Adviesraad voor Bioveiligheid
Conseil consultatif de Biosécurité

Advice of the Belgian Biosafety Advisory Council on the notification B/BE/25/BVW5 of the company AskBio for deliberate release in the environment of genetically modified organisms other than higher plants for research and development

Final version : 13/10/2025
Ref. SC/1510/BAC/2025_1211

Context

The notification B/BE/25/BVW5 has been submitted by AskBio to the Belgian Competent Authority in June 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 : *"A phase 2, adaptive, double-blinded, placebo controlled, randomized, multi-center trial to evaluate the efficacy, safety and tolerability of intracoronary infusion of AB-1002 in adult subjects with New York Heart Association (NYHA) Class III heart failure and non-ischemic cardiomyopathy".*

Congestive heart failure (CHF) is a major cause of morbidity and mortality throughout the world. Abnormal calcium handling plays a central role in the pathophysiology of a failing heart. Abnormal calcium handling is central to heart failure, with the SERCA2a-phospholamban (PLN) complex being key to maintaining calcium homeostasis in cardiomyocytes. Decreased SERCA2a expression and activity are linked to heart failure, and Inhibitor-1 (I-1) plays a regulatory role by enhancing SERCA2a activity. In failing hearts, levels of I-1 mRNA, protein are significantly reduced, contributing to impaired calcium regulation. Gene transfer of the constitutively active form of I-1 (I-1c) has been shown in several studies to have promising beneficial effects in alleviating HF in experimental rodent and porcine animal models.

The primary objective of this phase II study is to assess the efficacy, safety and tolerability of a single ntracoronary infusion of AB-1002 in adult subjects with New York Heart Association (NYHA) Class III heart failure and non-ischemic cardiomyopathy.
The active substance of AB-1002 consists of a chimeric cardiotropic AAV2/AAV8 vector capsid (AAV2i8) and a constitutively active form of protein phosphatase 1 Inhibitor 1 (I-1c) cDNA.

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 150 subjects with non-ischemic NYHA Class III heart failure will be included in this Phase II study, wherefore, five are expected in Belgium. AB-1002 will be administered at two different dose levels (low and high doses) as a single antegrade intracoronary artery infusion. A third group of subjects

will receive placebo. This study will be conducted at four clinical sites located in Flanders. The national territory is considered as the potential release area of AB-1002.

The dossier has been officially acknowledged by the Competent Authority on 11 July 2025 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 14 August 2025, 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 25 August 2025 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 04 September 2025. The answers of the notifier were received on 11 September 2025 and reviewed by the coordinator, after which a third list of questions was transmitted to the notifier on 18 September. The answers of the notifier were received on 06 October 2025, 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 three reactions from the public that did not require any comment.

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.
The AB-1002 drug substance is produced in a controlled environment, by the triple plasmid DNA transfection of the transgene, Rep/Cap and helper plasmids into human embryonic kidney (HEK) 293

cells. Given the high vector dose administered in this clinical trial, and considering that the transgene plasmid contains the ampicillin resistance (AmpR) gene, the applicant was asked to provide further details on the monitoring of bacterial backbone sequences in the vector production batches. As confirmed by the applicant, to maintain the integrity and safety of the clinical vector lots, each batch produced undergoes a specialized quantitative analysis.

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

Information related to the molecular characteristics of AB-1002 were adequately described in the dossier.

3. The conditions of the release

This phase II study will consist of three treatment groups (low or high dose or placebo). The GMO will be administered via antegrade intracoronary artery infusion, in hospital centres. After administration, subjects will stay at the study site for 18 to 24 hours, for safety and cardiac monitoring. 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 AB-1002 administration.

As no viral shedding analysis has been performed during the Phase 1 dose escalation gene therapy trial (NAN-CS101), samples including stool, semen (both depending on biological material availability), blood, urine, and saliva will be collected at 18-24 hours post dose, Day 4, Weeks 1, 2, 4, 8, 12, 24, 36 and 52. Additionally regarding biodistribution, participants will be asked to provide an optional right ventricular biopsy from the 24-week visit and/or donate heart tissue on the occasion of any heart procedure from week 4 to examine the levels of vector and insert mRNA.

Shedding data collected from the study will further contribute to a proper environmental risk evaluation. These shedding data will need to be evaluated in light of the observed quantity of shed viral vector material, and the period during which shedding is observed. Should the PCR analysis reveal detectable presence of vector genome, it will be important to determine whether the observed shed viral vector genome consists out of functional replication-deficient viral vector particles and to adapt the precautionary measures to be applied by the patients accordingly.

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

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 or other resistence genes. Like the wild-type AAVvirus, an AAV vector it 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 AB-1002 and wild-type AAV in case a triple infection by AB-1002, 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 would be too large in size 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.

All fertile women and men participating in the study, as well as their partners, must agree to use effective contraception for up to 6 months after drug administration. In addition, male participants must refrain from donating sperm for 6 months after receiving the product. As a precautionary measure, due to limited data on AAV2 and the fact that shedding analysis results will only become available 52 weeks post-treatment, the sponsor has agreed to extend both the contraception requirement and the sperm donation restriction to 12 months. This change will be implemented in the next protocol amendment.

Patients will also be prohibited from donating blood, tissues or cells. No specific timeframe for this restriction has been defined, also as precaution due to insufficient data on AAV2 and in alignment with guidance provided in the EU SmPCs of certain approved AAV-based gene therapies.

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 AB-1002 and the assumed lower amounts of shed and intact viral particles of AB-1002 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

To ensure clear and consistent guidance for healthcare personnel, the procedure for managing accidental spills or breakages has been aligned across all relevant documentation as follow: In the event of a spill, gloves and protective eyewear should be worn. The spilled solution should be contained and absorbed using an appropriate absorbent material. Once the spill has been covered by absorbent material, disinfectant shown to inactivate the GMO should carefully be poured over the absorbent material starting from the edge to the center. An appropriate contact time for the disinfectant to inactivate the GMO is required before removing all contaminated disposables into a suitable container. After removing the absorbent paper soaked in disinfectant, the area must be rinsed with water and dried.

While AAV particles are stable outside host organisms, replication of AAV cannot occur outside of a host cell. It is anticipated that AB-1002, like any other rAAV, is stable in a wide pH range (3-9) and like other non-enveloped viruses, is quite resistant to alcohol disinfectants. AAV is readily inactivated by disinfectants such as 1% sodium hypochlorite, 0.25% sodium dodecyl sulphate. As glutaraldehyde is

strictly restricted due to its toxicity and associated health risks for handlers, it has been removed from the list of proposed disinfectants, following BAC's request.
Thawing of frozen vials, dilution and preparation of syringes with the diluted product will be carried out in a vertical laminar flow biosafety cabinet.

Since propagation of AB-1002 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 expert, the Biosafety Advisory Council concludes that it is unlikely that AB-1002 developed to treat patients with NYHA Class III heart failure and non-ischemic cardiomyopathy, by means of endogenous production of Inhibitor-1c 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
    - AB-1002_CAF_AAV_Non Confidential_BE_25Aug25
    - AB-1002_CAF_AAV_Confidential
    - AB-1002_Safety Instructions for staff_v2_25Aug25
    - ASK-CHF2-CS201_GenePHIT_Patient Information Sheet_Viral Shedding Best Practices_v1.0_22Aug25

- As commited by the applicant, some documents still need to be updated as follows in the next amendment opportunity:
    - The protocol and all related documents will be updated in order to extent the contraception duration and restrictions on sperm donation to a period of up to 1 year post-treatment
    - IB (Investigator Brochure) : the dosing information, specifically whether the dose of 1E13 vg or 1E14 vg is per kg or per animal, will be clearly indicate throughout the entire document
    - IB : Biodistribution results from animals studies reported in section 4.4.1.3 of the IB appear to contradict data presented in section 4.3.2, which refers to systemic biodistribution studies of pre-clinical models from Asokan et al. (2010) suggesting that AAV2i8 is not expected to be distributed throughout the body in significant amounts. The applicant will update the relevant sections to ensure consistency and accuracy across the documentation
    - IB : As vector biodistribution is highly species-specific and differences in serotype and vector design strictly limit the extrapolation of preclinical data to humans, the applicant will avoid speculating in section 4.4.5 (p33/58) about the absence of persistence of vector DNA signal in the patients

- IB : in secion 6.6, “pregndoant” will be corrected into “pregnant”
- Protocol : in section 2.3.1 (p25/97), the following sentence “It is possible that AB-1002 could interact with other viruses with which the subject comes in contact, forming a new virus that could produce new side effects. However, this is unlikely to occur.” Will be corrected in a more appropriate phrasing such as : “The potential formation of a new virus via the interaction between AB-1002 and another exogenous or endogenous virus coming into contact with the patient, has the potential to provoke new side effects; however, this is highly unlikely to occur.”
- ICF_NL : on page 11/38, “een virus wordt gebruikt als vector” will be corrected into “een virale vector wordt gebruikt om genetische info over te brengen” and “genvirusproduct” will be changed to “AAV vector” or “viral vector”
- ICF_FR : on page 12/39, “une thérapie génique utilisant un virus comme vecteur” will be corrected into “un vecteur viral est utilisé pour transférer des informations génétiques” and on page 15/39, “Le produit viral du gène” will be changed to “Le vecteur AAV” ou « Le vecteur viral ».

- 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 AB-1002.

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/BVW5 (ref. SC/1510/BAC/2025_0978, SC/1510/BAC/2025_1048 and SC/1510/BAC/2025_1096

Adviesraad voor Bioveiligheid
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Compilation of comments of experts in charge of evaluating the dossier B/BE/25/BVW5
And comments submitted to the notifier

14 August 2025
Ref. SC/1510/BAC/2025_0978

Mandate for the Group of Experts: Mandate of the Biosafety Advisory Council (BAC) of 24 June 2025.
Coordinator: Karen Willard-Gallo (Jules Bordet Institute, ULB)
Experts: Rik Gijsbers (KULeuven), Liliane Tenenbaum (Lausanne University Hospital), Amaya Leunda Casi (SBB)
SBB: Sheela Onnockx

INTRODUCTION

Dossier **B/BE/25/BVW5** concerns a notification from AskBio 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 11 July 2025 and concerns a clinical trial entitled "Phase 2, adaptive, double-blinded, placebo-controlled, randomized, multi-center trial to evaluate the efficacy, safety and tolerability of intracoronary infusion of AB-1002 in adult subjects with New York Heart Association (NYHA) Class III heart failure and non-ischemic cardiomyopathy". The investigational medicinal product is a non-replicating, recombinant adeno-associated virus serotype 2 and 8 carrying the human Inhibitor-1 of protein phosphatase-1.

♦ 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 14-08-2025 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 transgene plasmid carries the amp resistance gene

The ampicillin resistance gene has been shown to contain an aberrant rescue site (Zhang et al., 2023; DOI: 10.1002/jmv.28433) enhancing illegitimate encapsidation of portions of the bacterial backbone sequences in a significant proportion (0.5 to 13 %) of the viral particles (Chadeuf,G., et al.. 2005 Mol. Ther., 12, 744–753).

Due to the presence of ITRs which have a promoter/enhancer activity, these sequences can be transcribed and translated (Taylor et al, 2024: doi.org/10.1016/j.omtm.2024.101295, Brimble et al., 2023: doi.org/10.1016/j.ymthe.2023.07.025).

Since high doses of AAV are used in this clinical trial, ampicillin resistance gene fragments or even the entire gene are expected to be contaminating the viral badges.

Although, transfer of the ampicillin resistance gene to the environment following accidental spillage or shedding has never been reported, it is important to document the percentage of illegimate encapsidation of backbone sequences. Did the applicant measure the percentage of such contaminants in their vector clinical badge?

SBB's comment:

The evaluation of the purity of the viral batches, such as a characterization of contaminating ampicillin resistance gene, are relevant for the quality assessment of the drug product but goes beyond the environmental risk assessment of AB-1002. From the perspective of the environmental risk assessment, non-target individuals (e.g. accidental exposure of heath care professionals at clinical trial site; exposure of close contacts because of shedding) will be exposed to much lower amounts of the drug product compared to the clinical dose.

Coordinator's comment:

I think she has a legitimate observation and it would be interesting to know if they did these measurements.

Comment 2

Has evaluated this item and has no questions/comments.

Comment 3

Has not evaluated this item

2.2. Demonstration of absence of formation of replication-competent virus

(e.g. assessment of risk of generation of replication competent AAV, test methods and test data, ….)

Comment 1
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.

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 not evaluated this item

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 not evaluated this item

2.5. Description of the insert

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

Comment 1
Has not evaluated this item

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

Comment 3

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

- It is not expected to be distributed throughout the body in significant amounts, based on systemic biodistribution studies that were completed in previous preclinical models (Asokan et al, 2010), as indicated p26/58 in B_BE_25_BVW5_Investigator s Brochure 10.0_04 Nov 2024.pdf. Still vector distribution is very species specific (on top different serotype and scAAV instead of ssDNA was used), and extrapolation to human would not be correct. Even though animal studies can show proof of concept for the genetic payload and reversal of phenotype, biodistribution info is not translatable. What evidence from the Ph1 trial is available that AB-1002 is not systemically administered upon human application, and is instead remaining present only locally? If not available, will this be assessed (biopt for other tissues?).

SBB comment:
According to page 26/58 of the Investigator's Brochure (IB), based on preclinical biodistribution studies, the administered viral vector is not expected to distribute systemically in significant amounts (Asokan et al, 2010).

Vector biodistribution is highly species-specific, and differences in serotype and vector design (e.g., scAAV vs. ssAAV) limit extrapolation of the animal data to humans. While preclinical models support proof of concept and therapeutic effect, they do not reliably predict human biodistribution. Furthermore, the administration via an antegrade catheter into the coronary artery of the patient guarantees there will be significant systemic distribution. Therefore, the applicant is requested to clarify whether data from the AB-1002 Phase I trial (NAN-CS101) is available demonstrating the distribution of AB-1002 following injection (both short and long-term). If direct biodistribution data are not currently available, what are the applicant's plans to determine this, such as the analysis of blood, urine, stool and if ethically possible tissue biopsies from non-target organs downstream of the catheter?

In the preclinical studies mentioned on page 30/58 of the IB, the applicant could be requested to clarify what was the threshold of detection? Was data collected from the Phase I trial (NAN-CS101) on AAV vector clearance from the blood post-injection from patients?

- In the few patients treated in Ph1, is any info available on clearence in the blood? It is not clear whether the dose indicated here is per kg, or per animal, and whether this is in the same ballpark as used in the clinical trial (or did I miss this info – please add info as done p33/58). Detail: the technology used is also not clear (ddPCR, or standard qPCR?)

SBB comment:
The first point of this question has been included in the proposed question here above.

The second point could be reported as a “Typos and other errors/omissions”: It is important that this is clarified: “the technology used is ddPCR or standard qPCR?”

As mentioned in section 4.4.1.3, page 30/58 of the IB, animals are dosed with two different vg concentrations. However, it is not specified whether the dose is per kg or per animal (one assumes the latter). The applicant is requested to update information throughout all documents for precision. Further, is there expected to be a differential effect based on body size if administration is a single set dose between patients that could range between ~60-120 kg?

According to page 30/58 of the IB, qPCR analysis was performed to detect AB-1002 clearance from the blood of all dosed animals at Day 5. Were any subsequent blood samples analyzed in the study?

- Results from animal studies reported in figure 9 on page p31/58 of the IB are contradictory to what was claimed p26/58.

SBB comment:
Stated in the Biodistribution section 4.3.2 of the IB, AB-1002 is not expected to be distributed throughout the body in significant amounts, based on systemic biodistribution studies that were completed in previous preclinical models (Asokan et al, 2010). Contradictory statements have indeed been formulated in the IB and the applicant is requested to correct all information where applicable for consistency and accuracy throughout the document.

- The applicant indicates p32/58 that, the risk of inadvertent germ line transmission is to be considered unlikely based on published data. Please provide a reference. To my knowledge this info is not available, especially not for this specific serotype and the dose envisioned. This should be framed differently. It is not correct to extrapolate data from other serotypes, exp in other species and doses. Each new serotype should be scrutinized and assessed in sufficient detail.

SBB comment:
The applicant is requested to provide specific references that support this statement? To our knowledge, such data are not publicly available - particularly for the serotype AAV2i8, at the intended clinical dose. Extrapolation from other serotypes, species, or dosing regimens is not scientifically robust. Each serotype, particularly when used at novel high doses, should undergo a thorough, case-specific evaluation of potential germline and developmental risks. The applicant is requested to address this aspect convincingly.

- The report reads “These doses are representative of the planned Phase 2 clinical doses, suggesting there will be no persistence of vector DNA signal in the clinical trial population as well.”. To me this is overinterpretation (see also comment made earlier). Assessment should be done. Instead, considering the disease to be treated here, I would dare to argue that the patient population in the HF clinical trials is in general at low risk for reproduction due to advanced age and decreased libido and frequency of sexual activity, as well as erectile dysfunction (as indicated, I would use this argument rather than claim there is no chance of shedding).

SBB comment:
Since vector biodistribution is highly species-specific and differences in serotype and vector design strictly limit the extrapolation of these preclinical data to humans, the applicant is asked not to speculate but to state clearly the findings as either pre-clinical studies or data from the AB-1002 Phase I trial (NAN-

CS101) with references to AB-1002 vector DNA persistence in humans based solely on results from the latter.

- Considering the age of some of the patients, it is key that family members taking care of them and healthcare providers will be informed well and will have all required PPE available to them, with clear guidelines on how to dispose and clean potentially contaminated materials.

SBB comment:
During the validation period of the dossier, the notifier commits to providing a patient information sheet that would explain and summarize all of the critical information and instructions for patients and their families as a safeguard against potential vector transmission to other people or release into the environment once patients leave the hospital setting. This document has not been received from the applicant, this is a firm request to provide a succinct take home summary (preferably a one or two page front and back plasticized document) that is readily available for patients and their families to consult for any information and instructions that they will need, written in language understandable by a layperson. At a minimum, the following information (with their duration) should be stated in the patient information sheet:

- Which bodily fluids are anticipated to contain viral vector genomes (even if at very low levels)
- Instructions on how good hygiene should be performed
- Guidance that will help to limit contact with the materials or surfaces that are most frequently contaminated with bodily fluids (e.g. use disposable tissues instead of cotton handkerchiefs, keep disinfectant wipes at hand in the toilet and bathroom, separate towels for the patient, etc…)
- A list of effective commercially available products that they can use to decontaminate contaminated surfaces, tissues, skin, …
- An absolute restriction on the donation of blood, organs, tissue and/or cells for transplantation
- A strict obligation to use contraceptives

Comment 3
Data on biodistribution and shedding of AB-1002 in humans are not available yet. The presence of the GMO will be sought in different biological fluids (semen, blood, urine, and saliva) and stool from patients in the framework of this study.
Available data are from pre-clinical studies on animals. On this basis and because the GMO administration will be performed by antegrade intracoronary artery infusion, transduction is expected to be mostly limited to cardiac cells.

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 not evaluated this item.

Comment 3
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 not evaluated this item.

Comment 3
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.

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
Has evaluated this item and has no questions/comments.

Comment 2
Has not evaluated this item.

Comment 3
Be coherent in the different given instructions.
About spill management
Investigator brochure p50 :
Accidental spill of the Gene therapy vector should be handled as follows:
• Gloves and protective eyewear should be worn. And a gown
• Use an absorbent material to contain/pick up the spilled solution.
• Once the absorbent is spent, place all contaminated disposables into a suitable container, seal, label and dispose as a biohazard material. The disinfectant should be poured on absorbent paper and surrounding surfaces.
• Wash spill site with freshly prepared with bleach (1:10 dilution of household bleach 5000 ppm of sodium hypochlorite) after material pickup is complete

CAF non-conf p16:
Accidental spillage of liquid samples must be contained using absorbent paper soaked in a suitable solution (to be selected according to the type of surface/material):
• Cover any area of spillage with absorbent paper or other absorbent material
• Soak with e.g., chlorine bleach 1% final active chlorine concentration or another suitable broad-spectrum disinfectant.
• Leave in contact for 20 minutes

Recommendation :
Cover the area of spillage with absorbent paper or other absorbent material and soak with e.g., 10% bleach (0.53% or ~5000 PPM sodium hypochlorite solution) with a minimum contact time of at least 20 minutes. The area should then be thoroughly rinsed with water and dried before cleaning with an appropriate cleaning solution. Disposable materials used in spill management should be discarded into a biohazard waste container.

SBB's comment:
It is important to provide clear and concise instructions to the health care personnel. The instructions provided in the Investigator brochure p50/58 and the CAF non-confidential document page 16 are not identical. The notifier is therefore requested to adapt these procedures throughout all pertinent documents so that it is clear what to do in the case of accidental spill or breakage. According to the Laboratory biosafety manual (Fourth edition) of the World Health Organization, in case of a potentially hazardous spill, all persons should immediately vacate the affected area and any exposed persons should be referred for medical evaluation. The room containing the spill should not be entered for a length of time that allows aerosols to be carried away and heavier particles to settle (a minimum of 30 minutes is generally necessary). Signs should be posted immediately to indicate that entry is forbidden. After the appropriate time, decontamination should proceed. Appropriate protective clothing and respiratory protection, such as a clean lab coat, gloves, glasses and a mask, should be worn during the decontamination procedure. The spill should be covered with towels and other absorbent material starting from the edge toward the centre. The appropriate disinfectant shown to inactivate the GMO (e.g., 10% bleach (0.53% or ~5000 PPM sodium hypochlorite solution) should carefully be poured over the absorbent material starting from the edge to the centre. An appropriate contact time (minimum 20 minutes) for the disinfectant to inactivate the GMO is required. Once the spill is treated, the absorbent towels and broken vials should be removed with tongs or forceps and discarded in a biohazard waste container. The PPE should be discarded in the biohazard bag. And lab coat and mask should be decontaminated before being handled further, although it is highly recommended that disposal protective clothing be worn and disposed of in the biohazard waste container. The medical staff must report the incident to the authority in charge and responsible at the site.

About staff safety during GMO manipulations
In the document "safety instructions for staff", Table 1, it's indicated that a horizontal laminar flow can be used to manipulate AB-1002. This type of laminar flow is only intended to protect the product from contaminations and do not protect personnel and environment form an exposure to the manipulated the product. The use of horizontal flow is thus forbidden.
It's recommended to remove it from the proposed protective measure or PEC.

SBB's comment:
This comment and the comment below could be combined as follow:

In the document “Safety Instructions for Staff” :

- Table 1 indicates that a horizontal laminar flow cabinet may be used for handling AB-1002. However, this class of laminar flow is designed to solely protect the product from contamination and does not provide protection for personnel or the environment. The use of a horizontal laminar flow cabinet is therefore inappropriate and should be prohibited. The use of “horizontal laminar flow cabinet” should be replaced with “vertical laminar flow cabinet” in the list of proposed protective measures (PECs) and any other pertinent documents to ensure that the best biosafety practices are applied.
- Table 2 indicates that 2% glutaraldehyde could be used as disinfectant for managing accidental spills. However, as this solution is strictly restricted due to its toxicity and associated health risks for handlers, glutaraldehyde should be removed from the list of proposed disinfectants, with all safe and equally effective alternatives listed.

Minor remark: in the same document, glutaraldehyde is proposed to manage an accidental spill (among other disinfectants). However the use of this substance is strictly restricted as it is recognised as a toxic molecule for users. It’s recommended to remove it from the proposed disinfectants.

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 point 2.1

Comment 2

- In B_BE_25_BVW5_Clinical Trial Protocol_V6_0.pdf p25/97 the applicant indicates “It is possible that AB-1002 could interact with other viruses with which the subject comes in contact, forming a new virus that could produce new side effects. However, this is unlikely to occur.” This wording is strange to me. I would advise to rephrase into “If AB-1002 would interact with other viruses with which the subject comes in contact, forming a new virus that could produce is unlikely to occur.” Same goes fot the txt in translation.

SBB comment:
This comment could be reported as a “Typos and other errors/omissions”:
In the Clinical Trial Protocol_V6_0.pdf p25/97, the applicant states: “*It is possible that AB-1002 could interact with other viruses with which the subject comes into contact, forming a new virus that could produce new side effects. However, this is unlikely to occur*.” This wording is somewhat unclear. A clearer and more appropriate phrasing could be:
“The potential formation of a new virus via the interaction between AB-1002 and another exogenous or endogenous virus coming into contact with the patient, has the potential to provoke new side effects; however, this is highly unlikely to occur.”

- In B_BE_25_BVW5_Environmental Risk Assessment_final.pdf (p2/12) the applicant provides info on an ongoing Ph1 trial in the US. The Ph2 study planned now, will specifically study shedding. saliva, feces, blood, semen and urine samples will be collected on day 1 following administration of AB-1002, day 4, then at weeks 1, 2, 4, 8, and 12, then months 6, 9 and 12. The dose applied here (>10e14 in the heart) seems very high compared to other trials. It is not clear how these doses are determined

(qPCR or ddPCR), and this should be better indicated, for example by indicating which method is considered (see Table 2 p 19/58 in B_BE_25_BVW5_Investigator s Brochure 10.0_04 Nov 2024.pdf).

SBB comment:

This comment could be reported as a "Typos and other errors/omissions":
In the Environmental Risk Assessment_final.pdf, page 6/12, the applicant provides information on the doses to be used in this trial, without specifying the method used for the dose determination (qPCR or ddPCR). To ensure completeness and clarity, the applicant is requested to update the ERA with explicit information regarding the method used to determine these doses. If both qPCR and ddPCR are used in different instances then this must be clearly stated.

- On p14/38 in B_BE_25_BVW5_ICF Core_NL_BE_Core_public.pdf it reads "Het anticonceptiemiddel moet worden gebruikt tot 6 maanden na toediening van het studiegeneesmiddel. Uw onderzoeker zal met u de verschillende doeltreffende voorbehoedsmiddelen bespreken." This should be amended with 'until at least two consecutive measurements are found to be negative'. Same for sperm donation, 'tot 6 maanden indien negative testen', otherwise this should go beyond 6 months.

SBB comment:

According to the inclusion criterion 7 of the protocol and the ICF (p15/38), male patients ~~capable of fathering a child~~ must agree to use barrier methods and not donate sperm for 6 months after receiving the investigational product. According to page 12/38 of the ICF, patients should refrain from donating blood, cells or tissues; although, no specific duration for this restriction is provided (but should be).
The applicant is requested to

- Clarify the rationale behind the six-month time frame for restricting contraception and sperm donation.
- Specify the duration on the prohibition of donating blood tissues, or cells in all relevant study documents.

Since this investigational drug is still under investigation and shedding data are not yet available, the applicant is requested to adapt the duration of restrictions to "until at least two consecutive shedding measurements are found to be negative".

Coordinator's comment:

It should not matter if they can father a child or not, if there is sexual contact they could potentially contaminate their partner.
And female patients? Equally they need to protect their partner. It has been shown that HIV can be transmitted from females to males through intercourse.

Comment 3
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 other questions/comments.

Comment 2

- In the txt the applicant regularly refers to ‘virus’ when ‘viral vector’, or ‘infecting, infection’ when ‘transducing/transduction’ is meant. This should be corrected. (eg B_BE_25_BVW5_Environmental Risk Assessment_final.pdf p2/12; shedding of virus – p5&6/12 the risk of infection via shedding).

SBB comment:
This comment could be reported as a “Typos and other errors/omissions”:
The applicant is requested to correct the text where applicable as follows:

- On page 3/12 of the ERA, “shedding of virus” is reported when “shedding of viral vector” is meant.
- On page 5/12 of the ERA, “AB-1002 is capable of transferring genes by infecting host cells” is reported, whereas “by transducing” should be the terminology used.
- On page 6/12 of the ERA, “The risk of infection via shedding of viral particles is low” is reported, whereas “The risk of transduction” should be the terminology used.
- On page 11/38 of the ICF_NL, “een virus wordt gebruikt als vector” is reported whereas “een virale vector wordt gebruikt om genetische info over te brengen” should have been reported
- On page 11/38 of the ICF_NL, “genvirusproduct” is confusing and should be changed to “AAV vector” or “viral vector”
- On page 12/39 of the ICF_FR, “une thérapie génique utilisant un virus comme vecteur” is reported, whereas “un vecteur viral est utilisé pour transférer des informations génétiques”
- On page 15/39 of the ICF_FR, “Le produit viral du gène” is confusing and should be changed to “Le vecteur AAV” ou « Le vecteur viral ».

- The same wrong wording is used in the txt for the study participants (eg p11/38 last paragraph in B_BE_25_BVW5_ICF Core_NL_BE_Core_public.pdf). The wording should be corrected. ‘een virus wordt gebruikt als vector’ is wrong. ‘een virale vector wordt gebruikt om genetische info over te brengen, …’

SBB comment:
This comment has been included in the question here above.

- At p14/38 in B_BE_25_BVW5_ICF Core_NL_BE_Core_public.pdf ‘genvirusproduct’ is used. This is confusing, stick to ‘AAV vector, or viral vector’.

SBB comment:
This comment has been included in the question here above.

- Typo p47/58: ‘pregndoant or lactating women’

SBB comment:
This comment could be reported as a “Typos and other errors/omissions”:
On page 47/58 of the IB, “pregndoant” is reported. The applicant is requested to correct the text.

Comment 3
Has no other questions/comments.

References

Brimble et al., 2023. Stowaways in the cargo: Contaminating nucleic acids in rAAV preparations for gene therapy. Mol Ther 31(10):2826-2838.

Chadeuf,G., et al. 2005. Evidence for encapsidation of prokaryotic sequences during recombinant adeno-associated virus production and their in vivo persistence after vector delivery. Mol. Ther., 12, 744–753

Taylor, NK et al., 2024. A self-complementary AAV proviral plasmid that reduces cross-packaging and ITR promoter activity in AAV vector preparations. Mol Ther Methods Clin Dev32(3) 101295.

Zhang J. et L., 2022. Cryptic resolution sites in the vector plasmid lead to the heterogeneities in the rAAV vectors. J. MED. Virol. Vol 25, issue 1. eE28433

Laboratory biosafety manual (Fourth edition) WORLD HEALTH ORGANIZATION Geneva 2020 https://www.who.int/publications/i/item/9789240011311

Adviesraad voor Bioveiligheid
Conseil consultatif de Biosécurité

Compilation of the expert's evaluations of the answers of AskBio on the list of questions for dossier B/BE/25/BVW5

04 September 2025
Ref. SC/1510/BAC/2025_1048

Coordinator: Karen Willard-Gallo (Institut Jules Bordet)
Experts: Rik Gijsbers (KULeuven), Liliane Tenenbaum (Lausanne University Hospital), Amaya Leunda Casi (SBB)
SBB: Sheela Onnockx

INTRODUCTION

Dossier **B/BE/25/BVW5** concerns a notification from AskBio for a clinical trial entitled "Phase 2, adaptive, double-blinded, placebo-controlled, randomized, multi-center trial to evaluate the efficacy, safety and tolerability of intracoronary infusion of AB-1002 in adult subjects with New York Heart Association (NYHA) Class III heart failure and non-ischemic cardiomyopathy".

On 14 August 2025, based on a list of questions prepared by the BAC (SC/1510/BAC/2025_0977), 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 25 August 2025. This complementary information was reviewed by the coordinator and the experts in charge of the evaluation of this notification.

Evaluation Expert 1

Thank you for providing Table 1.
However, additional information is needed to evaluate the risk of transfer of ampicillin resistance to environmental bacteria:
-The proportion of viral particles containing a full and functional AmpR gene, which is expected to be low;
-The level of shedding via body fluids which will be extensively evaluated during the Phase 2 trial but which has already been evaluated in pre-clinical experiments.

I understand that this issue has already been extensively evaluated in the IMPD dossier which has already been reviewed by the Belgian Health Authorities. Therefore my recommendation is to follow the assessment of the experts who have evaluated the IMPD dossier in order not to unnecessarily delay the start of the clinical trial.

SBB's comment:
In the IMPD, a section describes the evaluation of residual plasmid.

Evaluation Expert 2

Indeed, the measures outlined in the Laboratory Biosafety Manual (Fourth Edition) by the World Health Organization are recommended specifically in case of biological spills with a high initial risk, which is not the case for this study. Nevertheless, the measures proposed by the applicant could be improved by clarifying that once the spill has been covered by absorbent material, disinfectant shown to inactivate the GMO (e.g., 10% bleach (0.53% or ~5000 PPM sodium hypochlorite solution)) should carefully be poured over the absorbent material starting from the edge to the center. An appropriate contact time for the disinfectant to inactivate the GMO is required before removing all contaminated disposables into a suitable container. After removing the absorbent paper soaked in disinfectant, the area must be rinsed with water and dried.

SBB's comment:
In order to be consistent between the different DR dossiers related to the use of an AAV which have been evaluated by the BAC, this recommendation could be addressed to the applicant either as a second list of question or as a recommendation in the advice.

Coordinator's comment:
I agree that this needs to be a strong recommendation (i.e. they do not have a choice). This needs to be added to the draft.

Evaluation Expert 3

• Q1/Q2/Q7/Q8 reply: satisfactory reply

• Q3 reply: I'm a bit puzzled here. The notifier indicates that the drug dose per animal is used. In the argumentation, the notifier indicates that the dose is best correlated with the number of functioning cardiomyocytes, which remain constant. So injection can be better expressed as dose/heart, correct? Next the notifier argues that the structure of the heart is more relevant than the size of the heart (proportional to the body weight), but in the end this is not considered in the dosing if I understand well. Anyway, this may be beyond the ERA assessment.

SBB's comment:
The determination of AB-1002 doses to be administrated to the patients is relevant for the safety assessment of the drug product and goes behind the environmental risk assessment of AB-1002. To make sure the IB will be updated as requested, the following condition will be added in the final advise: the dosing information, specifically whether the dose of 1E13 vg or 1E14 vg is per kg or per animal, will be clearly indicate throughout the entire IB document

Coordinator's comment:
Yes this is important that it is clear and consistent. They must edit the document for consistency.

• Q4: I would appreciate more than consideration here, and expect implementation.

SBB's comment:
To make sure the IB is updated as requested, the following condition needs to be added in the final advise: Biodistribution results from animal studies reported in section 4.4.1.3 of the IB appear to contradict data presented in section 4.3.2. The applicant should update the relevant sections to ensure consistency and accuracy across all documentation.

Coordinator's comment:
Again, inconsistencies are just sloppy on their part and the document needs to be clear and consistent. Maybe a general statement with these points mentioned as examples is in order - if they re-read all of it for inconsistencies that would be doing their job.

• Q5: The sponsor believes that in the absence of empirical data that extrapolation from other serotypes, species, and dosing is scientifically robust. I do not agree. Even though some of the arguments are valid, this does not mean that extrapolation is sufficient to claim safety. Germ line transmission is not easy, but the product may be present in the seminal fluid for example, and as such transfer to the partner. The papers cited (one from 2003, a time where doses were not as high and pure as now, and detection was less sensitive) reported that AAV DNA was detected in testes and ovaries underscoring my concern. In addition, I would be cautious with translating rabbit and mouse data to human, and therefore be very careful with new serotypes. I agree with the statement, "To the best of our knowledge, over numerous clinical trials that examined the safety and efficacy of gene therapies, and over thousands of patients dosed with AAV capsids we do not find a documented case of germline transmission in humans.", but this is with other serotypes than the one tested here. I would like to refer to https://www.cell.com/molecular-therapy-family/molecular-therapy/fulltext/S1525-0016(25)00043-7, where the authors indicate 'transduction of unexpected tissues, including adrenals, testes, and ovaries.' following systemic transduction in mice (10e12gc/animal). Again, this is another species, but shows that we should be cautious, even though germ cell delivery was not detected, the vectors reach these tissues enter cells and express their payload.

SBB's comment:
As previously mentioned, extrapolation from other serotypes, species, or dosing regimens is not scientifically robust. Each serotype, particularly when used at novel high doses, should undergo a thorough, case-specific evaluation of potential germline and developmental risks.

Coordinator's comment:
Again, they need to remove all speculation from the IB.

• Q6, again I reckon adapting the txt is in order here

SBB's comment:
To make sure the IB will be updated as requested, the following condition will be added in the final advise: Vector biodistribution is highly species-specific and differences in serotype and vector design strictly limit the extrapolation of preclinical data to humans. It is essential that the applicant avoid speculating in section 4.4.5 (p33/58) of the IB about the lack of persistence of a vector DNA signal in patients.

Coordinator's comment:
Yes
• Q9: horizontal lam flows should not be allowed.

• Q10: even if horizontal transmission is low, there is substantial variation and therefore we should be cautious. I would advise to be more cautious in the beginning, since this investigational drug that is still under investigation and shedding data are not yet available. I would request to adapt the duration of restrictions to "until at least two consecutive shedding measurements are found to be negative".

SBB's comment:
As presented, the protocol appears to involve sample collection over a 52-week period for each patient following GMO administration with their analysis only done after this 52-week period. As the potential reproductive risks of the investigational drug are not fully understood (absence of empirical data of AB-1002 or AAV2i8 on the risk of inadvertent germline transmission), the contraception duration should be extended up to 1 year to alleviate this risk.

Coordinator's comment:
Clearly this needs to be a comment we make.