Adviesraad voor Bioveiligheid
Conseil consultatif de Biosécurité

Advice of the Belgian Biosafety Advisory Council on the notification B/BE/23/BVW2 of the company Janssen-Cilag International NV for deliberate release in the environment of genetically modified organisms other than higher plants for research and development

15/06/2023
Ref. SC/1510/BAC/2023_0547

Context

The notification B/BE/23/BVW2 has been submitted by Janssen-Cilag International NV to the Belgian Competent Authority in March 2023 for a request of deliberate release in the environment of genetically modified organisms (GMO) 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 entitled "*A Phase 2b, Randomized, Double-masked, Multicenter, Dose-ranging, Sham-controlled Clinical Trial to Evaluate Intravitreal JNJ-81201887(AAVCAGsCD59) Compared to Sham Procedure for the Treatment of Geographic Atrophy (GA) Secondary to Age-related Macular Degeneration (AMD)*". The primary objective of the proposed study is to evaluate whether a single administration of the gene therapy, JNJ-81201887, can slow down progression of GA in 60 years of age or older adults.

Age-related macular degeneration (AMD) is the leading cause of blindness in patients over 60 years of age in developed countries, affecting approximately 1 in 4 people over 70 years of age. AMD accounts for approximately 9% of all cases of blindness. The loss of vision occurs mostly in advanced cases of dry AMD with the formation and enlargement of GA.

The investigational medicinal product (AAVCAGsCD59) consists of a replication-incompetent recombinant AAV2 (rAAV2) encoding the soluble form of the human sCD59, a complement regulatory protein that protects the cells by inhibiting the formation of membrane attack complex (MAC), the terminal step of complement-mediated cell lysis. A high concentration of MAC correlates with an increased severity of AMD. The soluble form of CD59 lacks the membrane glycosylphosphatidylinositol anchor enabling sCD59 to diffuse.

Overall, approximately 300 patients will be included in this Phase IIb study, with approximatively 10 expected to be enrolled in Belgium. The volume of AAVCAGsCD59 that will be administered to a single eye corresponds to 0.1mL. Two dose levels of AAVCAGsCD59 will be tested; a low dose and a high dose. The IMP will be administered by intravitreal injection per standard local procedure and does not require a surgical vitrectomy.

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Vector shedding will be monitored at several time points after administration by qPCR from baseline and up to 6 months.

This study will be conducted at investigational clinical sites located in Flanders and Wallonia (2 subjects/site). The national territory is considered as the potential release area of AAVCAGsCD59.

The dossier has been officially acknowledged by the Competent Authority on 17 March 2023 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 assisted by 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 answered positively to this request. The SBB also took part in the evaluation of the dossier. The experts assessed whether the information provided in the notification was sufficient and accurate in order to state that the deliberate release of the genetically modified organism (GMO) 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 raised by the experts.

The scientific evaluation has been performed considering the 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.
- Good practice document on the assessment of GMO related aspects in the context of clinical trials with AAV clinical vectors developed by the national competent authorities and the Commission services available at https://ec.europa.eu/health/sites/health/files/files/advtherapies/docs/aavs_gp_en.pdf

The exclusive medical aspects concerning the efficacy of the IMP and its safety for the treated patient, as well as aspects related to social, economic or ethical considerations, are outside the scope of this evaluation.

On 20 April 2023, 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 03 May 2023 and transferred to the secretariat of the BAC on the day after. 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 15 May 2023. On 25 May 2023, upon a request of the notifier, an informal meeting took place with the coordinator of the dossier and the secretariat of the BAC. The answers of the notifier were received on 02 June 2023 and transmitted to the BAC, 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 to 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 of which some questions are related to biosafety issues.

Summary of the scientific evaluation

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

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

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

Although the glycosylphosphatidylinositol-anchored membrane protein CD59 acts as an inhibitor of the formation of the membrane attack complex (MAC) and thereby inhibits cell lysis, the recombinant soluble form of CD59, sCD59, from which the GPI membrane anchor has been deleted, has been proven to be a poor inhibitor of MAC both *in vitro* and *in vivo*.[1,2,3] To date, there is no reported link between soluble CD59 and carcinogenesis.

Information regarding the molecular characteristics of AAVCAGsCD59 was adequately described in the dossier.

3. The conditions of the release

The current study consists of three arms assigning participants either to a low AAVCAGsCD59 vector genomes/eye dose, or a high low AAVCAGsCD59 vector genomes/eye dose, or to a placebo (sham) arm.

Vector shedding will be monitored by qPCR AAV vector genome detection at several time points after administration. For the proposed study, samples will be collected from lacrimal fluid (tears) and saliva at days 1, 4, 6 and 18 and months 1, 3 and 6. Collection of shedding data will be stopped after three consecutive results that are at or below the limit of detection of the assay.

According to the information provided by the notifier all involved personnel on the respective sites are required to wear standard hospital protective equipment including coats and gloves during preparation and administration. The preparation of the IMP for administration is recommended to be conducted in a biological safety cabinet. The use of eye protection and mask are mandatory should the use of a biosafety cabinet not be available during the preparation of the IMP and during the administration of the IMP.

No shedding analysis has been obtained neither with the AAV vector AAVCAGsCD59 that will be used in this study, nor with intravitreal injection of any other recombinant AAV vector. However literature data can be found reporting low and transient presence of recombinant AAV2 vector particles in tears up to 3 days in patient undergoing subretinal injection (surgical procedure), a procedure which is more

[1] Siobhan MC, Ramo K, Kumar-Singh R. A Non Membrane-Targeted Human Soluble CD59 Attenuates Choroidal Neovascularization in a Model of Age Related Macular Degeneration. PLoS One, April 2011 | Volume 6 | Issue 4 | e19078

[2] Sugita Y, Ito K, Shiozuka K, Suzuki H, Gushima H, Tomita M, Masuho Y. Recombinant soluble CD59 inhibits reactive haemolysis with complement. Immunology. 1994 May;82(1):34-41.

[3] Vakeva A, Jauhiainen M, Ehnholm C, Lehto T, Meri S. High-density lipoproteins can act as carriers of glycophosphoinositol lipid-anchored CD59 in human plasma. Immunology. 1994; 82: 28–33.

invasive than intravitreal injection (no surgery required).[4,5,6] The notifier was requested to make sure precautionary measures for preventing contamination via tears, saliva, sputum, or cough will be applied. For up to 14 days after the intravitreal injection or sham patients will be instructed to wash their hands thoroughly after touching tears and nasal secretions, after using the bathroom, and before eating. Tissues and handkerchiefs used to wipe eye tearing or nasal secretions will be collected in sealed bags and put into the garbage waste closed. The notifier also met the BAC's request to provide a take-home summary with these instructions to the patients.

4. The risks for the environment or human health

AAVCAGsCD59 is a recombinant, replication deficient adeno-associated virus-based vector not harbouring any antibiotic or other resistance genes. Like its parental virus strain, it is considered not pathogenic. The genetic information (sCD59 cDNA) introduced in this AAV2-derived vector is not expected to confer the GMO with properties that could confer risks to the human population or the environment.

There is only a remote possibility of homologous recombination between the ITR-sequences of AAV2 in the IMP wild-type AAV in case a triple infection by AAVCAGsCD59, 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 AAV2 required for replication and encapsidation but would in turn lead to the loss of the current sCD59 transgene. Moreover, the genetic material from *rep* and *cap* genes together with the sCD59 transgene would be too large in size to be packed in AAV capsid, making it impossible to package this information, and thus to form a replication competent viral particles 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 cannot replicate and the 'dose' that may conceivably be transferred (from e.g. aerosol, splashing or fomites) will be several orders of magnitude lower than that received by patients. Worst case, the receiver will develop an immune respons to the AAV2 capsid.

Shedding data collected from the proposed 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 qPCR 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 for the patients accordingly, thereby preventing contamination via tears, saliva, sputum, or cough.

[4] Bainbridge JW, Smith AJ, Barker SS, *et al*. Effect of gene therapy on visual function in Leber's congenital amaurosis. N Engl J Med. 2008;358(21):2231-2239.

[5] Bainbridge JW, Mehat MS, Sundaram V, *et al*. Long-term effect of gene therapy on Leber's congenital amaurosis. N Engl J Med. 2015;372(20):1887-1897

[6] Maguire AM, Simonelli F, Pierce EA, *et al*. Safety and efficacy of gene transfer for Leber's congenital amaurosis. N Engl J Med. 2008;358:2240–2248.

Given the preliminary results from the phase 1 study involving the intravitreal injection of AAVCAGsCD59 in GA patients (81201887MDG1001), the BAC requested the notifier to apply restriction on blood, organs, tissues and cells donation. For up to 12 weeks after the intravitreal injection or sham, study participants will be advised to not donate blood, organs, tissues, and cells for transplantation. This recommendation is also included in the small take-home summary provided to the patients.

The BAC concludes that, based on the non-pathogenic and non-replicative nature of AAVCAGsCD59, on the assumed lower amounts of shed and intact viral particles of AAVCAGsCD59 as compared to the therapic dose, and on the strict implementation of the precautionary measures for preventing contamination via tears, saliva, sputum, or cough , 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 notifier

While rAAV vectors are non-replicating, it is anticipated that AAVCAGsCD59, 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. Following a remark of the BAC with respect to the disinfection solution, the notifier updated the documents by clarifying that 0.1M sodium hypochlorite in freshly prepared solution will be used to decontaminate any surface area exposed to the GMO.

On-site transportation of the clinical vector will be done by using double packaging, with the outer packaging a closed, easy to decontaminate, break- and leak-proof packaging.

Given the assessment of the likelihood of further propagation of AAVCAGsCD59, 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 for the intended trial.

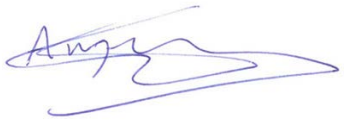
Conclusion

Based on the scientific assessment of the notification made by the Belgian experts, the Biosafety Advisory Council concludes that it is unlikely that AAVCAGsCD59, which is developed as an ocular gene therapy approach for the treatment of geographic atrophy (GA) secondary to age-related macular degeneration, will have 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 as described in the following updated documents (and for some still to be adapted in accordance with the conditions stipulated below):

- SNIF v6.0
- EU_CAF v2.0 (to be adapted in accordance with condition 4 stipulated below)
- EU_CAF confidential v2.0
- Safety statement AAV v2.0
- Take Home summary_v2.0
- Protocol version_Amendment 3

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

1. The notifier and the investigators must strictly apply the clinical trial protocol and the safety instructions as described in the dossier and the updated documents listed here above.
2. The notifier makes sure patients are well informed about the precautionary measures to be applied for preventing contamination via tears, saliva, sputum, or cough for 14 days post-injection. Patients should be informed before the start of the treatment and these measures must be recalled during the first visit after the treatment.
3. The notifier takes due account of its commitment to reinforce if necessary the precautionary measures according to the shedding results obtained from this study and the study 81201887MDG2001.
4. In the CAF document page 18/21, prohibition to donate blood, organs, tissues and cells for 12 weeks after intravitreal injection should be reported.
5. Any protocol amendment has to be previously approved by the Competent Authority.
6. 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 authorisations 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 the contained use of genetically modified micro-organisms.
7. The BAC should be informed within two weeks when the first patient starts the treatment and the last patient receives the last treatment.
8. At the latest six months after the last visit of the last patient included in the trial, the notifier must send the competent authority for the attention of the BAC a report with details concerning the biosafety aspects of the project. This report shall contain at least:
    a. The total number of patients included in the trial and the number of patients included in Belgium;
    b. A summary of all adverse events marked by the investigators as probably or definitely related to the study medication;
    c. A report on the accidental releases, if any, of AAVCAGsCD59.



Prof. 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/23/BVW2 (ref. SC/1510/BAC/2023_0359 and SC/1510/BAC/2023_0421)*
Annex II: *Answers to the public reaction to dossier B/BE/23/BVW2 in NL (ref. SC/1510/BAC/2023_0549) and FR (ref. SC/1510/BAC/2023_0548)*

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Compilation of comments of experts in charge of evaluating the dossier B/BE/23/BVW2
And comments submitted to the notifier

20 April 2023
Ref. SC/1510/BAC/2023_0359

Mandate for the Group of Experts: Mandate of the Biosafety Advisory Council (BAC) of 13 March 2023.
Coordinator: Rik Gijsbers (KULeuven),
Experts: Anton Roebroek (KULeuven), Willy Zorzi (ULiège), Aline Baldo (SBB)
SBB: Sheela Onnockx

INTRODUCTION

Dossier **B/BE/23/BVW2** concerns a notification from Janssen-Cilag International NV for the deliberate release in the environment of genetically modified organisms other than higher plants according to Chapter II of the Royal Decree of 21 February 2005.
The notification has been officially acknowledged on 03 March 2023 and concerns a clinical trial entitled "A Phase 2b, Randomized, Double-masked, Multicenter, Dose-ranging, Sham-controlled Clinical Trial to Evaluate Intravitreal JNJ-81201887(AAVCAGsCD59) Compared to Sham Procedure for the Treatment of Geographic Atrophy (GA) Secondary to Age-related Macular Degeneration (AMD).". The investigational medicinal product is a AAV2-derived recombinant replication deficient vector carrying the human CD59 cDNA (sCD59) that is truncated (deletion of the glycosylphosphatidylinositol (GPI) anchor to generate a soluble protein and driven by the CAG promoter (AAVCAGsCD59).

♦ 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 20-April-2023 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 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 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 comment:

According to the SNIF p15/17, viral shedding from patients who receive the GMO as part of the clinical trial will be assessed up to 6 months post administration.
According to the public CAF p18/21, samples will be collected from various matrices that may contain the viral vector including lacrimal fluid (tear), saliva, whole blood, serum, and aqueous humor. These samples will be collected at various time points during the study on days 4, 6, 18, and months 1, 3, 6, 12, and 18.
According to section 1.3, “Schedule of Activities” of the protocol p19/142, samples will be collected at days 1, 4, 6, 18 and Months 1, 3 and 6.
The notifier could be requested to adapt the documents where applicable in order to have consistent information throughout these documents.

Coordinator: I had also noted this discrepancy in my evaluation of the different documents. I agree to request clarification by the notifier.

Additional SBB comment:
According to the public CAF p12/21, in previous clinical studies of AAV2 vectors administered in the retina, shedding of vector could be detected in tears (*Boye et al, 2012*) and serum samples (*Manno et al, 2006*), which were transiently positive, but this resolved within a few days after the operation.
However, the article from Boye *et al* (2012) refers to the evaluation of another serotype, the AAV-5 vector containing the human rhodopsin kinase (hGRK1) promoter for its ability to target transgene expression to rod and cone photoreceptors when delivered subretinally in a nonhuman primate (NHP). This article does not correspond to results from a clinical trial and therefore, this reference reported in the sentence doesn't seem to be correct.
The notifier could be requested to adapt the first reference in this sentence and to provide us the article describing the shedding of the viral vector in tears since it would be useful to know how long shedding in tears has been observed.

Coordinator: I agree with this remark. The notifier should indicate that the paper refers to another serotype.

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
In the « 3.3 Storage of the clinical vector. » at the clinical site section of « B_BE_23_BVW2_Part 1B_CAF_EU_1.0 » file :
The applicant should provide information about the storage location, conditions of storage (including restrictions of access), and the maximal storage duration.[15]
Storage will be in line accordance with national legislation.
IP shipment should be stored at -80℃ ± 10°C (-70°C to -90°C) in a secure temperature controlled and monitored freezer. During storage, outer packaging must not be separated from inner packaging (e.g.,vials should not be removed from outer box). The packaging is designed to protect the drug from breakage and damage and parts should not be separated.

Comment: In this "Phase 2b, Randomized, Double-masked, Multicenter, Dose-ranging, Sham-controlled Clinical Trial", the notifier is invited to specify what are the total volumes of clinical GMO vector preparations stored in the freezer and to describe what are the stored units volumes (the largest volumes, the titers in clinical vector/parts of GMOs...). Is the used freezer specifically dedicated to this study or is it a shared freezer with the possibility of having other clinical vectors from other clinical studies stored in the same place

SBB Comment:
The notifier could be requested to specify what are the total volumes of clinical GMO vector preparations stored in the freezer and to describe what are the stored units volumes (the largest volumes, the titers in clinical vector/parts of GMOs...). According to the public CAF document page 16/21, the drug product is supplied as sterile solution of 0.1 mL of a high dose and low dose. The notifier could also be requested to clarify what will be both doses that will be administrated to the patient and which volume will be administrated as an intravitreal injection in the study eye.
Information related to the second part of the question will be provided and verified in the context of the biosafety dossier 'contained use' which is handed in for each of the study sites in accordance with the regulation implementing Directive 2009/41/EC on the contained use of GMOs and pathogens (contained use procedure).

In the « 3.4 Logistics for on-site transportation of the clinical vector. » section of « B_BE_23_BVW2_Part 1B_CAF_EU_1.0 » file:
In-house transport (i.e. at the clinical site) takes place according to local guidelines

Comment : Please describe more precisely « the In-house transport according to local guidelines »

SBB Comment:
Information related to the in-house transport will be provided and verified in the context of the biosafety dossier 'contained use' which is handed in for each of the study sites in accordance with the regulation implementing Directive 2009/41/EC on the contained use of GMOs and pathogens (contained use procedure).

Coordinator: I noted a similar comment. It is difficult to judge whether the conditions applied here are according to the guidelines, or could be improved if no details are provided. Could the notifier be requested to provide a minimal guideline?

SBB Comment:
This question will be sent to the notifier.

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
In the « 3.6 Measures to prevent dissemination into the environment. b) Personal protective equipment . » section of « B_BE_23_BVW2_Part 1B_CAF_EU_1.0 » file :
Medical personnel will follow standard hospital hygienic measures, standard hospital personal protective equipment will be worn, such as coats and gloves.

Comment : The notifier is advised to complete here, the list of PPE measures by adding : « For IP preparation and standard surgical vitrectomy, the use of eye protection and mask are mandatory during administration. » → As described p2 in the « Safety and security for handling, storage, transport and disposal of the IP » section of the « B_BE_23_BVW2_Safety statement AAV v1.7 BE » file

SBB Comment:
According to the SNIF, p15/17 and the public CAF p17/21, standard hospital personal protective equipment that will be worn by the clinical staff, includes coats and gloves. According to the Safety statement AAV p3/5, beside the standard hospital personal protective equipment, such as coats and gloves, the use of eye protection and mask are mandatory should the use of a biosafety cabinet not be possible during the preparation of the IMP and during the administration of the IMP. The notifier could be requested to adapt the list of PPE in both CAF and SNIF documents by adding that the use of eye protection and mask should the use of a biosafety cabinet not be possible during the preparation of the IMP and during the administration of the IMP.

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

Comment 3
Waste will be disposed of as specific hospital or GMO waste. Waste should be incinerated as medical waste and cannot be landfilled.

SBB Comment:
According to the SNIF, p15/17, all waste generated (material in contact with the GMO during the preparation and administration of the GMO) will be disposed of according to the local policy. Standard operating procedures for disposal within the medical facility will be consistent with the guidance given in the WHO Laboratory Biosafety Manual, 3rd Ed (2004) for BSL1/2. In the medical facility, this will

involve temporary containment in sharps bins or clearly marked bags (e.g. biohazard, medical waste) prior to autoclaving and/or incineration either on- or off-site as per local institutional guidelines for handling potentially biohazardous materials. It is not mentioned that waste will be landfilled.

Coordinator: All waste is expected to be treated as hospital waste, and thus incinerated.

Additional SBB Comment
According to the Safety statement AAV p3/5, after the standard surgical vitrectomy during which the viral vector will be injected, an ocular bandage and dressing will be placed on the treated eye. In the post-operative Day 1 visit, the ocular bandages and dressings will be removed by a health care worker and disposed according to local guidelines.

Though in previous clinical studies with AAV2 vectors administered in the retina, viral vector shedding in tears seems to be limited and transiently in human (Maguire *et al*, 2010; Bainbridge *et al*, 2015), shedding properties of AAVCAGsCD59 in humans are currently lacking. Therefore, as a precautionary measure, the notifier could be asked to consider extending the duration of wearing an eye bandage to 14 days post-injection in order to align patient's instructions with those given for the EU registered medicinal products Luxturna containing a recombinant AAV2 carrying the human retinal pigment epithelium protein (hRPE65) cDNA administrated by sub-retinal injection. According to the product information document (EPAR) for Luxturna, "Patients/caregivers should be advised to handle waste material generated from dressings, tears and nasal secretion appropriately, which may include storage of waste material in sealed bags prior to disposal. These handling precautions should be followed for 14 days after administration of voretigene neparvovec. It is recommended that patients/caregivers wear gloves for dressing changes and waste disposal, especially in case of underlying pregnancy, breast-feeding and immunodeficiency of caregivers."

Coordinator: I agree with this comment of the SBB. Unifying the post-intervention procedures is to be promoted. We should however be open to suggestions to restrict timings to shorter intervals when sufficient data/arguments are provided to do so.

SBB Comment:
The comment from the coordinator has been taken into account in the query that will be sent to the notifier.

Furthermore, the notifier could be requested to clarify whether patients will also undergo a standard surgical vitrectomy at the time of injection of the viral vector as this information is not reported in the Schedule of Activities table 1.3 of the protocol.

Finally, in order for patients and patient's family to adhere to and practice good hygiene, it is important to explain why measures are taken and what are the likely sources of contaminated material. Therefore, the notifier could be requested to provide a small take home summary (preferably one-page, plasticized document) to ensure that patients and patient's family easily can consult the information and all the instructions in an understandable format whenever needed.
The following information could be reported in this instruction sheet for the patient:
- The bodily fluids which are anticipated to contain viral vector genome
- The time points when eye pads may be removed and the instruction on how these eye pads should be changed and disposed of

- Instructions aimed at limiting contact with materials or surfaces frequently contaminated with bodily fluids (e.g. handkerchiefs, used eye pads)
- Instructions and effective solutions to decontaminate possible contaminated areas, tissues, skin, …
- The period during which these instructions must be followed

Coordinator: I agree with this addition. It would be good to provide here the info that was provided in an earlier dossier for an rAAV based therapy.

Additional SBB Comment

- Hypochlorite concentration in household bleach solutions varies by manufacturer. All decontamination procedures involving the use of sodium hypochlorite solution should thus specify the precise mass concentration (g/100 ml) or molar concentration (M or mol/l) of sodium hypochlorite in the final solution.
- Also, it should be specified that whenever hypochlorite solution is used (e.g. for the decontamination of work areas), attention should be given to the use of freshly prepared hypochlorite solution.

The notifier could be requested to adapt accordingly the following documents:

- Public CAF page 17/21
- Safety Statement AAV page 3/5
- SNIF page 6/17

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

The applicant says that no recommendations on donations by the clinical trial subsects are planned or considered necessary. Viral vector DNA is detectable in serum of 4/17 participants. The blood donation must therefore be prohibited during the clinical trial.

SBB Comment:

According to the Public CAF document p 18/21, based on the Good Practice on the assessment of GMO related aspects in the context of clinical trials with AAV clinical vectors, no recommendations on donations by the clinical trial subjects are planned or considered necessary. However, since there is a lack of experience with donation of blood or organs, tissues and cells for transplantation following AAV vector-based gene therapy, the notifier could be requested to revise the instructions regarding blood, organs, tissues and cells and to align these with the instruction given in the product information document (EPAR) of EU registered medicinal products containing recombinant AAV (Glybera, Zolgensma, Roctavian, Luxturna, Upstaza, Hemgenix) : 'Patients treated must not donate blood, organs, tissues, and cells for transplantation'.

Alternatively, the notifier is requested to give a rationale why instructions could deviate from measures commonly taken for current EU marketing authorized medicinal products containing recombinant AAV.

Coordinator: I also noted this remark. I would prefer to apply the instructions as stipulated for the currently registered products, and indeed request further clarification from the notifier in the event the notifier would prefer to deviate from the measures currently in place (and also sent to the notifier in the frame of the BVW5-2022 dossier).

6. OTHER INFORMATION

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

Comment 1
None

Comment 2
None

Comment 3
None

References

A.M. Maguire *et al*. Safety and Efficacy of Gene Transfer for Leber's Congenital Amaurosis. N Engl J Med. 2008 May 22; 358(21): 2240–2248. doi: 10.1056/NEJMoa0802315

J.W.B. Bainbridge *et al*. Long-Term Effect of Gene Therapy on Leber's Congenital Amaurosis. N Engl J Med. 2015 May 14; 372(20): 1887–1897. doi: 10.1056/NEJMoa1414221

Adviesraad voor Bioveiligheid
Conseil consultatif de Biosécurité

Compilation of the expert's evaluations of the answers of Janssen-Cilag International NV on the list of questions for dossier B/BE/23/BVW2

16 may 2023
Ref. SC/1510/BAC/2023_0421

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

INTRODUCTION

Dossier **B/BE/23/BVW2** concerns a notification from Janssen-Cilag International NV for a clinical trial entitled "A Phase 2b, Randomized, Double-masked, Multicenter, Dose-ranging, Sham-controlled Clinical Trial to Evaluate Intravitreal JNJ-81201887(AAVCAGsCD59) Compared to Sham Procedure for the Treatment of Geographic Atrophy (GA) Secondary to Age-related Macular Degeneration (AMD)".

On 20 April 2023, based on a list of questions prepared by the BAC (SC/1510/BAC/2023_0355), 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 03 May 2023. This complementary information was reviewed by the coordinator and the experts in charge of the evaluation of this notification.

Evaluation SBB

Q7-8:

The notifier provided three references on shedding of DNA AAV2 vector containing a gene encoding the protein RPE65 in tears: Bainbridge et al (2008) didn't isolated DNA from tears and saliva samples collected at day 1 and day 30 from 3 patients after subretinal injection of 1×10e11 recombinant AAV 2/2 vector. In another clinical trial described by Baindridge et al (2015) using the same recombinant AAV 2/2 vector, lacrimal fluid samples were weakly positive for vector DNA sequences at 1 day after surgery, but not at 30 days for one out of 12 participants receiving the lower dose (1×10e11 vg) and for 2 out of 12 participants receiving the higher dose (1×10e12 vg). Vector DNA was not detected in the participants' saliva. Finally, according to Maguire et al (2009), out of the 12 patients treated with one subretinal injection of AAV2 containing a gene encoding the protein RPE65, DNA vector was also transiently found in samples of tears up to 3 days after surgery: in 1 patient treated with 5×10e10 vg; in 3 treated with 4,8×10e10 vg; in 2 treated with 1,5×10e11 vg.

However, as pointed out by the notifier, these 3 references discuss subretinal injection of the viral vector, whereas in this study, intravitreal injection will be performed.

Given the above shedding results, given that as of now no shedding data are available for intravitreal injection, and given that shedding analysis with AAVCAGsCD59 has not been assessed neither during non-clinical studies nor during the previous phase I clinical trial, the shedding of rAAV particles in lacrimal fluid (tears) cannot be ruled out. Therefore, the notifier is requested to implement precautionary measures to make sure that the treated eye of the patients is covered by an eye bandage for at least 3 days post-injection until it is proven that there is no shedding detectable at this and later time-points post administration (shedding analysis 81201887MDG2001).

The results of the shedding analysis that will be performed during study 81201887MDG2001 will allow the notifier to reconsider the necessity of the precaution measures and alleviating these (or some of these).

Furthermore, patients and family should be advised to handle waste material generated from dressings, tears, and nasal secretion appropriately, which may include storage of waste material in sealed bags prior to disposal. It is also recommended that patients and family wear gloves for eye dressing changes and waste disposal, especially in case of underlying pregnancy, breastfeeding, and immunodeficiency of the family member.

Finally, in view of the above, the period of post-treatment time that the patients should adopt precautionary measures for preventing contamination via tears, saliva, sputum, or cough should be defined and justified. The notifier is requested to provide these recommendations as a small take home summary (preferably a one-page, plasticized document) to ensure that all important information in laymen terms is readily available to consult at any time.

The following information should be reported in this instruction sheet for the patient:

- The bodily fluids which are anticipated to contain viral vector genome (tears, saliva, nasal fluids)
- The time points when eye pads may be removed and the instruction on how these eye pads should be changed and disposed of
- Instructions aimed at limiting contact with materials or surfaces frequently contaminated with bodily fluids (e.g. handkerchiefs, used eye pads)
- Instructions and effective solutions to decontaminate possible contaminated areas, tissues, skin, …
- The period during which these instructions must be adhered to

Q9:

In the SNIF document page 6/17, the hypochlorite solution concentration is reported as “0.5%.1 M”. The notifier could be requested to correct this concentration either as a question in a second LOQ if applicable or as a requirement in the advice.

Additional SBB comment:

Since CD59 is widely expressed and has been shown to be overexpressed in most solid tumors, where it facilitates tumor cell escape from complement surveillance by inhibiting MAC formation (Fishelson Z et al. 2003), one could suppose that the possibility of oncogenic event has carefully been evaluated by the notifier.

We couldn't find in the documents provided by the notifier an evaluation of potential consequences of the overexpression of CD59 in the cells. In the IB p42/51, they developed the possibility of oncogenic events but only focused on to vector-mediated insertional mutagenesis.

Due to the potential oncogenic property of the insert, the notifier could be requested to substantiate its conclusion that the transgene is not harmful and to provide us with an evaluation of the potential consequence of overexpression of CD59 in humans.
However, we are wondering whether this request should be sent knowing that:

- The notifier indicated "Yes" on the question on p21/21 of the CAF_Public on page 21/21, meaning that the notifier considers that 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 vectors is applicable. Hence, one can assume that the notifier can demonstrate, among other things, the absence of formation of replication competent virus and that the transgene is not harmful.
- The dose that may be transferred to third will be orders of magnitude lower than that received by patients, any risk related to the transfer of vector to an unintended person is expected to be very low.

Coordinator's comment:
A reference dating from 2003 is very old. In the following two references a potential oncogenic driver is described, but these are very different cells and a lab context (https://doi.org/10.3892/ijo.2013.2007; https://doi.org/10.2217/fon-2017-0498). In cancer studies, CD59 emerges as a biomarker protein for certain cancers. CD59 would protect cancer cells against complement attack. We could ask for clarification from the notifier, even if it seems strong that the FAMHP would allow this product if it would be carcinogenic for the patient (and then possibly also for others after shedding).

Evaluation Expert 1

The notifier's responses can be considered as satisfactory.
I have no additional comment.

Evaluation Expert 2

I have reviewed the answers of the notifier and I consider these answers satisfactory, except for the answer on question 10 dealing with donation of blood etc.

The argument of the notifier, that because of the recommendation in the document EU Good Practice on the assessment of GMO related aspects in the context of clinical trials with AAV clinical vectors (Version 3, January 2022) no precautions are necessary unless analysis of the data of an ongoing Study 81201887MDG2001 data prove otherwise, I cannot agree with. It seems to me more logical, that precautions are only alleviated once is proven that this can be done. Given the fact, that only 8 patients will be enrolled in this clinical study in Belgium, the overall availability of donors in Belgium will not be effected and being more precautious than maybe necessary with respect to donation at this moment should not be an issue. If proven at the time of the MAA that this safety measure with respect to donation is not necessary, this precaution could be skipped.

SBB's Comment:
In the Investigator Brochure, section 4.2.1, qPCR results for AAVCAGsCD59 DNA on whole blood samples obtained from 17 participants in study 81201887MDG1001 are briefly developed with no numeric results. According to the notifier, no recommendations on blood, organs, tissues, and cells

donation are necessary unless analysis of the data of an ongoing Study 81201887MDG2001 prove otherwise. The BAC does not agree with this reasoning. Precautionary measures should be in place until the lack of shedding is proven. The notifier is therefore strongly encouraged to revise the instructions regarding donation of blood, organs, tissues and cells and to align these with the instruction given in the product information document (EPAR) of EU registered medicinal products containing recombinant AAV. If proven at the time of the MAA that this safety measure with respect to donation is not necessary, this precautionary measure can be lifted.

The applicant refers to Imlygic which is an attenuated herpes simplex virus type-1 (HSV-1) used in cancer medicine to treat adults with melanoma by intratumoral injection. Whereas, AAVCAGsCD59 is an adeno-associated viral vector. Since both viral constructions do not have the same viral origin, they will act differently and precautionary measures taken for each viral vector could differ.

Coordinator's comment:

Coordinator would omit this last part concerning Imlygic in the SBB comment. He understands that this is confusing for the applicant, and is himself surprised that when applying this approach blood donation would be allowed (Imlygic is a conditionally replicating HSV (in cancer cells)).

Evaluation Expert 3

J'ai lu les réponses du notifiant et je n'ai pas de remarques.

References:

Fishelson Z, Donin N, Zell S, Schultz S and Kirschfink M: Obstacles to cancer immunotherapy: Expression of membrane complement regulatory proteins (mCRPs) in tumors. Mol Immunol. 40:109–123. 2003.

Baijun Li Hui Lin Jian Fan Jiao Lan Yonglong Zhong Yong Yang Hui Li : CD59 is overexpressed in human lung cancer and regulates apoptosis of human lung cancer cells. Int J Oncol. 2013 Sep;43(3):850-8.

Ronghua Zhang, Qiaofei Liu, Quan Liao, Yupei Zhao: CD59: a promising target for tumor immunotherapy. Future Oncol. 2018 Apr;14(8):781-791.

Bainbridge JW, Smith AJ, Barker SS, et al. Effect of gene therapy on visual function in Leber's congenital amaurosis. N Engl J Med. 2008;358(21):2231-2239.

Bainbridge JW, Mehat MS, Sundaram V, et al. Long-term effect of gene therapy on Leber's congenital amaurosis. N Engl J Med. 2015;372(20):1887-1897

Maguire AM, Simonelli F, Pierce EA, et al. Safety and efficacy of gene transfer for Leber's congenital amaurosis. N Engl J Med. 2008;358:2240–2248.

Adviesraad voor Bioveiligheid
Conseil consultatif de Biosécurité

Réponse du Conseil consultatif de Biosécurité aux observations formulées pendant la consultation du public concernant la notification B/BE/23/BVW2 de Janssen-Cilag International NV pour l'introduction volontaire dans l'environnement, à des fins de recherche et développement, d'organismes génétiquement modifiés autres que les plantes supérieures

Adopté le 15/06/2023
Ref. SC/1510/BAC/2023_0548

Contexte

La notification B/BE/23/BVW2 a été soumise en mars 2023 par Janssen-Cilag International NV à l'autorité compétente belge pour une demande de dissémination volontaire dans l'environnement, à des fins de recherche et développement, d'organismes génétiquement modifiés autres que les plantes supérieures, conformément au chapitre II de l'arrêté royal du 21 février 2005. La notification a été lancée par l'autorité compétente (AP) le 17 mars 2023, après que le notifiant aie suffisamment répondu aux questions de validation.
Conformément à l'article 17 de l'arrêté royal, l'AC a organisé une consultation du public pendant une période de 30 jours. À la suite de cette consultation, l'AC a transmis les observations du public au Conseil consultatif de biosécurité, parmi lesquelles un certain nombre d'observations pertinentes en matière de biosécurité.
Conformément à l'article 16§2 de l'arrêté royal, ces observations ont été prises en compte lors de la préparation de l'avis du Conseil consultatif de Biosécurité (référence BAC_2023_0547). La réponse à ces observations est donnée ci-dessous.
Les questions/observations du public qui ne sont pas pertinentes en matière de biosécurité (telles que les questions liées au patient, les questions économiques ou éthiques) ne sont pas prises en compte par le Conseil de Biosécurité.

Question 1: Nous constatons qu'aucune mesure concernant l'excrétion n'est décrite dans le dossier public ou technique, bien qu'une dissémination du transgène dans l'environnement ne soit pas exclue (mais peu probable). Les dossiers précédents décrivaient souvent une forme de quarantaine ou de protection du site d'administration. L'absence de telles mesures constitue-t-elle un problème à vos yeux ? Nous n'avons pas trouvé de fiche d'information pour les patients dans le dossier, je n'ai donc pas pu m'appuyer sur cette information.

Commentaire du SBB :
Le vecteur viral AAVCAGsCD59, réplicatif déficient et non pathogène, est administré aux patients par injection intravitréenne. En raison du faible volume d'injection, du site d'injection et de la méthode d'injection (injection intravitréenne, pas d'intervention chirurgicale - sensiblement différente de l'injection sous-rétinienne comme dans la plupart des autres GT sur le marché), on peut estimer que l'excrétion virale par le patient sera faible. En outre, comme le virus parental dont est issu le vecteur

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viral n'est pas pathogène (et de surcroît omniprésent dans la population) et comme le transgène codant pour une protéine humaine CD59 rendue soluble ne devrait pas conférer au vecteur viral des propriétés susceptibles de présenter des risques pour la population humaine ou l'environnement, des mesures de quarantaine ne semblent pas nécessaires pour ces patients, et ce même si l'exposition d'un tiers à l'excrétion par les patients ne peut être exclue. Cependant, comme aucune analyse d'excrétion n'ayant été réalisée à ce jour, ni avec le vecteur AAV qui sera utilisé dans cette étude, ni avec l'injection intravitréenne de tout autre vecteur AAV recombinant, il a été demandé au notifiant de proposer des précautions proportionnées aux risques éventuels associés aux propriétés non-réplicatives et non pathogènes des vecteurs viraux.

Question 2: Les informations sur l'immunogénicité et la tumorigénicité sont manquantes. Ce dernier nous semble particulièrement important pour estimer correctement le risque pour l'environnement, étant donné que le vecteur devrait avoir des effets inhibiteurs de la mort cellulaire. Quelle est votre évaluation du risque en termes d'immunogénicité et surtout de tumorigénicité ?

Commentaire du SBB:
Pour ce dossier-ci, le demandeur a rempli le document spécifique formulaire commun de demande pour les vecteurs cliniques AAV (CAF_AAV) qui se concentre sur l'évaluation des risques environnementaux du vecteur recombinant et dans lequel les données sur l'immunogénicité et la tumorigénicité ne doivent pas être détaillées.

Ce document fait référence à un questionnaire d'évaluation des risques environnementaux spécifiques à l'utilisation des vecteurs viraux AAV dans les applications cliniques, qui est intitulé « Good Practice on the assessment of GMO related aspects in the context of clinical trials with AAV clinical vectors. Cette évaluation spécifique de risques ne peut s'appliquer aux vecteurs cliniques AAV que si le demandeur démontre l'absence de formation de virus capables de se répliquer et que le transgène n'est pas nocif. Au travers de ce document ERA spécifique, les dangers potentiels pour la santé humaine et l'environnement liés à l'utilisation d'un vecteur AAV et son insert sont caractérisés et analysés.
En répondant « oui » à la question de la section 5 « Environmental risk assessment » du CAF-AAV, le notifiant considère que l'évaluation des risques environnementaux comme décrit à la section 2 du document « Good Practice » est applicable et a été réalisée. Dans ce cas, plusieurs aspects du ERA ne doivent plus être développés dans le CAF-AAV.

Immunogenicity :
Le produit transgénique et la capsule virale sont les seules sources d'antigène étranger censées être capables de déclencher une réponse immunitaire. La protéine humaine sCD59 est une version recombinante soluble d'une molécule liée à la membrane qui protège les cellules hôtes en inhibant l'étape finale de la lyse cellulaire médiée par le complément.

Des tests d'immunogénicité ont été effectués chez la souris et le singe pour détecter les anticorps dirigés contre la capside AAV2 et le transgène sCD59 du vecteur viral AAVCAGsCD59. Les réponses immunitaires cellulaires et humorales contre la capside AAV2 et la protéine transgène sCD59 seront évaluées au cours de l'essai clinique proposé.

Tumorigenicity:
La protéine membranaire CD59, ancrée à la membrane par un glycosylphosphatidylinositol, est une protéine régulant le complément (CRP) qui inhibe l'assemblage du complexe d'attaque membranaire (MAC) et empêche ainsi la lyse cellulaire. La forme recombinante soluble de CD59 utilisée dans cet essai clinique s'est avérée être un mauvais inhibiteur de MAC *in vitro* en présence de sérum (Sugita et

al., 1994 ; Vakeva et al., 1994) et *in vivo* où elle est rapidement éliminée dans les reins (Siobhan et al., 2011). À ce jour, aucune association n'a été rapportée entre la protéine sCD59 soluble et le cancer.

En cas de transmission du vecteur à un receveur immunocompétent non ciblé, les risques devraient être nettement inférieurs au risque potentiel pour le participant, car le vecteur est incapable de se répliquer et la « dose » potentiellement transmise (par exemple par aérosol, éclaboussures ou fomites) sera bien inférieure à la dose reçue par les patients. Dans le pire des cas, le receveur développe une réponse immunitaire contre la capside AAV2.

De plus, un lien de causalité entre la tumorigénicité et une mutagénèse insertionnelle est une possibilité théorique qui a déjà été étudiée dans des études pré-cliniques et qui n'a pas encore été démontré jusqu'à présent. De même, une telle corrélation n'a pas encore été observée dans aucune étude clinique réalisée avec des vecteurs cliniques AAV jusqu'à ce jour. C'est pourquoi, la probabilité qu'une mutagénèse insertionnelle se produise lors d'un transfert accidentel ou d'une exposition involontaire au vecteur clinique AAV peut être considérée comme négligeable (Sabatino *et al*. 2022). Le fait que de faibles quantités soient utilisées ici et que l'injection dans l'œil soit effectuée dans le cadre d'une procédure non chirurgicale ne fait que réduire ce risque négligeable de mutagenèse.

References:

Good Practice on the assessment of GMO related aspects in the context of clinical trials with AAV clinical vectors (2019) https://health.ec.europa.eu/system/files/2022-01/aavs_gp_en.pdf

D.E. Sabatino, F.D. Bushman, R.J. Chandler, R.G. Crystal, B.L. Davidson, R. Dolmetsch, K.C. Eggan, G. Gao, I.Gil-Farina, M.A. Kay, D.M. McCarty, E. Montini, A. Ndu, and J.Yuan, The American Society of Gene and Cell Therapy (ASGCT) Working Group on AAV Integration
Evaluating the state of the science for adeno-associated virus integration: An integrated perspective. Mol Ther. 2022 Aug 3;30(8):2646-2663. doi: 10.1016/j.ymthe.2022.06.004.

Siobhan M. Cashman, K. Ramo, R. Kumar-Singh. A Non Membrane-Targeted Human Soluble CD59 Attenuates Choroidal Neovascularization in a Model of Age Related Macular Degeneration. PLoS One, April 2011 | Volume 6 | Issue 4 | e19078

Sugita Y, K Ito, K Shiozuka, H Suzuki, H Gushima, M Tomita, Y Masuho Recombinant soluble CD59 inhibits reactive haemolysis with complement. Immunology. 1994 May;82(1):34-41..

Vakeva A, Jauhiainen M, Ehnholm C, Lehto T, Meri S (1994) High-density lipoproteins can act as carriers of glycophosphoinositol lipid-anchored CD59 in human plasma. Immunology 82: 28–33.

Adviesraad voor Bioveiligheid
Conseil consultatif de Biosécurité

Antwoorden van de Adviesraad voor Bioveiligheid op opmerkingen gekregen tijdens de publieksraadpleging over de kennisgeving B/BE/23/BVW2 van Janssen-Cilag International NV voor doelbewuste introductie in het leefmilieu van genetisch gemodificeerde organismen met uitzondering van hogere planten voor onderzoek en ontwikkeling

Goedgekeurd op 15/06/2023
Ref. SC/1510/BAC/2023_0549

Contexte

De kennisgeving B/BE/23/BVW2 werd in maart 2023 door Janssen-Cilag International NV bij de Belgische bevoegde overheid ingediend voor een verzoek om doelbewuste introductie in het leefmilieu van genetisch gemodificeerde organismen, met uitzondering van hogere planten voor onderzoek en ontwikkeling, overeenkomstig hoofdstuk II van het koninklijk besluit van 21 februari 2005. De kennisgeving kon opgestart worden door de bevoegde overheid (BO) op 17 maart 2023 nadat de kennisgever de validatievragen voldoende beantwoord had.
Volgens artikel 17 van het koninklijk besluit organiseerde de BO een openbare raadpleging van het publiek voor een periode van 30 dagen. Als resultaat van deze raadpleging heeft de BO de opmerkingen van het publiek doorgestuurd naar de Adviesraad voor Bioveiligheid, waarvan een aantal opmerkingen betreffende bioveiligheid.
Overeenkomstig artikel 16§2 van het koninklijk besluit zijn deze opmerkingen in beschouwing genomen bij het uitbrengen van het advies van de Adviesraad voor Bioveiligheid (referentie BAC_2023_0547). Het antwoord op deze opmerkingen wordt hieronder gegeven.
Vragen/opmerkingen van het publiek die niet relevant zijn inzake bioveiligheid (zoals patiënt gerelateerde vragen, economische of ethische kwesties) worden door de Bioveiligheidsraad niet in aanmerking genomen.

Vraag 1: We merken dat er geen maatregelen rond shedding beschreven staan in het publieke of het technische dossier, hoewel een vrijgave van het transgen naar het milieu niet uitgesloten is (maar onwaarschijnlijk). In voorgaande dossiers was een vorm van quarantaine of bescherming van de plaats van toediening vaak wel beschreven. Is het ontbreken van zulke maatregelen een probleem wat jullie betreft? Een eventuele informatiefiche voor patiënten vonden we niet direct terug in het dossier, op deze informatie heb ik me dus niet kunnen baseren.

SBBs Comment:
De replicatie-deficiënte, niet-pathogene AAVCAGsCD59 virale vector wordt aan patiënten toegediend door middel van een intravitreale injectie. Vanwege het kleine injectievolume, de injectieplaats en de injectiemethode (intravitreale injectie, geen chirurgische procedure – substantieel verschillend met subretinale injectie zoals in meeste andere GT met marketaccess) kan worden aangenomen dat de uitscheiding van de virale vector door de patiënt laag zal zijn. Aangezien het virus waarvan de virale

vector is afgeleid, niet pathogeen is (en bovendien alomtegenwoordig is in de bevolking) en aangezien het transgen dat codeert voor een oplosbaar gemaakt humaan CD59-eiwit (sCD59) geen eigenschappen vertoont die een risico kunnen vormen voor de menselijke bevolking of het milieu, lijken quarantainemaatregelen voor deze patiënten niet nodig, zelfs als de blootstelling van een derde aan uitscheiding door de patiënt niet kan worden uitgesloten. Omdat er tot nu toe nog geen uitscheiding analyse gedaan is, noch met de AAV vector die in deze studie zal worden gebruikt, noch met intravitreale injectie van enige andere recombinante AAV vector, werd de kennisgever gevraagd voorzorgsmaatregelen voor te stellen die in verhouding staan tot de mogelijke risico's verbonden aan de niet-replicerende en niet-pathogene virale vectoren.

Vraag 2: Hier ontbreekt er info over immunogeniciteit en tumorigeniciteit. Zeker het laatste lijkt ons van belang om het risico voor het leefmilieu correct in te schatten, gezien de vector celdood-inhiberende effecten zou moeten hebben. Wat is jullie inschatting van het risico wat betreft immunogeniciteit en in het bijzonder tumorigeniciteit?

SBBs Comment:
Voor het huidige dossier, heeft de aanvrager de specifieke Common Application Form voor klinische vectoren van AAV (CAF_AAV) document ingevuld dat gericht is op de milieurisicobeoordeling van de recombinante vector en waarbij gegevens over immunogeniciteit en tumorigeniciteit niet verder gedetailleerd moeten worden. Dit invulformulier verwijst naar een specifieke milieurisicobeoordeling voor gebruik van AAV virale vectoren in klinische toepassingen, de zogenaamde "Good Practice on the assessment of GMO related aspects in the context of clinical trials with AAV clinical vectors". Deze specifieke milieurisicobeoordeling kan alleen op klinische AAV-vectoren worden toegepast als de aanvrager aantoont dat geen competent virus wordt gevormd en dat het transgen niet schadelijk is. In deze specifieke ERA worden de potentiële gevaren voor de menselijke gezondheid en het milieu in verband met het gebruik van een AAV-vector en het transgen gekarakteriseerd en geanalyseerd.

Door "ja" te antwoorden op de vraag in sectie 5 "Environmental risk assessment" van het CAF_AAV document geeft de kennisgever aan dat de beoordeling van specifieke milieurisico's zoals beschreven in sectie 2 van het bovenvermelde Good Practice document is uitgevoerd. In dat geval moeten de verschillende aspecten van de ERA niet meer worden beschreven in de CAF_AAV.

Immunogeniciteit :
Het transgenproduct en het viruskapsel zijn de enige bronnen van vreemd antigeen waarvan wordt aangenomen dat ze een immuunrespons kunnen opwekken. Het menselijke eiwit sCD59 is een oplosbare recombinante versie van een membraangebonden molecule dat gastheercellen beschermt door de laatste stap van de complement-gemedieerde cel lyse, af te remmen.

Immunogeniciteitstests werden zowel bij de muis als bij de aap uitgevoerd om antilichamen tegen het AAV2-capside en het sCD59-transgen van de AAVCAGsCD59-virale vector te detecteren. Zowel cellulaire als humorale immuunreacties tegen het AAV2-capside en het sCD59-transgeneiwit zullen tijdens de voorgestelde klinische studie worden beoordeeld.

Tumorigeniciteit :
Het glycosylfosfatidylinositol-geankerde membraaneiwit CD59 is een complement reguleerde eiwit (CRP) dat de assemblage van het membraanaanvalscomplex (MAC) remt en daardoor cel lyse verhindert. Het werd bewezen dat de recombinante oplosbare vorm van CD59 die in deze klinische proef wordt gebruikt, een slechte remmer is van MAC *in vitro* in aanwezigheid van serum (Sugita *et al*., 1994; Vakeva *et al*., 1994) en *in vivo* waar het snel wordt opgeruimd in de nieren (Siobhan *et al*., 2011). Tot op heden is er geen gerapporteerd verband tussen oplosbaar sCD59 en kanker.

In het geval van overdracht van de vector aan een niet gericht immuun-competente ontvanger, zullen de risico's naar verwachting aanzienlijk kleiner zijn dan het potentiële risico voor de deelnemer, aangezien de vector niet in staat is om te repliceren en de "dosis" die mogelijk wordt overgedragen (door bijvoorbeeld aerosol, spatten of fomieten) veel lager zal zijn dan de dosis die de patiënten ontvangen. In het ergste geval ontwikkelt de ontvanger een immuunrespons tegen de AAV2-capside.

Bovendien is een causaal verband tussen tumorigeniciteit en AAV-gemedieerde insertionele mutagenese een theoretische mogelijkheid die reeds in preklinische studies is onderzocht en nog niet is aangetoond. Een dergelijk verband is ook nog niet waargenomen in klinische studies met AAV-vectoren. Daarom kan de kans op insertionele mutagenese bij accidentele overdracht of onopzettelijke blootstelling aan de klinische AAV-vector als verwaarloosbaar worden beschouwd (Sabatino *et al*. 2022). Het feit dat hier lage hoeveelheden worden gebruikt, en injectie in het oog gebeurt in een procedure zonder chirurgische ingreep maakt deze verwaarloze kans op mutagenese alleen maar kleiner.

References:

Good Practice on the assessment of GMO related aspects in the context of clinical trials with AAV clinical vectors (2019) https://health.ec.europa.eu/system/files/2022-01/aavs_gp_en.pdf

D.E. Sabatino, F.D. Bushman, R.J. Chandler, R.G. Crystal, B.L. Davidson, R. Dolmetsch, K.C. Eggan, G. Gao, I.Gil-Farina, M.A. Kay, D.M. McCarty, E. Montini, A. Ndu, and J.Yuan, The American Society of Gene and Cell Therapy (ASGCT) Working Group on AAV Integration
Evaluating the state of the science for adeno-associated virus integration: An integrated perspective. Mol Ther. 2022 Aug 3;30(8):2646-2663. doi: 10.1016/j.ymthe.2022.06.004.

Siobhan M. Cashman, K. Ramo, R. Kumar-Singh. A Non Membrane-Targeted Human Soluble CD59 Attenuates Choroidal Neovascularization in a Model of Age Related Macular Degeneration. PLoS One, April 2011 | Volume 6 | Issue 4 | e19078

Sugita Y, K Ito, K Shiozuka, H Suzuki, H Gushima, M Tomita, Y Masuho Recombinant soluble CD59 inhibits reactive haemolysis with complement. Immunology. 1994 May;82(1):34-41..

Vakeva A, Jauhiainen M, Ehnholm C, Lehto T, Meri S (1994) High-density lipoproteins can act as carriers of glycophosphoinositol lipid-anchored CD59 in human plasma. Immunology 82: 28–33.