Adviesraad voor Bioveiligheid
Conseil consultatif de Biosécurité

Advice of the Belgian Biosafety Advisory Council on the notification B/BE/25/BVW9 of the company VectorY Therapeutics B.V. for deliberate release in the environment of genetically modified organisms other than higher plants for research and development

FINAL version : 12/05/2026
Ref. SC/1510/BAC/2026_0449

Context

The notification B/BE/25/BVW9 has been submitted by VectorY Therapeutics B.V. to the Belgian Competent Authority in December 2025 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 : *"phase 1/2 investigation of novel experimental regimen in amyotrophic lateral sclerosis (PIONEER-ALS): An open-label, uncontrolled, multicenter study to assess the safety and tolerability of two doses of VTx-002 in participants with ALS".*

Amyotrophic lateral sclerosis is a rapidly progressive and fatal neurodegenerative disease that primarily affects motor neurons (MNs) in the cerebral motor cortex, brainstem, and spinal cord. In most cases, the disease is characterized by dysregulation of the nuclear transcriptional repressor TDP-43, leading to its mislocalization and pathological aggregation within neuronal cells. This causes motor neurons in the brain and spinal cord to die off. This leads to loss of strength and muscle paralysis in ALS patients, making it increasingly difficult to move, swallow and breathe, among other things. There is currently a large unmet need for a therapy that can slow or halt ALS disease progression.

The primary objective of this phase I/II study is to assess the safety and tolerability of increasing doses of a single administration of VTx-002 into the cisterna magna in adult patients.
VTx-002 is a gene therapy being developed to express a humanised anti-TDP-43 single chain variable fragment (scFv) transgene in the central nervous system. This transgene is designed to bind pathological forms of TDP-43, found in the nervous system of ALS patients. By achieving this sustained intracellular production of the anti-TDP-43 scFv, disease progression could potentially be slowed or halted by targeting the underlying pathology.

Compared to the wild-type AAV virus, the AAV vector lacks the *rep* and *cap* viral sequences rendering it unable to replicate, even in the presence of a helper virus. The vector will therefore persist as episome.

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Overall, up to 12 subjects will be included in this Phase I/II study, wherefore, three are expected in Belgium. VTx-002 will be administered at two different dose levels (low and high doses) as a single dose via intracisterna magna injection in adult participants with amyotrophic lateral sclerosis (ALS). This study will be conducted at one clinical site located in Flanders. The national territory is considered as the potential release area of VTx-002.

The dossier has been officially acknowledged by the Competent Authority on 04 February 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. Four experts from the common list of experts drawn up by the BAC and the Service Biosafety and Biotechnology (SBB) of Sciensano 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 02 March 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 16 March 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 26 March 2026. The answers of the notifier were received on 08 April 2026 and reviewed by the coordinator, after which a third list of questions was transmitted to the notifier on 15 April. The answers of the notifier were received on 29 April 2026, after which the BAC was able to come to a conclusion 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 required 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.

VTx-002 is produced in a baculovirus/insect cell-based production system by a triple plasmid DNA transfection of the vector genome plasmid carrying the transgene, an AAV trans plasmid encoding the AAV *rep* and *cap* genes required for the encapsidation and an adenovirus helper plasmid containing essential adenoviral helper genes required for rAAV replication. With the exception of the ITRs, all wt genetic AAV elements are absent from the clinical vector. VTx-002 genomic construct is encapsidated by a chimeric AAV5.2 capsid derived from adeno-associated virus (AAV) serotype 5, with the VP1 unique region derived from AAV serotype 2.

Despite the present of a chimeric AAV5.2 capsid, the well-characterized and highly efficient AAV2 ITRs have been used in this AAV gene therapy vector which is efficiently recognized by AAV2 Rep protein.

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

Information related to the molecular characteristics of VTx-002 were adequately described in the dossier.

AAV5.2 is a chimeric capsid in which only the VP1 unique region is derived from AAV2, while VP2 and VP3 remain from AAV5. The VP1u region includes functional motifs involved in intracellular processes such as endosomal escape, nuclear trafficking, and post-entry viral processing, which occur only after cellular uptake and do not affect receptor binding or tissue targeting. Non clinical data in mice, minipigs and non-human primates with an AAV5.2 vector showed biodistribution and safety profiles consistent with conventional AAV5-based vectors, without evidence of altered tissue tropism or increased toxicity.

3. The conditions of the release

This phase I/II study will consist of two treatment groups (low or high dose of VTx-002). The GMO will be administered via a single intracisterna magna injection, in hospital centres. After administration, participants will have a 24 hour inpatient stay directly following the procedure. Subjects will be monitored for 52 weeks to assess treatment outcomes. Afterwards, all the subjects will continue the study for long-term follow-up for a total of five years after VTx-002 administration.

Viral shedding analysis in blood, saliva, urine and fecal samples will be assessed via immunohistochemistry/quantitative polymerase chain reaction/digital droplet PCR/southern blotting/in situ hybridization/next-generation sequencing to monitor the duration of viral vector shedding in the environment via biofluid. Although testing nasal secretions and tears could be considered relevant, the applicant has clearly justified why such sampling will not be included in the protocol. This rationale is supported by multiple literature references, results from a NHP GLP toxicology study, and by a prior clinical trial (B/BE/21/BVW3) of an ICM-administered AAV9 gene therapy product, which received Belgian GMO approval and assessed shedding in saliva, urine, and stool without including nasal or tear samples.

As a safeguard against potential vector transmission to other people or release into the environment once patients leave the hospital setting, the notifier provided a Patient Information Sheet explaining and summarizing all the critical information and instructions for patients and their families. So, information on bodily fluids which are anticipated to contain viral vector genome have been updated by adding nasal secretions to saliva, urine, blood and stool. As rAAV are non-enveloped, it is now clearly specified in

the Participant Hygiene Leaflet that alcohol-based disinfectants are not suitable for cleaning any visible soiling on surfaces. These handling precautions will be followed for 6 months after administration.

Vector mobilization and secondary transmission, particularly replication-dependent mobilization, require the persistence of intact recombinant AAV (rAAV) genomes in transduced cells, the presence of functional AAV Rep proteins in trans, and co-infection with a helper virus providing the necessary replication functions. The likelihood of all these conditions occurring simultaneously within the same cell population is low and is not expected under normal clinical conditions. While passive shedding of vector particles may occur at low levels, rAAV mobilization remains a theoretical and highly constrained risk. Overall, the available data support the conclusion that there is no meaningful risk to public health.

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 vector not harbouring any antibiotic resistence 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 the GMO any 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 VTx-002 and wild-type AAV in case a triple infection by VTx-002, wild type AAV (providing the *rep* and *cap* functions) and a helper virus occurs in exposed persons. Such recombination event would result in gain of functional genes of AAV required for replication and encapsidation but would in turn lead to the loss of the transgene. 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 would contain the transgene and the *rep* and *cap* genes necessary for multiplication.

According to the CAF, women of childbearing potential (WOCBP), as well as male participants with female partners who are WOCBP, must use a highly effective method of contraception for a fixed period of one year following administration of study drug, applicable to all treated subjects irrespective of continued study participation or early discontinuation.

All participants must refrain from donating blood, organs, tissues, and cells for transplantation for the rest of their lives. Furthermore, women treated with VTx-002 must not donate eggs and men must not donate sperm for the rest of their lives.

In the case of transfer of vector to an unintended immune-competent human recipient, the risks are expected to be considerably reduced as compared to any potential risk for the participant, since the vector is not able to replicate and the transferred ‘dose’ (from e.g. aerosol, splashing or fomites) will be orders of magnitude lower than that received by patients. Worst case, the receiver develops an immune response to the AAV capsid proteins.

The BAC concludes that, based on the non-pathogenic and non-replicative nature of VTx-002 and the assumed lower amounts of shed and intact viral particles of VTx-002 as compared to the therapic dose,

the overall risk associated to exposure and transmission to other individuals can be considered negligible.

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

Following SBB request, the Pharmacy Manual and all related documents have been updated to explicitly include all information requested regarding the mandatory personal protective equipment (PPE), sample-handling instructions, and detailed procedures for spill management and occupational exposure. The notifier also provided a 2 pages technical sheet 'Instructions for study site personnel' giving an overview of all relevant handling instructions, detailed instructions in case of spill or inadvertent exposure of human, waste management and other risk management measures.

In the event a spill of the IP occurs, the spill will be contained, and the area will be decontaminated with an approved disinfectant such as freshly prepared 6000 ppm (mg/L) chlorine (~1% sodium hypochlorite) solution. To maintain chlorine strength and ensure bleach effectiveness, sodium hypochlorite solution must be freshly prepared and stored in a dark bottle. For needle sticks or contamination of intact skin aseptic soap is considered more appropriate for decontamination.

Since propagation of VTx-002 is very unlikely, the BAC supports the view that, in terms of risk for the environment or human health, the proposed measures as 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 VTx-002 developed to treat patients with amyotrophic lateral sclerosis, by means of endogenous production of a humanised anti-TDP-43 single chain variable fragment (scFv) in the central nervous system, 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
    - Lateste version of the SNIF
    - CAF_non confidential_v5
    - CAF_Confidential_Annex v3
    - Participant Instructions_Participant Hygiene Leaflet V2.0Global_BEL
    - Study Staff Instructions_Pharmacy Manual_V6.0 Belgium
    - Study Staff Instructions_Handling Instruction V3.0Global

- To ensure participants know which effective solutions to use for decontaminating potentially contaminated areas, examples of suitable wipes or disinfectant for cleaning any visible soiling on surfaces should be included in the Participant Hygiene Leaflet.

- As commited by the applicant, the Protocol and the ICF will be updated at the next amendment by adding the recommendation to refrain from donation of blood, organs, tissues, and cells for the rest of their life. Furthermore, women treated with VTx-002 in Belgium must not donate eggs and men must not donate sperm for the rest of their lives.

- As commited by the applicant, the protocol, ICF and any other relevant study documents will be updated by clearly indicating that participants who are women of childbearing potential (WOCBP), as well as male participants with female partners who are WOCBP, must use a highly effective method of for a fixed period of one year following administration of study drug, applicable to all treated subjects in Belgium irrespective of continued study participation or early discontinuation.

- Any protocol amendment has to 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 included in the trial, the notifier must send to the competent authority at the attention of the Biosafety Advisory Council a report with details concerning the biosafety aspects of the project. This report will at least contain:
    - The total number of patients included in the trial and the number of patients included in Belgium;
    - A summary of all adverse events marked by the investigators as probably or definitely related to the study medication;
    - A report on the accidental releases, if any, of VTx-002.

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/25/BVW9 (ref. SC/1510/BAC/2026_0217, SC/1510/BAC/2026_0330 and SC/1510/BAC/2026_0392)

Adviesraad voor Bioveiligheid
Conseil consultatif de Biosécurité

Compilation of comments of experts in charge of evaluating the dossier B/BE/25/BVW9
And comments submitted to the notifier

02 March 2026
Ref. SC/1510/BAC/2026_0217

Mandate for the Group of Experts: Mandate of the Biosafety Advisory Council (BAC) of 15 January 2026.
Coordinator: Véronique Fontaine (ULB)
Experts: Rik Gijsbers (KULeuven), Anton Roebroek (KULeuven), Nicolas van Larebeke-Arschodt (UGent, VUB), Willy Zorzi (ULiège)
SBB: Sheela Onnockx

INTRODUCTION

Dossier **B/BE/25/BVW9** concerns a notification from VectorY Therapeutics B.V 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 February 2026 and concerns a clinical trial entitled “phase 1/2 investigation of novel experimental regimen in amyotrophic lateral sclerosis (PIONEER-ALS): An open-label, uncontrolled, multicenter study to assess the safety and tolerability of two doses of VTx-002 in participants with ALS”. The investigational medicinal product is a recombinant AAV-5 vector coding for a single-chain variable fragment that binds the pathological human TDP-43.

♦ 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 02-03-2026 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

The origin of the capsid protein of the clinical vector is described in detail in the CAFs. The explanation of the name of AAV5.2 capsid is however only given in the common CAF section 4.2. This information could also be added to the confidential CAF. With respect to the origin of Rep detailed information is not clearly mentioned in the CAFs. The origin of the rep sequences should however be AAV2, because in the assay to demonstrate absence of replication competent AAV, rcAAV detection is performed using a qPCR directed to the AAV Rep2 gene. Otherwise the results of the assay would not be valid. The origin of Rep should be clearly mentioned in the CAFs

SBB comment:

The following question could be sent to the applicant: To ensure clear and comprehensive information regarding the origin of the different elements used for the viral vector, the applicant is requested to explicitly specify in the confidential CAF document:

- The origin of the capsid protein of the clinical vector : According to section 2.4 of the public CAF document, the capsid is described as a chimeric capsid. The precise origin of the capsid components should therefore be clearly detailed.
- The origin of the Rep gene : Based on the rcAAV detection method, it may be assumed that the Rep gene is derived from AAV2, as rcAAV detection is performed using a qPCR directed to the AAV Rep2 gene. Otherwise the results of the assay would not be valid. However, the origin of Rep should clearly be specified in the CAF document

Coordinator comment:

I agree with this proposition, this question could be send as proposed.

Comment 2

It is stated that “Recombination of the VTx-002 genome with wild type AAV to produce RCV is not possible because there are no homologous AAV sequences in the vector except the flanking AAV ITRs. All AAV sequences have been removed from the VTx-002 genome”. Firstly, it is not because no homologous sequences are not present that other processes could not lead to a recombinant replication competent virus, and secondly, the ITR could maybe suffice for recombination. I wonder whether the phenomena mentioned by Song (2020) (see point 6) can be related to this problem.

SBB comment:

There is only a remote possibility of homologous recombination between the ITR-sequences of VTx-002 and wild-type AAV in case a triple infection by VTx-002, wild type AAV (providing the rep and cap functions) and a helper virus occurs in exposed persons. Such recombination event would result in gain of functional genes of AAV required for replication and encapsidation but would in turn lead to the loss of the transgene. Furthermore, 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 would contain the transgene and the rep and cap genes necessary for multiplication.

Coordinator comment:
I agree

Comment 3
Has evaluated this item and has no questions/comments.

Comment 4
- What is the downstream process that is used in the drug product production system? Affinity column and subsequent polishing on an AEX column? Is the ratio between full and empty rAAV5.2 particles determined?
- In the Public info, the drug product is indicated to be AAV5 virus derived. Even though this is not incorrect, it would be more correct to mention that it is a hybrid carrying an AAV2 VP1 protein sequence.

SBB comment:
- First point of the question regarding the downstream purification process and the ratio of full to empty rAAV5.2 particles relates to the manufacturing and quality control of the medicinal product, which are considered outside the scope of the environmental risk assessment.
- The document “Information for the public” could indeed be improved by specifying that the drug product corresponds to a hybrid viral vector AAV5 carrying an AAV2 protein sequence.

Coordinator comment:
Point 1: I agree
Point 2 : I agree, this should then be asked to the applicant

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
See comment 2.1

Comment 2
“The VTx-002 rAAV vector cannot replicate in the presence of a helper virus as it lacks the AAV Rep and Cap genes required for replication.” However, it could acquire the AAV Rep and Cap genes by recombination with wild type AAV viruses.

SBB comment:

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 would contain the transgene and the rep and cap genes necessary for multiplication.

Coordinator comment:
Ok

Comment 3
Has evaluated this item and has no questions/comments.

Comment 4
Has evaluated this item and has no questions/comments.

2.3. Diagram (map) of the clinical vector

Comment 1
Has evaluated this item and has no questions/comments.

Comment 2
Has evaluated this item and has no questions/comments.

Comment 3
Has evaluated this item and has no questions/comments.

Comment 4
Has evaluated this item and has no questions/comments.

2.4. Molecular characterisation of the clinical vector
(e.g. annotated sequence of the genome, genetic stability, ….)

Comment 1
Has evaluated this item and has no questions/comments.

Comment 2
Has evaluated this item and has no questions/comments.

Comment 3
Has evaluated this item and has no questions/comments.

Comment 4
The AAV5.2 serotype has not been tested in human setting before and should therefore be treated with caution. It is presented as a chimeric capsid, but I cannot find any info on it online or in publications. The AAV5.2 chimeric capsid has the viral protein 1 (VP1) amino acid sequence of AAV2 and the VP2 and VP3 amino acid sequences of AAV5. Biodistribution, safety and toxicology studies of VTx-002 following ICM administration in mice/minipig or NHP animal models are not relevant for human translation, anatomical and physiological similarities to humans, and should be considered a best-case

scenario (see Fig 13 p48/82 in B_BE_25_BVW9_IB_Ed1.0_10Oct2025.pdf).

SBB comment:
According to section 1.2 of the IB, biodistribution has been assessed in mice upon intra cisterna magna (ICM) administration of VTx-002, in minipigs with a AAV5.2 vector with the same capsid as VTx-002 and in non-human primate upon ICM administration of AAV5.2-GFP and VTx-002.
For this first-in-human clinical trial, indeed no clinical data are available for VTx-002. According to section 8.3.8.3 of the protocol, during this phase I/II clinical trial, blood, saliva, urine and fecal samples will be collected for the detection of potential viral shedding via immunohistochemistry/quantitative polymerase chain reaction/digital droplet PCR/southern blotting/in situ hybridization/next-generation sequencing.

Coordinator comment:
I agree with expert comment. I am not familiar with biodistribution, safety and toxicology studies but the phase I/II study that will be performed on human should be performed taking all care of assessing all potential available data. Sample collections should therefore be performed as much as possible for further studies by immunochemistry or pPCR

SBB comment:
The following question could be sent to the applicant:
AAV5.2 serotype has not yet been tested in humans and is presented as a chimeric capsid with limited or no published data. Given the anatomical and physiological differences between mice, minipigs, or NHPs animal models and humans, and that preclinical studies may represent a best-case scenario, the applicant is requested to clarify what additional precautions or studies are planned to ensure safe and reliable translation to humans and potential biosafety risks, both to human health and to the environment, are adequately assessed and mitigated.

Coordinator comment:
Ok, thank you

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

Comment 1
Has evaluated this item and has no questions/comments.

Comment 2
“VTx-002 carries the transgene expression cassette encoding a humanized antibody fragment, flanked by AAV2 ITRs.” Would it not be better to use type 5 ITRs? See paper by Hewitt stating as a conclusion “. “Based on these data and the relative prevalence of wt AAV serotypes in the population, we propose that TR5 vectors have a significantly lower risk of mobilization and should be considered for clinical use” It should be noted that there are concerns about the influence AAV sequences could act as both promoters and enhancers in hepatocytes, in particular the 3′ inverted terminal repeat (ITR), which was previously shown to have promoter properties and presumably also contains an enhancer element

SBB comment:
The reference mentioned by the expert corresponds to “Reducing the risk of adeno-associated virus (AAV) vector mobilization with AAV type 5 vectors” by F.C. Hewitt (2009). J Virol. 2009 Apr;83(8):3919-29. doi: 10.1128/JVI.02466-08

Coordinator comment:
Expert is right, it is a pity they didn't use AAV5 ITR. I suspect that the only thing we could ask at this step, is to ask the applicant why they use this ITR sequence and if they could analyze the additional risks considering the higher risk of mobilisation (Hewitt et al, 2009) and gene expression activity (Lu Y, Ling C, Shoti J, Yang H, Nath A, Keeler GD, Qing K, Srivastava A. Enhanced transgene expression from single-stranded AAV vectors in human cells in vitro and in murine hepatocytes in vivo. Mol Ther Nucleic Acids. 2024 Apr 23;35(2):102196. doi: 10.1016/j.omtn.2024.102196. PMID: 38766527; PMCID: PMC11101737.)

SBB comment:
The vector VTx-002 carries a transgene expression cassette encoding a humanized antibody fragment flanked by AAV2 ITRs. Considering published data suggesting that AAV5 ITRs may reduce the risk of vector mobilization (Hewitt et al., 2009) and enhanced transgene expression in hepatocytes (Lu et al., 2024), the applicant is requested to provide the rationale for the choice of AAV2 ITRs instead of AAV5 ITRs and supporting data or analyses addressing the assessment of potential impact or risks on human health and the environment.

Coordinator comment:
Ok, thank you

Comment 3
Has evaluated this item and has no questions/comments.

Comment 4
Has evaluated this item and has no questions/comments.

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
The sentence in common CAF (2.6., page 10 of 19) "However, given that similar levels were measured in samples from control animals, it is unclear whether the levels detected in dosed animals are truly representative of actual vector shedding in these animals." needs further explanation. Is there a problem with respect to the sensitivity of the assay to detect shedding?

SBB comment:
In the shedding study, it is noted that similar levels of vector signal were detected in samples from control animals. As a result, it is unclear whether the levels measured in dosed animals accurately reflect actual vector shedding. Therefore, the following question could be sent to the applicant:
According to section 2.6 of the CAF document (p10/19), "Shedding of VTx-002 was observed in several samples collected at various timepoints during follow up […] However, given that similar levels were measured in samples from control animals, it is unclear whether the levels detected in dosed animals are truly representative of actual vector shedding in these animals." As it is unclear whether the levels measured in dosed animals accurately reflect actual vector shedding, the applicant is requested to

provide further explanation by clarifying if there was a problem with respect to the sensitivity of the assay to detect shedding, by clarifying the origin of the signals observed in control animals, by explaining whether these shedding results in animals can be reliably interpreted and by providing additional shedding analysis carried out or planned to accurately assess vector shedding? Please also clearly indicate in which animals these results were obtained.

Coordinator comment:
Excellent proposition. I agree

Comment 2
Based on shedding analysis by qPCR vector seems to remain in the central nervous system during a long period. I do not understand how the amount of VTx-002 vector DNA can remain stable between the 3 and 6 month time periods without rounds of DNA synthesis.
The applicant considers that the vector has a zero risk of spreading. However, in a paper by Song et al.(2020) “Adeno-Associated Virus Vector Mobilization, Risk Versus Reality” one can read ““ In a mobilization assay, a sizeable amount of rAAV recovered from infected 293 cell lysate remained intact and competent for a secondary round of infection (termed Ad-independent mobilization). In rAAV-infected cells coinfected with Ad and wt AAV, rAAV particle production was increased >50-fold compared with no Ad conditions. In addition, Ad-dependent rAAV vectors mobilized and resulted in >1,000-fold transduction upon a subsequent second-round infection, highlighting the reality of these theoretical safety concerns that can be manifested under various conditions. Overall, these studies document and signify the need for mobilization-resistant vectors and the opportunity to derive better vector production systems.”

SBB comment:
According to section 4.5.1.4 (Biodistribution and safety in NHPs) of the IB, Shedding analysis indicated that vector was rapidly cleared from the CSF and dropping below the lower limit of quantification by Day 90. Recombinant AAV vectors are replication-defective. The observed persistence in non-dividing CNS cells could likely reflects stable episomal forms of the vector genome rather than ongoing rounds of DNA synthesis.
It should be noted that the *in vitro* studies reported by Song et al. (2020) demonstrating that rAAV can be mobilized under specific artificial conditions (e.g., 293 cells coinfected with adenovirus and wild-type AAV), do not represent the clinical context.

Coordinator comment:
Good analysis. No need for question here then as it is found in differentiated cells.

Comment 3
Has evaluated this item and has no questions/comments.

Comment 4
- Biodistribution, safety and toxicology studies of VTx-002 following ICM administration in mice/minipig or NHP animal models are not relevant for human translation, anatomical and physiological similarities to humans, and should be considered a best-case scenario (see Fig 13 p48/82 in IB_Ed1.0_10Oct2025.pdf). In addition to all the work done (which is greatly appreciated and thoroughly conducted/analysed) also tears, seminal fluid should be tested. I would be very cautious with aligning this serotype with previous work and conclusions drawn for rAAV5. (e.g. as done in 5.5.6 p64/82 in IB_Ed1.0_10Oct2025.pdf).

SBB Comment:
Indeed, data obtained in animals are not readily extrapolable from animals to human, in particular when different routes of administration are used or no data have been collected in larger animals, such as non-human primates. However, valuable information and insights into the toxicity, biodistribution and shedding pattern of viral vectors can be obtained from non-clinical studies. As compared to clinical studies, animal studies are more amenable to be conducted in the early stages of the development of investigational viral vector.

Given that the viral vector used for this study, VTx-002, is an AAV5.2 hybrid viral vector, with limited prior characterization, the applicant could be requested to indicate whether, other biological samples such as tears and seminal fluid were also evaluated for vector shedding.
If such analyses were conducted, the applicant could be requested to provide the corresponding results.
If not, to justify why these additional matrices were not considered relevant for the shedding assessment.

Coordinator comment:
I agree with this proposition. Indeed, applicant should provide a complete assessment for this hybrid vector.

- I do not understand that individuals in same sex relationships are not required to use contraception. I would advise the use of condom at least for the duration of the study.

SBB Comment:
According to the protocol section 10.4.2, men and WOCBP must use a highly effective method of contraception consistently and correctly for the duration of the study including the long-term follow-up.

Coordinator comment:
OK

- In B_BE_25_BVW9_Part 2_Common Application Form.pdf p10/19 the applicant reports: “However, given that similar levels were measured in samples from control animals, it is unclear whether the levels detected in dosed animals are truly representative of actual vector shedding in these animals.”
This should be explained. Which animals are referred to here?

SBB Comment:
This comment was also raised by Expert 1 (in the same section here above), and has already been addressed by the SBB.

Coordinator comment:
Indeed

- The PIONEER-ALS study to investigate the safety and efficacy of ICM administered VTx-002 in ALS patients is designed to collect data on biodistribution and viral shedding. Please provide more info on which bodily fluids will be samples and for how long post injection.

SBB Comment:

According to the schedule of activities (Table 1 on page 26) of the protocol, blood, saliva, urine and fecal samples will be collected for the detection of potential viral shedding. Sample collection will be performed at Day 1, Weeks 1, 4, 8, 12, 24 and 52. Sampling will continue for a specific matrix until three consecutive negative samples have been detected for the participant for that matrix

Coordinator comment:
This should be amended considering the valuable remark of expert 4.

- Considering the long duration of shedding of DP material, the caretakers and family members should also be informed on how to deal with bodily fluids. Please provide (in line with earlier advice) a specific portfolio with stepwise instructions on how to deal with this.

SBB Comment:
Given that the vector is a novel hybrid AAV5.2 construct with limited prior clinical experience and given that shedding analysis from Non-human Primate body fluids from GLP toxicology study (IV#017) indicates detectable vector genome levels in saliva and nasal samples on Day 180 after administration, the applicant could be requested to propose precautionary hygiene measures for patients and patient's family at home and to explain why measures are taken.
Therefore, the notifier could be requested to provide a small take home summary (preferably one-page, plasticized document) to ensure that patients and patient's family easily can consult the information and all the instructions in an understandable format whenever needed.
The following information should be reported on this instruction sheet for the patient :
- The bodily fluids which are anticipated to contain viral vector genome
- Instructions aimed at limiting contact with materials or surfaces frequently contaminated with bodily fluids (avoid sharing drinking glasses, utensils, toothbrushes, dispose of tissues immediately after use, frequently handwashing…)
- Instructions and effective solutions to decontaminate possible contaminated areas, tissues, skin, …
- The period during which these instructions must be followed

Coordinator comment:
I agree with this comment

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
Has evaluated this item and has no questions/comments.

Comment 2
Has evaluated this item and has no questions/comments.

Comment 3
Has evaluated this item and has no questions/comments.

Comment 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

Has evaluated this item and has no questions/comments.

Comment 2

Has evaluated this item and has no questions/comments.

Comment 3

1) In page 16 of the protocol, it written that the wound or skin should be disinfected with alcohol
2) In p15 of the CAF, it is written that VTx-002 is a non-enveloped virus and resistant to alcohol-based disinfectant
3) An in Article J. Korte et al. 2021 “inactivation of Adeno-Associated Viral vectors by oxidant-based disinfectants, as well reported by the applicant, it is written that:

as adenovirus. In summary, our data reinforce the recommendation to not use 70% ethanol or formulations based on low concentrations of hydrogen peroxide as AAV disinfectants. At the indicated concentrations, sodium hypochlorite is the only tested substance ensuring rapid and complete capsid degradation (necessarily resulting in complete particle inactivation) of even the most thermostable serotype AAV5, whereas potassium peroxymonosulfate requires at least a 30-min incubation time to yield similar effects. We therefore recommend to use potassium perox-

Following the three 1), 2), 3), preceding points, it is clearly stated that 70% ethanol or 70% isopropyl alcohol is not suitable for eliminating VTx-002 in cases of laboratory surface disinfection, needle stick injury or contamination of intact skin.

The applicant is invited to propose other alternative disinfection treatments for needle sticks or contamination of intact skin.

SBB Comment:

As mentioned in section 3.6.c of the CAF document page 15/19, VTx-002 is a non-enveloped virus and resistant to alcohol-based disinfectants (Korte et al., 2021). However, according to page 16 of the “Staff Instructions_Pharmacy Manual_V2.0”, in case of accidental exposure of the skin with VTx-002, area should be “wash-off with a gauze soaked in a 0.5% chlorohexidine in 70% isopropyl alcohol solution and subsequently wash with water”. As 70% of alcohol should not be used as AAV disinfectant, the applicant could be requested to propose other alternative disinfection treatments for needle sticks or contamination of intact skin.

Coordinator comment

I agree with this comment

Comment 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
Has evaluated this item and has no questions/comments.

Comment 2
Has evaluated this item and has no questions/comments.

Comment 3
Has evaluated this item and has no questions/comments.

Comment 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
With respect to recommendations on donation of blood/cells/tissues/organs (including sperm and oocytes) by the clinical trial subject the dossier should be corrected as the BAC stipulates in similar dossiers that patients treated must not donate blood, organs, tissues and cells for transplantation. All relevant documents like SNIF, public CAF, etc. should be updated with this requirement.

SBB comment
In order to be consistent with our recommendations provided for other clinical trials using an AAV viral vector as drug product, the following request could be sent to the applicant:
As mentioned in section 3.6.g of the CAF (p17/19), “women must not donate eggs for the duration of the study (and long-term follow-up) and men must not donate sperm for the same period. Otherwise, no other recommendations on donations by the clinical trial subjects are planned or considered necessary”, based on the Good Practice on the assessment of GMO related aspects in the context of clinical trials with AAV clinical vectors document. However, according to 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’.
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 and to either align these with the instruction given in the EPAR, or to give a rationale why instructions could deviate from measures commonly taken for current EU marketing authorized medicinal products containing recombinant AAV.
All relevant documents like SNIF, public CAF, etc. should be updated accordingly.

Coordinator comment

I agree, of course, with this obvious comment

Clear instruction should be given to the treated patient and patient's family with respect to good hygiene practices in order to minimize spread of shedded vector to other people and the environment. Such instructions should be provided in a short readable format document that will be given to each patient.

SBB comment:
This comment was also raised by Expert 4 (in section 2.6 here above), and has already been addressed by the SBB.

Coordinator comment
OK

Use of anticonception by a sexually active treated patient is only mentioned in the ICFs, whereas such requirement should also be included in the SNIF and CAF and potentially other relevant documents of the dossier. The suggested period to use anticonception (up to 90 days after administration) seems short in comparison with other dossiers dealing administration of AAV-based clinical vectors, in which use of anticonception is advised for longer periods. Maybe the BAC should come up with a consistent opinion about the length of the period to use anticonception (potentially depending on amount of clinical AAV vector administered and systemic injection or injection into a particular organ/tissue (e.g. intravitreal injection).

SBB comment:
According to the protocol section 10.4.2, men and WOCBP must use a highly effective method of contraception consistently and correctly for the duration of the study including the long-term follow-up.
However, according to the inclusion criteria 10 of the protocol and section 6.54 of the ICF, women of childbearing potential (WOCBP) and male participants with female partners who are WOCBP (based on gender assignation at birth) must agree to use highly effective (<1% failure rate) contraception for at least 30 days prior to the first dose of study medication, during the study, and for 90 days following end of treatment [EOT]/end of study [EOS].
Section 10.4.2 requires contraception for the duration of the study including long-term follow-up, while Inclusion Criterion 10 specifies contraception until 90 days after EOT/EOS. There appears to be an inconsistency between Section 10.4.2 and Inclusion Criterion 10 of the protocol regarding the required duration of contraception. Therefore, the applicant could be requested to clarify the intended duration of contraception and provide the scientific justification for the selected timeframe.
The protocol and ICF should be updated accordingly. In addition, the SNIF and CAF documents should be revised to ensure that this information is consistently reflected throughout the dossier.

Coordinator comment
OK, I agree

The instructions on how to handle accidental spills or breakage of a GMO containing vial should be worked out in detail (which disinfectant and how to apply, etc.) and not only in merely general terms referring to local legislation. Documents like the SNIF, public CAF and other relevant documents (Pharmacy Manual) etc. should be updated with this detailed information. All medical personnel involved need to receive an overview (1 to 2 page instruction sheet) of all relevant handling instructions, detailed instructions in case of spill, waste management and other risk management measures.

SBB comment:
The following question could be sent to the applicant:
The procedures for managing accidental spills or breakage of vials containing the GMO should be described in detail. Specifically, the documentation should clearly indicate the type of disinfectant to be used, its concentration, the required contact time, and the method of application, rather than referring only in general terms to compliance with local legislation.

In addition, the documentation should explicitly specify the mandatory personal protective equipment (PPE) required for healthcare workers handling the product (as reported in section 3 of the pharmacy manual p7/27), as well as clear instructions on the collection and handling of samples (blood, saliva etc.) to prevent dissemination to the environment.

The SNIF, the public CAF, the Pharmacy Manual, and any other relevant documents should be updated accordingly to ensure that these information are consistently and clearly reflected throughout the dossier.

Furthermore, we recommend 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.

Providing such a consolidated document would greatly assist medical personnel in their daily practice, as it would ensure that all essential information is readily accessible in a clear and practical format, thereby facilitating safe and compliant handling of the product.

This sheet should include all relevant handling instructions, detailed procedures to handling a spill including appropriate disinfectants, waste management and other risk management measures:
- 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 blood, urine, vomit or other bodily fluids from patients in the initial period after administration of the IMP
- waste management

Coordinator comment
I totally agree
All caretakers involved in the collection and handling of samples (blood, saliva etc.) should receive clear instructions to prevent dissemination to the environment. Special instructions might be necessary with respect to waste treatment in case of home visits in order to collect samples.

SBB comment:
The expert's remark has been implemented in the proposed request to the applicant under section 3.6 Expert 1.
According to the Table 1 of the protocol, "Schedule of activities", no samples collection will be performed during the remote visits.

Coordinator comment:

Point 1 : Indeed

Point 2 : Let see what they might decide, considering that we are asking for additional information on biodistribution and shedding

Comment 2

Apparently the applicant considers that administration of the (VTx-002) vector carries not the slightest risk for the patient nor for humans around the patient. However, this might be not completely true. In view of the fact that more and more therapeutic interventions will make use of AAV vectors, and that there is very likely a kind of uncertainty concerning the long term consequences of these therapies, it seems necessary to include a systematic follow-up of what happens with the patients and possibly with people around them. This type of information should be provided to the patients, and a possibility of contact with responsible medical researchers even after a very long period after the administration of the vector should be provided. In a paper by Sant'Ana & Araujo in Virology Journal 2000 doi: 10.1186/s12985-022-01900-4 one reads "Even though AAV has been studied for over 50 years, little is known about the virus's natural infection. This fact may be even more surprising given that anti-AAV antibodies are found in up to 80% of the human population [6, 7]. AAV has been found in human blood cells, cervix uteri, penis, semen, liver, epithelial cell brushings, endometrium, amniotic fluid, and abortion material [43–46]. AAV may be transmitted through direct contact with an infected individual or indirect contact with the contaminated environment. Transmission routes include respiratory, gastrointestinal, and possibly sexual transmission. A concern for vertical transmission from mother to fetus also exists [46–48]." So AAV are very present in our populations and are well transmissible.

I Have some doubts concerning the statement " Based on the risk assessment, as outlined in this document, as well as safety from other similar gene therapy products that are administered though ICM, additional recommendations given to the clinical trial subjects to prevent dissemination are not applicable." This in view of the following text from a paper by by Sant'Ana & Araujo in Virology Journal 2000 doi: 10.1186/s12985-022-01900-4: ""Even though AAV has been studied for over 50 years, little is known about the virus's natural infection. This fact may be even more surprising given that anti-AAV antibodies are found in up to 80% of the human population [6, 7]. AAV has been found in human blood cells, cervix uteri, penis, semen, liver, epithelial cell brushings, endometrium, amniotic fluid, and abortion material [43–46]. AAV may be transmitted through direct contact with an infected individual or indirect contact with the contaminated environment. Transmission routes include respiratory, gastrointestinal, and possibly sexual transmission. A concern for vertical transmission from mother to fetus also exists [46–48]". So apparently AAV are well transmissible, and maybe the vector could also be transmissible too some extent.

SBB comment:

We agree that, although AAV vectors have been used in clinical research for many years, continued vigilance remains warranted, particularly in view of the increasing number of therapeutic applications and the evolving scientific understanding of their long-term effects.

In this context, and following the comments of Expert 4, we have suggested in Section 2.6 that the applicant further specify the precautionary measures to be followed by patients after leaving the hospital premises. These measures aim to minimize potential exposure of close contacts and to ensure appropriate risk management.

Concerning the transmissibility of wild-type AAV, as discussed in the literature (e.g., Sant'Ana & Araujo), it is acknowledged that AAV is widespread in the human population. However, the vector used in this study is a replication-defective recombinant AAV, which differs biologically from wild-type AAV.

Coordinator comment:
OK, I agree with the answer and also the ask for additional precautionary measure, considering it is a new hybrid vector

Comment 3
Has evaluated this item and has no questions/comments.

Comment 4
- P15/19 in B_BE_25_BVW9_Part 2_Common Application Form.pdf: PPE should include goggles to protect the eyes.
- Would be good to indicate as well that the hypochlorite solution should be freshly prepared.
- Recommendations to donate organs, blood, sperm/eggs should be prohibited, or at least extended in my opinion beyond the study duration (also this is not clear what timing the applicant has in mind here). Is 5 years the timing (see 3.7b - P18/19 in B_BE_25_BVW9_Part 2_Common Application Form.pdf)? Considering the disease, these organs are probably not considered any way for donation.

SBB comment:
Point 1 was also raised by Expert 4 in section 2.6 here above, and has already been addressed by the SBB.

Point 2: the following question could be sent to the applicant:
Given that to maintain chlorine strength and ensure bleach effectiveness, it is crucial to prepare the solution just before use to avoid loss of effectiveness over time, the notifier is requested to complete the information by indicating that this sodium hypochlorite solution must be freshly prepared and stored in a dark bottle.

Point 3 was also raised by Expert 1 in section 3.6 here above, and has already been addressed by the SBB.

Coordinator comment:
I am not familiar with goggles , if it is a good suggestions, you can add this in the letter to the applicant
Ok

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
See 3,6. With correct recommendations and compliance to these recommendations the overall environmental risk will be negligible.

Comment 2

As to the risks associated with infections with AAV, it was reported that AAV sequences can play a role in the induction of hepatic carcinoma. See paper by Russell & Grompe mentioned under point 6

Comment 3
Has evaluated this item and has no questions/comments.

Comment 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
Has no further questions/comments.

Comment 2

- It is certain that the AAV vector technology has tremendous potential to help in the fight against many human diseases. An important question relating to the increasing use of AAV in treatment of human diseases is whether, and to what extent, the apprentice sorcerer aspects of this approach are accompanied by adverse effects. A paper by Puetz (2023) Emergent data influences the risk/benefit assessment of hemophilia gene therapy using recombinant adeno-associated virus (DOI: 10.3389/fmed.2023.1256919 carries the following message “”Emergent data is showing that gene therapy may not be as beneficial as hoped and more toxic than planned. At a minimum, a reassessment of risk/benefit estimate of gene therapy for hemophilia is needed.
- A paper by Moroncini et al (2023) https://doi.org/10.1002/art.42746 reports that Adeno-Associated Virus TypeIn a paper by Song et al. 5 Infection via PDGFRα Is Associated With Interstitial Lung Disease in Systemic Sclerosis
- In a paper by Song et al.(2020) “Adeno-Associated Virus Vector Mobilization, Risk Versus Reality” DOI: 10.1089/hum.2020.118 one reads “ In a mobilization assay, a sizeable amount of rAAV recovered from infected 293 cell lysate remained intact and competent for a secondary round of infection (termed Ad-independent mobilization). In rAAV-infected cells coinfected with Ad and wtAAV, rAAV particle production was increased >50-fold compared with no Ad conditions. In addition, Ad-dependent rAAV vectors mobilized and resulted in >1,000-fold transduction upon a subsequent second-round infection, highlighting the reality of these theoretical safety concerns that can be manifested under various conditions. Overall, these studies document and signify the need for mobilization-resistant vectors and the opportunity to derive better vector production systems.”
- A paper by Russell & Grompe Adeno-associated virus finds its disease DOI: 10.1038/ng.3407 states that “Adeno-associated virus (AAV) vectors have been widely adopted for use in gene therapy. A new study raises concerns regarding this approach, reporting that chromosomal insertions of AAV serotype 2 seem to activate proto-oncogenes in human hepatocellular carcinoma” Several proto-oncogenes have been found to be overexpressed in human HCC and are thought to be causally related to cancer development. A subset of these genes, including TERT (telomerase reverse transcriptase), CCNE1 (cyclin E1) and KMT2B (lysine-specific methyltransferase 2B), are associated with HBV insertions2, with TERT activation being the most frequent genetic event in all HCCs and presumably an early event in the ontogeny3. In this issue

of Nature Genetics, Jessica Zucman-Rossi and colleagues add to this story by sequencing human HCC samples and finding clonal integrations of sequences derived from wild-type AAV2 at several known HCC driver genes4. In vitro modeling demonstrated that these insertions increased the expression of the proto-oncogenes and that the partial AAV sequences could act as both promoters and enhancers in hepatocytes (Fig. 1). These data strongly suggest that the AAV integrations actually caused the tumors, similarly to the scenario with HBV integrations. The results are surprising in several ways, especially because AAV has long been considered a nonpathogenic virus that even has anti-oncogenic properties5. Most of the insertions included a 3′ portion of the AAV2 capsid gene and the 3′ inverted terminal repeat (ITR), which was previously shown to have promoter properties6 and presumably also contains an enhancer element, as implied by the reverse orientation of some of the insertions. Approximately 6% of the HCCs studied contained clonal AAV insertions at proto-oncogene loci, with 21% of matched non-tumor liver specimens containing nonclonal AAV sequences. The HCCs with AAV insertions were enriched in patients without underlying cirrhosis, suggesting that AAV-induced inflammation was not a major contributor to oncogenicity (unlike in HBV-associated HCC). More than 50% of the population of the United States is thought to be infected with AAV, but no chronic hepatitis seems to have developed as a result. The absence of chronic liver injury in AAV infections may explain why the relative risk of developing HCC is lower than with HBV. Nonetheless, these results clearly indicate that insertional mutagenesis by AAV can cause malignant transformation in the liver, apparently without additional insults. This is remarkable given that AAV is generally considered a respiratory virus and requires a helper virus, such as adenovirus, for productive infection. The frequent presence of viral sequences in liver specimens suggests that the virus can also enter the bloodstream and infect internal organs at high levels. It remains to be seen whether similar oncogenic insertions occur in other types of human tumors. Does the finding that wild-type AAV integration can lead to human HCC shed light on the risks of gene therapy? In one sense, this study is reassuring because the critical 3′ capsid gene fragment present in wild-type AAV insertions is absent from AAV vectors. However, a larger concern is the novel observation that an enhancer-promoter element packaged in an AAV virion can integrate and activate a proto-oncogene in human hepatocytes, as most AAV vectors by necessity include a strong enhancer-promoter that is active in the target tissue. In liver-directed gene therapy, integration in as few as 0.1% of hepatocytes would still result in tens of millions of integration events. Consequently, it is likely that some patients will have vector sequences inserted at proto-oncogene loci. The tumorigenic impact of these insertions is difficult to predict because multiple oncogenic hits may be required for transformation. Even so, it is hard to imagine an exposure to wild-type AAV that is equivalent to the intravenous delivery of >1013 vector particles that a patient undergoing gene therapy typically might receive, and we now know that, in some cases, exposure to wild-type AAV might be enough to cause a tumor.

- Moving forward, there are several steps that gene therapists can take to improve the safety of AAV vectors. Careful design of enhancer and promoter elements may minimize the risks of insertional mutagenesis, as demonstrated for integration in the mouse Rian locus11. Eventually, the field may adopt promoterless vectors that integrate site specifically, as suggested by the recent demonstration of clotting Factor IX expression from an albumin locus knock-in vector12. When targeting tissues other than liver, one should choose vector serotypes with reduced liver tropism and enhancer and promoter elements that are not active in hepatocytes. Chronic hepatic inflammation and cirrhosis are clear contributors to HCC evolution, and patients with such conditions might not be suitable candidates for liver-directed gene therapy. Notably, obesity is a frequent cause of chronic hepatitis13 and thus could also be a risk factor for AAV-mediated oncogenesis. Similarly, AAV integrations occur more frequently in dividing cells14, so the risk of

tumor formation could be higher in any setting with hepatocyte proliferation, especially in young children with growing livers. Finally, there should be renewed efforts to eliminate even low levels of contaminating replication-competent AAV from clinical-grade vector stocks, as these particles could deliver the oncogenic capsid gene element. Close follow-ups of patients treated with AAV vectors will shed light on some of these issues, and renewed research into the potential oncogenicity of AAV vectors is now more important than ever.

- A paper by Janelidze (2014) DOI: 10.1002/jgm.2779 concludes Our findings indicate that intracerebral AAV2-based gene therapy is compromised in rats with pre-existing immunity to AAV2. By contrast, a local neuroinflammatory response, caused by intrastriatal a 6-OHDA injection, does not affect viral vector-mediated transgene expression. Our results emphasize the importance of monitoring circulating AAV-specific neutralizing antibodies in patients undergoing intracerebral gene therapy using AAV vectors.
- In a paper by Smith et al. Phase I/II trial of adeno-associated virus-mediated alpha-glucosidase gene therapy to the diaphragm for chronic respiratory failure in Pompe disease: initial safety and ventilatory outcomes one reads:“One subject demonstrated a slight increase in anti-GAA antibody that was not considered clinically significant. “
- In a paper byVan Der Marel doi: 10.1002/ibd.21673 one reads: “However, neutralizing antibodies (nAb's) made in response to wildtype AAV have been associated with a partial to complete block of transduction in case of reexposure
- In a paper by Hewitt DOI: 10.1128/JVI.02466-08 one reads: “ Current adeno-associated virus (AAV) gene therapy vectors package a transgene flanked by the terminal repeats (TRs) of AAV type 2 (AAV2). Although these vectors are replication deficient, wild-type (wt) AAV2 prevalent in the human population could lead to replication and packaging of a type 2 TR (TR2)-flanked transgene in trans during superinfection by a helper virus, leading to "mobilization" of the vector genome from treated cells. More importantly, it appears likely that the majority of currently characterized AAV serotypes as well as the majority of new novel isolates are capable of rescuing and replicating AAV2 vector templates. To investigate this possibility, we flanked a green fluorescent protein transgene with type 2 and, the most divergent AAV serotype, type 5 TRs (TR2 or TR5). Consistent with AAV clades, AAV5 specifically replicated TR5 vectors, while AAV2 and AAV6 replicated TR2-flanked vectors. To exploit this specificity, we created a TR5 vector production system for Cap1 to Cap5. Next, we showed that persisting recombinant AAV genomes flanked by TR2s or TR5s were mobilized in vitro after addition of the cognate AAV Rep (as well as Rep6 for TR2) and adenoviral helper. Finally, we showed that a cell line containing a stably integrated wt AAV2 genome resulted in mobilization of a TR2-flanked vector but not a TR5-flanked vector upon adenoviral superinfection. Based on these data and the relative prevalence of wt AAV serotypes in the population, we propose that TR5 vectors have a significantly lower risk of mobilization and should be considered for clinical use.
- A paper by Nault et al. “Recurrent AAV2-related insertional mutagenesis in human hepatocellular carcinomas” DOI: 10.1038/ng.3389 reports “Hepatocellular carcinomas (HCCs) are liver tumors related to various etiologies, including alcohol intake and infection with hepatitis B (HBV) or C (HCV) virus. Additional risk factors remain to be identified, particularly in patients who develop HCC without cirrhosis. We found clonal integration of adeno-associated virus type 2 (AAV2) in 11 of 193 HCCs. These AAV2 integrations occurred in known cancer driver genes, namely CCNA2 (cyclin A2; four cases), TERT (telomerase reverse transcriptase; one case), CCNE1 (cyclin E1; three cases), TNFSF10 (tumor necrosis factor superfamily member 10; two cases) and KMT2B (lysine-specific methyltransferase 2B; one case), leading to overexpression of the target genes. Tumors with viral integration mainly developed in non-cirrhotic liver (9 of 11 cases) and without known risk factors (6 of 11 cases), suggesting a pathogenic role for AAV2 in these patients. In

conclusion, AAV2 is a DNA virus associated with oncogenic insertional mutagenesis in human HCC."

- Adeno-associated virus infection and its impact in human health: an overview. Sant'Anna TB, Araujo NM.Virol J. 2022 Oct 31;19(1):173. doi: 10.1186/s12985-022-01900-4.PMID: 36316711Free PMC article.Review.

Coordinator comment

Point 1

Logical for any therapeutic intervention

Point 2

I am not a medical doctor but this publication is interesting and the applicant should assess potential adverse side effect, considering this publication. However, here we are only assessing biosafety for human and environment, not the balance risk/benefit, so this aspect should be more investigated by the AFMPS and ethical committee. This reference and aspect could nevertheless be mentioned in an adequate file of the applicant submission, so that this aspect could not be forgot during the balance risk/benefit assessment.

Point 3

This publication is aiming at being more cautious about rAAV production. The probability of such event is low (here it is in human, mainly neuronal, tissue and not in the Hek393 immortalized cell line as reported in the article). Nevertheless, I have no opposition to ask the applicant how they are taking this risk into account.

Point 4

Same remark as above: We are only assessing biosafety for human and environment, not the balance risk/benefit, so this aspect should be more investigated by the AFMPS and ethical committee. This reference and aspect could nevertheless be mentioned in an adequate file of the applicant submission, so that this aspect could not be forgot during the balance risk/benefit assessment.

Point 5

This is outside the scope of our assessment. We should not advise them how to make their products, only take care that the risk for the environment and human is under control during the use of these GMO . Nevertheless, as this is a new chimeric hybrid vector, more samples should be collected for analyses during the clinical trial phase 1 and broader analyses should be performed in order to have a more complete view of the biodistribution and shedding of this new vector.

Point 6-7-8

Again nice literature research by Expert, but this is out the scope of this assessment, as it is more adapted for the analysis of the balance risk/benefit

Point 9

This is already discussed in point 2.5. by the same expert and I already proposed an answer already to this point above

Points 10-11

Same remark as above: We are only assessing biosafety for human and environment, not the balance risk/benefit, so this aspect should be more investigated by the AFMPS and ethical committee.

SBB comment:

Based on comment on point 3, the following question could be raised:

Song et al. (2020) report that in a mobilization assay, a sizeable amount of rAAV recovered from infected 293 cell lysate remained intact and competent for a secondary round of infection. Furthermore, in rAAV-infected cells coinfected with Ad and wild-type AAV, rAAV particle production increased more than 50-fold compared with conditions without Ad. Moreover, Ad-dependent rAAV vectors mobilized and

produced more than 1,000-fold transduction during a second round of infection. These findings highlight that the theoretical safety concerns regarding vector mobilization can indeed manifest under certain conditions and indicating the imperative for the development of mobilization-resistant vectors and optimized vector production systems.
The applicant is requested to evaluate the risk of rAAV mobilization within the framework of biosafety regulations and environmental risk assessment, particularly with regard to vector shedding, secondary transmission, recombination, and potential public health impact.

Coordinator comment:
Ok, thank you

Comment 3
Has no further questions/comments.

Comment 4
- In B_BE_25_BVW9_IB_Ed1.0_10Oct2025.pdf p19/82 The applicant mentions "nonclinical development package of VTx-002 comprises a series of in vitro efficacy and in vivo biodistribution, and safety studies, summarized in Table 1 and Table 2." Table 2 also contains in vitro info. Not clear what is the purpose here. This is probably misplaced and should be included in Table1?

SBB comment:
This remark could be reported as a "Typos and other errors/omissions" as follow:
In the IB page 24, table 2 provides an overview of *in vivo* biodistribution and safety studies with VTx-002 and other GFP expressing AAV5.2 vectors. However, some *in vitro* results are also present in this table. Please make sure this information is correctly reported or consider to move it to table 1 which provides an overview of *in vitro* nonclinical studies with VTx-002.

Coordinator comment
Ok, I agree

- P15/19 in B_BE_25_BVW9_Part 2_Common Application Form.pdf: VTx-002 is referred to as "a non-enveloped virus". This is not correct. Should be viral vector, or virus-derived vector.

SBB comment:
This remark could be reported as a "Typos and other errors/omissions" as follow:
The following sentence can be found in section 3.6.c of the CAF document (page 15/19) : "VTx-002 is a non-enveloped virus and resistant to alcohol-based disinfectants (Korte et al., 2021)". However, the term "virus" is used where "viral vector" or "virus-derived vector" is meant. The applicant is requested to update the wording.

Coordinator comment
Ok
- Same goes for B_BE_25_BVW9_Protocol_V2.0_Final_01Oct2025.pdf: viral shedding for example is used, would be more correct to use 'viral vector shedding' to distinguish the DP, potential recombinant virus and the parental virus.

SBB comment:
This remark could be reported as a "Typos and other errors/omissions" as follow:

The term “viral shedding” is used several times throughout the protocol document whereas ‘viral vector shedding’ should have been used to distinguish the DP, potential recombinant virus and the parental virus. The applicant is requested to update the wording where applicable in the protocol.

Coordinator comment
Ok

References

Hewitt FC, Li C Gray SJ, Cockrell S, Washburn M, Samulski RJ. 2009. Reducing the risk of adeno-associated virus (AAV) vector mobilization with AAV type 5 vectors”. J Virol. 2009 Apr;83(8):3919-29.

Lu Y, Ling C, Shoti J, Yang H, Nath A, Keeler GD, Qing K, Srivastava A. 2024. Enhanced transgene expression from single-stranded AAV vectors in human cells in vitro and in murine hepatocytes in vivo. Mol Ther Nucleic Acids. 2024 Apr 23;35(2):102196.

Song L, Samulski RJ, Hirsch ML. 2020. Adeno-Associated Virus Vector Mobilization, Risk Versus Reality. Hum Gene Ther. 2020 Oct;31(19-20):1054-1067.

Adviesraad voor Bioveiligheid
Conseil consultatif de Biosécurité

Compilation of the expert's evaluations of the answers of VectorY Therapeutics B.V on the list of questions for dossier B/BE/25/BVW9

26 March 2026
Ref. SC/1510/BAC/2026_0330

Coordinator: Véronique Fontaine (ULB)
Experts: Anton Roebroek (KULeuven), Rik Gijsbers (KULeuven), Nicolas van Larebeke-Arschodt (UGent, VUB), Willy Zorzi (ULiège)
SBB: Katia Pauwels and Sheela Onnockx

INTRODUCTION

Dossier **B/BE/25/BVW9** concerns a notification from VectorY Therapeutics B.V 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 February 2026 and concerns a clinical trial entitled "phase 1/2 investigation of novel experimental regimen in amyotrophic lateral sclerosis (PIONEER-ALS): An open-label, uncontrolled, multicenter study to assess the safety and tolerability of two doses of VTx-002 in participants with ALS". The investigational medicinal product is a recombinant AAV-5 vector coding for a single-chain variable fragment that binds the pathological human TDP-43.

On 2 March 2026, based on a list of questions prepared by the BAC (SC/1510/BAC/2026_0219), 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 16 March 2026. This complementary information was reviewed by the coordinator and the experts in charge of the evaluation of this notification.

Evaluation Expert 1

I do agree with most of the replies of the applicant to the questions raised by the BAC. I however do have reservations for some of the answers as clarified below.

In the answer to Q4, the applicant replies '*the AAV5.2 chimeric capsid used for VTx-002 has the viral protein 1 (VP1) unique region amino acid sequence (first 102 Aa) of AAV2 and the VP2 and VP3 amino acid sequences of AAV5. The resulting AAV particle only has AAV5 capsid amino acid residues displayed on the outer surface of the capsid thereby determining its biodistribution and humoral immunogenic properties'*. Please provide a reference for this statement.

'*The rationale for including the AAV2 VP1 pertains to molecular advantages upon cell entry where the VP1 from AAV2 is more potent than AAV5 VP1 in escape of the vector from the endosome and*

trafficking to the cellular nucleus, steps necessary for potent expression of the transgene. These improved intracellular steps do not affect the biosafety aspects of the vector'. Again, a reference/paper should be provided for this statement.

SBB comment

If agreed, the following follow-up question could be posed to the applicant :

In its answer to Q4 the applicant states that 'the *AAV5.2 based vector only has AAV5 capsid amino acid residues displayed on the outer surface of the capsid and that these determine the biodistribution and humoral immunogenic properties of the viral vector particle.'* Secondly, the applicant states that *'the choice of using the VP1 from AAV2 over the AAV5 VP1 is based on improved intracellular processes, which are not affecting the biosafety aspects of the vector.*'
The applicant is requested to provide supporting data or literature references that can substantiate both statements.

Coordinator comment

Perfect, I agree, thank you

'AAV5-based vectors have been used in gene therapy clinical studies, such as in Huntington's disease (NCT04120493; (Rodrigues and Wild, 2020) and in the marketed products Hemgenix and Roctavian (Blair, 2022), whereas an AAV5.2 based vector has been used in a clinical study in osteoarthritis (NCT04875754). Therefore, the AAV5.2 vector has been tested in humans previously without any biosafety risks both to human health or the environment.' This last statement may be correct, but should be supported by proper referencing to literature or a study description paper.

SBB comment

Based on the reference *NCT04875754, a* search upon clinical.gov and brief literature search points to the findings of Heald. A *et al*., 2025, which provides the results of a single phase 1/2a study of 8 subjects after intra-articular injection of a *AAV5.2 based vector (ICM-203). Heald et al.,* reports that no significant safety or tolerability concerns arose.

Ref

Heald A. et al. (2025). A First-in-human phase 1/2a clinical study of ICM-203 AAV gene therapy: promising signals as a dmoad candidate. Osterarthritis Imaging ((1), 100291 https://doi.org/10.1016/j.ostima.2025.100291

Coordinator comment

OK, so no additional referencing is required for the applicant. Thank you for the reference.

As to the reply for Q5: As a result, interpretation of vector clearance kinetics in these matrices is limited. The clients claims: "*Nevertheless, given the very low magnitude of the detected signals and the absence of higher level findings, increased vector shedding via nasal or salivary routes is not expected*." This claim cannot be translated form animals models to human.
The animals these studies were performed in (which was asked to be clarified but it was not done in the answer) is not clear to me; in translation to human we must be careful and thus, shedding should be assessed prior to concluding that it is not expected.

SBB comment

It can be noted that section 4.5.1.4 of the IB (p45-49) presents VTx-002 shedding data in Non-human primate body fluids (including from nasal and saliva samples). Overall, biodistribution and shedding data have been obtained in mice , pigs and non-human primates.

Here again, while relevant non-clinical biodistribution and shedding data are provided in the IB, the applicant tend to give overly conclusions. and could be advised to stick to evidence-based wording throughout the dossier.

Coordinator comment

My proposal is presented here :

It can be noted that section 4.5.1.4 of the IB (p45-49) presents VTx-002 shedding data in Non-human primate body fluids (including from nasal and saliva samples). Overall, biodistribution and shedding data have been obtained in mice , pigs and non-human primates, but not on human.

Here again, while relevant non-clinical biodistribution and shedding data are provided in the IB, the applicant tend to give overly , not evidence-based, conclusions. The applicant is required to provide a more complete shedding and biodistribution assessment study with his new chimeric vector on human, at short, intermediate and long time points (including on nasal and tear samples).

SBB comment

An additional question for the applicant is proposed so as to include nasal secretions and tears as sample type for the shedding study.

It is not common to conduct a biodistribution study in human , as it would mean in theory that organ and other tissues should be investigated. A shedding study can suffice and is useful for the ERA-related questions for *VTx-002.*

The timepoints as currently formulated in the protocol for shedding analysis are at Day 1, Week 1, Week 4, Week 8, Week 12, Week 24, Week 52. In order to have better insights in the shedding profile shortly after administration of VTx-002, we may suggest to add timepoints for sampling at D3 and Week 2.

Proposed additional question for the applicant :

ON THE APPLICANT'S RESPONSE ON QUESTION 5

Question

Section 4.5.1.4 of the IB (p45-49) presents VTx-002 shedding data in non-human primate body fluids (including from nasal and saliva samples). Overall, biodistribution and shedding data have been obtained in mice, pigs and non-human primates. In section 8.3.8.3 (viral genome detection) of the protocol, it is proposed that ' *Blood, saliva, urine and fecal samples will be collected for the detection of potential viral shedding via immunohistochemistry/quantitative polymerase chain reaction/digital droplet PCR/southern blotting/in situ hybridization/next-generation sequencing*.' On p27 it is indicated that '*Viral genome detection: blood, urine, saliva, feces. Sampling should continue for the individual participant and for a specific matrix until three consecutive negative samples have been detected for the participant for that matrix'*.

Given the neurotropic use of the VTx-002 administered via the intracisternal magna, testing nasal secretions and tears may be relevant. The applicant is requested to include nasal and tear samples for viral genome detection and to add two timepoints (at Day3 and Week2) for all sample types. Sampling of blood, urine, saliva, feces, nasal secretions and tears should continue until three consecutive negative results are obtained.

As to Q6: see also Q4. The reported study trial NCT04875754 is sponsored by ICM Co., Ltd. and is part of a growing field of intra-articular AAV gene therapies for chronic joint pain. The bio distribution in this trial is different due to the different route of administration (see https://clinicaltrials.gov/study/NCT04875754).

The applicant states '*VTx-002 is administered via intracisternal magna (ICM) injection in the PIONEER-ALS trial, which the applicant expects to result in substantially lower systemic exposure than intravenous (IV) administration. Reduced systemic exposure following non intravenous administration is expected to further decrease the likelihood of vector dissemination, relative to intravenous dosing, as demonstrated in preclinical biodistribution and shedding studies (Aruda VR, et al 2001, Okai T, et al, 2025 and Hinderer C, et al, 2018, Sawyer 2019*)'.
I tried to check the papers referred to and these refer to AAV2 serotype, or experiments in animal models; these data cannot be readily translated. These expectations should be supported by data, and I would therefore request more details as to the biodistribution and shedding in the current trial, or at least for a plan to assess and collect samples in the planned trial.

SBB comment
In the protocol, in the 8.3.8. 3 (viral genome detection), it is stated that ' Blood, saliva, urine and fecal samples will be collected for the detection of potential viral shedding via immunohistochemistry/quantitative polymerase chain reaction/digital droplet PCR/southern blotting/in situ hybridization/next-generation sequencing.' On p27 it is indicated that '*Viral genome detection: blood, urine, saliva, feces. Sampling should continue for the individual participant and for a specific matrix until three consecutive negative samples have been detected for the participant for that matrix'*.
So, while the applicant indeed refers to a ongoing clinical trial study with intra-articular injection of a AAV5.2 based vector (NCT04875754), which is a different route of administration than the planned trial, it is noticed that the applicant foresees a shedding study in the planned trial.

Coordinator comment
See my previous comment on the response to Q5. The shedding and biodistribution assessment should be performed with this new vector on all samples, especially nasal samples

SBB comment
Agreed, an additional question for the applicant is proposed here above.

Evaluation Expert 2

Remark on answer to question 12:

Regarding the required duration of contraception use the different documents are still not consistent:

Clinical study protocol (page 16):
'Women of childbearing potential (WOCBP) and male participants with female partners who are WOCBP (based on gender assignation at birth) must agree to use highly effective (<1% failure rate) contraception for at least 30 days prior to the first dose of study medication, during the study, and for 90 days following end of study [EOS], including the long term follow-up.'

CAF (page 17):

'*Participants who are women of childbearing potential (WOCBP), as well as male participants with female partners who are WOCBP, must use a highly effective method of contraception for the entire duration of the study, including the longterm followup period.'*

ICF (page 7 and 24)
'You must agree to use highly effective birth control starting 30 days before you receive the IMP injection until 3 months (90 days) after your end of trial visit (including long-term follow-up).'

Questions:
Why is a period of 90 days (extra) – not consistently used – necessary, since the long term follow-up period is 5 years?
Is "end of study" the same as "end of trial visit"?
No arguments are presented why the use of contraception should last (more than) 5 years. This is extremely long compared to other studies. In the previous round of evaluation I judged a period of only 90 days after administration (my initial interpretation of "after your end of treatment/end of trial visit") as being short compared to other studies (e.g. 1 year in study B_BE_25_BVW3);
In the ICF, the sentence referred to above is only used in the part regarding female participants and in fact not in the part regarding male participants. Although a similar sentence is used regarding donation of sperm.
What about the update of the SNIF regarding this issue? The updated SNIF is not provided.

SBB comment
The expert's remark is supported and could be formulated towards the applicant as follows (a page number has been added to the protocol and the first-person pronouns have been eliminated) :

Regarding the required duration of contraception use the different documents are still not consistent:

In the Clinical study protocol (page 16, and page 44, it is stated :
'Women of childbearing potential (WOCBP) and male participants with female partners who are WOCBP (based on gender assignation at birth) must agree to use highly effective (<1% failure rate) contraception for at least 30 days prior to the first dose of study medication, during the study, and for 90 days following end of study [EOS], including the long term follow-up.'

In the CAF (page 17), is it stated:
'Participants who are women of childbearing potential (WOCBP), as well as male participants with female partners who are WOCBP, must use a highly effective method of contraception for the entire duration of the study, including the longterm follow-up period.'

In the ICF (page 7 and 24), it is stated:
'You must agree to use highly effective birth control starting 30 days before you receive the IMP injection until 3 months (90 days) after your end of trial visit (including long-term follow-up)'

The applicant is requested to clarify why a period of 90 days (extra) is not consistently mentioned and why it is actually necessary, since the long term follow-up period is 5 years? Referring to the inconsistency between the protocol and the ICF, is "end of study" the same as "end of trial visit"?
While a period of only 90 days after administration may seems short as compared to for example a period of 1 year seen in other studies, no arguments are presented why the use of contraception should last (more than) 5 years, which seems extremely long.

Also, in the ICF, the sentence referred to above is only used in the part regarding female participants and in fact not in the part regarding male participants. Although a similar sentence is used regarding donation of sperm. Could the applicant clarify ?
No update of the SNIF has been provided. The applicant is requested to provide the update of the SNIF -document.

Coordinator comment
I agree with the proposed text.

Remark with respect to use of 10% bleach
Sodium hypochlorite solution mentioned in several documents: 10% bleach (10 x diluted household bleach (?)) might not be the same as 1% sodium hypochlorite. Use a freshly prepared 6,000 ppm (mg/L) sodium hypochlorite solution.

SBB comment
This remark can be related to question 10. The expert touches upon a valid point regarding the percentage of sodium hypochlorite that may possibly vary between different brands of household bleach products. As suggested by the expert, recommendations on the use of bleach solutions are preferably expressed in terms of % sodium hypochlorite or ppm (mg/L).

In addition, while it is acknowledged that instructions for ensuring the effectiveness of the bleach solution have been adapted in the CAF public and section 12 of the Pharmacy manual, no such instruction were added on p9/49 of the Pharmacy Manual, nor in the two-page document '10_StudyStaff Instructions_Pharmacy Manual_V2.0".

Therefore the following question could be communicated to the applicant: :

While it is acknowledged that instructions for ensuring the effectiveness of the bleach solution have been adapted in the CAF public and section 12 of the Pharmacy manual, no such instruction were added on p9/49 of the Pharmacy Manual, nor in the two-page document '10_StudyStaff Instructions Pharmacy Manual_V2.0". Moreover, recommendations on the use of bleach solutions are preferably expressed in terms of % sodium hypochlorite or ppm (mg/L) as the percentage of sodium hypochlorite may possibly vary between different brands of household bleach products. The applicant is requested to update all relevant documents as appropriate.

Coordinator comment
I agree with the proposed text.

Evaluation Expert 3

Question 3
In my opinion, the applicant's justification for the choice of AAV2 ITRs is very weak; it is not really indicated why the AAV2 ITRs are preferable to the AAV5 ITRs.
This justification is limited to generalities such as "*The sponsor acknowledges the continuous efforts of the AAV field on fundamental research into the functioning of AAV vector design*", to references to the absence of negative experiences with AAV5-based products using ITRs from AAV2, and to preferences that are not clearly substantiated, such as "*molecular compatibility with Rep & Cap (in which part of the VP1 is derived from AAV2*)" and "*robust manufacturing"* en *"supported by an extensive release and*

characterization packages that ensure VTx-002 genome purity and safety". The fact that experiences with products such as Roctavian and Hemgenix have not yielded any detected problems to date is of only very limited significance, because, for example, insertional carcinogenic effects are evidently very rare phenomena, yet they do have serious consequences.
The essence of the question is not addressed.

SBB comment
In its answer, the applicant points to the use of AAV2 ITR in several clinical -grade AAV-based gene therapies, some of which have reached marketing authorization and argues that no confirmed cases of clinically relevant vector mobilization attributable to AAV2 ITR-mediated rescue have been demonstrated. The applicant further points to the mitigating measures minimizing the risk of formation replication competent AAV during the manufacturing process and the choice of AAV2 ITR in terms of integrity and purification yields. With this, valid points for the choice of the AAV2 ITR have been provided, both considering possible outcomes of mobilization *in vitro* (manufacturing processes) and *in vivo* (clinical experience with studied recombinant AAV carrying AAV2 ITR).
If correctly understood, the expert expected a further discussion of the significance of the findings of Hewitt et al., 2009 for *in vivo* scenario's, this is upon administration of VTx-002 to individuals. However, it is likely that the applicant can only give a theoretic discussion.

Coordinator comment
I agree. I understand the remark of the expert but it will be to difficult for the patient if the applicant has to investigate for recombination and mobility event. This is not the good patient population (already vulnerable) to perform such a study. Furthermore, balance risk/benefit of this treatment will be assessed by the medical team surrounding the patient.

Question 4

The applicant refers to doses in animal experiments on non-human primates. These animals tolerated doses 3 or 9 times higher than the therapeutic dose very well. That safety margin does not seem convincing to me.

SBB comment
No follow-up question for the applicant can be clearly identified from the expert's comment.

Coordinator comment
I can't provide an answer myself, I suspect that is why clinical study level phase 1 are performed on a small number of patients (eventually with increasing doses chronologically).

Question 5
Given the observations in control animals and the answer provided, the question arises whether these measurements were performed with due care.

SBB comment
No follow-up question for the applicant can be clearly identified from the expert's comment.

Coordinator comment
I agree

Question 6

It should be noted that, in view of the neurotropic use of the VTx-002 administered via intracisternal magna, testing of the tears might be relevant.

SBB comment

When a drug is injected into the **cisterna magna**, it enters the **cerebrospinal fluid**, which circulates around the brain and spinal cord and eventually drains into the bloodstream. From there, the drug can potentially appear ("be shed") in several body fluid. In the protocol, in the 8.3.8. 3 (viral genome detection), it is stated that blood, saliva, urine and fecal samples will be collected for the detection of potential viral shedding.

Viral genome detection in tear samples are usually conducted upon intravitreal injection or subretinal injection in the treatment of ocular diseases.

Coordinator comment

Since it is a new vector and administrated into the cisterna magna, I believe the proposition of the expert is relevant. I however already included this in the response of Q5.

SBB comment

Agreed, an additional question for the applicant is proposed here above.

Question 7

The answer is largely based on rather vague estimates such as "M*oreover, in vector mobilization studies, mobilization typically relies on high multiplicities of infection, forced Rep expression, and strong helper virus input, conditions that do not reflect physiological exposure scenarios*" and "*The probability that all three occur concurrently in the same cell population is low, and these conditions are not met under normal clinical circumstances.*"

It is also implicitly stated that helper viruses will be absent in the clinical context and that "*Even when present, helper virus infection must overlap temporally and spatially with cells harboring persistent rAAV genomes, which is unlikely in a therapeutic context*."

All in all, the conclusion "*Together, within the framework of biosafety regulations and environmental risk assessment, rAAV mobilization represents a theoretical but highly constrained risk, and the provided assessment supports the conclusion that there is no meaningful risk for public health*." therefore does not appear to be truly scientifically substantiated.

SBB comment

Please see SBB comment on Question 3 (expert Van Larebeke) here above.

Please notice the overall conclusions of the applicant on the environmental risks for human health and environment corroborates with the '*Good Practice on the assessment of GMO related aspects in the context of clinical trials with AAV clinical vectors* ' which is available at https://health.ec.europa.eu/system/files/2022-01/aavs_gp_en.pdf and which is a document that has been agreed upon among several Member states

Coordinator comment

I agree

<u>Some general consideration</u>

I also do not consider it positive that my remark that long-term follow-up of the patients involved can be very important is not being addressed. I agree with Russel & Grompe (DOI: 10.1038/ng.3407) who state: "*Close follow-ups of patients treated with AAV vectors will shed light on some of these issues, and renewed research into the potential oncogenicity of AAV vectors is now more important than ever*."

I am of the opinion that once more this experimental therapy represents very likely a great progress, but that the potential risks associated with adeno-associated virus therapies are not taken seriously enough.

SBB comment

We refer to the compilation of comments of experts and the review by the coordinator and SBB, *SC/1510/BAC/2026_0217, which was communicated to the experts on 2 March 2026.*

Coordinator comment

Agreed, I believe we already ask an improved and more complete assessment of shedding and biodistribution in our previous responses

Evaluation Expert 4

By this way, I would like to let you know that the notifier addressed correctly and satisfactorily all the comments/questions except for question 9.

Regarding the question 9, the applicant was asked to suggest alternative disinfection treatments for needlestick injuries or contamination of intact skin.
In the answer to this question, we could suggest replacing the generic term "soap" with "aseptic soap" in the sections on "Needle-stick injury and Skin Contact" as this is more appropriate for disinfection procedures.

SBB comment

Question 9 relates to the proposed measures described in the document '10_Study Staff Instructions_Pharmacy Manual_V2.0". In its answer, the applicant proposes disinfection treatment other than the use of alcohol-based disinfection procedures in case of needle-stick injury and skin contact. While the applicant addressed the question satisfactorily, the applicant could be invited to replace the generic term "soap" with "aseptic soap" in the sections on "Needle-stick injury and Skin Contact" as this is a more appropriate for disinfection procedures.
The same remark applies to the two-page document '10. Study Staff Instructions_handling_Instruction V1.0 Global(en)_11Mar 2026' and the document 11' Participant Instructions_participant_Hygiêne_Leaflet_V01 Global(en)_10_March2026.

Additional remarks by SBB

Regarding answer to question 8

The applicant provided a concise, user-friendly two-page document '11. Participant instructions_Participant Hygiene_Leaflet_V01Global(en)_10March2026' with a summary of instructions for the clinical trial patient. To further ameliorate the clarity and efficacy of the proposed measures, the applicant is requested to adapt in the document the following elements :

- P1, first paragraph : 'AAV gene therapy uses a harmless virus' should read 'AAV gene therapy uses a viral vector derived from a harmless virus… '

- P1, in the second paragraph ‘ it is stated that AAV may be found in saliva, urine, blood and stool. The applicant is requested to add ‘nasal secretions’
- P1, in the fourth paragraph, it is recommended to implement the precautionary measures for at least the first 8 weeks after treatment. Given that shedding analysis from Non-human Primate body fluids from GLP toxicology study (IV#017) indicates detectable vector genome levels in saliva and nasal samples on Day 180 after administration’ , the applicant is asked to give the rational to limit the duration of the precautionary measures until 8 weeks after the treatment.
- In the document, the use of disinfectant wipes is recommended for cleaning visible soiling on surfaces, cleaning the toilet seat and surrounding areas during bathroom use. Recommendations should sufficiently be clear in order to avoid the use of alcohol-based wipes as rAAV are non-enveloped and resistant to alcohol-based disinfectants (Korte et al., 2021). It should be made clear that commonly available 70% alcohol wipes (like 70% isopropanol wipes) will not properly disinfect spills or contaminated surfaces.

Regarding answer to question 11

With respect to the recommendations on refraining donation of blood, organs, tissues, and cells for transplantation, it is acknowledged that the CAF document (p17/20, section 3.6 (g) as well as the CSP document has been adequately adapted. However no update of the SNIF has been provided.
The applicant is requested to provide the update of the SNIF-document.
In addition, the applicant is requested to consider the addition of a recommendation on refraining from donation of blood, organs, tissues and cells for the duration of the study and the follow-up in the IB, for example under section 6.8.’special warnings and special precautions for use’.

Regarding answer to question 13

The modifications in the CAF and the pharmacy manual as well as the provision of a concise two-page overview document ‘10. Study Staff Instructions_Handling Instruction V1.0Global(en)‘ are acknowledged and are greatly appreciated.
It the latter document it is noticed that the implementation of precautionary measures when there is a contact with bodily fluids of the patients treated with VTx-002, as well as the duration for implementation of measures related to PPE, are proposed to be implemented for 8 weeks after a trial participant’s dosing. The applicant is requested to justify the duration of 8 weeks considering that shedding analysis from Non-human Primate body fluids from GLP toxicology study (IV#017) indicates detectable vector genome levels in saliva and nasal samples on Day 180 after administration.

Regarding the translation of documents #14, 18 and 19 as referred to in table on p17/18

*The applicant’s commitment to provide a translation to Dutch and French of documents ‘*09. SIS and ICF_Main_V2.0BEL3.0(en) ; ‘ 10. Study Staff Instructions_Handling Instruction V1.0Global(en) ‘ and document ‘11. Participant Instructions_Participant Hygiene Leaflet V1.0Global(en)’ is acknowledged.

Coordinator comment
Agreed

Adviesraad voor Bioveiligheid
Conseil consultatif de Biosécurité

Compilation of the expert's evaluations of the answers of VectorY Therapeutics B.V on the list of questions for dossier B/BE/25/BVW9

14 April 2026
Ref. SC/1510/BAC/2026_0392

Coordinator: Véronique Fontaine (ULB)
Experts: Anton Roebroek (KULeuven), Rik Gijsbers (KULeuven), Nicolas van Larebeke-Arschodt (UGent, VUB), Willy Zorzi (ULiège)
SBB: Sheela Onnockx

INTRODUCTION

Dossier **B/BE/25/BVW9** concerns a notification from VectorY Therapeutics B.V 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 February 2026 and concerns a clinical trial entitled "phase 1/2 investigation of novel experimental regimen in amyotrophic lateral sclerosis (PIONEER-ALS): An open-label, uncontrolled, multicenter study to assess the safety and tolerability of two doses of VTx-002 in participants with ALS". The investigational medicinal product is a recombinant AAV-5 vector coding for a single-chain variable fragment that binds the pathological human TDP-43.

On 26 March 2026, based on a list of questions prepared by the BAC (SC/1510/BAC/2026_0331), 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 8 April 2026. This complementary information was reviewed by the coordinator and the experts in charge of the evaluation of this notification.

Evaluation Expert 1

I'm concerned about the replies stating that they detect vector genome levels in untreated control animals, and that these signals are in line with dosed animals, whereas naive in house animals appeared to be negative, and the source of the contamination could not be identified. I'm not sure how the applicant concludes that this implies that saliva and nasal swab data do not provide reliable evidence of true long-term persistence. Considering the results "not-interpretable for ERA" seems incorrect to me. Instead of qPCR, other orthogonal techniques could be used, such as ddPCR or sequencing.
The fact that no difference can be made between treatment related shedding and background is not a correct conclusion, since 'background' is negative in naive in-house animals.(see Response p10/12 and 5/12)

Additionally, in the response on p5/12, several of the papers used to underscore that shedding in nasal secretion and tears are not primary informative matrices refer to papers where a different serotype or dose is used. I understand that it is difficult to include these additional samples in the study. Still, it is confusing to me that the applicant does not want to add samples to monitor shedding in tears and saliva, but proposes to adhere to the precautionary principle and will extend the precautionary measures for participants and staff personnel to 6 months post dose.

SBB comment

This comment is related to the applicant's comment : "II: On the applicant's response on question 5 (first round)". The applicant notes inconsistencies and uncertainties regarding the interpretation of vector genome detection in shedding studies. Specifically, vector genome signals were reported in untreated control animals at levels comparable to dosed animals, while naïve in-house animals tested negative, and the source of this signal could not be identified.
It is therefore not clear whether the low level of qPCR signals detected in saliva and nasal swab samples up to Day 180 post-dose are attributable to vector shedding, as comparable levels were observed in untreated control animals. This could suggest that the detected signals are likely at or near the assay's limit of detection.

A few references have been provided that confirmed that AAV vector genomes were largely confined to CNS tissues after ICM administration. However, it appears that no studies have evaluated AAV vector shedding in nasal or lacrimal secretions after intracisternal magna (ICM) injection. This indicates a knowledge gap as such shedding can neither be confirmed nor excluded based on the currently available data.
However, rAAV vectors are non-replicative and if any shedding occurs, it is expected to be limited to very low quantities.

Risk assessment is based on the principle: Risk = hazard × exposure.
This means that if the hazard is negligible, the associated risk can also be considered negligible, despite a real possibility of exposure. Conversely, if exposure is estimated to be negligible, the risk will be negligible, even if a hazard were to be identified.
In the context of this dossier, there are no data indicating a hazard or adverse effects for non-patients, given that the parental virus from which the viral vector is derived is non-pathogenic (and, moreover, ubiquitous within the population) and the encoded protein exhibits no toxic properties. Even if exposure of close contacts via shedding by patients cannot be ruled out, there is no data indicating a real risk to close contacts.

The clinical study B/BE/21/BVW3 with PR006 (LY3884963), an ICM-administered gene therapy product with the AAV9 serotype, has received Belgian GMO approval, whereas excretion was assessed only in saliva, urine, and stool, without sampling of nasal or tear fluid.

The proposed precautionary measures (extension to 6 months and providing instruction on hygiene and handling instructions for nasal secretions) seem sufficient from an environmental risk perspective.

Coordinator comment

Were vector genome signals reported in shedding analysis in human materials ?
How to be sure exposure can be estimated to be negligible?
Indeed, the encoded protein exhibits no toxic properties.
Indeed too late to change

I agree that the proposed precautionary measures seem sufficient from an environmental risk perspective

SBB's comment:

This is a Phase 1/2 clinical trial and shedding analysis will be performed for the first time in human during this clinical trial.

The precautionary measures implemented during a clinical trial aim to significantly reduce the risk of exposure. The risk will not be zero, but it will be greatly reduced.

Coordinator comment

Ok

Evaluation Expert 2

Remarks with respect to the answer to "VII: On the applicant's response on question 12 (first round)" (regarding the required duration of contraception use)

It is appreciated that most of the inconsistencies identified with respect to the use of contraception and condom between the different documents are resolved.
With respect to the required time period to use contraception and/or a condom (SINF page, 15; SIS and ICF, page 26-29; CSP, page 16-19) , the addition of 'including the long-term follow-up period' results in an extremely long period of 5 years to use contraception or a condom with an unnecessary long period of burden to the patient (male or female) or the (female) partner of a treated male. Like in most other AAV-based gene therapies an advised period of 1 year after administration of the vector should be sufficient to reduce the risks of shedding for the partner or the risks for the unborn child to an acceptable level (very low to negligible). If not reduced to a 1-year period, a female patient or the female partner of a male patient is not allowed to get pregnant in the second to the fifth year after administration. In this time period she might loose her fertility due to aging. It is questionable whether you can ask this from a patient considering the remaining risks after a 1-year period.
In the response it is furthermore stated that "In the updated version of the protocol, a 90 days period is linked to either End of Study or Early Termination visit in case the subject discontinues the study prematurely.". This 90-days period is far to short in case the subject discontinues the study prematurely very early on (e.g. second week after administration). Furthermore, it is very contradictory to have far more severe safety restrictions for patients who continue to participate in the study than patients that leave the study. Patients should agree to comply to the measurements to prevent pregnancy and exposure to the partner at least for a period of one year after administration of the vector even in case of discontinuation the study. This should be also be clearly mentioned in the ICF.

SBB comment

SBB can support expert's comment and the following request could be sent to the applicant:
With respect to the required time period to use contraception and/or a condom, the inclusion of the wording "including the long-term follow-up period" results in an overall duration of up to 5 years. This is considered excessively long and may impose an unnecessary burden on patients (male or female) as well as on the (female) partners of treated male patients. If the 5-year duration is maintained, this would imply that female patients—or female partners of treated male patients—would be unable to become pregnant for a prolonged period of up to five years post-treatment, during which time fertility may decline due to age. It is therefore questionable whether such a requirement is proportionate given the residual risks beyond a 1-year period.

Furthermore, the response indicates that "in the updated version of the protocol, a 90-day period is linked to either the End of Study or Early Termination visit in case the subject discontinues the study prematurely." This 90-day period appears insufficient in cases where a subject discontinues participation shortly after dosing (e.g., within the first weeks following administration). In such scenarios, a 90-day requirement may not adequately cover the period of potential risk.

Also, it is very contradictory to have more severe safety restrictions for patients who continue to participate in the study than patients that leave the study.

In line with most other AAV-based gene therapies, a post-administration contraception period of 1 year is generally considered sufficient to reduce the risks of vector shedding and potential exposure to the partner or unborn child to an acceptable level (very low to negligible). Therefore, all subjects, regardless of study participation status, should be required to comply with contraception and exposure prevention measures for at least 1 year following vector administration. This obligation should be clearly and explicitly stated in all applicable documents such as the Informed Consent Form (ICF).

Coordinator comment
I agree with this comment

In the SNIF (page 15) it is stated that all participants must refrain from donating blood, organs, tissues, and cells for transplantation for the duration of the study and long term follow up. Since alternative donors should be available, the participants should refrain from donation for the rest of their life (as is specifically required in most other AAV-based gene therapies). In case of donation for transplantation one can choose for absolute safety without in fact causing any burden for anyone. With respect to the wish to get pregnant (see above) a safe limitation of the period to use contraception should be defined as there are in fact no in vivo alternatives for a couple to have a child of their own in case there would be a lifetime restriction.

SBB comment
According to question 11 from our first List of questions, it was requested to either align the recommendations with the instruction given in the EPAR ('Patients treated must not donate blood, organs, tissues, and cells for transplantation'), or to give a rationale why instructions could deviate from measures commonly taken for current EU marketing authorized medicinal products containing recombinant AAV. Since the recommendations deviated from the EPAR (all participants must refrain from donating blood, organs, tissues, and cells for transplantation for the duration of the study and long term follow up, instead of for the rest of their life), the applicant could be requested to provide a rationale why instructions could deviate.

Coordinator comment
OK with comments

Evaluation Expert 3

In my opinion, the company's response regarding the AAV2 and AAV5 issue is incomplete. No argumentation is provided to demonstrate that "which are not affecting the biosafety aspects of the vector" is indeed credible.

SBB comment
This comment is related to the applicant's comment : "I: On the applicant's response on question 4 (first round)". Beyond structural and functional considerations, it is important to explicitly address whether incorporation of the AAV2 VP1 unique region introduces any new biosafety risks. The answer from the applicant demonstrates that using VP1 from AAV2 into AAV5 capsid should not affect tropism and humoral immunogenicity. It is indeed unclear whether the substitution of the VP1 unique region could alter the biodistribution profile of the vector. Furthermore, it is unclear whether the incorporation of the AAV2 VP1 unique region introduces new or unforeseen biological risks such as increased toxicity, unintended tissue targeting or increased pathogenicity.

Coordinator comment

It was therefore interesting to verify presence of vector in tears and nasal secretions, but it seems too late for asking this

As for the answer regarding "shedding," the observations concerning the presence of viral sequences in tear and nasal fluid certainly do not credibly indicate the absence of shedding via these bodily fluids.

SBB comment

This comment is related to the applicant's comment : "II: On the applicant's response on question 5 (first round)". See SBB comment to expert 1 here above.

Coordinator comment

Indeed

Evaluation Expert 4

After examining the notifier's responses on the second list of questions of the Biosafety Advisory Council for dossier B/BE/25/BVW9 (clinical trial submitted by VectorY Therapeutics B.V. related to the use of recombinant AAV for subjects with Amyotrophic Lateral Sclerosis), we would like to let you know that the notifier did not respond correctly and satisfactorily to the comments/questions, particularly for question n°10:

1)In p8/12, of the document "Response to questions Round 2", it is written that :

V: On the applicant's response on question 10 (first round)

Requirement

While it is acknowledged that instructions for ensuring the effectiveness of the bleach solution have been adapted in the CAF public and section 12 of the Pharmacy manual, no such instruction were added on p9/49 of the Pharmacy Manual, nor in the two-page document '10_StudyStaff Instructions Pharmacy Manual_V2.0". Moreover, recommendations on the use of bleach solutions are preferably expressed in terms of % sodium hypochlorite or ppm (mg/L) as the percentage of sodium hypochlorite may possibly vary between different brands of household bleach products. The applicant is requested to update all relevant documents as appropriate.

Response:

The requested updates have been implemented. Instructions to ensure the effectiveness of the bleach solution have been added to the Pharmacy Manual (Version 5.0). In addition, bleach solution recommendations have been revised to specify concentrations in % sodium hypochlorite to account for variability between commercial bleach products.

2) On page 16/26 of the document "Study Staff Instruction Pharmacy Manual", it is written that :

Spillage

In case of an indoor or outdoor spill, isolate and soak up the liquid with paper towels. If the spill is outside a biological safety cabinet, avoid breathing in aerosols. Disinfect area with 10% bleach (1% sodium hypochlorite)* or detergent-based disinfectant to deactivate virus and let stand for 10 minutes. Discard soaked materials into biohazardous waste, to be picked up by a licensed disposal company, or autoclave at 125°C for a minimum of 30 minutes before regular trash disposal.

* where sodium hypochlorite is used, it is crucial to prepare the solution just before use and to ensure it is stored in a dark bottle to avoid loss of effectiveness over time.

Following the sections 1) and 2), we consider that the applicant has not well understood the question / request n°10 of BAC council :

A) In order to clarify the request, the following explanation could be provided :
The usual concentration for decontamination of Adenoviruses is 6000 ppm (see ref 1). Ready-to-use solutions can contain between 5 and 36°Chl in Belgium. The dilution (1:10 or 10%) of Household Bleach (for the "USA") depends on this initial concentration. We invite the applicant to eliminate the ambiguous terms "10% bleach solution (1% sodium hypochlorite)" and replace it with: "6000 ppm (mg/L) chlorine (~1% sodium hypochlorite)".

Indeed, this titer "6000 ppm (mg/L) of chlorine" corresponds to 0.615% of sodium hypochlorite and can be extrapolated to "1% of sodium hypochlorite" for common use.

Ref 1 :

Antimicrob Agents Chemother
. 2006 Apr;50(4):1419–1424. doi: 10.1128/AAC.50.4.1419-1424.2006
Efficacy of Hospital Germicides against Adenovirus 8, a Common Cause of Epidemic Keratoconjunctivitis in Health Care Facilities
William A Rutala [1,2,*], Jeffrey E Peacock [3], Maria F Gergen [1], Mark D Sobsey [3], David J Weber [1,2]

Ref 2 :

For the "USA" :

Expected Chlorine Concentrations by Various Dulitions of Household Bleach (5.25-6.15% sodium hypochlorite)

Dilution	Chlorine (ppm)
None	52,500–61,500
1:10	5,250-6,150
1:100	525-615
1:1000	53-62

source :

Accessible version: https://www.cdc.gov/infection-control/hcp/disinfection-and-sterilization/index.html

Guideline for Disinfection and Sterilization in Healthcare Facilities, 2008

Update: June 2024

William A. Rutala, Ph.D., M.P.H.[1,2], David J. Weber, M.D., M.P.H.[1,2], and the Healthcare Infection Control Practices Advisory Committee (HICPAC)[3]

B)
Please note that the time required to treat a spill depends on its composition (viscous or not), and its concentration and on the disinfectants (composition and concentration) used. therefore, the procedure cannot be simplified by reducing it to "10 minutes to stand".
C)
The treatment by autoclave is not appropriate in order to avoid potential generation of toxic vapors by heating bleach or detergent-based disinfectants. The latter can typically contain complex chemical mixtures, including volatile organic compounds, mainly used as solvents and perfumes, preservatives and disinfectants.

Taking in account these 3 remarks A), B), C), we propose to modify the section as following :
« Disinfect area with a 1% sodium hypochlorite solution, freshly prepared and in dark bottle stored or detergent-based viricidal disinfectant. Depending on its composition and concentration, leave the disinfectant solution acting on the spill for the appropriate time. Discard soaked materials into biohazardous waste, to be picked up by a licensed disposal company. For this biohazardous waste, the treatment by autoclave is not appropriate to avoid the potential generation of toxic vapors by heating waste previouly-treated with bleach or detergent-based viricidal disinfectants: the latter can typically contain complex chemical mixtures, including volatile organic compounds, mainly used as solvents and perfumes, preservatives and disinfectants.

SBB comment:
The proposed question by the expert could be sent to the applicant by supplementing it with the following comment from the expert (point A) :
The usual concentration for decontamination of Adenoviruses is 6000 ppm (see ref 1). Ready-to-use solutions can contain between 5 and 36°Chl in Belgium. The dilution (1:10 or 10%) of Household Bleach (for the “USA”) depends on this initial concentration. We invite the applicant to eliminate the ambiguous terms "10% bleach solution (1% sodium hypochlorite)" and replace it with: "6000 ppm (mg/L) chlorine (~1% sodium hypochlorite)".

Coordinator comment
OK thank you

Evaluation coordinator

I quickly checked for the first two answers. I am not satisfied with the first response (about Q4), but I don’t know whether this is important to tackle.
Most published studies are performed *in vitro*. There is a lack of data on *in vivo* biodistribution, especially when dealing with chimeric capsids. Although VP3 is the major capsid protein, VP1 and VP2 have unique N-terminus. Their N-terminal regions contain conserved elements required for AAV infectivity such as a phospholipase A2 (PLA2) domain or a calcium-binding domain. Some of those might play a role in secondary receptor interaction, so consequently in infectivity and biodistribution. As such, a capsid might, after natural viral modification (i.e. hydrolysis by VP1 or VP2) show different conformation to allow interaction with an additional secondary receptor. Indeed, structural differences described

between AAVs must allow for different receptor attachments beside transduction efficiency, and antigenic diversity between the serotypes (AAV-2 and AAV-5 are not using same host primary receptor for infection and I have no data about secondary receptors, nevertheless these information are usually obtained in vitro and potentially not representative of natural infections). I find no evidence in references for these affirmations of the applicant:

- "VP3 protein sequence forms the entire antigenic outer surface of AAV vectors"
- "While VP1 and VP2 are essential for intracellular trafficking and productive infection, they do not typically dictate organ level biodistribution".
- "VP1u region of AAV2 mediating intracellular processes" (ONLY?)

SBB comment:

Biodistribution and tissue tropism directly influence shedding, transmission potential, and environmental exposure. In this context, the use of a chimeric capsid introduces additional uncertainty compared to well-characterized serotypes. Moreover, some affirmations of the applicant regarding capsid biology do not appear to be sufficiently supported by the provided references. This concern is further reinforced by the lack of *in vivo* data, which is particularly important when attempting to extrapolate the environmental behavior of the vector.

Therefore, the following question could be sent to the applicant:

The applicant states that (1) VP3 protein sequence forms the entire antigenic outer surface of AAV vectors, and (2) VP1 and VP2 do not typically dictate organ-level biodistribution and (3) VP1 unique region of AAV2 seems to mediate only intracellular processes.

However, considering that VP1 and VP2 contain unique N-terminal domains (e.g. PLA2 and calcium-binding domains) potentially involved in infectivity and receptor interactions, and given the limited availability of *in vivo* biodistribution data for chimeric capsids, the scientific basis for these 3 statements remains unclear and need to be further developed.

Coordinator comment

OK thank you

About the response on Q5…their answer is clever. I believe we cannot go further (it is too late…)

SBB comment:

See SBB comment to expert 1

Coordinator comment

OK thank you

For the additional answers, I have no problem (quickly read).