves, mask and protective goggles (used for handling IMP)

The described PPE here described is not harmonized with the other documents of this dossier. Please synchronize it and consider that FFP2 mask is also appropriate for the management of spill cases and ATN-201 preparations outside the biosafety cabinet.

SBB comment:
The expert's observation is well-founded and could be sent to the applicant as follows.
In both CAF documents page 14, section 3.6.a, it is stated that: “The personnel performing the dilution of the GMO will use universal precautions and appropriate PPE (as applicable): personal apron, gloves, mask and protective goggles (used for handling IMP)”. In the SNIF, section F.4.c., the safety procedures specify that “the 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”.

- The applicant is requested to ensure that the PPE requirements described in the CAF and SNIF documents are aligned and consistent.
- In addition, the applicant is requested to consider the use of FFP2 mask during spill management activities and during IMP preparation procedures performed outside a biosafety cabinet, as this level of respiratory protection would also be appropriate in these circumstances.

- Finally, it should be noted that the use of an apron alone as such is not considered sufficient. A laboratory coat should be worn as a minimum requirement. An apron may be used in combination with a lab coat.

The applicant is requested to revise the relevant sections of the CAF and SNIF accordingly and ensure that the PPE requirements are consistently reflected throughout both documents.

Coordinator's comment

I agree with the expert and suggestion by the SBB.

In the document CAF confidential p13-14, in section 3.6.c:

> Standard hospital hygienic measures will be effective during reconstitution, handling and administration of all vectors. Medical personnel will follow standard hospital hygienic measures. Non-disposable material will be autoclaved or disinfected with an appropriate validated disinfection detergent. 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, for instance a 1000 ppm chlorine solutions.

In this section the applicant proposed a treatment by autoclave : is it sterilization by saturated steam under pressure or "Autoclaving oxygen-releasing solution (i.e. Descogene 3% for 30 minutes)" as reported In the document SNIF p22, point 4(b). Treatment of waste

SBB comment:

As mentioned here above by the same expert, autoclaving and chemical disinfection such as Descogene 3% are different methods. However, the applicant could be requested to include an appropriate disinfectant as follows:

Section 3.6.c of both CAF documents about "waste treatment" should be improved as followed:

1- A description of the expected "non-disposable material" should be included
2- Example(s) of "validated disinfection detergent" should be included

III) In the document CAF confidential p12, section 3.5

During the reconstitution of the ATSN-201 batches for subretinal injection, needles and syringes are used. So the risk of needle-stick injury or a splash in the eyes (liquid under-pressure handling) must be considered and specific procedures (ex: specific skin care disinfectant) must be described in a list as follows:

We recommend that all healthcare personnel involved in the study receive a concise instruction sheet (1-2 page) summarizing all relevant procedures. The document should provide clear and practical guidance on the safe handling of the product, including detailed instructions in event of accidental exposure or spills, waste management requirements, and other risk management measures.

Providing such a consolidated document would facilitate daily practice by ensuring that all essential information is readily accessible to health care personnel, thereby promoting safe and compliant handling of the investigational product.

This instruction sheet should include at minimum all elements listed below:

- the use of personal protective equipment for health care workers (e.g. specify which PPE are mandatory)

- procedure in the event of accidental occupational exposure through a splash in the eyes, mucous membrane, needle-stick injury or contact with skin and clothing
- procedures for treatment of accidental spill (disinfectant, concentration of disinfectant, contact time)
- procedures to prevent and to deal with direct exposure to fluidic samples from patients in the initial period after administration of the ATSN-201
- waste management procedures

SBB comment:
The expert's comment is acknowledged and could be communicated to the applicant as is

Coordinator's comment
I agree with the expert and suggest some adaptations.

SBB comment:
Proposed adaptations have been taken into account in the text here above and in the question that will be sent to the applicant

Comment 2 (**Expert 2**)
1. Decontamination:

In the public CAF (page 14 of 27) and the SNIF (page 23 of 27) a 1000 ppm chlorine solution is mentioned as an appropriate validated detergent for inactivation of the AAV vector. This is questionable, because usually a solution of 5000 to 6000 ppm hypochlorite is used to inactivate AAV. Furthermore, is the use of Descogen® 3% for 30 minutes really validated in order to inactivate AAV?

It should be noted that recommendations on the use of chlorine solutions are preferably expressed in terms of % sodium hypochlorite or ppm (mg/L). Also, it should be remarked that the use of 6000 ppm (mg/L) bleach solution can generate ocular irritation or oropharyngeal, oesophageal, and gastric burns and should be reserved for minor surface spill treatment. The applicant is recommended to provide a list of disinfecting agents with viricidal activity against adenovirus- associated virus other than 6000 ppm (mg/L) bleach solution, because the use of bleach at this concentration for the decontamination must be strictly limited to treat small surfaces whose composition is chemically compatible and without stainless steel, aluminium and the most rubbers.

With respect to the use of such viricidal disinfecting agents with activity against adenovirus- associated virus other than 6000 ppm (mg/L) bleach solution, it should be noted, that clear instructions should be provided how to use these agents safely. Spraying of these agents should be avoided as much possible, since they typically contain complex chemical mixtures, including volatile organic compounds, primarily used as solvents and fragrances, preservatives, and disinfectants. Consequently, inhalation exposure is likely higher after spray application than other liquid application methods (Kumbhar & Geddugol, 2025).

Kumbhar & Geddugol (2025). Prevalence of Health Effects Due to Disinfectant Exposure and its Impact on Selected Physiological Parameters Among Class D Workers : A Descriptive Cross-Sectional Study. Cureus 17(3): e79994. DOI 10.7759/cureus.79994.

SBB Comment 1

Regarding the use of chlorine solution, the expert's remark is supported and could be communicated to the applicant as follows:
In the public CAF (page 14/27) and the SNIF (page 23/27), a 1000 ppm chlorine solution is identified as an appropriate validated disinfectant for inactivation of the AAV vector. This claim appears questionable, as concentrations of 5000–6000 ppm sodium hypochlorite are generally reported in the literature for effective AAV inactivation.

For clarity and consistency, recommendations regarding chlorine-based disinfectants should preferably be expressed as sodium hypochlorite concentration (% or ppm [mg/L]) rather than as "chlorine solution".

It should also be noted that the use of sodium hypochlorite at concentrations of approximately 6000 ppm (mg/L) may cause ocular irritation as well as oropharyngeal, oesophageal, and gastric burns. Consequently, its use should be restricted to the treatment of small surface spills and only on materials that are chemically compatible, as bleach at this concentration may damage stainless steel, aluminium, and many rubber materials.

The applicant is therefore requested to justify the proposed use of a 1000 ppm chlorine solution for AAV inactivation by providing appropriate validation data. In addition, the applicant is encouraged to identify alternative disinfectants with demonstrated virucidal activity against AAV that may be suitable for routine decontamination procedures and that present fewer compatibility and safety concerns than high-concentration sodium hypochlorite solutions.

For any disinfectant proposed for AAV decontamination, clear instructions for safe handling and application should be provided. In particular, spray application should be avoided whenever possible, as many commercial disinfectants contain complex chemical mixtures, including volatile organic compounds used as solvents, fragrances, preservatives, and active ingredients. As a result, spray application may lead to greater inhalation exposure than other liquid application methods (Kumbhar & Geddugol, 2025).

SBB Comment 2
Regarding the use of Descogen®, the following comment could be sent to the applicant:
The applicant indicates that Descogen® 3% with a contact time of 30 minutes is used for decontamination. However, no evidence has been provided demonstrating that this disinfectant is effective against AAV. Given the known resistance of AAV to many commonly used disinfectants, the applicant is requested to provide scientific evidence or product-specific validation demonstrating that Descogen® 3% under the proposed conditions effectively inactivates the clinical AAV vector, or alternatively to use a disinfectant with published efficacy against AAV.

Furthermore, it should be recommended that all medical personnel involved in the study receive a concise overview (a 1–2-page instruction sheet) summarizing all relevant handling procedures, including detailed instructions in case of accidental spills, waste management requirements, and other risk management measures.

SBB Comment
See previous comment from expert 1 here above

Coordinator's comment
I agree with the expert and SBB.

2. Personal protective equipment:

In the public CAF (page 14 of 27) the use of an apron as protective clothing is mentioned. An apron as such is not sufficient, a lab coat should be used. Although an apron could be used in combination with a lab coat.

SBB Comment

This comment has been included in previous comment from expert 1 here above

3. Recommendations given to clinical trial subjects to prevent dissemination (where applicable):

Based on the recommendation provided in the EPAR document foatsenarm EMA for Luxterna, an AAV vector-based gene sub-retinal therapy for the treatment of adult and paediatric patients with vision loss, the applicant is asked to ensure that the treated eye of the patient is adequately protected by an eye bandage for at least 7 days post-injection until it is proven that no shedding is detectable at this and later time points post-administration.
Patients and family should be advised on how to handle waste material generated from dressings, tears, and nasal secretion appropriately, which may include storage of waste material in sealed bags prior to disposal. It is also recommended that patients and their family members wear gloves when changing eye dressing and handling waste, particularly in cases where a family member is pregnant, breastfeeding, or immunocompromised.

SBB Comment

The expert's remark is supported and could be communicated to the applicant as follows:
As stated in the CAF document page 10, "Atsena did not collect shedding samples from the nonclinical studies. However, available nonclinical and clinical data with other rAAV vectors using the same ROA have shown minimal vector shedding in tears and nasal swabs 3 to 4 days after subretinal administration".
According to the article from Seitz (2017), shedding via the tear film showed a pronounced dose-dependent effect, both groups (intravitreal and subretinal injection) showed quantifiable shedding one week after treatment.
In light of these results and based on the recommendation provided in the EPAR document from EMA for Luxterna, an AAV2 vector-based sub-retinal gene therapy for the treatment of adult and paediatric patients with vision loss, the applicant is asked to ensure that the treated eye of the patient is adequately protected by an eye bandage for at least 7 days post-injection until it is proven that no shedding is detectable at this and later time points post-administration.
Patients and family should be advised on how to handle waste material generated from dressings, tears, and nasal secretion appropriately, which may include storage of waste material in sealed bags prior to disposal. It is also recommended that patients and their family members wear gloves when changing eye dressing and handling waste, particularly in cases where a family member is pregnant, breastfeeding, or immunocompromised. All these recommendations should clearly be reported either in the applicable documents (ICF…) or in a dedicated instruction sheet for the patient.

Coordinator's comment

I agree with the expert and SBB. I additionally would like to include that the serotype here (AAV44.9 (E531D) Cap) has never been described for human use. AAV44.9 is a naturally occurring AAV isolate whose sequence is closely related to AAVrh.8 and AAVrh.8R. AAV44.9 is a variation (T179S S473N

S483C and E531D) of AAVrh.8 (Di Pasquale, G., *et al.*, Transduction of Salivary Gland Acinar Cells with a Novel AAV Vector 44.9. Molecular Therapy – Methods & Clinical Development, 2020. 19: p. 459-466), which falls between clade D and E and has homology to AAVRh8R, also had transduction activity for acinar cells in the parotid gland. It has attracted interest because of its efficient transduction of specific tissues, including photoreceptors in the retina. A new serotype requires additional caution, and shedding info and off-target transduction should be assessed in shedding studies instead of assuming that their biosafety level is in line with earlier serotypes.

SBB comment:
The following remark could be provided to the applicant:
The serotype (AAV44.9 (E531D) Cap) has never been described in human use previously. AAV44.9 is a naturally occurring AAV isolate whose sequence is closely related to AAVrh.8 and AAVrh.8R. Specifically, AAV44.9 differs from AAVrh.8 by four amino acid substitutions (T179S S473N S483C and E531D) (Di Pasquale, G., *et al.*, 2020), which falls between clade D and E, has homology with AAVrh8R, also has transduction activity for acinar cells in the parotid gland. AAV44.9 has attracted interest due to its efficient transduction of specific tissues, including retinal photoreceptors. A new serotype requires additional caution, and shedding info and off-target transduction should be assessed in shedding studies instead of assuming that their biosafety level is in line with earlier serotypes.
The use of a novel serotype warrants additional caution. Biodistribution, shedding, and potential off-target transduction should be evaluated specifically for ATSN-201 rather than assuming that its biosafety profile is comparable to that of previously characterized AAV serotypes. Modified or redirected tropism may result in shedding patterns that differ substantially from those of parental or naturally occurring serotypes, including both increased and decreased shedding. Furthermore, engineered vectors may exhibit tissue-targeting profiles that are not adequately predicted by conventional animal models, including potential transduction of reproductive tissues in humans.

Consequently, biodistribution and shedding data from earlier clinical studies involving different AAV serotypes cannot be considered fully representative of, or directly extrapolated to, ATSN-201. The safety characteristics of this vector should therefore be assessed based on its own biological properties and supported by vector-specific biodistribution and shedding data.

Female participants of WOCBP and partners of trial participants must agree to use a highly effective method of contraception for at least 12 months post ATSN-201 administration. Male trial participants must agree with their partner to use 2 forms of contraception, including 1 barrier method for at least 12 months following ATSN-201 administration.
All these recommendations and restrictions should clearly be reported in the applicable documents (CAF, SNIF and SIS and ICF).

SBB Comment
The expert's remark is supported and could be communicated to the applicant as follows:
According to the CAF document (page 15), women of childbearing potential and fertile men must be willing to use barrier contraception methods or abstain from sexual intercourse from the time of informed consent until 3 months following the completion of study treatment administration. This is consistent with inclusion criterion 5 of part A and B of the protocol (page 53), which requires participants to use barrier contraception methods or abstain from sexual intercourse for a period of 3 months following study treatment administration.
However, page 17 of the ICF states that patient must agree to use a double contraception method for up to 6 months after surgery. In addition, IC3 for Part C requires women of childbearing potential and

fertile men to use protocol-required contraception methods or abstain from sexual intercourse from the time of informed consent until 6 months following study treatment administration.
The applicant is requested to verify the required contraception duration and ensure that this information is consistent across all study documents (CAF, SNIF and SIS and ICF).
Furthermore, as Atsena did not collect vector shedding samples in non-clinical studies and as shedding data from previous clinical trials involving different AAV serotypes cannot be considered fully representative of or extrapolatable to ATSN-201, it is strongly recommended that the notifier considers extending the post-administration contraception period to 12 months. This approach is consistent with that adopted for most other AAV-based gene therapies and is generally considered sufficient to reduce the risk of vector shedding and potential exposure to the partner or unborn child to an acceptable level (very low to negligible).

Coordinator's comment
Should we propose here 'until two consecutive AAV ddPCR tests have shown a negative result'?

SBB Comment
This option has been added in the question to the applicant in the list of questions

4. Recommendations on donation of blood/cells/tissues/organs by the clinical trial subject:

It should be recommended to avoid donation of sperm, oocytes, blood, tissue, cell or organs for any subjects for 1 year following the administration of ATSN-201.
After this period, the decision is left to the subject, in consultation with the investigator and/or primary care provider, as appropriate.

All these recommendations and restrictions should clearly be reported in the applicable documents (CAF, SNIF and SIS and ICF).

SBB Comment
The expert's remark is supported and could be communicated to the applicant as follows:
According to the CAF document, patients will be advised to consult with their physician before they choose to donate blood, organs, tissues or cells for transplantation, at any time after GMO administration. The Main ICF only specifies that it is not allowed to do egg/ovum donation during and after your participation in the study for up to 6 months after the surgery with ATSN-201 intake and to donate sperm for 6 months after surgery. Nothing has been mentioned regarding donation of blood, organs or any tissues or cells. No corresponding information is provided in the SNIF.
Since there is a lack of experience with donation of blood or organs, tissues and cells for transplantation following AAV vector-based gene therapy, the notifier is requested to revise the instructions regarding blood, organs, tissues and cells in the different documents and to align these with the instruction given in the product information document (EPAR) of EU registered medicinal products containing recombinant AAV (Glybera, Zolgensma, Roctavian, Luxturna, Upstaza, Hemgenix) : 'Patients treated must not donate blood, organs, tissues, and cells for transplantation'.
Alternatively, the notifier is requested to give a rationale why instructions (a restriction for at least 12 months following ATSN-201 administration) could deviate from measures taken for current EU marketing authorized medicinal products containing recombinant AAV.

Comment 3 (**Expert 3**)
Has evaluated this item and has no questions/comments.

Comment 4 (**Expert 4**)
It is mentioned that ATSN-201 will not be released in the environment (SNIF). However, it is also said that ATSN-201 shedding is expected via participant tears and saliva. Are there any measures foreseen to avoid such dissemination (covering the treated eyes; not sharing cutlery, glasses, …)? What are these measures and for how long should they be applied?

SBB Comment
The expert's remark is supported and could be communicated to the applicant as follows:
As stated in the Public CAF document page 10/22, Atsena did not collect shedding samples from the nonclinical studies. However, based on available nonclinical and clinical data (for other rAAV serotypes) using the same route of administration, low levels of vector shedding in tears and nasal secretions may be expected for up to 3-4 days following subretinal administration. The applicant is therefore requested to clarify which measures are foreseen to minimise potential dissemination of the vector during this period. These measures should be described in detail (e.g. covering or dressing of the treated eye, appropriate handling and disposal of tissues or dressings, avoidance of sharing personal items such as towels, glasses, or cutlery, and reinforcement of hand hygiene practices), by specifying the duration for which such precautionary measures should be applied.

Furthermore, according to the Public CAF document, page 15, "patients are advised to maintain certain hygiene and safety protocols to minimise potential exposure". However, no details have been presented on how clinical trial subjects should avoid dissemination of the product.
For patients, caretakers and patient's family to practice and adhere to good hygiene, it is important to explain why measures are taken and what are the likely sources of contaminated material. Therefore, it is strongly recommended to develop a take home leaflet (preferably one-page, plasticized document) which would bring together all information and instructions for patients and patient's family to avoid potential transmission of the viral vector to other people or to the environment, if any, when patients are leaving the hospital setting.
The following information (with their duration) should be reported in this instruction sheet for the patient:
- Which bodily fluids are anticipated to contain viral vector genome (albeit very low levels)
- Instructions aimed at limiting contact with materials or surfaces frequently contaminated with bodily fluids
- At which time points eye pads may be changed or removed, the procedure for changing eye dressing and how these eye pads should be disposed of.
- Instruction on good hygiene to be practiced
- Instruction and effective solutions for decontaminating potentially contaminated areas, tissues, skin…
- The obligation to use contraceptive methods

5. ENVIRONMENTAL RISK ASSESSMENT

(applicability of the specific environmental risk assessment provided for in Section 2 of the '*Good Practice on the assessment of GMO related aspects in the context of clinical trials with AAV clinical*' taking into account the specific characteristics of the investigational medicinal product)

Comment 1 (**Expert 1**)
Has evaluated this item and has no questions/comments.

Comment 2 (**Expert 2**)

Has evaluated this item and has no questions/comments.

Comment 3 (**Expert 3**)
Has not evaluated this item.

Comment 4 (**Expert 4**)
Has evaluated this item and has no questions/comments.

6. OTHER INFORMATION

Do you have any other questions/comments concerning this notification that are not covered under the previous items?

Comment 1 (**Expert 1**)
Has no further questions/comments.

Comment 2 (**Expert 2**)
Typo (see page 4 of 27 in the public CAF): should pXX6-80 not be PXX6-807?

SBB Comment
This remark could be reported as a "Typos and other errors/omissions" as follow:
According to page 4 of the Public CAF document, the adenovirus 5 derived plasmid, pALD-X80 is a described as a kanamycin resistant version of pXX6-807. However, "pXX6-80" has also been used in the same section. The applicant is requested to verify the name of the plasmid and to correct data as appropriate

Comment 3 (**Expert 3**)
Has no further questions/comments.

Comment 4 (**Expert 4**)
Has no further questions/comments.

References

Di Pasquale, G., *et al*., Transduction of Salivary Gland Acinar Cells with a Novel AAV Vector 44.9. Molecular Therapy – Methods & Clinical Development, 2020. 19: p. 459-466

Douglas B Howard & Brandon K Harvey (2017). Assaying the Stability and Inactivation of AAV Serotype 1 Vectors. Hum Gene Ther Methods. 2017 Feb;28(1):39-48. doi: 10.1089/hgtb.2016.180.

Kumbhar & Geddugol (2025). Prevalence of Health Effects Due to Disinfectant Exposure and its Impact on Selected Physiological Parameters Among Class D Workers: A Descriptive Cross-Sectional Study. Cureus 17(3): e79994. DOI 10.7759/cureus.79994

Adviesraad voor Bioveiligheid
Conseil consultatif de Biosécurité

Compilation of the expert's evaluations of the answers of Atsena Therapeutics Inc. on the list of questions for dossier B/BE/26/BVW4

11 August 2026
Ref. SC/1510/BAC/2026_0674

Coordinator: Rik Gijsbers (KULeuven)
Experts: Anton Roebroek (KULeuven), Willy Zorzi (ULiège), Liliane Tennenbaum (Lausanne University Hospital)
SBB: Sheela Onnockx

INTRODUCTION

Dossier **B/BE/26/BVW4** concerns a notification from Atsena Therapeutics Inc. for the deliberate release in the environment of genetically modified organisms other than higher plants according to Chapter II of the Royal Decree of 21 February 2005.
The notification has been officially acknowledged on 04 April 2026 and concerns a clinical trial entitled "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)". The investigational medicinal product is a recombinant AAV vector carrying human retinoschisin 1 (hRS1) expression cassette.

On 09 July 2026, based on a list of questions prepared by the BAC (SC/1510/BAC/2026_0608), the Competent Authority requested the notifier to provide additional information about the notification. The answers from the notifier to these questions were received by the Competent Authority on 31 July 2026. This complementary information was reviewed by the coordinator and the experts in charge of the evaluation of this notification.

Evaluation Expert 1

By this way, we would like to let you know that the notifier addressed correctly and satisfactorily the comments/questions that have been raised except for the 3 following minor points:

1- Page 18 of the SNIF, the wording « universal » in the indicated sentence is not appropriate:

(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.
- The personnel performing the dilution of the GMO will use universal precautions and appropriate PPE (as applicable): labcoat, personal apron, gloves, mask and protective goggles (used for handling IMP). ~~During spill management the personnel will use in addition, a FFP2 mask. 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.~~

SBB comment:
The expert's comment could be sent to the applicant as follows:
According to page 18 of the SNIF, the safety procedures include the application of "universal precautions"; however, these precautions are not further described. To ensure clarity, the applicant is requested to specify in this section the universal precautions that are expected to be implemented.

Coordinator comment:
I agree with the comment raised by the expert that the wording should be clarified.

2- Page 22 of the SNIF,

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.

Descogen Liquid (manufactured by Antiseptica) is formulated without conventional detergents or surfactants. It is an oxygen-releasing surface and medical device disinfectant concentrate whose primary active ingredient is potassium peroxymonosulfate (caroat).
Composition and Properties :
-Active Ingredient: 100 g of Descogen Liquid contains 20 g of Caroate (active oxygen). Detergent
-Content: Free of added perfumes, colorants, and conventional surfactant/detergent systems.
-Action: Acts purely as an oxygen-releasing disinfectant rather than a combined detergent-disinfectant.
-Application : If it is planning to clean and disinfect a specific surface or medical device with this product, please analyse what type of material or soiling you are dealing and if a pre-cleaning step with a detergent is recommended first.

The notifier is invited to modify in all the documents of this dossier the wrong description of Descogen as detergent and to limit its description as disinfection solution.

SBB comment:
The expert's comment could be sent to the applicant as follows:
In the SNIF document, Descogen is described as an appropriate validated disinfection detergent. However, Descogen Liquid (manufactured by Antiseptica) is formulated without conventional detergents or surfactants. It is an oxygen-releasing disinfectant solution for surface and medical device whose primary active ingredient is potassium peroxymonosulfate (caroat). The applicant is therefore requested to revise all relevant documents (SNIF, CAF…) to correct the description of Descogen by limiting its description as disinfection solution.

Coordinator comment:
I agree with the comment raised by the expert. Descogen® is a disinfectant brand (Antiseptica Dr. Hans-Joachim Molitor GmbH). Several Descogen products, including Descogen I, Descogen Liquid, and Descogen Liquid R.F.U., are listed by Antiseptica. The fact that Descogen Liquid R.F.U. is labeled "virucidal" under EN standards (https://www.antiseptica.com/descogen_liquid_konz) does not automatically mean it has been validated for AAV vectors, which are notoriously resistant.

Whether Descogen Liquid reliably inactivates AAV depends on its active ingredients and contact time, and I could not find any published AAV-specific validation data for Descogen Liquid. For laboratory biosafety involving AAV, the agents with the strongest published evidence remains sodium hypochlorite (bleach).
Only for Descogen I potassium peroxymonosulfate (active oxygen) is indicated as a major active ingredient. There is one published study found that 0.45% potassium peroxymonosulfate effectively inactivated AAV2 and AAV5 vectors, including effects on capsid integrity and infectivity (https://pubmed.ncbi.nlm.nih.gov/33023320/). They studied alternative approaches for proper decontamination of tools and surfaces based on reliable AAV inactivation. Although sodium hypochlorite is recommended for AAV decontamination, it is not compatible with all surfaces found in the laboratory or clinical environment due to its corrosive nature. Washing a surface will result in removal of the AAV particles form the surface, but it does not inactivate them.

Korte et al. studied the impact of five disinfectants on virus capsid integrity, viral genome integrity, and infectivity upon different exposure times was tested on AAV2 and AAV5, two serotypes with highly different thermostability. While sodium hypochlorite, potassium peroxymonosulfate, and PAA successfully inactivated AAV2 after 1, 5, and 30 min, respectively, ethanol and hydrogen peroxide did not show significant effects on AAV2 even after exposure for 30 min. For AAV5, only sodium hypochlorite and potassium peroxymonosulfate proved efficient capsid and genome denaturation after incubation for 1 and 30 min, respectively. Consequently, ethanol or hydrogen peroxide should not be considered for routine laboratory or clinical use, while 0.45% potassium peroxymonosulfate and 0.5% sodium hypochlorite represent suitable and broadly effective disinfectants for AAV inactivation. Yet, this study did not test the commercial Descogen formulations against AAV.
Despite the mechanistic rationale that oxygen-based Descogen products containing potassium peroxymonosulfate could be effective against AAV, efficacy would still depend on: the working concentration, contact time, AAV serotype and its concentration on the surface, and the surface/material being treated.

For a biosafety protocol, I would still rely primarily on validated AAV decontamination data (e.g., sodium hypochlorite or potassium peroxymonosulfate-based procedures with documented contact times) rather than extrapolating from the product name alone.

SBB comment:
Based on coordinator's comment, the following recommendation could be added to the request:
Korte et al. (2021) investigated the effect of five disinfectants on AAV capsid integrity, viral genome integrity, and infectivity at different exposure using AAV2 and AAV5, two serotypes with highly different thermostability. For AAV2, sodium hypochlorite, potassium peroxymonosulfate, and PAA successfully inactivated the virus after 1, 5, and 30 min of exposure, respectively. For AAV5, only sodium hypochlorite and potassium peroxymonosulfate proved efficient capsid and genome denaturation after incubation for 1 and 30 min, respectively.
However, this study did not evaluate the efficacy of the commercial Descogen formulations against AAV. Despite the mechanistic rationale that oxygen-based Descogen products containing potassium peroxymonosulfate could be effective against AAV, their efficacy cannot be assumed solely on the basis

of their active ingredient. It is likely to depend on several factors, including working concentration, contact time, AAV serotype and its concentration on the surface, and the surface/material being treated. Therefore, we recommend that AAV decontamination procedures be selected primarily on the basis of validated efficacy data against AAV, rather than relying on the product name or formulation composition alone.

3- Page 23 of the SNIF:

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.

The wording « Autoclaving » is too vague. Please describe the conditions of use: temperature, pressure and time of treatment.
In the same way, the wording « 0.25% peracetic acid, iodine, 10% sodium hypochlorite » are too vague. Please describe the conditions of use as the application time of treatment and for « iodine », the usual concentration.

SBB comment:
The expert's comment could be sent to the applicant as follows:
According to page 23 of the SNIF, the methods proposed for the removal of the GMO(s) from potentially affected areas include “autoclaving, 0.25% peracetic acid, iodine, or 10% sodium hypochlorite”. To ensure clarity, the applicant is requested to specify in this section the conditions of use for each method, where applicable, including temperature, pressure, time of treatment and/or concentration.

Coordinator comment:
I agree with the comment raised by the expert and the reply of SBB. Additionally, it is important to keep in mind that the serotype affects stability and that procedures should be reassessed for novel serotypes.

SBB comment:
The following recommendation could be added to the question:
It is important to take into account that the stability of the GMO may be influenced by the serotype. Therefore, as the GMO under assessment contains a novel serotype, the applicant should ensure that the proposed procedures have been appropriately assessed and are effective for this specific serotype.

Evaluation Expert 2

I have evaluated the responses of the applicants and I consider them satisfactory.

Evaluation Expert 3

Comment on general comment:

Due to lack of overview and insight in the procedures and legislation in the context of the Belgian Contained Use authorization, the expert is not able to determine whether the arguments used by the applicant to propose not to revise the CAF at this stage, are acceptable for the BAC and the SBB.
In the evaluation of the answers to the questions, the expert assumes for the moment that this proposition is acceptable.

SBB Comment:
The following comment could be sent to the applicant:

The applicant's justification for not revising the CAF is noted. However, it should be clarified that, under the Belgian Contained Use (CU) procedure, the CAF document is not formally reviewed or approved. Instead, the assessment focuses on the site-specific information, with the CAF serving as additional supporting information.

In contrast, the CAF is subject to a dedicated scientific review during the Deliberate Release procedure by the experts of the Belgian Biosafety Advisory Council (BAC). Consequently, the comments provided during this procedure should be addressed by updating the CAF accordingly.

The requested revisions remain necessary for the following reasons:

- Several statements in the current CAF are inaccurate and could mislead healthcare professionals. These statements should therefore be corrected.
- Some wording lacks sufficient clarity regarding the appropriate handling procedures. If left unchanged, this could result in incorrect handling of the investigational product.
- Certain relevant information is currently missing from the CAF and should be included to ensure that the document is complete.

Although the CAF is not intended to be provided directly to patients, it is a reference document for healthcare professionals involved in the handling and administration of the investigational product. Healthcare personnel may also rely on the information contained in the CAF when informing patients. It is therefore essential that the document is accurate, clear, and complete.

The applicant is therefore requested to implement all requested revisions (see questions 1, 2, 4, 8, 9, 10, 12, 15, 16 and Typo) and provide the investigational sites with an updated version of the CAF.

Coordinator comment:
I agree with the comment and the reply of SBB.

Comment on answer to question 1
The answer is acceptable, assuming no update of present CAF necessary at this moment.

Comment on answer to question 2
The expert agrees that the referred section already provides the estimated percentage of rcAAV in a clinical dose, namely < 0.000001% rcAAV, which, in the Applicant's view, adequately reflects the magnitude of the difference between rcAAV and non-rcAAV particles. However, in the opinion of the expert the use of 'mostly' to describe the overwhelming amount of non-rcAAV, namely >99.999999%, remains weird and could easily be resolved by describing the relative amount of rcAAV as extremely low.
However, the answer is acceptable, assuming no update of present CAF necessary at this moment.

Comment on answer to question 3
The answer is acceptable, assuming no update of present CAF necessary at this moment.

Comment on answer to question 4
The answer is acceptable, assuming no update of present CAF necessary at this moment.

Comment on answer to question 5
The answer is acceptable, assuming no update of present CAF necessary at this moment. SNIF updated. However, does it make sense to monitor vector biodistribution and viral shedding during the Predose phase (Day -90 to -8) (SNIF, section H6, page 22 of 27)? How many samples during this period?
Furthermore, on page 22 of 27, section H6, Frequency of monitoring (SNIF): "…….shedding will be during……." Should be corrected in "…….shedding will be monitored during……."

SBB comment:
This remark could be reported as a "Typos and other errors/omissions" as follows:
On page 22 of the SNIF, the following sentence should be revised by adding the word "monitored" :
"*Vector biodistribution and viral shedding will be during the Predose phase*"

Coordinator comment:
I support the comment raised by the expert and the reply of SBB.

Comment on answer to question 6
The answer is acceptable and sufficient, assuming no update of present CAF necessary at this moment.

Comment on answer to question 7
The answer is acceptable, assuming no update of present CAF necessary at this moment. SNIF adequately updated.

Comment on answer to question 8
The answer is acceptable, assuming no update of present CAF necessary at this moment.

Comment on answer to question 9
The answer is acceptable, assuming no update of present CAF necessary at this moment. SNIF adequately updated.

Comment on answer to question 10
The answer is acceptable, assuming no update of present CAF necessary at this moment. SNIF adequately updated.

Comment on answer to question 11
The answer is acceptable, assuming no update of present CAF necessary at this moment. SNIF adequately updated.

Comment on answer to question 12
The answer is acceptable, assuming no update of present CAF necessary at this moment. SNIF adequately updated.

Comment on answer to question 13
The answer is acceptable, assuming no update of present CAF necessary at this moment. SNIF adequately updated.

Comment on answer to question 14
The answer is not acceptable. The shedding data do not give any information about course of shedding between day 1 and day 7. As shedding is observed for some patients at 24h, and consequently possibly the days after, the proposed precautionary measures remain necessary.

SBB comment:
The expert's remark is supported and could be communicated to the applicant as follows:

The applicant states that "the currently available shedding data for ATSN-201 do not indicate persistent or detectable shedding beyond 24 hours post-administration." However, this conclusion is not supported by the available data.
Shedding assessments were performed only on Day 1 and Day 7 post-administration. As no samples were collected between these two time points, no conclusions can be drawn regarding the presence or absence of vector shedding during this interval. Consequently, it cannot be excluded that vector shedding persisted or was undetectable beyond 24 hours. In addition, AAV vector DNA was detected in tears from the treated eye in some patients on Day 1, indicating that ocular shedding in tears occurred in a substantial proportion of treated patients.
Therefore, the original recommendation remains applicable. In line with the recommendations included in the European Medicines Agency (EMA) EPAR for Luxturna, an AAV2 vector-based subretinal gene therapy indicated for the treatment of inherited retinal dystrophy, the applicant is requested to ensure

that the treated eye is adequately covered with an eye bandage for at least 7 days following administration.
Furthermore, patients and family should be informed on how to handle waste material generated from dressings, tears, and nasal secretion appropriately, which may include storage of waste material in sealed bags prior to disposal. It is also recommended that patients and their family members/care takers wear gloves when changing eye dressing and handling waste, particularly in cases where a family member is pregnant, breastfeeding, or immunocompromised. All these recommendations should clearly be reported in the applicable documents (ICF…) and in the dedicated instruction sheet for the patient.

Coordinator comment:
I agree with the comment raised by the expert and the reply of SBB.

Comment on answer to question 15
Based on the ATSN-201-specific data currently available, the expert can accept a contraception period of only 6 months. This is an exception compared to the general advice for AAV gene therapies. But given the shedding data for ATSN-201 after subretinal administration this is acceptable in comparison with a contraception period of 12 months for gene therapies involving systemic injection of far larger quantities of vector.

Comment on answer to question 16
The answer is acceptable, assuming no update of present CAF necessary at this moment.

Comment on answer to question 17
Patient instruction material remains necessary. See also comment to answer to question 14.

SBB Comment
The expert's remark is supported and could be communicated to the applicant as follows:

Although the Patient Instruction Sheet is still under development, the submission of a draft version as part of the current procedure would be highly valuable. This would enable the experts to assess whether the information and instructions intended for patients and their family members/caregivers are accurate, complete, and appropriate for minimizing the risk of viral vector transmission to other individuals or to the environment following hospital discharge.

Coordinator comment:
I align with the comment raised by the expert and the reply of SBB.

References:

J. Korte et al. 2021. Inactivation of Adeno-Associated Viral Vectors by Oxidant-Based Disinfectants. Hum Gene Ther. 2021 Jul;32(13-14):771-781.

Adviesraad voor Bioveiligheid
Conseil consultatif de Biosécurité

Compilation of the expert's evaluations of the answers of Atsena Therapeutics Inc. on the list of questions for dossier B/BE/26/BVW4

07 September 2026
Ref. SC/1510/BAC/2026_0749

Coordinator: Rik Gijsbers (KULeuven)
Experts: Anton Roebroek (KULeuven), Willy Zorzi (ULiège), Liliane Tenenbaum (Lausanne University Hospital)
SBB: Sheela Onnockx

INTRODUCTION

Dossier **B/BE/26/BVW4** concerns a notification from Atsena Therapeutics Inc. for the deliberate release in the environment of genetically modified organisms other than higher plants according to Chapter II of the Royal Decree of 21 February 2005.
The notification has been officially acknowledged on 04 April 2026 and concerns a clinical trial entitled "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)". The investigational medicinal product is a recombinant AAV vector carrying human retinoschisin 1 (hRS1) expression cassette.

On 09 July 2026, based on a list of questions prepared by the BAC (SC/1510/BAC/2026_0608), the Competent Authority requested the notifier to provide additional information about the notification. The answers from the notifier to these questions were received by the Competent Authority on 31 July 2026. This complementary information was reviewed by the coordinator and the experts in charge of the evaluation of this notification.

Evaluation Expert 1

After analysis, I would like to let you know that the notifier addressed correctly and satisfactorily the comments/questions that have been raised.

Evaluation Expert 2

I analyzed the responses of the notifier and I believe that these are satisfactory except one:
Question 2: Percentage of rc AAV.
According to the request of the committee, the notifier changed the phrasing: the word "mostly" was removed leaving only "0.000001%" However, they did not provide a statistical test and a p-value, as requested.

In the new version of CAF annex, the applicant states that “very low amounts of rcAAV obtained in the analyses of three ATSN-201 batches (see section 2.2 of confidential annex for exact value)., corresponding to 0.000001% of rcAAV particles in a clinical dose.

However, in the confidential annex paragraph 2.2, the data are confusing.
The applicant states that rcAAV per standardized quantity vg were detected, suggesting that the values were under the detection threshold.
However, they then say that “a certain number of replication-competent AAV-positive samples were extracted and sequenced” and provide a qualitative analysis of the sequencing data. Therefore, my interpretation is that there were rcAAVs above the detection threshold in at least some of the lots produced.
Are the lots retained for clinical trials containing no rcAAV under the detection threshold? In this case, there is indeed no statistical test to be done.
Alternatively, if the clinical lots contain a detectable amount of rcAAV, providing numbers and statistical analysis is feasible.

SBB comment:
Information reported in the public CAF appears to be a shortened restatement of the quantitative conclusion reported in the confidential CAF document. The reported level of rcAAV per standardized quantity vg and the resulting estimated maximum exposure of rcAAV per clinical dose indicate a very low potential exposure. However, clarification could be needed as to whether rcAAV was actually detected in the clinical lots, and, if so, whether the detected levels were above the assay's limit of detection and/or limit of quantification.
If a question should be sent to the applicant, the following request could be formulated:
Based on section 2.2 of the CAF document, the reported level of rcAAV per standardized quantity vg and the resulting estimated maximum exposure of rcAAV per clinical dose indicate a very low potential exposure. However, clarification is needed as to whether rcAAV was actually detected in the clinical lots, and, if so, whether the detected levels were above the assay's limit of detection and/or limit of quantification. The assay LOD/LOQ and the individual results for the clinical lots should therefore be provided to establish the actual potential exposure to rcAAV and to support the biosafety risk assessment.

Coordinator comment:
I understand the concern of the expert and align with the way SBB addressed it. It is key to keep in mind that a positive result in qPCR for Rep2 sequences is a proxy. It indicates there is DNA present that can get amplified, it does not proof there is AAV that is effectively rc.

SBB comment:
Following a discussion with the FAGG Agency, we have obtained additional information regarding the evaluation of rcAAVs via the IMPD. We therefore take the view that, while the question is relevant, it is an intrinsic part of the evaluation by the Agency's quality control expert team.

Coordinator reply:
I agree. Reading through the IMPD it is however not clear what the final target sequence is for the ddPCR to estimate rcAAV and the AAV DP. The AAV DP seems to be quantified using the target GOI, RS1, to be amplified (p99/165 in the IMPD), whereas earlier (p106/165 in the IMPD) the rcAAV is indicated to be positive after double positive ddPCR tests targeting both Rep2 and Cap sequences.
But I agree this is the responsibility of the FAGG

Evaluation Expert 3

According to me the notifier addressed correctly and satisfactorily the comments/questions that have been raised.