Adviesraad voor Bioveiligheid
Conseil consultatif de Biosécurité

Advice of the Belgian Biosafety Advisory Council on the notification B/BE/25/BVW3 of the company CSL Behring LLC for deliberate release in the environment of genetically modified organisms other than higher plants for research and development

17/06/2025
Ref. SC/1510/BAC/2025_0767

Context

The notification B/BE/25/BVW3 has been submitted by CSL Behring LLC to the Belgian Competent Authority in April 2025 for a request of deliberate release in the environment of genetically modified organisms (GMOs) other than higher plants for research and development according to Chapter II of the Royal Decree of 21 February 2005.

The planned activity concerns a clinical trial with the title : *"Phase 3, Open-label, Single-dose, Multicenter Study Investigating Efficacy, Safety, and Tolerability of CSL222 (Etranacogene Dezaparvovec) Administered to Adolescent Male Subjects (≥ 12 to < 18 Years of Age) with Severe or Moderately Severe Hemophilia B".*

Congenital haemophilia B is a X-linked recessive inherited bleeding disorder characterized by an increased bleeding tendency due to either a partial or complete deficiency of the essential blood coagulation protein, factor IX (FIX), resulting from mutations of the respective clotting factor gene. There is no cure for hemophilia B. The primary goals of hemophilia B therapy are the prevention of bleeding episodes. CSL222 (previously termed AMT-061; HEMGENIX®; International Nonproprietary Name: etranacogene dezaparvovec) is a somatic gene therapy product that aims to deliver a nucleic acid expression cassette capable of driving expression and synthesis of functional FIX to the liver of patients living with hemophilia B. One-time treatment with CSL222 allows the patient to continuously produce functional hFIXco-Padua version of the FIX protein at levels which modify the severity of their hemophilia B disease.
CSL222 is authorized for use in adults with hemophilia B in the US, the European Union, Great Britain, and other countries and has shown a favorable benefit-risk balance for adults with hemophilia B with a severe bleeding phenotype.

The primary objectives of this Phase III study is to generate the clinical data needed to assess an advancement of CSL222 gene therapy in the adolescent population of patients with severe or moderately severe hemophilia B. Efficacy and safety aspects upon systemic administration of CSL222 belong to the secondary objectives.

CSL222 is a recombinant, replication-deficient adeno-associated virus-based vector (AAV5-based vector) containing the codon-optimised human Padua factor IX expression cassette under the control of a human liver-specific promoter (LP1). Compared to its parental counterpart, the AAV5-based 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, approximately 18 adolescent male subjects (≥ 12 to < 18 years of age) with severe or moderately severe hemophilia B will be included in this Phase III study, one of which is expected in Belgium. As for adults, CSL222 will be administered at a dose level of 2×10^{13} gene copies/kg as a one-time infusion in a peripheral vein. This study will be conducted at one clinical site located in Flanders. The national territory is considered as the potential release area of CSL222.

The dossier has been officially acknowledged by the Competent Authority on 22 April 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. Three experts from the common list of experts drawn up by the BAC and the Service Biosafety and biotechnology (SBB) of Sciensano answered positively to this request. The experts assessed whether the information provided in the notification was sufficient and accurate to state that the deliberate release of the genetically modified organism would not raise any problems for the environment, animal health or human health (people coming in contact with the treated patient and/or with the GMO) in the context of its intended use. See Annex I for an overview of all the comments from the experts.

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

On 23 May 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 04 June 2025 and transmitted to the secretariat of the BAC on the same day, 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 no reaction 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.

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

Information related to the molecular characteristics of CSL222 were adequately described in the dossier.

3. The conditions of the release

The study consists of four periods: Screening, Lead-in, Treatment, and Posttreatment Follow-up. All subjects will receive the same dose level of CSL222. The GMO will be administered intravenously to adolescent (≥ 12 to < 18 years of age) male subjects with severe or moderately severe hemophilia B, in hospital centres. After administration, subjects will be monitored at the clinical trial site for infusion reactions throughout the infusion period and for at least 3 hours after the end of the infusion. Subjects will then be followed for a total of 5 years in the post-treatment follow-up phase with weekly visits for the first 12 weeks followed by monthly visits from month 4 to 12 and visits twice a year from month 12 to 60.

Based on the shedding results assessed in the marketing authorisation procedure, some shedding of vector DNA is expected to occur in bodily fluids/excreta for several days after administration. Subjects must agree to use barrier contraception protection for 1 year after CSL222 treatment. For the treatment of adolescent subjects no additional risks to the environment or risks to human health is expected. As agreed with the European Medicines Agency (EMA) Paediatric Committee (PDCO) during the development of the PIP, the assessment of shedding in semen will not be included due to the age of the adolescent study population.
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 its parental strain it is not known to be pathogenic. The genetic modification introduced in the AAV5-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 CSL222 and the wild-type AAV in case a triple infection by AAV5-hFIXco-Padua, 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 AAV5 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 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 AAV5-hFIXco-Padua and the assumed lower amounts of shed and intact viral particles of AAV5-hFIXco-Padua 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

According to the notifier, a general guidance compatible with the handling of Risk Group 1 biological agents will be provided to the sites. The Biosafety Officer of the Belgian site has confirmed that the procedure followed at the site for the management of spills or breakages with biological agents is based on the Belgian Biosafety Advisory Council guidelines. Materials which will potentially be in contact with Etranacogene Dezaparvovec will be decontaminated with an appropriate disinfectant with viricidal activity (e.g. a chlorine releasing disinfectant like hypochlorite containing 0.1% available chlorine (1000 ppm)). As high temperature and pressure of the autoclave can cause chlorine gas to be released, which is toxic and can cause serious respiratory problems, the site confirms that autoclaving will not be used as decontamination approach in this study. Potentially contaminated disposable material will be disposed of as high-risk medical waste (RMA) and sent to an incinerator designated for RMA. Surface decontamination will be performed with a decontaminant effective against the GMO at a given concentration and contact time specified by the manufacturer of the decontaminant.

Given the assessment of the likelihood of further propagation of AAV5-hFIXco-Padua, 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 CSL222 developed to treat haemophilia B patients by means of endogenous production of FIX-Padua variant 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_2023-505805-18
    - CSL222_3004_SNIF_V2.0

- As committed by the notifier, the unit of time (day or week) indicated in the title of Table 15 in the IB, which summarizes the time to first shedding negativity in Study CSL220_1001, will be adjusted to align with the time unit used in the accompanying text describing the results.

- 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 CSL222.

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/BVW3 (ref. SC/1510/BAC/2025_0732 SC/1510/BAC/2025_0733)

Compilation of comments of experts in charge of evaluating the dossier B/BE/25/BVW3
And comments submitted to the notifier

06 June 2025
Ref. SC/1510/BAC/2025_0732

Mandate for the Group of Experts: Mandate of the Biosafety Advisory Council (BAC) of 11 April 2025.
Coordinator: Rik Gijsbers (KULeuven)
Experts: Jozef Anné (KUL), Anton Roebroek (KULeuven), Willy Zorzi (ULiège)
SBB: Sheela Onnockx

INTRODUCTION

Dossier **B/BE/25/BVW3** concerns a notification from CSL Behring, LLC 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 22 April 2025 and concerns a clinical trial entitled "Phase 3, open-label, single-dose, multicenter study investigating efficacy, safety, and tolerability of CSL222 (Etranacogene Dezaparvovec) in adolescent male subjects (≥ 12 to <18 years of age) with severe or moderately severe hemophilia B". The investigational medicinal product is a non-replicating, recombinant adeno-associated virus serotype 5 (AAV5) based vector containing a codon-optimised cDNA of the human coagulation Factor IX variant R338L (FIX-Padua) gene under the control of a liver-specific promoter (LP1).

♦ 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 22-05-2025 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
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.

2.2. 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 evaluated this item and has no questions/comments.

2.4. Molecular characterisation of the clinical vector

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

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

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

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

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.

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
Has evaluated this item and has no questions/comments.

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

Additional SBB's comment:

According to the SNIF document page 15/16, "the presence of vector DNA sequences will be determined in serum and semen from the treated patients". However, in table "Schedule of activities" in the protocol, section 1.3, it seems that no collection of sperm is planned after the administration of the treatment. The applicant could be requested to clarify this discrepancy.

Comment coordinator:
Semen samples were analysed in Section 6.3.1.7. in NHP, showing positive results. Still, positive PCR results do not indicate the presence of viral vector particles that can transduce cells. Further, based on Table15 there does not seem to be a correlation between AAV vector dose and semen presence of AAV signal (Cohort2 received 4x the amount of AAV vector, and showed only 64% of the GC compared to Cohort1).
A remark would be that Table15 is not clear. In the txt it is indicated that #weeks to negative shedding is indicated, whereas the title says Days. This should be updated.

SBB's comment:
This comment could be reported as a "Typos and other errors/omissions" :
Table 15 of the IB summaries the time to first shedding negative in Study CSL220_1001. According to the title of the table, this time is given in Days. However, according to the text just above the table, Table 15 provides a summary of the number of weeks to first negative shedding. The notifier is requested to update the document where applicable.

3. INFORMATION RELATED TO THE CLINICAL TRIAL

3.3. Storage of the clinical vector at the clinical site

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

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

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

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

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

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

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

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

Comment 3
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
The final GMO is not released in the environment but will be administered to patients in a controlled area (clinical site). In case of accidental spills or breakage of a vial containing the GMO, the medical staff should alert people in the area of the spill, remove contaminated clothes and leave the area for 30 min. He/she should close the area and post “DO NOT ENTER”. After 30 min, he/she must wear clean PPE (as described). He/she must cover the spill with towels and other absorbent material starting from the edge toward the centre. He/she must carefully pour the appropriate disinfectant over the absorbent material starting from the edge to the centre. It must allow a sufficient contact time for the disinfectant to inactivate the GMO. After that, he/she must remove the paper towels and broken vials with tongs or forceps and discard in a biohazard waste bag. This procedure with absorbent materials and disinfectant should be performed twice. All materials in contact with the product should be disposed of as hospital waste according to local biosafety guidelines, as described in the Investigator’s Brochure Date: 30 January 2025.
Wild type AAV is not known to be involved in environmental processes and none of the genetic modifications made to wild type AAV during construction of etranacogene dezaparvovec is expected to have any impact on this property.

SBB’s comment:
Each vial contains an extractable volume of 10 mL of concentrate for solution for infusion. Hemgenix is delivered as a single dose of 2 mL/kg body weight. Therefore, the volumes handled during the preparation of the IMP are not so negligible. In order to provide clear and concise instruction to health care personnel, the notifier could be requested to adapt the procedure in case of accidental spill or breakage by clarifying as suggested by the expert in the comment above.

Comment coordinator:
I agree with the comment of the SBB and align with the suggestions made by the expert.

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

Comment 3
1) p22 in the B_BE_25_BVW3_EU SMPC CSL222 document, it is written that :

Precautions to be taken for the disposal of the medicinal product

Work surfaces and materials which have potentially been in contact with etranacogene dezaparvovec must be decontaminated with appropriate disinfectant with viricidal activity (e.g. a chlorine releasing disinfectant like hypochlorite containing 0.1% available chlorine (1000 ppm)) after usage and then autoclaved, if possible.

Please amend this sentence to:
« Work surfaces and materials which have potentially been in contact with etranacogene dezaparvovec must be decontaminated after usage, with appropriate disinfectant with viridal activity (e.g., a chlorine-releasing disinfectant, such as freshly prepared hypochlorite containing 0.1% active chlorine (1000 ppm).
Absorbent paper or other materials soaked in chlorine must not be autoclaved due to the risk of generating toxic chlorine vapour.
Materials without a hypochlorite-based disinfectant may be autoclaved after testing for compatibility with autoclaving treatment. »

2) in p 43-44 of the B_BE_25_BVW3_EU SMPC CSL222 document :

Same remark as before in 1)

3) Concerning the section I Information on post-release and waste treatment p15-16 In the B_BE_25_BVW3_Part2_SNIF_final_10Apr25 document, it is written that :

3. (b) Treatment of waste

Sharps such as needles will be disposed of in adequate sharp containers and incinerated. Disposables such as syringes, tubing and catheters will be decontaminated by immersion in a chemical disinfectant with virucidal activity before incineration.

All the surgical materials (surgery tools, linens) and surgery waste (gloves, compresses) will be collected and autoclaved before washing and sterilisation or incineration. All non-disposable surgical equipment will be cleaned using a chemical disinfectant with proven virucidal activity (e.g. hypoclorite solution) and then sterilised by autoclaving according to standard practices of the institution.

Please note that non-disposable surgical equipment must be sterilized by autoclaving after disinfection with a hypochlorite solution only after vigorous washing with water, to remove all traces of chlorine in this equipment and on its surface. This method is recommended due to the risk of generating toxic chlorine vapours during autoclaving sterilization.

SBB's comment:

Indeed, materials soaked in chlorine should not be autoclaved, as the high temperature and pressure of the autoclave can cause chlorine gas to be released, which is toxic and can cause serious respiratory problems. The following recommendation could be provided :

According to pages 22 and 44 of the EU_SMPC_CSL document, "Work surfaces and materials which have potentially been in contact with etranacogene dezaparvovec must be decontaminated with appropriate disinfectant with viricidal activity (e.g. a chlorine releasing disinfectant like hypochlorite containing 0.1% available chlorine (1000 ppm)) after usage and then autoclaved, if possible." Similarly, according to the SNIF page 16, "all non-disposable surgical equipment will be cleaned using a chemical disinfectant with proven virucidal activity (e.g. hypoclorite solution) and then sterilised by autoclaving according to standard practices of the institution".

As high temperature and pressure of the autoclave can cause chlorine gas to be released, which is toxic and can cause serious respiratory problems, materials soaked in chlorine should not be autoclaved due to the risk of generating toxic chlorine vapour. Materials and non-disposable surgical equipment must be sterilized by autoclaving after disinfection with a hypochlorite solution only after washing with water, to remove all traces of chlorine in this equipment and on its surface or could be directly autoclaved if autoclaving process has previously been validated.

Comment coordinator:
I agree with the suggestion provided by the SBB.

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
Has evaluated this item and has no questions/comments.

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 further questions/comments.

Comment 2
Has no further questions/comments.

Comment 3
Has no further questions/comments.

References
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Compilation of the expert's evaluations of the answers of CSL Behring, LLC on the list of questions for dossier B/BE/25/BVW3

06 June 2025
Ref. SC/1510/BAC/2025_0733

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

INTRODUCTION

Dossier **B/BE/25/BVW3** concerns a notification from CSL Behring, LLC for a clinical trial entitled "Phase 3, open-label, single-dose, multicenter study investigating efficacy, safety, and tolerability of CSL222 (Etranacogene Dezaparvovec) in adolescent male subjects (≥ 12 to <18 years of age) with severe or moderately severe hemophilia B".

On 22 May 2025, based on a list of questions prepared by the BAC (SC/1510/BAC/2025_0694), 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 04 June 2025. This complementary information was reviewed by the coordinator and the experts in charge of the evaluation of this notification.

Evaluation Expert 1

By this way, I would like to let you know that I reviewed the answers of the notifier to the questions raised in May 2025 by the Biosafety Council for the dossier B/BE/25/BVW3 (clinical trial submitted by CSL Behring, LLC related to the use of recombinant AAV for subjects with Hemophilia B) and that the notifier addressed correctly and satisfactorily all the comments/questions.

Evaluation Expert 2

I have reviewed the documents and have no further comment. Answers to the questions asked seem logical to me.

Evaluation Expert 3

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