Adviesraad voor Bioveiligheid
Conseil consultatif de Biosécurité

Advice of the Belgian Biosafety Advisory Council on the notification B/BE/26/BVW3 of the company GENETHON for deliberate release in the environment of genetically modified organisms other than higher plants for research and development

FINAL version: 21/05/2026
Ref. SC/1510/BAC/2026_0463

Context

The notification B/BE/26/BVW3 has been submitted by GENETHON to the Belgian Competent Authority in February 2026 for a request of deliberate release in the environment of genetically modified organisms (GMOs) other than higher plants for research and development according to Chapter II of the Royal Decree of 21 February 2005.

The planned activity concerns a clinical trial with the title: *"A phase I/II/III study with a dose determination part followed by an efficacy and safety evaluation, quadruple blind placebo-controlled part and then by a long-term safety follow up part, in ambulant boys with Duchenne Muscular Dystrophy".*

Duchenne muscular dystrophy (DMD) is a X-linked degenerative neuromuscular disease caused by mutations in the dystrophin gene. It predominantly affects boys. The lack of functional dystrophin protein results in progressive muscle weakness and wasting. Ultimately heart and respiratory muscles are affected, causing premature death of DMD patients. There is no cure for the disease yet, but treatments such as corticosteroids and gene therapy can slow down its progression.

The primary objective of this phase I/II/III study is to determine the dose of IMP and to assess the efficacy, the safety and tolerability of a single peripheral intravenous (IV) infusion of GNT0004 compared to placebo in ambulatory boys aged six to ten inclusive with Duchenne muscular dystrophy. GNT0004 is a gene therapy being developed to provide patients with an optimized microdystrophin protein in order to significantly slow down or inhibit the progression of the disease.
GNT0004 is a replication-incompetent recombinant AAV-8 containing the shortened functional human dystrophin gene (hMD1). GNT0004 is a gene therapy targeting skeletal and cardiac muscles developed to increase the expression of microdystrophin in the patients in order to strengthen the muscles and slow down or halt the damage caused by the disease.

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.

Overall, up to 70 subjects will be included in this all-in-one clinical study, wherefore, ten are expected in Belgium. In this clinical trial, a single dose of the investigated medicine GNT0004 will be administered through peripheral intravenous (IV) infusion to boys with Duchenne muscular dystrophy. This study will

Biosafety Advisory Council - Secretariat • Service Biosafety and Biotechnology (SBB)
Sciensano • Rue Juliette Wytsmanstraat 14 • B-1050 Brussels • Belgium
T + 32 2 642 52 93 • bac@sciensano.be • www.bio-council.be

be conducted at three clinical sites located in Walonia, Flanders and Brussels. The national territory is considered as the potential release area of GNT0004.

The dossier has been officially acknowledged by the Competent Authority on 04 March 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. Two experts from the common list of experts drawn up by the BAC and the Service Biosafety and Biotechnology (SBB) of Sciensano and one expert from the SBB answered positively to this request. The experts assessed whether the information provided in the notification was sufficient and accurate to state that the deliberate release of the genetically modified organism would not raise any problems for the environment, animal health or human health (people coming in contact with the treated patient and/or with the GMO) in the context of its intended use. See Annex I for an overview of all the comments from the experts.

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

On 02 April 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 14 April 2026 and transmitted to the secretariat of the BAC on 20 April 2026. 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 27 April 2026. The answers of the notifier were received on 06 May 2026 and reviewed by the coordinator, 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 didn’t receive any reactions from the public.

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.
rAAV vector production involves transient transfection of HEK293T cells using three plasmids that carry the therapeutic cDNA, AAV rep/cap genes, and helper adenoviral genes. As the HEK293T cells contain the SV40 Large T-antigen coding sequence to enable higher transfection efficiency and increased protein expression, the applicant confirms that the potential presence of the SV40 large T antigen has

been evaluated in each clinical drug substance batches and that the risk associated to residual cellular DNA SV40 large T antigen can be considered low.

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

Information related to the molecular characteristics of GNT0004 were adequately described in the dossier. Identity tests on GNT0004 viral genome has been performed to determine the nucleotide sequence of the vector with different sequencing methods.

3. The conditions of the release

This all-in-one clinical trial will consist of three parts: Part 1 consists of a phase I/II part to determine the appropriate dose level. Part 2 consists of a phase III placebo-controlled part to confirm the efficacy and assess the safety of the selected dose of IMP. Part 3 consists of a long-term follow-up evaluating safety and efficacy for up to five years after GNT0004 administration in participants previously enrolled and treated in Parts 1 and 2. Participants will be in the trial for up to a maximum of 6 years. This includes 52 weeks (Parts 1 and 2) followed by a minimum of 5 years of long-term follow-up after the GNT0004 dosing date. The GMO will be administered via a single peripheral intravenous infusion, in hospital centres. After administration, participants will be monitored for a minimum of 3 hours post-administration.

Shedding data collected from the study will further contribute to a proper environmental risk evaluation. These shedding data will need to be evaluated considering the observed quantity of shed viral vector material, and the period during which shedding is observed. In non-clinical studies, vector DNA was quantifiable in urine samples in dogs and rats in studies. In this clinical trial, clearance of GNT0004 vector genomes in blood, urine, saliva and fece samples will be assessed before and after GNT0004 administration. The frequency of samples post administration is every week until week 8, then monthly and every 3 months up to 1 year. This post administration vector shedding follow-up lasts until the clearance of vector (defined as indetectable results are obtained in two consecutive sample timepoints) in each type of biologic excretion/fluid for each individual patient who was enrolled in Part 1.

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 hygiene and precautions guidance explaining and summarizing all the critical information and instructions for patients and their families.

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 (rAAV) 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 GNT0004 and wild-type AAV in case a triple infection by GNT0004, 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.

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

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 GNT0004 and the assumed lower amounts of shed and intact viral particles of GNT0004 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

Transport between the hospital pharmacy and the ward must take place in a double packaging with the outer packaging consisting of a leak-proof container.
To protect footwear from potential biological contamination caused by visible spills or undetected microspills on the floor, as well as from exposure to chemical disinfectants used during decontamination, overshoes have been added to the existing list of personal protective equipment which already included laboratory coats, gowns, gloves, safety glasses.
For needle sticks or contamination of intact skin, aseptic soap, other than alcohol, is considered more appropriate as first aid measure for decontamination. First aid measures in case of splash on the skin, in the eyes, inhalation and puncture or needle stick injury have correctly been reported in the different documents.
A spill kits will be available at all times and during all steps of handling the investigational product, including reception, storage, preparation, transport, administration, and disposal of contaminated materials.

Effective disinfectants such as freshly prepared 1% active chlorine, equivalent to 10 500 ppm sodium hypochlorite solution, will be used for decontamination of the GNT0004 preparation area, the administration room after completing administration and in the event of spill. To maintain chlorine strength and ensure bleach effectiveness, solution will be prepared just before use and stored in a dark bottle to prevent loss of effectiveness over time. The applicant is aware that hypochlorite solution cannot be proposed as a universal decontaminant or disinfectant because it can corrode or damage stainless steel, aluminum and the most rubbers components of surfaces and has proposed a list of adequate of decontamination / disinfection solutions for areas that cannot be decontaminated with bleach. In event of an accidental spillage of the IMP, materials soaked with bleach and used to contained the spill will not be autoclaved, but will be disposed of as infectious clinical waste.

The notifier also provided an updated version of the 'Instructions for study site personnel' sheet 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.

Since propagation of GNT0004 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 GNT0004 developed to treat patients with Duchenne muscular dystrophy (DMD), by means of an optimized microdystrophin protein, will have any adverse effects on human health or on the environment in the context of the intended clinical trial provided that all the foreseen safety measures are followed.

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

- The notifier and the investigators must strictly apply the clinical trial protocol, and all the safety instructions as described in the following documents:
    - Latest version of the ICF
    - Latest version of the Protocol
    - SNIF_06May2026
    - CAF_ NC_BEL_06May 2026
    - CAF_ C_BEL_06May 2026
    - DR_Study Staff Instructions_06May 2026

- As committed by the applicant in his response of 14 April 2026 to our first list of questions, it will be clearly indicated in the **ICF** that patients who receive treatment must not donate blood, organs, tissues, or cells for transplantation in order to be consistent with recommendation provided in the EPAR of EU registered medicinal products containing recombinant AAV

- 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 enrolled 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 contain as a minimum:
    - The total number of patients enrolled in the trial and the number of patients from Belgium;
    - A summary of all adverse events documented by the investigators as likely or definitely related to the study medication;
    - A report on accidental releases, if any, of GNT0004.

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

Annex I: Compilations of comments of experts in charge of evaluating the dossier B/BE/26/BVW3 (ref. SC/1510/BAC/2026_0372, SC/1510/BAC/2026_0416 and SC/1510/BAC/2026_0458)

Adviesraad voor Bioveiligheid
Conseil consultatif de Biosécurité

Compilation of comments of experts in charge of evaluating the dossier B/BE/26/BVW3
And comments submitted to the notifier

02 April 2026
Ref. SC/1510/BAC/2026_0372

Mandate for the Group of Experts: Mandate of the Biosafety Advisory Council (BAC) of 17 February 2026.
Coordinator: Rik Gijsbers (KULeuven)
Experts: Anton Roebroek (KULeuven), Willy Zorzi (ULiège)
SBB: Sheela Onnockx

INTRODUCTION

Dossier **B/BE/26/BVW3** concerns a notification from GENETHON 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 March 2026 and concerns a clinical trial entitled "A phase I/II/III study with a dose determination part followed by an efficacy and safety evaluation, quadruple blind placebo-controlled part and then by a long-term safety follow up part, in ambulant boys with Duchenne Muscular Dystrophy". The investigational medicinal product is a recombinant AAV8 (rAAV8) vector carrying codon-optimized human Micro-Dystrophin 1 (hMD1-co) expression cassette.

♦ INSTRUCTIONS FOR EVALUATION

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

List of comments received from the experts

Remark: The comments below have served as basis for a list of questions that the Competent authority forwarded on 02-04-2026 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

Has evaluated this item and has no questions/comments.

Comment 2

Has evaluated this item and has no questions/comments.

Additional coordinator comment:

Production is done on HEK293T cells. Is this an issue? The large T Ag is a key protein produced by certain polyomaviruses, most notably the SV40 virus. It is a critical oncogene that transforms cells and induces cancer by inhibiting human tumor suppressor proteins, specifically p53 and pRb (retinoblastoma protein). I did not come across an assessment for the absence of large T sequences in the DP. Maybe this is beyond ERA?

I was convinced all other productions are done in HEK293 cells instead.

SBB comment:

Given the role of SV40 large T antigen in cellular transformation, an evaluation of the potential presence of residual or packaged sequences and their implications for environmental release or unintended exposure would be relevant within the scope of this assessment.

Coordinator comment:

I would include a question to inquire about the way(s) the applicants is assessing this.

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

Has evaluated this item and has no questions/comments.

Comment 2

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

In table 1 in the confidential CAF, different (page 7 of 76) the sizes of the ITRs in plasmid is described reported between pages 7, 40 and 43 of 76 and in table 40 (page 45 of 76). What is the explanation of this difference: result of the replication mechanism involving the ITRs or a mistake?

SBB Comment

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

Coordinator comment:

For me beyond ERA, but can be asked. Agreed.

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.

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

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

Has evaluated this item and has no questions/comments.

Comment 2

In the SNIF (page 3 of 16 , A.7.5.) it is stated that in non-clinical studies VOI-04, VOI-04LT and 20180053TRP studies no shedding was observed in urine. This is not correct, because in the Investigator Brochure (page 51 of 105) shedding was detectable in urine in these studies. Furthermore, in recent clinical studies shedding could be observed up to 39 weeks in patients (confidential CAF, page 47 of 76). The SNIF should be corrected and updated accordingly.

SBB Comment

The expert's remark is supported and could be formulated towards the applicant as follows :

According to page 3/16 of the SNIF, "to date from all doses administered in VOI-04, VOI-04-LT and 20180053TRP studies no vector DNA was detectable in urine samples". However, according to the results reported in the CAF confidential document, page 46/76 and in section IV.3 of the IB (p51/105),

vector DNA was detectable in urine was quantifiable in dogs and rats from study VOI-04, study VOI-04-LT and GLP study 20180053TRP. The applicant is requested to update the SNIF document to align with the results reported both in the IB and the CAF confidential documents.

Additional SBB comment

The results from pre-clinical studies in dogs and rats, as reported in the CAF confidential document, page 46/76 and in section IV.3 of the IB (p51/105), refer only to shedding analyses performed on urine samples. It is unclear whether additional sample types were evaluated for shedding. The applicant is requested to clarify whether other samples were analysed and, if so, to provide the corresponding results. If no further analyses were conducted, a justification should be provided.

Coordinator comment:

I agree with these comments. Shedding is dependent on the dose used and the application route together with the serotype of the AAV used and the host species. I appreciate the efforts by the applicant. Still, I agree with SBB and the expert that all info available should be provided. Primarily results in patients are most relevant since biodistribution and shedding is species-dependent.

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.

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.

Additional SBB comment

There are no specific instructions for on-site transportation of the prepared clinical vector in the Study Staff Instruction. The applicant is requested to specify in relevant document such as the Study Staff Instruction and other documents such as SNIF or CAF that transport must take place in a double packaging with the outer packaging consisting of a leak-proof container.

Coordinator comment:

Agreed.

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.

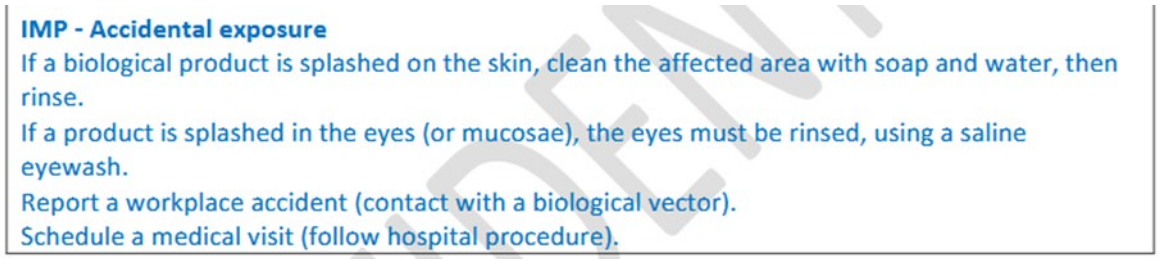
3.6. Measures to prevent dissemination into the environment (e.g. control measures, PPE, decontamination/cleaning measures after administration or in the case of accidental spilling, waste treatment, recommendation given to clinical trial subjects, …)

Comment 1
In the B_BE_26_BVW3_Part 2 _Confidential CAF_18Feb2026 document,
1)p53, it is written in point c) that personal protective equipment includes gloves, coats, and protective glasses.
In the procedures relating to accidental spills, we propose to keep overshoes in the composition of PPE in order to protect workers' footwear during decontamination of the floor, thus avoiding any biological contamination from the distinguishable spill or indistinguishable microspill on the floor or any chemical contact with the disinfectant used to treat the spill.

SBB comment:
The expert's remark is supported and could be formulated towards the applicant as follows :
In section 3.6.c of the CAF, in the procedures relating to accidental spills, the use of overshoes is not mentioned in the list of personal protective equipment (PPE) to be worn by site staff involved in the disposal of contaminated materials following spill clean-up. However, overshoes are important to protect footwear from potential biological contamination arising from visible spills or undetected microspills on the floor, as well as from contact with chemical disinfectants used during decontamination. Therefore, the applicant could be requested to add overshoes in the list of PPE in both CAF documents, the SNIF and the Study Staff Instruction document.

Coordinator comment:
Agreed.

2)p54, further in point c), it is written that:


IMP - Accidental exposure
If a biological product is splashed on the skin, clean the affected area with soap and water, then rinse.
If a product is splashed in the eyes (or mucosae), the eyes must be rinsed, using a saline eyewash.
Report a workplace accident (contact with a biological vector).
Schedule a medical visit (follow hospital procedure).

We could suggest replacing the generic term "soap" with "aseptic soap" in the sentence concerning the scenario of a biological product splashing onto the skin, as this is more appropriate for disinfection procedures.

SBB comment:
The expert's remark is supported and could be formulated towards the applicant as follows :
The first aid measure described in the event of a biological product being splashed on the skin is reported as : 'clean the affected area with soap and water, then rinse'. However, the use of an 'aseptic soap' is considered more appropriate for this type of decontamination procedure. Therefore, the applicant is requested to update the instructions accordingly in section 3.6.c of both CAF documents, section 2 of the Study Staff Instruction document and section J1 of the SNIF.

Coordinator comment:
Agreed.

We could also propose adding the case of "needle stick injury" to the list of accidental exposure scenarios, with the following first aid procedure:
Induce bleeding from the wound and wash the area with aseptic soap, then rinse with water.
This case of scenario could occur during the reconstitution steps of the solution for infusion or during the intravenous infusion act

SBB comment:
The expert's remark is supported and could be formulated towards the applicant as follows :
In the SNIF p15/16, section J1, and in the CAF section 3.6.c, p13/16, first aid measures have been reported in case of splash on the skin and in the eyes. Please complete this section by reporting first aid measures in case of inhalation and puncture or needle stick injury as already reported in the Study Staff Instruction document.

Coordinator comment:
Agreed.

Comment 2

No recommendation is given on the donation of blood/cells/tissues/organs by the clinical trial subject (confidential CAF (page 55 of 76) and CAF page 15 of 16)). 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'. The notifier should be 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. In case of revision such instruction should be included in all relevant documents (CAFs, SNIF, ICF etc.).

SBB comment:
The expert's comment is supported and could be sent to the applicant as is.

Coordinator comment:

I had a similar comment in my personal notes reading through the documents. Still, we should consider that for Duchenne patients, the donation of blood, tissues, or cells/organs is most probably not an option. Agreed to provide the comment to the applicant to be consistent with earlier dossiers.

The present Study staff instructions document contains presently no more than the info in the MATERIAL SAFETY DATA SHEET OF GNT0004). It should be recommended that all medical personnel involved in the study receive a concise overview (a 1–2 page instruction sheet) summarizing all relevant handling procedures, including detailed instructions in case of accidental spills, waste management requirements, and other risk management measures. Furthermore, a Study Staff Instructions Pharmacy Manual seems to be missing although detailed instructions are presented in the SNIF and the CAFs. These instruction s should be worked out in a separate Pharmacy Manual.

SBB comment:
The expert's remark is supported and could be formulated towards the applicant as follows :
The current Study Staff Instructions document does not provide additional information beyond what is already included in the Material Safety Data Sheet of GNT0004. The applicant is therefore requested to provide a concise and user-friendly overview in the form of a short document (1–2 pages) containing only the essential procedural information. This summary should facilitate practical use in clinical settings and should include key instructions on handling procedures including:
- 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, ingestion, inhalation, needle-stick injury or contact with skin and clothing
- procedures for treatment of accidental spill (disinfectant, concentration of disinfectant, contact time, procedure to be followed)
- 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
- on-site transportation

Coordinator comment:
Agreed, to be in line with earlier reports I support to include this remark.

Spill kits should be present in the pharmacy and the hospital room where the medical product will be administrated to the patient. With respect to procedures for treatment of accidental spills, disinfectant, concentration of disinfectant, contact time should be described in detail. E.g. chlorine bleach (Javel water) with 1% final active chlorine might be misunderstood (hypochlorite concentration in household bleach solutions (=" 10% bleach solution"?) varies by manufacturer). All decontamination procedures involving the use of sodium hypochlorite solution should therefore specify the precise mass concentration (6000 ppm (mg/l)) of sodium hypochlorite in the final freshly prepared solution.

SBB comment:
The expert's remark is supported and could be formulated towards the applicant as follows :
According to the SNIF, an appropriate spill kit will be available in the areas where GNT0004 is prepared (pharmacy) and administered (hospital rooms). Please update the Study Staff Instruction accordingly to include this information as well.

Regarding procedures for the treatment of accidental spills, the disinfectant, its concentration, and the required contact time should be described in detail. For example, the use of chlorine bleach ("Javel water") at 1% final active chlorine may be misinterpreted, as the hypochlorite content in household bleach solutions (often labelled as "10% bleach solution") varies by manufacturer. Therefore, all decontamination procedures involving sodium hypochlorite should specify the exact mass concentration in the freshly prepared solution, for instance, 6000 ppm (mg/L) of sodium hypochlorite.
Furthermore, to maintain chlorine strength and ensure bleach effectiveness, it is crucial to prepare the solution immediately prior to use as effectiveness is lost 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.

Coordinator comment:
Agreed.

Additional SBB comment:
In case of spill, it is recommended to first leave the area of spill for a minimum of 30 minutes to allow agents to settle down before initiating the spill procedure. Medical personnel should first remove possibly contaminated personal protective equipment (PPE) and then evacuate the area. A sign "DO NOT ENTER" should be posted on the door. Please improve section J1 of the SNIF (p15/16), section 3.6.c of both CAF documents and section 6 "Accidental release measures" of the Study Staff Instructions document by adding these steps before initiating the spill response procedure.

Coordinator comment:
I do personally not agree with this sentence. Volumes are minimal, and this sentence does not align for me with the next one. One cannot leave to settle the spill, while removing protective garment => this would result in possible spreading of the DP (rAAV).
In all, AAV is BSL1 level, not pathogentic and most healthy individuals have preexisting Abs.

SBB comment
This comment was introduced to ensure alignment with the procedure described in previous AAV dossiers. Given that the quantities released are very small, this initial step may not be necessary and could potentially be omitted.

Coordinator comment:
I agree to leave the first senstence out for clarity.

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
Has evaluated this item and has no questions/comments.

Comment 2
See 3,6. With correct recommendations, instruction documents and compliance to these recommendations and instructions the overall environmental risk will be negligible.

Coordinator comment:
Agreed.

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
Has no further questions/comments.

Additional SBB comment:
Since one additional Belgium site will be included in this trial, the applicant is requested to update all the documents for this Deliberate Release procedure by reporting this new site.

References
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Adviesraad voor Bioveiligheid
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Compilation of the expert's evaluations of the answers of GENETHON on the list of questions for dossier B/BE/26/BVW3

27 April 2026
Ref. SC/1510/BAC/2026_0416

Coordinator: Rik Gijsbers (KULeuven)
Experts: Anton Roebroek (KULeuven), Willy Zorzi (ULiège)
SBB: Sheela Onnockx

INTRODUCTION

Dossier **B/BE/26/BVW3** concerns a notification from GENETHON for the deliberate release in the environment of genetically modified organisms other than higher plants according to Chapter II of the Royal Decree of 21 February 2005.
The notification has been officially acknowledged on 04 April 2026 and concerns a clinical trial entitled "A phase I/II/III study with a dose determination part followed by an efficacy and safety evaluation, quadruple blind placebo-controlled part and then by a long-term safety follow up part, in ambulant boys with Duchenne Muscular Dystrophy". The investigational medicinal product is a recombinant AAV vector carrying human Micro-Dystrophin 1 (hMD1) expression cassette.

On 2 April 2026, based on a list of questions prepared by the BAC (SC/1510/BAC/2026_0371), 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 14 April 2026. This complementary information was reviewed by the coordinator and the experts in charge of the evaluation of this notification.

Evaluation Expert 1

According to me, the applicant did not sufficiently respond to all the questions in the list. Some changes and additions mentioned in the response letter I could not find in the updated documents.

Question 1-8, 10, 11 and 13: OK

Question 9: SNIF and CAF not updated with the restriction not to donate blood, tissue, cells etc.

SBB comment:
The expert's remark is supported and could be formulated as follows:
According to the sponsor's answers, the SNIF, the CAF and ICF will be updated. However, neither the SNIF nor the CAF have been revised as requested. The applicant is therefore requested to ensure these documents are updated accordingly by clearly stating that patients who receive treatment must not donate blood, organs, tissues, or cells for transplantation, to ensure consistency with the recommendations provided in the EPAR of EU registered medicinal products containing recombinant AAV. In addition, beyond updating the ICF, the applicant is requested to ensure that the study protocol is revised accordingly to reflect this requirement.

Coordinator comment:
I came to the same conclusion and align with the expert's remark.
I agree with the formulation proposed by the SBB. It is a pity that this results in additional administrative work.

Question 12: The updated Study Staff Instructions specify, that 1% active chlorine is equivalent to 10 500 ppm sodium hypochlorite at the second time 1% active chlorine is mentioned in this text. Why not at the first time or better both times. Preferably the specification of 1% active chlorine being equivalent to 10 500 ppm sodium hypochlorite should also be added in the SNIF and CAF whenever 1% active chlorine is mentioned.

SBB comment:
The expert's remark is supported and could be formulated as follows:
The updated Study Staff Instructions specify that 1% active chlorine is equivalent to 10,500 ppm sodium hypochlorite only at the second occurrence of the term. The applicant is requested to ensure consistency by including this specification at the first occurrence as well, or preferably at all occurrences within the document.

Furthermore, it is recommended that the equivalence of 1% active chlorine to 10,500 ppm sodium hypochlorite be consistently stated in the SNIF and CAF wherever 1% active chlorine is mentioned, to ensure clarity and harmonization across all study documentation.

Coordinator comment:
I had a similar remark.
I agree with the formulation proposed by the SBB.
Inactivation of rAAV is indicated in https://vnl.princeton.edu/safety-and-handling to be inactivated using 0.5%. "*To disinfect material exposed to AAV you can use 0.5% sodium hypochlorite, 2% glutaraldehyde, or autoclave for 30 minutes."* 1% is already an excess. Still, on stainless steal surfaces, this is corrosive. Maybe we can provide a uniform description that was added earlier to previous reports?

SBB comment:
The following remark and request could be added to the previous requirement:
Please be informed that according to the recommendation provided by Princeton University, 0.5% sodium hypochlorite can be used to disinfect material exposed to AAV (https://vnl.princeton.edu/safety-and-handling).
Furthermore, hypochlorite solution cannot be proposed as a universal decontaminant or disinfectant because it can corrode or damage stainless steel, aluminium and the most rubbers components of surfaces. A list of adequate of decontamination / disinfection solutions for areas that cannot be decontaminated with bleach is required.

Evaluation Expert 2

By this way, we would like to inform you that the notifier's responses do not yet correctly and satisfactorily address the comments/questions, particularly:

In p15-16 of the SNIF document, it is written that :
"*Soak with chlorine bleach (Javel water) 1% final active chlorine concentration or Virkon 1% (volume identical to the spilt volume).*
Leave in contact for 20 minutes.
Pick up all material, working from the outside in, destroy the contaminated objects by autoclaving followed by incineration or immersion in chlorine bleach (Javel water) with 1% final active chlorine for 20 minutes, then dispose of as infectious clinical waste."
The following sentence «Pick up all material, working from the outside in, destroy the contaminated objects by autoclaving... » could be misleading.

Indeed, as described previously, the absorbant paper or other absorbant material is used to cover the area of the spillage and the broken pieces of vial (if applicable) and this material can be associated to the «contaminated objects treated by autoclaving».

This material is soaked with chlorine bleach (1% final active chlorine concentration) or with Virkon 1%.

However, it is generally accepted that:
1)Virkon solution should not be autoclaved.
Autoclaving Virkon-impregnated materials is dangerous because the high temperatures can cause the disinfectant to decompose, potentially releasing toxic sulphur dioxide gas.
2)Autoclaving bleach-impregnated materials is dangerous and hazardous for several reasons:
-Toxic Fumes: The high heat of the autoclave causes bleach to release toxic chlorinated gases into the laboratory atmosphere, such as chloramines, which can irritate the eyes and respiratory system.
-Damage to Autoclave: bleach heating is highly corrosive and can damage the stainless steel chamber and internal components of the autoclave.

Consequently, we propose to modify the sentence as following : « Pick up all material, working from the outside in and then dispose of as infectious clinical waste.»

SBB comment:
The expert's remark is supported and could be formulated towards the applicant as proposed by the expert.

Coordinator comment:
Agreed. Additionally, have been looking into specific info on disinfectants for rAAV viral vectors for GT. Where enveloped vectors are easy to inactivate, rAAV is a true challenge. Eggers et al. (Expert Committee on Virus Disinfection of the German Association for the Control of Viral Diseases (DVV) https://pmc.ncbi.nlm.nih.gov/articles/PMC10675031/) summarizes the available methods for evaluating the virucidal activity of chemical disinfectants against genetically modified organisms (GMOs) using current European standards, including the activity against highly resistant parvoviridae such as the adeno-associated virus (AAV), and provides guidance on the selection of disinfectants for pharmaceutical manufacturers, laboratories, and clinical users. The correct products are not available according to this publication. Also elsewhere in literature, I could not find any scientific reference to the use of Virkon for inactivation of AAV. I would therefore also ask for supportive papers, or ask to omit the Virkon reference.

SBB comment:
The following request could be added to the previous requirement:
Recombinant adeno-associated virus (rAAV) is known to be highly resistant to inactivation compared to enveloped viruses. Therefore, AAVs represent a challenge for virucidal efficacy and only a limited number of disinfections are suitable and validated disinfectants against AAV (Eggers et al. 2023).
According to the Study Staff Instructions document, the CAF and SNIF, Virkon 1% has been proposed as an alternative to hypochlorite solution. Although, while Virkon has shown efficacy against other viruses (e.g., adenovirus), no scientific reference could be found supporting the use of Virkon for inactivation of AAV. The applicant is therefore request to provide supporting scientific references demonstrating the efficacy of Virkon against AAV, or to remove the Virkon from the list if such evidence is not available.

On p16 of the SNIF document, it is written that :
"In case of needle-stick injury do not bleed. Immediate cleaning of the injured skin area with soap and water and rinsing. Antisepsis with chlorinated derivative (Dakin® or chlorine bleach with 2.6% active chlorine diluted 1/5) or polyvidone iodine in dermal or defective solution, 70 ° alcohol (at least 5 min)."

Korten et al (2021) reported that:
"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."

Following this article, it is clearly stated that 70% ethanol or 70% isopropyl alcohol is not suitable for eliminating AVV in cases of laboratory surface disinfection, needle stick injury or contamination of intact skin.
Therefore, we propose to remove the following words from the sentence: « defective solution 70% alcohol ».

SBB comment:
If agreed, the following follow-up question could be posed to the applicant :
According to page 16 of the SNIF, in case of needle-stick, 70° alcohol has been proposed as antiseptic solution. However, as mentioned by Korte et al. (2021), 70% ethanol or 70% isopropyl alcohol is not suitable for eliminating rAAV in cases of laboratory surface disinfection, needle stick injury or contamination of intact skin. The applicant is therefore requested to remove 70 ° alcohol from the list of proposed antiseptic solution.

Coordinator comment:
Agreed

References:

Eggers M. et al (2023). Suitable Disinfectants with Proven Efficacy for Genetically Modified Viruses and Viral Vectors. Viruses. 2023 Oct 30;15(11):2179. doi: 10.3390/v15112179

Korte Joelle et al (2021). Inactivation of Adeno-Associated Viral Vectors by Oxidant-Based Disinfectants. Hum Gene Ther. 2021 Jul;32(13-14):771-781. doi: 10.1089/hum.2020.120. Epub 2020 Nov 6.

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Compilation of the expert's evaluations of the answers of GENETHON on the second list of questions for dossier B/BE/26/BVW3

18 May 2026
Ref. SC/1510/BAC/2026_0458

Coordinator: Rik Gijsbers (KULeuven)
Experts: Anton Roebroek (KULeuven), Willy Zorzi (ULiège)
SBB: Sheela Onnockx

INTRODUCTION

Dossier **B/BE/26/BVW3** concerns a notification from GENETHON for the deliberate release in the environment of genetically modified organisms other than higher plants according to Chapter II of the Royal Decree of 21 February 2005.
The notification has been officially acknowledged on 04 April 2026 and concerns a clinical trial entitled "A phase I/II/III study with a dose determination part followed by an efficacy and safety evaluation, quadruple blind placebo-controlled part and then by a long-term safety follow up part, in ambulant boys with Duchenne Muscular Dystrophy". The investigational medicinal product is a recombinant AAV vector carrying human Micro-Dystrophin 1 (hMD1) expression cassette.

On 27 April 2026, based on a second list of questions prepared by the BAC (SC/1510/BAC/2026_0415), 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 06 May 2026. This complementary information was reviewed by the coordinator and the experts in charge of the evaluation of this notification.

Evaluation Anton Roebroek

I am satisfied with the answers of the applicant on the second list of questions, except with the applicant's response on question 9. The requirement for patients not to donate does not end in my view at the end of the study timeframe. Unless proven to be no longer necessary, this restriction is a lifetime restriction. While I can live with the proposition to include the precaution that patients who receive treatment must not donate blood, organs, tissues, or cells for transplantation only in a next, updated protocol for future clinical studies, I can see no reason why this restriction could not be included in the ICF, meaning that both the researcher and the parents (responsible person(s)) are aware of this restriction and agree to it. Addition to include this requirement also into the ICF was requested according to the LOQ2, but apparently not incorporated into an updated ICF.

SBB comment:
According to applicant's answers to our first list of questions sent out on 02 April 2026: The ICF already contained wording in page 15 for blood and organs. It will be updated to include tissues and cells as well, and it will be submitted in CTIS with protocol version 9 amendment.

The following sentence can indeed be found in the ICF for Belgium: "*Als uw kind gentherapie krijgt, is het niet meer mogelijk om bloed of organen te doneren*".
To make sure, the ICF for Belgium is updated accordingly at the next amendment, the following condition could be added in the Advice:
As committed by the applicant in his response of 14 April 2026 to our first list of questions, it will be clearly indicated in the ICF that patients who receive treatment must not donate blood, organs, tissues, or cells for transplantation in order to be consistent with recommendation provided in the EPAR of EU registered medicinal products containing recombinant AAV.

Coordinator:
I agree with the expert, and align with the reply of SBB.
I highlighted in grey what can be added to the advice

Evaluation Willy Zorzi

Regarding requirement 3: I would like to correct a requirement sent to the notifier regarding the lack of references proving the effectiveness of Virkon S on rAAV, as here is a reference that proves this product is effective:

Comparative Medicine
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Pages 215–221

Overview

Viral Vector Biosafety in Laboratory Animal Research

Dalis E Collins,[1] Jon D Reuter,[2] Howard G Rush,[3] and Jason S Villano[3,*]

were recoverable by cell culture for 3 and 14 d respectively.[44] In an evaluation of several disinfectants for in vitro efficacy against viral vectors (lentiviral, adenoviral, and AAV), only Virkon S (Dupont, Wilmington, DE) demonstrated robust surface disinfection and minimal aversion, making it preferable for use on surfaces that rodents contact.[8] For many studies, sanitation through a cage

SBB comment:
According to this article, Virkon S does indeed seem to be effective against the AAV viral vector in laboratory animal research. Furthermore, as shown by Korte et al (2021), 0.45% potassium peroxymonosulfate and 0.5% sodium hypochlorite represent suitable and broadly effective disinfectants for AAV inactivation. This information will be included in the advice.

Comment coordinator:
Collins et al does not show data, but merely reports a statement and refer to https://pmc.ncbi.nlm.nih.gov/articles/PMC4783637/pdf/jaalas2016000175.pdf. In my opinion this statement is not supported by any data. In the latter paper refers to AAV and indicates that the AAV vectors are in the end excluded from the experiments performed. In this paper Disinfectant D was Virkon-S (DuPont, Wilmington DE), a 21.4% potassium peroxymonosulfate soluble concentrate diluted to 1% in tap water.

water and then cleaned with unscented soap (Seventh Generation, Burlington, VT), followed by another thorough rinsing. Disinfectants A through D were tested, all prepared as described above. Tap water was used as a negative control. This test was done with each of the 3 mouse lines listed earlier.

Results

In vitro efficacy testing. When each of the disinfectants was diluted in CCM at a ratio of 1:1000 and exposed to HEK-293T cells, none of the diluted disinfectants had negative effects on

Vol 55, No 2
Journal of the American Association for Laboratory Animal Science
March 2016

cell morphology or survival as detected using cell counting and flow cytometry. Therefore, after the viral vectors were exposed to disinfectants, the resultant mixture could be effectively neutralized by employing a 1:1000 dilution before cell exposure, without leading to disinfectant-induced cell toxicity. In contrast, when disinfectants were diluted at a 1:100 ratio and exposed to cells, results were more variable between disinfectants, with indication of decreased cell survival. In the absence of disinfectants, viral titers were still sufficiently high at a 1:1000 dilution of lentivirus and adenoviral vectors to transduce GFP into HEK-293T cells. This dilution factor was therefore used for all disinfection assays, with the exception of the dried adenovirus on a nonporous surface, where a slight variation in the protocol required a maximum dilution of 1:300. In addition, the initial titer of adeno-associated viral vector was too low to yield measurable numbers of GFP-expressing cells after a 1:1000 dilution, therefore this vector was excluded from the study.

Efficacy of disinfectants against lentiviral and adenoviral vectors in suspension. When lentiviral vectors in suspension were

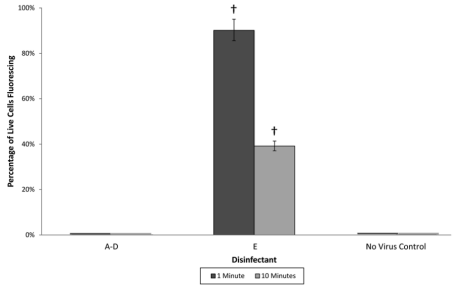

Figure 2. Percentage of live HEK-293T cells expressing GFP after incubation with adenoviral vector that had undergone exposure to disinfectants A through E for contact times of either 1 or 10 min. Percentages for exposures to disinfectants A through D were negligible and equivalent to the GFP expression of cells unexposed to virus. Data are expressed as means ± SE; †, value significantly ($P < 0.01$) different from baseline fluorescence of cells unexposed to virus.

And in the conclusion

Vol 55, No 2
Journal of the American Association for Laboratory Animal Science
March 2016

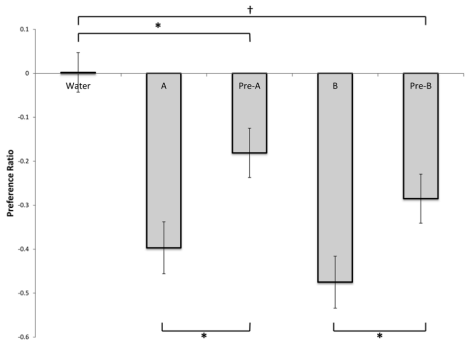

Figure 8. Preference scores for innate aversion testing of C57Bl/6 mice with disinfectants A and B, with either no prior exposure (A, B) or a history of previous exposure (Pre-A, Pre-B). Scores were calculated by using the following equation: (time on the agent side – time on the tap-water side) / (time on the agent side + time on the tap-water side). A

factors such as desiccation. The adenoviral vector, a partially lipophilic nonenveloped virus, was difficult to eliminate, but a hydrophilic nonenveloped pathogen, such as a parvovirus, is even less susceptible to disinfectants.[31] The GFP-expressing adeno-associated viral vector is an example of a parvovirus that may be available for testing by using the described methods, but the titer available for this study was insufficient to allow its use. This finding brings to light another important factor—the amount of pathogen being disinfected. As the number and diversity of pathogens increases, the susceptibility to disinfection decreases, due to synergistic effects, such as aggregation and the formation of biofilms, which greatly increase the amount and complexity of the organic load that the disinfectant must overcome.[58,59,63,67,90] Lastly, recent findings suggest that there may be differences in susceptibility among viruses of similar envelope structure when disinfectants that do not specifically damage the viral genome, including many oxidizing agents, are used[89] and some pathogens may adapt to become less sensitive to disinfectants through repeated exposure.[60]

Korte et al. indeed show effectiveness of a 0.45% potassium peroxymonosulfate solution, yet, in the abstract (I do not have access to the full paper to look at the data), it is indicated that *"While sodium hypochlorite, potassium peroxymonosulfate, and PAA successfully inactivated AAV2 after 1, 5, and 30 min, respectively, ethanol and hydrogen peroxide did not show significant effects on AAV2 even after exposure for 30 min. For AAV5, only sodium hypochlorite and potassium peroxymonosulfate proved efficient capsid and genome denaturation after incubation for 1 and 30 min, respectively."* This sentence indicates that there are substantial differences in the inactivation potential for different AAV serotypes *=> potassium peroxymonosulfate inactivated AAV2 after 5 min, and for AAV5, potassium*

peroxymonosulfate proved efficient capsid and genome denaturation after incubation for 30 min. To me this means that Virkon solution should cover the AAV5 for 30'.

In conclusion, I would propose to not come back to the reply of the applicant, and I agree with the statement made by the applicant, "*Given the lack of scientific references demonstrating the efficacy of Virkon® against recombinant AAV, the applicant has, as requested, removed Virkon® from the list of decontamination/disinfection solutions. The documentation has been updated to include alternative, scientifically supported decontamination agents (see Question 3).*"

References:

Dalis E Collins et al. 2017. Viral Vector Biosafety in Laboratory Animal Research. Comp Med. 2017 Jun 1;67(3):215-221.

Korte Joelle et al (2021). Inactivation of Adeno-Associated Viral Vectors by Oxidant-Based Disinfectants. Hum Gene Ther. 2021 Jul;32(13-14):771-781. doi: 10.1089/hum.2020.120. Epub 2020 Nov 6.