Adviesraad voor Bioveiligheid
Conseil consultatif de Biosécurité

Advice of the Belgian Biosafety Advisory Council on the notification B/BE/22/BVW5 of the company F. Hoffmann-La Roche Ltd for deliberate release in the environment of genetically modified organisms other than higher plants for research and development

17/07/2023
Ref. SC/1510/BAC/2023_0693

Context

The notification B/BE/22/BVW5 has been submitted by F. Hoffmann-La Roche Ltd to the Belgian Competent Authority in October 2022 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 and the title of the notification is: ***"A two-part, open-label systemic gene delivery study to evaluate the safety and expression of RO7494222 (SRP-9001) in subjects under the age of four with Duchenne Muscular Dystrophy".***

The purpose of this study, also called ENVOL study, is to assess the safety and the efficacy of the study treatment RO7494222 (SRP-9001) in male subjects under the age of 4, with a genetic diagnosis of Duchenne Muscular Dystrophy (DMD).

Duchenne muscular dystrophy (DMD) is a X-linked degenerative neuromuscular disease caused by mutations in the dystrophin gene and predominantly affects boys. The lack of functional dystrophin protein results in progressive muscle weakness and wasting. Ultimately heart and respiratory muscles are affected, causing premature death of DMD patients.

As a gene therapy product, RO7494222 (SRP-9001) has the potential to deliver functional truncated dystrophin, called micro-dystrophin, in cardiac and skeletal muscle, thereby addressing the root cause of the disease. The non-replicating, recombinant adeno-associated virus (rAAV) serotype rh74, isolated from Rhesus macaque, contains an abbreviated version of the human dystrophin gene referred to as "micro-dystrophin" under the control of the MHCK7 promotor/enhancer that has been optimized for driving expression in cardiac and skeletal muscle (Rodino-Klapac *et al*. 2013)[1].

1. Rodino-Klapac, L. R., P. M. Janssen, K. M. Shontz, B. Canan, C. L. Montgomery, D. Griffin, K. Heller, L. Schmelzer, C. Handy, K. R. Clark, Z. Sahenk, J. R. Mendell, and B. K. Kaspar. 2013. 'Micro-dystrophin and follistatin co-delivery restores muscle function in aged DMD model', Hum Mol Genet, 22: 4929-37

Overall, approximately twenty one patients will be included in this Phase II study and it is estimated that four patients will be included in Belgium, each receiving a single dose intravenous infusion into a peripheral limb vein. This study will be conducted in one clinical site located in Wallonia.

The use of the same GMO in a clinical trial, SRP-9001, also referred to as delandistrogene moxeparvovec, has already been assessed by the BAC in the framework of notification B/BE/21/BVW5[1], submitted by Sarepta Therapeutics.

The dossier has been officially acknowledged by the Competent Authority on 21 October 2022 and forwarded to the Biosafety Advisory Council (BAC) for advice.
Within the framework of the evaluation procedure, the BAC, under the supervision of a coordinator and with the assistance of its Secretariat, contacted experts to evaluate the dossier. Four experts, from the common list of experts drawn up by the BAC answered positively to this request.
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 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 patient, as well as aspects related to social, economic or ethical considerations, are outside the scope of this evaluation.

On 28 November 2022, 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 17 March 2023 and transmitted to the secretariat of the BAC on the same day. This complementary information was reviewed by the BAC and resulted in a second list of questions, which was transmitted to the notifier on 31 March 2023. The answers of the notifier were received on 7 July 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 did receive two reactions from the public with 5 questions related to biosafety issues. According to Article 16 §2 of the Royal Decree of 21 February 2005, the comments that are relevant for biosafety received in the framework of the public consultation, have been taken into account in the preparation of the advice below.

[1] Advice of the Belgian Biosafety Advisory Council on the notification B/BE/21/BVW5 - *Ref. SC/1510/BAC/2022_0677*

Summary of the scientific evaluation

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

The BAC is of the opinion that, the donor, recipient and parental organisms are adequately described in the dossier.

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

The production of SRP-9001 is accomplished via cellular transfection using three DNA-containing plasmids: the “transfer vector” which contains the therapeutic gene of interest (GOI) - pAAV.MHCK7.Micro-Dystrophin, the “rep/Cap” plasmid - pNLREP2-Caprh74, and a “helper plasmid” which contains some adenovirus genes. The three plasmids are well described in the confidential documents. Taking into account additional information from the notifier regarding the assessment of potential presence of illegitimate plasmid DNA as part of the release test of clinical batches, the production system was deemed sufficiently described from an environmental risk point of view.
The assays to demonstrate the absence of replication-competent virus in the clinical batch were mentioned in the public Common application form (CAF) and detailed in the confidential annex of the CAF but were found difficult to interprete. Upon BAC’s request to increase the readability and consistency, the notifier provided a revised and improved confidential annex of the CAF and a corresponding public CAF.

3. The conditions of the release

The study consists of four cohorts consisting of respectively approximately 10 participants who are equal or above three years of age and under 4 years of age (cohort A), approximately 4 participants who are equal or above two years of age and under 3 years of age (cohort B), approximately 4 participants who are above 6 months of age and under 2 years of age (cohort C) and approximately 3 participants who are equal or less than 6 months of age (cohort D). All subjects will receive intravenous (IV) one-single dose (1,33 x 10^{14} vector genome/kg) and will be followed for 260 weeks following infusion. Patients will be monitored closely and stay for a minimum of 6 hours after completion of the infusion where after patients can leave the hospital.

No shedding analysis will be planned during this clinical trial because biological samples from saliva, urine and stool have been collected in Sarepta study SRP-9001-103 which is using the same IMP at the same dose. Results on time for peak vector genome DNA detected in saliva, urine and feces were presented, yet no further information was available on the nature of observed shed vector DNA to distinguish between integrated vector DNA in shed cells or whether it consists out of remaining or rescued replication-deficient viral vector particles. Furthermore, the notifier was asked to report in the public CAF document that shedding may occur through bodily fluids after infusion.

4. The risks for the environment or human health

RO7494222 (SRP-9001) is a recombinant, replication deficient adeno-associated virus-based vector not harbouring any antibiotic or other resistance genes. Like its parental virus strain, isolated from Rhesus macaque, it is not known to be pathogenic. The genetic modification introduced in this AAVrh74

derived vector does not 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 AAVrh74 in the IMP and wild-type AAV, in case a triple infection by SRP-9001, 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 AAVrh74 required for replication and encapsidation but would in turn lead to the loss of the current abbreviated version of the human dystrophin transgene. Moreover, the genetic material from rep and cap genes together with the micro-dystrophin transgene would be too large in size to be packed in AAV capsid, making it impossible to form a replication competent viral particle that would contain the transgene and the rep and cap genes necessary for multiplication.

In the case immune-competent human recipient are unintendedly exposed to the viral vector, the risks are expected to be considerably reduced as compared to any potential risk for the participant. This is because the viral vector is not able to replicate and that the amount at which third persons may be exposed (from e.g. aerosol, splashing or fomites) will be orders of magnitude lower than that received by patients. Worst case, the third person will develop an immune response to the AAVrh74 capsid.

While the assessment of potential insertional mutagenesis of RO7494222 (SRP-9001) predominantly pertain to the clinical safety assessment of the trial and the safety of the patient, the potential for germline transmission pertains to the environmental risk assessment. The latter has been evaluated previously in the context of B/BE/21/BVW5. Moreover, in the context of the current trial application, only patients under 4 year of age will considered for inclusion in the clinical trial, which also explains why no contraceptive requirements have been set.

After injection, patients and caregivers will be provided with detailed instructions in order to avoid potential transmission of the virus to other people or to the environment when patients are leaving the hospital setting. With respect to the duration of period to apply post-injection hygiene measures for patients and caregivers, a period 4 weeks was agreed upon following the assessment of shedding data that were provided on 20 Dec 2022 in the context of a substantial amendment for dossier B/BE/21/BVW5 (EMBARK study, also using SRP-9001). This period of 4 weeks was also deemed sufficient for the current proposed ENVOL study. Upon BAC's request, the Post infusion hygiene guidance was also improved with more detailed instructions on the procedures for the use and disposal of gloves and other contaminated material. Because the updated guidance mentioned the use of an alcohol-based hand sanitizer as an alternative for washing hands and the BAC considers it is not a suitable and effective disinfectants for AAV, the notifier was requested to modify the guidance accordingly. All instructions for the patients and caregivers with respect to good hygiene practices have been detailed in a short, readable format document that will be provided to each patient.

With respect to the instructions regarding blood, organs, tissues and cells donation, the BAC remarked that proposed instructions differ from instructions given in the product information document of EU registered medicinal products containing AAV. In its response the notifier took due account of the remark and updated relevant text in the SNIF and the public CAF by stipulating that 'Patients treated must not donate blood, organs, tissues, and cells for transplantation'. The notifier further commits to implement the text in the same way in a protocol version for Belgium and to distribute a protocol clarification letter to all sites immediately following the approval of the clinical trial.

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

In addition to the Pharmacy manual, the notifier provided a one page sheet (doc 4.1a handling RG1-IMP-pharmacie clinical trial sector) giving an overview on how the IMP should be handled in the storage room, the preparation zone and the infusion room, including instructions for transport between the different areas. This one page document has the benefit that it can be used as a hands-one document. Upon BAC's request more detailed handling instructions for the personnel in case of spill or other risk management measures were provided in an addendum to the pharmacy and dose administration manual, including description of the personal protective equipment for personnel (lab coats, safety goggles, gloves, hair covers and overshoes) and detailed procedure for the use of decontamination/disinfection solutions (sodium hypochlorite solution and others).

Given the assessment of likelihood of further spreading into the environment of RO7494222 (SRP-9001), 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.

Conclusion

Based on the scientific assessment of the notification made by the Belgian expert, the Biosafety Advisory Council concludes that it is unlikely that RO7494222 (SRP-9001) developed as a gene therapy approach for the treatment of Duchenne Muscular Dystrophy disease 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 documents making part of the notification and the following updated documents:

- 4.1 2022-000691-19 20221010_BN43881 EU Common application form - Belgium Public – Clean (sent 6 July 2023)
- 4.1.1 2022-000691-19 20221010_BN43881 EU Common application form - Belgium Confidential Annex – Version 2 , June 2023
- 4.2 2022-000691-19 BN43881 – SNIF – Belgium Clean (sent 6 July 2023)
- 4.3 2022-000691-19 SRP-9001 BELGIUM Summary of information for the public_FINAL_– and version NL_clean and FR_clean
- 5.1 2022-000691-19 BN43881_Belgium Hygiene Guidance v3.0 – Final_18April2023
- 5.3 2022-000691-19 BN43881_Final_Belgium_Addendum to pharmacy & dose administration manuals v2.0_FINAL

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 (BN43881 Protocol version 2), and all the safety instructions as described in the dossier and the updated and new documents listed here above. Regarding the instruction for patients with respect to donation of *blood, organs, tissues, and cells for transplantation*, and referring to the notifier's commitment, relevant text in the protocol needs to be adapted and protocol clarification letter shall be distributed to all sites immediately following the approval clinical trial.
- Any protocol amendment has to be previously approved by the Competent Authority.

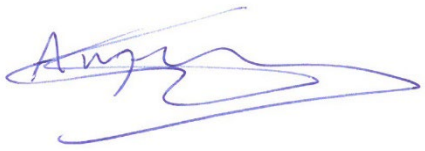
- The notifier is responsible to verify that the study centre has qualified personnel experienced in handling infectious material and that the investigator has the required authorizations to perform the clinical trial activities inside the hospital (laboratory, pharmacy, hospital room, consultation room...) according to the Regional Decrees transposing Directive 2009/41/EC on Contained use of genetically modified micro-organisms.

- At the latest 15 days after the start of the trial, the notifier should provide, along with the delivery of the control sample, a detailed protocol for the method of conservation and analysis of the control sample.

- 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 shall 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 RO7494222 (SRP-9001);

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/22/BVW5 (ref. SC/1510/BAC/2022_1375 and SC/1510/BAC/2023_0300)

Annex II: Answers to the public reaction to dossier B/BE/22/BVW5 in NL (ref. SC/1510/BAC/2023_0309) and FR (ref. SC/1510/BAC/2023_0310)

Adviesraad voor Bioveiligheid
Conseil consultatif de Biosécurité

Compilation of comments of experts in charge of evaluating the dossier B/BE/22/BVW5
And comments submitted to the notifier

28 November 2022
Ref. SC/1510/BAC/2022_1375

Mandate for the Group of Experts: Mandate of the Biosafety Advisory Council (BAC) of 14 September 2022.
Coordinator: Anton Roebroek (KULeuven)
Experts: Rik Gijsbers (KULeuven), Nicolas van Larebeke-Arschodt (UGent, VUB), Willy Zorzi (ULiège), Liliane Tenenbaum (Lausanne University Hospital)
SBB: Sheela Onnockx and Katia Pauwels

INTRODUCTION

Dossier **B/BE/22/BVW5** concerns a notification from F. Hoffmann-La Roche Ltd 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 21 October 2022 and concerns a clinical trial entitled "*A two-part, open-label systemic gene delivery study to evaluate the safety and expression of ro7494222 (srp-9001) in subjects under the age of four with Duchenne Muscular Dystrophy*". The investigational medicinal product, also known as SRP-9001, is a AAV rh74-derived recombinant replication deficient vector carrying a truncated dystrophin encoding gene.

♦ 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 28-11-2022 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.

Comment 4

The description of the 3 plasmids used for transfection as well as their quality control is described in file "4.1 2022-000691-19 20221010_BN43881 EU CAF- BEL CONFIDENTIAL". However I could not find the description of the viral vector production, purification, titration and quality control, except for the assessment of the presence replication-competent viral particles.

SBB's comment:

Viral vector production, purification, titration and quality control except for the assessment of the presence of replication-competent viral particles are addressed within the quality assessment of the proposed trial and goes beyond the scope of the environmental risk assessment or the biosafety assessment of the proposed trial.

Coordinator's comment:

Agreeing with the SBB's comment.

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.

Comment 4

Since the rep2/cap rh74 plasmid has a large intron sequence in the middle of the rep sequence, as expected, the applicant did not find any rc virus in their assay based on adenovirus-infected HEK293 cells. However, they used as a positive control a wt AAV2 virus which is well known to infect HEK293 cells. Did the applicant tested whether wt AAV rh74 replicates on HEK293 cells in the presence of Ad5? This is crucial to validate the assay.

SBB's comment:

The expert's comment pertains to the validation of the assay to assess the potential of generating replication competent particles. As pointed out by the expert, the following question could be addressed to the notifier:

No replication–competent particles were found using an assay based on adenovirus-infected HEK293 cells. This was expected since the rep2/cap rh74 plasmid has a large intron sequence in the middle of the rep sequence. However, it is also noted that wt AAV2 has been used as a positive control which is well known to infect HEK293 cells. Did the notifier tested whether wt AAV rh74 replicates on HEK293 cells in the presence of Ad5? This is considered crucial to validate the assay.

Coordinator's comment:

In the coordinator's opinion, paragraph 2.2 of the confidential CAF (page 6) describes an assay to determine the presence of rcAAV in SRP-9001 via detection of AAVrh74 CAP DNA sequences by qPCR. Wild-type AAVrh74 control is used as positive control in the assay, but also for determination of the Limit of Detection (LOD). Wild-type AAVrh74 is also used as spike control in this assay to ensure the absence of inhibition.

Paragraph 2.2.1 of the confidential CAF (page 6) describes in fact a second assay to determine the presence of rcAAV in SRP-9001 via detection of Rep2 DNA sequences by qPCR. Here, wtAAV2 is used as positive control and spike. It should be noted, that in this paragraph the target of the qPCR (Rep2 DNA sequences) was not specified, but had to be deduced from the text presented in paragraph 2.2.2 of the confidential CAF.

The coordinator's interpretation of paragraph 2.2 clearly answers the question by the expert: wt AAVrh74 replicates on HEK293 cells in the presence of Ad5.

In the coordinator's opinion the text of paragraph 2.2 is difficult to interpret, especially also because the title of paragraph 2.2.1 is misleading. This title suggests that the actual description of the method in detail is presented in this paragraph, whereas paragraph 2.2 should be read as a general introduction to the assay to determine presence of rcAVV. Paragraph 2.2 presents, however, important details on a first part of the assay (P1 = first part of a two-part passage assay?; P2 described in 2.2.1?). The text could surely be improved.

SBB's comment on the coordinator's comment

The coordinator's comment clarifies the answer asked by the expert and make the first SBB comment obsolete. It also shows that the readability of section 2.2 of confidential CAF should be improved.
The notifier could be asked to improve the text, taking into account that :

- it needs to be clear that in paragraph 2.2.1 a second assay is described, which aims at detecting Rep2 DNA, instead of detecting AAVrh74CAP as described for the first assay in the introduction of 2.2.

- the first sentence of paragraph 2.2.1 ' *This assay is used'* is particularly misleading as the assay described in 2.2.1 is not the same as the one described in the introduction of 2.2. The subtitle 2.2.1 is also misleading because one could erroneously think that paragraph 2.2.1 describes the method for the assay detecting CAPrh74 that is described in the introduction of 2.2.
- The sentence In the introduction of 2.2. '*The use of a wild-type AAVrh74 control enables the Limit of Detection (LOD) of the assay to be determined'* could be erroneously associated with the numeric value of the LOD/LOQ in Tabel 1 of paragraph 2.2.2
- Can the notifier provide a numeric value for the LOD associated with the assay targeting CAPrh74 (introduction 2.2)?

2nd Coordinator's comment on additional SBB comment

In the public CAF (2.2, page 5) literally the same text is presented as in the confidential CAF. This part of the public CAF also needs improvement in the context of the improvement of this part in the confidential CAF.

The text in confidential CAF 2.2.1 is literally identical to a text in document '5.01 2019-003374-91 SRP-9001-QIMPD v03' (paragraph 2.16, page 277) of dossier B_BE_21_BVW5 (Substantial Amendment : Investigator Brochure for B/BE/21/BVW5 – (EMBARK study)- ddl 13 oktober). It should be noted that in this document no assay is mentioned to use detection of AAVrh74CAP by qPCR as means to analyze the presence of rcAAV in SRP-9001 as is referred to in the confidential CAF 2.2. Is this assay for the detection of AAVrh74CAP by qPCR described in another part of the SRP-9001 dossiers?

N.B. the document '5.01 2019-003374-91 SRP-9001-QIMPD v03' was not communicated to the experts evaluating dossier B_BE_2022_BWV5.

2.3. Diagram (map) of the clinical vector

Comment 1

Has evaluated this item and has no questions/comments.

Comment 2

Can the REP gene not be reconstituted in the HEK cells that are used to produce the rAAV (SRP-9001) (be it only rarely) by excision of the human collagen intron resulting in the presence of this gene in an AAV particle or even in the presence of replication competent AAV?

SBB's comment:

According to the confidential CAF document, page 10/27, the pNLREP2-Caprh74 is an AAV RepCap plasmid which encodes wild-type AAV2 rep proteins and wild-type AAV capsid proteins from serotype rh74. A 3kb human collagen intron has been introduced into the middle of Rep coding sequence, to prevent formation of replication competent AAV during SRP-9001 production.

According to the confidential CAF document, page 5/27, encapsidated AAV vector DNA has been completely sequenced as part of clinical product release testing. Rep reconstitution is expected to be revealed during the sequencing and may therefore not be accepted for release.

Also section 2.2. of the confidential CAF document briefly describes the detection and quantification of replication competent AAV as part of the acceptance criteria for drug product release.

Coordinator's comment:
Agreeing with the SBB's comment. The tests performed do not reveal excision of the human collagen intron resulting in the presence of the Rep gene in an AAV particle present in SRP-9001.

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

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

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

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

Comment 2
Is it conceivable that in the process of loading the AAV particles with micro-dystrophin gene other genes might be incorporated in the AAV particles? Is there a possibility to asses quantitatively this occurrence? Possibly the statements "It can't be elucidated whether the potential presence of co-packaged/encapsidated plasmid or host cell derived DNA is accounted for in the current testing strategy – this should be addressed." And "The percent of rAAV that can contain co-packaged DNA different to the desired one, such as residual plasmid DNA "in the EMA advice (EMADOC-1700519818-74104) relates to this issue.

How come the mention "pending" in tables 7, 9 and 11?

SBB's comment:
According to the confidential CAF document, pag5/27, encapsidated AAV vector DNA has been completely sequenced as part of clinical product release testing.
The requirements for RCA testing are determined by the European Pharmacopoeia, and are within the remit of the quality control assessment of the clinical trial application (see https://www.ema.europa.eu/en/documents/scientific-guideline/guideline-quality-non-clinical-clinical-aspects-gene-therapy-medicinal-products_en.pdf) and go beyond the tasks of the Biosafety Advisory Council. It is also noticed that the EMA scientific advice, although it is part of the documents that has been submitted along with the dossier, should be seen as a back ground document and is confidential.

Tables 7, 9 and 11 of the confidential CAF document refers to stability data of the three plasmids used to manufacture SRP-9001 AAV vector and is information belonging to the quality aspect of the product.

Coordinator's comment:
Agreeing with the SBB's comment.

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

Comment 4

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
See 2.4

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

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

2.6. Biodistribution and shedding

(e.g. shedding data, administered dose, route of administration, biodistribution data, methods used for detection of viral shedding….)

Comment 1
On p109/115 in 3.1 2022-000691-19 Delandistrogene moxeparvovec IB Version 7.0 - 16May2022.pdf the applicant provides info that shedding and transmission patterns are currently being assessed. The info available should be included. They indicate that shedded rAAV is detected 'several weeks' following injection. This is subjective wording, please provide specific fluids and time frames for shedding and detected concentration (with method of detection) to allow proper judgements whether shedding is relevant for ERA.

SBB's comment:
Section 2.6 "Biodistribution and shedding" of the confidential CAF document provides preliminary shedding results from clinical study 103 encompassing data on mean peak DNA vector (concentration and time indication) in saliva, urine and feces.
In addition to these preliminary results, the notifier could be asked to give the most recent update on shedding analysis of the ongoing study.

From an ERA perspective it is also relevant to answer the question whether the observed shed vector DNA (or a remnant thereof) reflects integrated vector DNA in shed cells or whether it consists out of remaining or rescued replication-deficient viral vector particles.
It is probable that more shedding data as well as the nature of shed vector DNA were not available yet at the time of the current biosafety dossier submission. Therefore, the notifier could be requested to provide an update of shedding data and to inform whether any other analysis will be undertaken to examine the nature of the shed vector DNA.

In section 2.6 "Biodistribution and shedding", on page 5/17 of the Public CAF document, nothing has been reported regarding the shedding results. The notifier should have briefly mentioned in the public CAF document that shedding is occurring. It is also noted that the document 5.3 Belgium Hygiene

Guidance, which provides instructions for patients, close contacts and caregivers, explicitly states that viral particles can be passed through bodily fluids for several weeks after infusion.

Coordinator's comment:
Agreeing with the SBB's comment.

Further, subject are not allowed to give blood for 2 years, which is not relevant I guess since subjects are under 4 years. The latter info (2 years no donation of blood) is also contradictory to the info provided 4.1 2022-000691-19 20221010_BN43881 EU CAF- BEL CONFIDENTIAL.pdf (p22/27), where it is indicated that they cannot donate blood/cells/tissues, …(here it should be indicated whether this is live-long or limited in time).

SBB's comment:
According to the SNIF, page 13/19, patients are prohibited from donating blood for two years following the vector injection. However, according to the protocol, page 38/122, participants are prohibited from donating blood and blood products for 1 year following the infusion. The public CAF document mentions on page 12/17 that trial subjects are not eligible for donating blood/cells/tissues/organs per the clinical protocol but no period of time is reported. The notifier could be requested to adapt the documents where applicable to render the information regarding the donation of blood, cells, tissues and organs and the duration of prohibition as consistent as possible throughout the different documents.

Coordinator's comment:
Agreeing with the SBB's comment.

At the same page: as to the potential of cancer development and integration of the rAAV vector, (even though not explicitly ERA), the current phrasing at p109 is euphemistic. I would surely consider the most recent FDA/ASGCT recommendations (https://asgct.org/global/documents/advocacy/2021-fda-liaison-meeting/final-aav-integration-slides.aspx), particularly when high doses are applied. In the current disease the benefits probably outweigh the risk, however, this cannot per se be extrapolated to any other rAAV treated condition.

SBB's comment:

The expert is referring to p109 of the Investigator's Brochure in which a section is dedicated to carcinogenicity. This section is referring to rAAV2 vectors used in haemophilia gene therapy studies.

We also refer to the SBB's comment under section 6, comment 2 here below for further considerations on the environmental risk aspects.

Coordinator's comment:
Agreeing with the SBB's comment.

At p110/6.12.3. the applicant indicates that "the AAVrh74 vector in conjunction with the concommitant viral infection could lead to replication of SRP-9001". This is not correct. SRP-9001 can be packaged but will not replicate. Please adapt wording.

SBB's comment:

There is a potential hazard that the clinical vector would acquire replication competence as a result of simultaneous triple infection of a cell with the clinical vector, a wild-type AAV virus and a helper virus, such as adenovirus, herpes simplex virus, pseudorabies virus and human papilloma virus. The likelihood of simultaneous triple infection can be considered very low and rescue experiments have also been discussed at p110 of the IB.

Coordinator's comment:
Agreeing with the SBB's comment. SRP-9001 is by definition replication-defective, but its genome can be replicated and subsequently packaged by co-infection/superinfection by particular other viruses.

On p16/27 in 4.1 2022-000691-19 20221010_BN43881 EU CAF- BEL CONFIDENTIAL.pdf the vector doses are indicated wrongly ("treatment dose of 1.33x1014 vg/kg" should be 10e14). Again in this document the info on shedding is incomplete.

SBB's comment:
The wrongly indicated dose (1.33x1014 vg/kg" should be 1.33 x 10e14) could be addressed to the notifier under the section '**Typos and other errors/omissions'.**
With respect to the remark that shedding data are incomplete, we would like to refer to SBB's comment under section 2.6 , comment 1 (here above).

Coordinator's comment:
Agreeing with the SBB's comment.

Comment 2
- To what extent the MHCK7 promoter is specific for muscles?
- 2.6.1".Biodistribution of non-muscle cells should not result in transgene expression due to the use of a skeletal and cardiac specific promoter." There will probably be some expression
- I suppose that NHP means Non-Human Primates

SBB's comment:
- According to Skopenkova et al (2021), the chimeric promoter MHCK7 was developed to achieve a high expression level of the transgene in the cardiac muscle. The promoter MHCK7 ensured a transgene expression level comparable to those for the CMV and RSV promoters in skeletal and cardiac muscles. Low expression levels were observed in the liver, lungs, and spleen after AAV6 had been intravenously injected to mice. The MHCK7 promoter was 400 and 50 times more active in the heart and the diaphragm, respectively, than promoter CK6.
- According to section 2.6.1 of the confidential CAF, page 16/27, "biodistribution of non-muscle cells should not result in transgene expression due to the use of a skeletal and cardiac specific promoter". Since low expression cannot be excluded, the notifier could be requested to adapt the sentence accordingly.
- Indeed, NHP means Non-human primate.

Coordinator's comment:
Agreeing with the SBB's comment.

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

Comment 4

The dose administered (1,33 x10e14) is considered as a very high AAV dose which has proven to be toxic in other clinical trials (ref Kishimoto and Samulski, 2022. DOI: 10.1080/14712598.2022.2060737 and references therein).

The clinical safety is not considered in the present evaluation. However, with such high intravenous doses, the amount of virus shed in the body fluids is expected to be high. The applicant shows that virus can still be found in urine and saliva at 4 weeks after administration. They mention that the shedding study is still underway. It will be important to evaluate whether the measures taken, such as eliminate diapers in a double bag and washing hands with soaps are sufficient to prevent dissemination. For example, are the patients kept at distance from other children for 4 weeks (or longer)? Even if the probability of an adverse effect in siblings is extremely low if not absent, a contamination with AAV rh74 capsids could potentially elicit an antibody response that would preclude inclusion in a gene therapy trial.

SBB's comment:

The notifier provided a document 5.3 2022-000691-19 BN43881_Belgium Hygiene Guidance v1.0_Final_15June22 describing the instructions patients and caregivers should follow during the first 4 weeks after infusion of the IMP, including double-bagging of dirty diapers before disposal and washing hands thoroughly with soap and water after handling potentially contaminated materials. One of the recommendations is also to minimize contact of untreated sibling with the treated child (for example, sharing foods or drinks) and to ensure that the treated child plays with his own toys and doesn't share them with friends or family.

According to the SNIF page 18/19, the public CAF page 11/17, the protocol pages 38/122 and 106/122 and Post-Infusion Hygiene Guidance (Belgium) document, family members and caregivers will be instructed to practice good hand-hygiene after the product administration for up to 4 weeks after RO7494222 (SRP-9001) administration. However, given that Zaidy et al (2019) recommend caregivers to practice good hand hygiene for approximately 60 days after the injection with adeno-associated viral (AAV) vector containing the human SMN gene, the BAC recommended in its advice for biosafety dossier B/BE/21/BVW5 also using SRP-9001, to increase the period of time to practice appropriate hand hygiene for at least 60 days. As a precautionary measure and as it has already been adapted for the dossier B/BE/21/BVW5, the notifier could be requested to increase the period of time to practice appropriate hand hygiene to at least 60 days following IMP injection and to adapt the Post-Infusion Hygiene Guidance (Belgium) document and the Investigator's Brochure (p109, section 6.12.1) accordingly.

According to point 5 of the Post infusion hygiene guidance (Belgium) document, the disposal of double-bagged diapers should follow hospital instructions. Since there is only one hospital involved for Belgium, the notifier could be asked to specify what will be the recommendation given to caregivers in this regard and to report it directly in the Post infusion hygiene guidance (Belgium) document.

According to point 6 of the Post infusion hygiene guidance (Belgium) document, hands should be washed with soap after handling potentially contaminated cloth and launderable materials, such as clothing, linens, pillow, and blankets. Since gloves should be used when disposing of potentially contaminated materials, the notifier could be requested to mention that when handling potential contaminated cloths and launderable materials, gloves should also be worn.

Another remark pertains to the disposal of used gloves. According to the public CAF document, page 11/17, family members and caregivers will be instructed to use appropriate protective gloves if coming

into direct contact with bodily fluids and waste of the treated individual. However, no instruction has been provided on how to manage potential contaminated gloves. The notifier could be asked to specify in the Post infusion hygiene guidance (Belgium) document how the gloves used by the patients, patient's family and caregivers should be disposed of.

Coordinator's comment:
Agreeing with the SBB's comments

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
On p18/27 in 4.1 2022-000691-19 20221010_BN43881 EU CAF- BEL CONFIDENTIAL.pdf storage is explained. It is not clear whether this is an ATMP compatible area. What is the BSL of the room? How will the product be thawed (LAF?)?

SBB's comment:
Containment level 1 (L1 and HR1) has been allocated to the different rooms where the IMP will be handled (storage, preparation and administering). This information has been provided in the context of the biosafety dossier 'contained use' that 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's comment:
Agreeing with the SBB's comment. Details on the safe storage and handling of the IMP are also described in 5.1 2022-000691-19 BN43881_Pharmacy Manual_V2.0_Final_19May2022

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

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

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

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

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

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

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

Comment 3
See the comments in point 3.6.Measures to prevent dissemination into the environment of this evaluation

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

3.5. Reconstitution, finished medicinal product and administration to the patients

(e.g. mode of administration, information on dosing and administration schedule, information on concomitant medication,…)

Comment 1
On p19/27 in 4.1 2022-000691-19 20221010_BN43881 EU CAF- BEL CONFIDENTIAL.pdf : it is not clear why the product should be provided to the within 12 hrs. Since there is a single vial per patient, the timing is not adequate and should be substantially shortened (thereby preventing other issues such as spill following preparation).

SBB's comment:
It could be considered to add the expert's consideration (to shorten the time frame to less than 12h) as a minor remark to the list of questions/remarks addressed to the notifier.

Coordinator's comment:
Agreeing with the SBB's comment.

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

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

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

3.6. Measures to prevent dissemination into the environment

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

Comment 1
On p20/27 in 4.1 2022-000691-19 20221010_BN43881 EU CAF- BEL CONFIDENTIAL.pdf : 'Any spills and accidents (e.g. accidental needle stick) resulting in potential exposure of AAV to individuals will be reported to the Principal Investigator and the site Occupational Health Services equivalent within 24 hours. More details are described in the pharmacy manual.' This information should be clearer. The personnel should know how to clean up spills and PPE should be explained well in advance. In particular, a 24 hrs time-frame is not acceptable, potential exposure should be reported as soon as possible as to initiate treatment of the personnel as quickly as possible after having judged the potential risk.

SBB's comment:
It is proposed to include the expert's remark into a request to the notifier to draft a 2-4 page technical sheet (see text proposal below).

Coordinator's comment:
Agreeing with the SBB's comment.

Double gloving should not be considered but included as a standard. I would not leave open to the personnel to decide how PPE is applied.

SBB's comment:
It is proposed to include the expert's remark into a request to the notifier to draft a 2-4 page technical sheet (see text proposal below).

Coordinator's comment:
Agreeing with the SBB's comment.

Please specify the concentration of the active ingredient in the bleach solution. (point c.3)

SBB comment:
A concentration of active ingredient in the bleach solution has been mentioned on p18/27 of the Pharmacy manual in the context of decontamination of surfaces: a 1:10 dilution of 5.25% sodium hypochlorite (bleach) solution, per policy. Ideally, information on the concentration of the active ingredient in the bleach solution should be indicated where ever the use of bleach solution is indicated. In line with the expert's remark the notifier could be requested to draft a 2-4 page technical sheet (see text proposal below,) in which concentration of active ingredient in the bleach should be sufficiently detailed.

Coordinator's comment:
Agreeing with the SBB's comment.

Text proposal for requesting 2-4 page technical sheet comprising relevant handling instructions

In addition to doc 5.1 Pharmacy manual, the notifier provided a one page sheet (doc 4.1a handling RG1-IMP-pharmacie clinical trial sector) giving an overview on how the IMP should be handled in the storage room, the preparation zone and the infusion room, including instructions for transport between the different areas. The one page document has the benefit that it can be used as a hands-one document. However, as pointed out by the expert, it fails to give more detailed handling instructions for the personnel in case of spill or other risk management measures. Therefore, notifier could be asked to provide a 2-4 page technical sheet comprising all relevant handling instructions, which can be used as a hands-on document for the personnel, and which can easily be consulted in addition to the pharmacy manual.

In meeting this request of providing a 2-4 page technical sheet, and also considering the remarks of other experts here below (see comment 2 and 3), the notifier could be asked to give due consideration to :

- 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.

-Bleach solution and alcohol can react and can produce toxic vapors such as chloroform. The combination of bleach and the use of alcohol wipes should be avoided (cfr step 3 of procedure of handling spills : '*Carefully pour disinfectant (bleach solution followed by alcohol wipes) over the absorbed spill, again starting at the edges. Saturate the area with disinfectant)*. The notifier is requested to verify and include the procedure cited in the « NIH/CDC guidance for handling of biosafety level 1 agents » used as reference in the present section. The notifier is also requested to specify the disinfectant.

- Hypochlorite solution cannot be proposed as a universal decontaminant or disinfectant because contaminated work surfaces may have different properties: porous and nonporous materials, stainless steel, solid surface, floor or table. A list of adequate of decontamination / disinfection solutions should be provided.

- the use of personal protective equipment for health care workers (e.g. which PPE are mandatory).

-The use of double gloves should be standard, and not only considered as currently stated on p20 in document CAF- BEL CONFIDENTIAL (point 3.6.b) and on p18 in the Pharmacy manual.

- Lab coats should be personal, and dedicated for the specific room where the rAAV is applied to prevent spreading to the environment.

- procedure in the event of accidental occupational exposure : the 24 hrs time-frame is not acceptable. Reporting should be reported as soon as possible in order to initiate treatment of the personnel as quickly as possible after having judged the potential risk.

- the procedure in case of accidental spill should be more detailed than in the Pharmacy manual and indicate that

- o before readily evacuating the area, contaminated PPE like labcoat, shoes, or others clothing, should be removed and should not leave the area.
- o after having evacuated the area, a sign should be posted indicating that entry is forbidden and that wearing of appropriate protective clothes is mandatory when entering again the area to initiate cleaning the spill and decontaminate the surfaces.

- risk management procedures used in case of needle-stick injury or the formation of aerosols.

- procedures to prevent and to deal with exposure to blood, urine, vomit or other bodily fluids from patients in the initial period where there are high numbers of transduced cells after infusion

These instructions should be provided as a separate document so as to ensure that study staff can use it as an hands-on document.

For consistency reasons, the notifier should also amend, detail or align all relevant documents making part of the dossier in accordance with the information that is requested as part of the requested 2-4 page technical sheet so as to obtain consistency throughout all the documents. In particular, this includes a verification of the following documents :

- o 4.1 2022-000691-19 20221010_BN43881 EU CAF- BEL CONFIDENTIAL

- 4.1 2022-000691-19 20221010_BN43881 EU CAF- BEL PUBLIC
- 5.1 2022-000691-19 BN43881_Pharmacy Manual_V2.0_Final_19May2022
- 4.3 2022-000691-19 SRP-9001 BELGIUM Summary of information for the public_clean – version in EN, FR and Dutch.

Coordinator's comment:
Agreeing with all the SBB's comments and suggested requests to the notifier as discussed above.

Comment 2

3.6.a "The administration site will be disinfected to minimize the environmental spread of the recombinant organism before preparing the final volume required based on the patient weight." I do not understand what is actually meant here

SBB's comment:
The expert's comment relate to p19/27 of the document 4.1 2022-000691-19 20221010_BN43881 EU CAF- BEL CONFIDENTIAL. Possibly, 'administration site' refers to the place of intravenous injection of the IMP by means of a dedicated catheder.

Coordinator's comment:
Agreeing with the SBB's comment. The drug product consists consists of a target of 1.33 x 10E13 vg/mL of viral vector RO7494222 (SRP-9001). The final volume administered depends on the weight of the patient (1.33 x 10E14 vg/kg).

3.6.c "allow agents to settle for a minimum of 30 minutes " ." I do not understand what is actually meant here
According to the Laboratory biosafety manual (Fourth edition) of the World Health Organization, in case of potentially hazardous spill, all persons should immediately vacate the affected area and any exposed persons should be referred for medical evaluation. The room containing the spill should not be entered for a length of time that allows aerosols to be carried away and heavier particles to settle (30 minutes are generally required). Signs should be posted indicating that entry is forbidden. After the appropriate time, decontamination should proceed. Appropriate protective clothing and respiratory protection should be worn during the decontamination procedure.
The notifier could be requested to detail the procedure for accidental spill handling by including a waiting period just after the spill before the start of the decontamination steps, by posting a sign indicating that entry is forbidden and by recommending the wearing of appropriate protective clothes when entering again the area to initiate for cleaning the spill and decontaminate surfaces. See Text proposal for requesting 2-4 page technical sheet under comment 1, here above.

Coordinator's comment:
Agreeing with the SBB's comment.

3.6.e "of which the majority, if not all, is not considered "infectious"" Seems to me a strange statement as the viral vector is infectious

SBB's comment:

The IMP is a replication-defective AAV that is able to transduce cells. The IMP will remain replication defective after transduction as it lacks the rep and cap genes. It is inappropriate to state that the IMP, the viral vector, is infectious.

Coordinator's comment:

Agreeing with the SBB's comment.

Comment 3

p20 in the 4.1 2022-000691-19 20221010_BN43881 EU CAF- BEL CONFIDENTIAL document, in the section *3.6. Measures to Prevent Dissemination into the Environment. a.Control measures during reconstitution (if applicable), handling and administration. point c. Decontamination/cleaning measures after administration or in the case of accidental spilling (i.e. decontamination /cleaning measures of potentially contaminated materials, surfaces and areas). In addition, the disinfection procedures applied should be justified by providing evidence that the chosen method is sufficiently active against the clinical vector,* it is stated :

In case of accidental spillage of RO7494222 (SRP-9001) during the dose preparation and administration to the patient at the health-care provider, instructions provided by the Sponsor´s pharmacy manual will be followed to contain and immediately disinfect the spill to prevent further spread. All contaminated materials will be disposed of locally by incineration or autoclaving. All other places will be cleaned, according to normal decontamination procedures as per the NIH/CDC guidance for handling of biosafety level 1 agents and the Pharmacy Manual.

1. Evacuate area, remove contaminated PPE and allow agents to settle for a minimum of 30 minutes. Initiate spill response procedure.

2. Cover the spill with absorbent material. Starting at the edges and work towards the center.

3. Carefully pour disinfectant (bleach solution followed by alcohol wipes) over the absorbed spill, again starting at the edges. Saturate the area with disinfectant.

Expert's remarks:

1)It is written that: *In case of accidental spillage of RO7494222 (SRP-9001) during the dose preparation and administration to the patient at the health-care provider, instructions provided by the Sponsor´s pharmacy manual will be followed to contain and immediately disinfect the spill to prevent further spread.* Please precise the terms "immediately disinfect" and/or the procedure must be changed and/or completed by an addition of an awaiting step just after the spill, outside the contaminated zone (30-60min: time depending on the size of the spill that generates aerosols in order to avoid inhalation of aerosols or droplets) before the start of the decontamination steps.

SBB's comment:

It is remarked that the instruction to evacuate the area, to remove any contaminated PPE and allow agents to settle for a minimum of 30 minutes has been specified on p19/37 of the Pharmacy manual in the context of handling spills.

In line with the expert's remark the notifier could be requested to draft a 2-4 page technical sheet (see text proposal above, section 3.6 , under comment 1) in which procedures for handling spills are sufficiently detailed.

Coordinator's comment:

Agreeing with the SBB's comment.

2) In the step 3 of procedure in case of spill here described, please consider that bleach solution and alcohol can react and produce toxic vapour as chloroform…Therefore, the association between the bleach and alcohol wipes could not be recommended.
Concerning this point, could the notifier produce the procedure cited in the « NIH/CDC guidance for handling of biosafety level 1 agents » used as reference in the present section?

SBB's comment:
The expert's remark is implemented in the proposal to request the notifier to draft a 2-4 page technical sheet (see text proposal above, section 3.6 , under comment 1) in which procedures for using bleach solutions should be sufficiently detailed.

Coordinator's comment:
Agreeing with the SBB's comment.

3) Bleach solution cannot be proposed as universal treatment because the work surfaces to be disinfected/decontaminated can present different properties: porous and nonporous materials, stainless steel, solid surface, floor or table…

4)Please precise the disinfectant used in the step 3 of the present procedure: is it again Alcohol (if yes, which one) or if, in case of a different one, which disinfectant?

5)Please complete by a list of decontamination / disinfection solutions with their protocols and application scope.

SBB's comment:
Remarks 3, 4 and 5 of the expert are implemented in the proposal to request the notifier to draft a 2-4 page technical sheet (see text proposal above, section 3.6 , under comment 1).

Coordinator's comment:
Agreeing with the SBB's comment.

p5 of the 4.3 2022-000691-19 SRP-9001 BELGIUM Summary of information for the public_clean English patient number highlighted document in the section 6. The proposed measures to limit the potential risks, to control and to ensure follow-up of the deliberate release. point Decontamination/cleaning measures after administration or in the case of accidental spilling (i.e. decontamination /cleaning measures of potentially contaminated materials, surfaces and areas). In addition, the disinfection procedures applied should be justified by providing evidence that the chosen method is sufficiently active against the clinical vector.
The following paragraph is structured with the same contents as mentioned here above (repetition) and is to be modified following the same recommendations reported in the 5 remarks here above.

p7 of the "4.3 2022-000691-19 SRP-9001 BELGIUM Summary of information for the public_clean_FR-BE PR clean" document in point 6. Mesures proposées pour limiter les risques potentiels, contrôler et assurer le suivi de la libération délibérée in the section Mesures de décontamination/nettoyage après l'administration ou en cas de déversement accidentel (c.-à-d. mesures de décontamination/nettoyage

des matériaux, surfaces et zones potentiellement contaminés). De plus, les procédures de désinfection appliquées doivent être justifiées en apportant la preuve que la méthode choisie est suffisamment active contre le vecteur clinique.
The following paragraph is structured with the same contents as mentioned above (repetition) and is to be modified following the same way (see the 5 remarks above).

p19 of the "5.1 2022-000691-19 BN43881_Pharmacy Manual_V2.0_Final_19May2022" document in the section "Handling Spills" point 3. Carefully pour disinfectant (bleach solution followed by alcohol wipes) over the absorbed spill, again starting at the edges. Saturate the area with disinfectant.
For the comment, please refer to Remarks 2) to 5) above.

SBB's comment
The last three remarks of the expert here above are implemented in the proposal to request the notifier to draft a 2-4 page technical sheet (see last paragraph of text proposal under section 3.6, under comment 1).

Coordinator's comment:
Agreeing with the SBB's comment.

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

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 not evaluated this item

Comment 2
I regret that no specific data are provided. See my note under paragraph 6

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

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

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

Comment 1
None

Comment 2

Although this gene therapy approach is promising, I am of the opinion that rAAV SRP-9001 is not yet ready for large scale use on humans.

I am, on the basis of the available information not convinced that transmission through the germ line is excluded. Also I think that issues related to fertility and toxicity to early life are not properly addressed. But my main point of concern are possible genotoxic and carcinogenic effects. Nakai et al. (2003) found that AAV serotype 2 vectors preferentially integrate into active genes in mice. A small but growing number of murine studies have documented that adeno-associated viral gene delivery can result in insertional mutagenesis (Chandler et al., 2017). This insertional mutagenesis might contribute to the risk of cancer. That insertional mutagenesis by Adeno associated viruses can indeed lead to carcinogenesis un humans is suggested by the findings of Nault et al. (2015). Nault et al. (2015) observed clonal integration of adeno-associated virus type 2 (AAV2) in 11 of 193 human hepatocellular carcinoma's. These AAV2 integrations occurred in known cancer driver genes, namely CCNA2 (cyclin A2; four cases), TERT (telomerase reverse transcriptase; one case), CCNE1 (cyclin E1; three cases), TNFSF10 (tumor necrosis factor superfamily member 10; two cases) and KMT2B (lysine-specific methyltransferase 2B; one case), leading to overexpression of the target genes. Tumors with viral integration mainly developed in non-cirrhotic liver (9 of 11 cases) and without known risk factors (6 of 11 cases), suggesting a pathogenic role for AAV2 in these patients. They concluded that AAV2 is a DNA virus associated with oncogenic insertional mutagenesis in humans. In an interesting recent review by 14 authors, many of them linked to pharmaceutical firms, it was considered that overall, the frequency of AAV integration is low and the risk of malignancy appears to be theoretical given that no cases of cancer associated with rAAV have been reported in humans to date (Sabatino et al., 2022). Sabatino et al. also considered that, despite their extensive use in biomedical research and drug development, animal models can be poor predictors of human disease. However, we need to realise that in terms of carcinogenesis, one of the most fundamental diseases of multicellar organisms, mammals and humans are very similar. That no cases of cancer associated with rAAV have been reported in humans to date has only a limited meaning in view of the long latency period of cancer in humans and of the fact that duration of exposure to a carcinogenic agent is much more important than the dose (Peto, 1986; Peto et al., 1991a; Peto et al., 1991 b)

SBB's comment

Should the IMP reach marketing authorisation, it is intended as a gene therapy for the treatment of Duchenne Muscular dystrophy, which is a X-linked recessive condition and rare disease with an incidence of 19.7 per 100,000 male live births and 1 in 5076 live born males (Kariyawasam *et al*., 2022).
In the conclusion, Sabatino et al 2022 acknowledge the importance of the ongoing discussion of rAAV integration and the need to better understand any potential risk of genotoxicity for human beings. As pointed out by another expert (see section 2.6, comment 1), the most recent FDA/ASGCT recommendations (https://asgct.org/global/documents/advocacy/2021-fda-liaison-meeting/final-aav-integration-slides.aspx) could also be considered in this respect.
Integration site analysis of samples of participants in clinical trials, encompassing samples from different tissues, could aid to have a better insight in the assessment of rAAV integration and its clinical significance, provided due account is given to the possible effect of the dose, rAAV vector design and mitotic potential of transduced cell that may impact conclusions on potential of genotoxicity.
The above considerations predominantly pertain to the clinical safety assessment of the trial and the safety of the patient. From the perspective of environmental risk assessment (ERA), individuals other than the patient will be exposed to much lower concentrations of viral vector particles as compared to the vector dose administered by intravenous injection for patient treatment. From an ERA perspective, possible adverse effects for the human population at large (environmental risk) associated to the clinical

trial and the probability of insertional mutagenesis should focus on the potential of germline transmission. However, in the context of the current trial application, only patients under 4 year of age will considered for inclusion in the clinical trial, which also explains why no contraceptive requirements have been set.
The studies that have been performed to examine possible germline transmission have been summarized in Tabel 4 of the Investigator's Brochure version 7.0 (cfr study SR-20-14 and study SR-20-15). It is also remarked that during the evaluation of dossier B_BE_21_BVW4 (for a clinical trial involving the use of SRP-9001 in patients ≥ 4 to < 8 years of age) the notifier elaborated on the approach taken and the study design of SR-20-14 (e.g. timepoint of samples taken, statistical power, etc) and on numerical data of SR-20-15) as a response to two additional question of the Biosafety Advisory Council.

Coordinator's comment:
Agreeing with the SBB's comment.

Comment 3
None

Comment 4
None

Typos and other errors/omissions :

SNIF page 8/19: RO7494222 (SRP-9001) is a non-replicating, recombinant adeno-associated virus (rAAV) containing a human micro-dystrophin gene under the control of the MHCK7 promotor/enhancer, has been optimized for driving expression in cardiac and skeletal muscle. The word "that" is missing before "has been optimized". The notifier could be requested to update this sentence.

Coordinator's comment:
Agreeing with the SBB's comment.

References

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Zaidy & Mendell : Clinical Trials to Clinical Practice: Practical Considerations for Gene Replacement Therapy in SMA Type 1. Pediatric Neurology Volume 100, November 2019, Pages 3-11.

Adviesraad voor Bioveiligheid
Conseil consultatif de Biosécurité

Compilation of the expert's evaluations of the answers of F. Hoffmann-La Roche Ltd on the list of questions for dossier B/BE/22/BVW5

31 March 2023
Ref. SC/1510/BAC/2023_0300

Coordinator: Anton Roebroek (KULeuven)
Experts: Rik Gijsbers (KULeuven), Nicolas van Larebeke-Arschodt (UGent, VUB), Willy Zorzi (ULiège), Liliane Tenenbaum (Lausanne University Hospital)
SBB: Katia Pauwels

INTRODUCTION

Dossier **B/BE/22/BVW5** concerns a notification from F. Hoffmann-La Roche Ltd for a clinical trial entitled "*A two-part, open-label systemic gene delivery study to evaluate the safety and expression of ro7494222 (srp-9001) in subjects under the age of four with Duchenne Muscular Dystrophy*".

On 28 November 2022, based on a list of questions prepared by the BAC (SC/1510/BAC/2022_1376), 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 17 March 2023. This complementary information was reviewed by the coordinator and the experts in charge of the evaluation of this notification.

Remark: The comments below have served as basis for a list of questions that the Competent authority forwarded on 03-04-2023 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.

Evaluation SBB

Q1

While the overall readability of section 2.2.1 (p11) of the revised CAF confidential annex has been improved and a numeric value of LOD/LOQ has been included, it is noticed that the target sequences detected by qPCR for rcAAV testing are not mentioned anymore.

Section 2.2.2 of the same document refers to Table 5 whereas it should refer to Table 1 as included.

Coordinator's comment:

Agreed with the SBB's comment.

Q2

The SBB remarks that the applicant does not provide any new information on the nature of shed vector genome data. Instead, the applicant refers to data on vector genome DNA in saliva, urine, feces that were already included in a vector shedding report presented in the context of a substantial amendment of dossier B/BE/21/BVW5 (EMBARK study, also using SRP-9001). The coordinator for the EMBARK study and the secretariat of the Biosafety advisory Council (BAC) assessed this SRP-9001 Vector Shedding Report (shedding data obtained with an earlier study 9001-13), which was provided on 20 Dec 2022.

Coordinator's comment:

Correct.

Q5

The coordinator for the current ENVOL study and the secretariat of the Biosafety advisory Council (BAC) assessed shedding data that were provided on 20 Dec 2022 in the context of a substantial amendment for dossier B/BE/21/BVW5 (EMBARK study, also using SRP-9001). As a result, the proposal to limit the period to apply post-injection hygiene measures for caregivers to only 4 weeks was agreed upon. This period of 4 weeks was also deemed sufficient for the other studies with the SRP-9001 Vector (thus also for the current application B-BE-22-BVW5 study).

Coordinator's comment:

Correct.

Evaluation Rik Gijsbers

The applicant provided a document with replies, even though the large part of several replies was not indicated in the response document (which was a pity).
In the document, still the word 'virus' is used when 'viral vector' should be. This has been mentioned earlier, and in my opinion is important, especially in documents that are used to communicate with the public such as 5.2 2022-000691-19_BN43881_Belgium Hygiene Guidance v3.0_Final_Hospital instruction tracked changes.pdf, which indicates 'Although the risk is low, to protect yourself and caregivers and to minimize the possibility of spreading the virus to others'. This should be replaced by rAAV viral vector.

Q1. The applicants answered point by point and readability has improved.

Q2. The applicant indicates that SRP-9001 is not expected to survive when eliminated intact from the treated patient. I agree with the multiply and disperse point, but rAAV are quite stable and thus the product will 'survive' when shed. I agree with the applicant that the concentration of product is low relative to the amount injected, still the number seem substantial. Currently, it is difficult to judge what the concentration of rAAV in saliva, urine and faeces mean that are indicated in the Table (is this per ml sampled urine/saliva/faeces or is this in the conc in the prepared sample for qPCR (and what are the numbers then in the original sample?). Could this be provided in the legend of the Table? At p13 of 4.1.1 2022-000691-19_SRP-9001 BN43881 EU Common Application Form - Belgium Confidential Annex - FINAL.pdf it reads 'In feces, the mean peak vector DNA concentration was 133000000 vgc/ug at week 1 and declined at week 4 to 10622.7 vgc/ml.' How should be compare vgc/ug to vgc/ml?

Even though there is substantial drop in signal detected by week4, it is difficult to judge what this amount could do: would this be sufficient to elicit an immune response in a sibling (that thus will no longer be able to receive GT)?

Also, in the last sentence of the answer, again virus should be viral vector.

SBB's further information :

With respect to the 'vgc/ug to vgc/ml' conversion aspect, no further relevant information was found in a vector shedding report, which was provided on 20 Dec 2022 in the context of a substantial amendment of dossier B/BE/21/BVW5 (EMBARK study, also using SRP-9001). The same sentence is mentioned : '*In feces, the mean peak vector DNA concentration was 133,000,000 vgc/ug at Week 1 and declined at Week 4 to 10,622.7 vgc/ml"* . , Noteworthy, in a section describing shedding studies from a GLP study in mice in the same vector shedding report, and in section 2.6.2 of the revised confidential CAF document, the following is indicated : " *SRP9001 concentrations in excreta samples were determined by qPCR, reported in units of copies/100 µl for urine and* ***copies/ug DNA for feces****. The analyses were evaluated using validated DNA extraction and validated qPCR methodology following the FDA guidance for preclinical assessment of investigational cellular and gene therapy products (FDA 2013)."*

Coordinator's comment:
The coordinator also has the opinion that here (Week 4 to 10,622.7 vgc/ml) mistakenly the wrong unit was used.

With respect to the consequences of exposure to shed viral vector , it can be added that the elicitation of an immune response in a sibling following exposure is a potential hazard only in the case when the sibling would also be receiver of a AAVrh74 based or dystrophin based gene therapy. With respect to the overall risk evaluation, this potential hazard should also be considered along with the instructions for gene therapy receivers and their caregivers in the 5.2 2022-000691-19_BN43881_Belgium Hygiene Guidance v3.0_Final_Hospital instruction tracked changes.pdf. The instruction '*If your child has a sibling with DMD that has not received gene therapy, it is recommended for the untreated sibling to minimize contact with the treated child (e.g., sharing foods or drinks)'* is of particular relevance here.

Coordinator's comment:
Agreed with the SBB's comment.

Q3. Answered sufficiently. Still, as a minor comment, although important, I would like to mention that the document still contain reference to 'virus/viral' in sections where 'viral vector' should be used. For example in section 2.6.2 on shedding (viral load shed, viral shedding) on p13&14 in 4.1.1 2022-000691-19_SRP-9001 BN43881 EU Common Application Form - Belgium Confidential Annex - FINAL.pdf.

SBB comment:
If it is decided to finalize the advice of the BAC on this dossier and not to send a second list of questions to the notifier, the BAC could include in its advice a general consideration on the documents provided by the applicant by stating that there are still inaccuracies in the wording (e.g use viral vector instead of virus/viral; do not state that shed vector particles are not able to survive as recombinant AAV vectors are non-enveloped and quite stable in the environment).

Coordinator's comment:

Agreed with the SBB's comment.

Q4. The adaptation of the protocol to allow blood donation already at 6 months, whereas organ donation is disadvised seems odd to me. Also, the update is confusing. The applicant indicates to aim at aligning the period for blood, cells, tissues and organ donation across all documents, but only proposes to state a period of 6 months for blood, and to avoid organ donation. What about tissue and cells? What is the rationale for the 6 months period for blood and the avoiding of organ donation (can the applicant decide independently?

SBB comment :

Referring to the product information document (EPAR) of all EU marketing authorized medicinal products containing recombinant AAV (cfr EPAR document for Glybera, Zolgensma, Roctavian, Luxturna, Upstaza, Hemgenix), all indicate that 'Patients treated must not donate blood, organs, tissues, and cells for transplantation'.

As pointed out by the expert, the notifier could be asked to give a rationale why instructions could deviate from measures commonly taken for current EU marketing authorized medicinal products containing recombinant AAV.

Alternatively, the notifier could be asked to revise the instructions regarding blood, organs, tissues and cells and to align these with those that are prevailing for EU marketing authorized medicinal products containing recombinant AAV.

Coordinator's comment:

In the coordinator's opinion this issue should be addressed in a second list of questions.

Q5. This point requires specific attention. In my opinion, we cannot apply a general rule for all rAAV therapies about the timing to be considered. The shedding will be proportional to the dose provided to the patient (and volume) together with the route of entry. The dose for the current therapy is very high (and volumes large) compared to rAAV-SMN doses or doses supplied for Luxturna (in the eye). Even though I cannot propose a correct timing (if there would be any), I would apply a safety principle and be rather cautious then bold, and increase the period rather than lowering it (see also Q2).

Q6/7/8. The advice is thus to discard the diapers in normal house-hold waste, even in the hospital? Is this common practice? The double bagging indeed seems a good addition to protect also cleaning personnel that may take out the waste-bags.

SBB comment

The product information document (EPAR) of EU marketing authorized medicinal product Zolgensma involving the intravenous administration of recombinant AAV9 vectors (dose 1,1x 10E14 vg/kg) and reporting clearance of shedding in stool of patients within 30 days post administration also indicate that '*Disposable nappies can be sealed in double plastic bags and disposed of in household waste*'.

It is important that caregivers and families maintain and adhere to the instructions for 4 weeks long. As patients are likely to leave the setting soon after administration of the IMP, it could be beneficial to have instructions that are more likely to be adhered to during the 4-weeks period by caregivers and family, rather than impose measures that are less feasible to be implemented for this period of time.

Coordinator's comment:

Agreed with the SBB's comment.

Q9. Maybe I missed it, but I did not find the bleach details adapted (for example in 4.2 2022-000691-19 20220524_BN43881_SNIF - Belgium FINAL TC.pdf).

SBB's Comment:

The requests regarding the preparation and proper use of bleach solutions for decontamination has been detailed on p3 of the document 5.3 2022-000691-19 BN43881 Final Belgium_Addendum to pharmacy & dose administration manuals V2.0 final.

Coordinator's comment:
Agreed with the SBB's comment.

Evaluation Liliane Tenenbaum

Description of the production system and environmental risk assessment

The production system is well described. The final product has been evaluated for the presence of rcAAV. However, I could not find an evaluation of the presence of illegitimate plasmid DNA (illegitimate encapsidation of DNA from non vector sequences such as the kanamycin-resistance gene. See for a review: Magalie Penaud-Budloo, 2018, Mol Ther Meth and Clin Dev, Phamacology of recombinant Adeno-associated virus production. Few percent of such impurities, usually detected in rAAV batches, are relevant to dissemination of antibiotic-resistance genes into the environment. This information should be provided.

SBB's Comment:

An IMPD document is not explicitly requested in the frame of the environmental risk assessment of the IMP, hence the information package provided to the experts in charge of evaluating the ERA of this dossier does not comprise the IMPD document.

However, a document on quality aspects for SRP-9001, 5.01 2019-003374-91 SRP-9001-QIMPD v03, was made available to the coordinator and the SBB along with a substantial amendment for B/BE/21/BVW5 – (EMBARK study). This document encompasses data on the quality assessment of the manufacturing processes and drug product batches SRP-9001. According to 5.01 2019-003374-91 SRP-9001-QIMPD v03, vector plasmid titer in drug product batches were assessed and determined in terms of Total quantity, total packaged, and total free DRP (drug released product) /ml (Drug Product batch analysis – process A : table 2, p308/430; table 3, p310/430, table 4, p315/430). A quantification of residual plasmid DNA of the three plasmids used during the production of the IMP has also been performed (Drug Product batch analysis – process B : Table 7 p321/430, Table 8 p323, Table 9 p326, Table 10p331). It indicates that quality assessment data for drug product batches or SRP-9001 comprises determination of vector plasmid titer and a quantification of residual plasmid DNA in the drug product.

If deemed relevant, the notifier of the current ENVOL study can be asked to confirm that the above described quality parameters were verified for the clinical batches that are intended to be used for the proposed clinical trial.

Coordinator's comment:
The coordinator suggests to include this question in the second list of questions.

With respect to the environmental risk assessment it could also be considered that only the fraction of DNA fragments containing full and intact antibiotic resistance genes would justify an environmental risk assessment.

Measures to prevent dissemination into the environment
Good hygiene hand washing" is advised consisting in using soap regularly. Gloves will be weared during manipulation of diapers and other contaminated material. The elimination of these materials will be performed using double plastic bags.

The caregivers and family members should nevertheless be informed that soap does not inactivate AAV so that they are aware that additional attention should be given not to touch potentially contaminated material.

SBB comment

In addition to the experts comment it is remarked that the novel Post-Infusion Hygiene Guidance (BN43881 BEL Hygiene Guidance v3.0_15March2023_English Final) mentions the use of an alcohol-based hand sanitizer as an alternative for washing hands with soap and warm running water. This alternative was not mentioned in the previous version of the *BN43881: Post-Infusion Hygiene Guidance (Belgium)*. However, according to Korte *et al*., 2021, a commonly available disinfectants such as 70% ethanol is not found appropriate as a suitable and effective disinfectants for AAV.

Therefore the applicant could be asked to modify the *BN43881: Post-Infusion Hygiene Guidance (Belgium)* so as to

- avoid the recommendation of alcohol-based desinfectants

- make patients and their caregivers aware of the fact that the investigational drug might not be easily inactivated by water and soap and that focus should remain on avoiding contact with potentially contaminated material.

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

Coordinator's comment:
The coordinator suggests to include this in the second list of questions.

Evaluation Nicolas van Larebeke - Arschodt

No further comments

Evaluation Willy Zorzi

Comment 1:
In the file « 4.1.1 2022-000691-19_SRP-9001 BN43881 EU Common Application Form - Belgium Confidential Annex – FINAL », the « TABLE OF CONTENTS » seems not to follow the order of the real page numbers corresponding with the different sections of the docum

Comment 2:
In the file « 4.1 2022-000691-19_SRP-9001 BN43881 EU Common Application Form - Belgium Public -FINAL – PDF »,
p12, point c : *"Personnel should not work with AAV, if skin is cut or open sores. Accidental exposure to SRP-9001 must be avoided. Advice will be provided in the event of exposure to skin or eyes as reflected in the Pharmacy Manual.*
Decontamination/cleaning measures after administration or in the case of accidental spilling (i.e. decontamination /cleaning measures of potentially contaminated materials, surfaces and areas). In addition, the disinfection procedures applied should be justified by providing evidence that the chosen method is sufficiently active against the clinical vector **".**
"In case of accidental spillage of RO7494222 (SRP-9001) during the dose preparation and administration to the patient at the health-care provider, instructions provided by the Sponsor´s pharmacy manual will be followed to contain and immediately disinfect the spill to prevent further spread. All contaminated materials will be disposed of locally by incineration or autoclaving. All other places will be cleaned, according to normal decontamination procedures as per the NIH/CDC guidance for handling of biosafety level 1 agents and the Pharmacy Manual ".

In regard of question 9 addressed to the notifier requesting that :

> *the procedure in case of accidental spill should be more detailed than in the Pharmacy manual and indicate that*
> *o before readily evacuating the area, contaminated PPE like labcoat, shoes, or others clothing, should be removed and should not leave the area.*
> *o after having evacuated the area, a sign should be posted indicating that entry is forbidden and that wearing of appropriate protective clothes is mandatory when entering again the area to initiate cleaning the spill and decontaminate the surfaces*

The notifier is invited to modify the sentence " immediately disinfect the spill to prevent further spread " The term " immediately " is not appropriate.

to remember :
According to normal decontamination procedures as per the NIH/CDC guidance for handling of biosafety level 1 agents and the Pharmacy Manual.
1. Evacuate area, remove contaminated PPE and allow agents to settle for a minimum of 30 minutes. Initiate spill response procedure.
2. Cover the spill with absorbent material. Starting at the edges and work towards the center.
3. Carefully pour disinfectant over the absorbed spill, again starting at the edges. Saturate the area with disinfectant.

Comment 3 :
p4 of the « 5.3 2022-000691-19_BN43881_Final Belgium_ADDENDUM TO PHARMACY & DOSE ADMINISTRATION MANUALS v2.0_FINAL » document, it is writing that :

"*personnel should follow existing site spill response procedures (spill management guidelines are available on the hospital intranet and are also known by the trial nurses – Biosafety e-learning is available for all personnel working with GMO's) or exposure control plan to manage such an incident* "
Please detail the spill response procedures described in the Biosafety e-learning available for all personnel working with GMO's. Is this procedure in compliance with the procedure described previously in this present evaluation (see points 1, 2, 3)

SBB's Comment:
The aspects addressed in the expert's comment 2 and 3 have been evaluated in the context of the biosafety dossier that was handed in for CHR de la Citadelle - the only study site in Belgium where the proposed clinical trial will be conducted - in accordance with the regulation implementing Directive 2009/41/EC on the contained use of GMOs and pathogens (contained use procedure). It can be confirmed that the spill response procedures handed in for CHR de la Citadelle take into account the 3 steps as recommended by the expert . A time period of 20 à 30 minutes to vacate the room after a spill incident is described and before evacuating the area, contaminated PPE like labcoat, overshoes, mask, gloves are removed and do not leave the area.

Coordinator's comment:
Thus: issue solved.

Adviesraad voor Bioveiligheid
Conseil consultatif de Biosécurité

Antwoorden van de Adviesraad voor Bioveiligheid op opmerkingen gekregen tijdens de publieksraadpleging over de kennisgeving B/BE/22/BVW5 van F. Hoffmann-La Roche Ltd voor doelbewuste introductie in het leefmilieu van genetisch gemodificeerde organismen met uitzondering van hogere planten voor onderzoek en ontwikkeling

Goedgekeurd op 05/04/2023
Ref. SC/1510/BAC/2023_0309

Context

De kennisgeving B/BE/22/BVW5 werd in oktober 2022 door F. Hoffmann-La Roche Ltd 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 21 oktober 2022 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_0693). 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: Het dossier vermeldt niet direct de mogelijkheid van replicatie bij een ‘cellular genotoxic stress response’ (UV, carcinogenen,…) of bij een superinfectie. Is dit een serieuze mogelijkheid in dit dossier, met de aanwezige genen en omstandigheden van de proef ?

Antwoord :
Yalkinogglu *et al*., toonde in 1988 aan dat replicatie van wilde type adeno-geassocieerde virus (wt AAV) kon plaatsvinden in de afwezigheid van helpervirus in cellijnen die werden blootgesteld aan een verscheidenheid van genotoxische stoffen zoals chemische kankerverwekkende stoffen, UV of hitteschok. In 2012, toonde Nicolas *et al*. echter aan dat deze helpervirus-onafhankelijke wt AAV replicatie als reactie op een verscheidenheid aan behandelingen slechts in zeer beperkte mate optreedt en afhangt van de synergetische bijdrage van verschillende extrinsieke factoren zoals de aanwezigheid van de SV40LTAg of de aanwezigheid van adenovirus afgeleide contaminanten.
AAV wordt beschouwd als niet pathogeen voor de mens. Bij recombinante AAVs, zoals deze die zullen worden toegediend bij de aanvraag van de klinische proef, zijn de genetische sequenties *rep* en *cap*

bovendien niet aanwezig, wat de kans op replicatie onder gelijkaardige condities ook kleiner maakt (zie ook antwoord op vraag 5.1).

Blootstelling van een derde (een persoon verschillend van de te behandelen persoon), bijvoorbeeld door accidentele blootstelling van gezondheidswerkers op de locatie van de klinische proef of de blootstelling van nauwe contacten als gevolg van de uitscheiding van virale vectorpartikels door de behandelde patiënten, kan niet worden uitgesloten. Maar deze derde zal blootgesteld worden aan veel lagere hoeveelheden van het geneesmiddel in vergelijking met de klinische dosis. Bovendien zullen patiënten en zorgverstrekkers instructies krijgen om de blootstelling van derden en het milieu te beperken.

Vraag 2: Op p. 4 van het technisch dossier vinden we het volgende over de opbouw van de vector: '*These are the only adeno-associated viral sequences included in this vector, which are required for viral DNA replication and packaging of the recombinant AAV vector genome'*, in document 4.2 wordt gespecifieerd dat het over de AAV Inverted Terminal Repeats gaat. Is er een risico verbonden aan dit onderdeel van de plasmide of wat is de concrete functie hiervan in de behandeling?

Antwoord :
De virale vector bevat de coderende sequentie voor een functioneel ingekorte dystrofine (micro-dystrofine), dat naast de MHCK7 promoter werd ingebouwd. Samen vormen ze een expressiecassette die door twee AAV2 afgeleide Inverted Terminal Repeats (ITR) wordt geflankeerd. De twee AAV2 ITR zijn de enige AAV virale sequenties die in het genoom van de virale vector zijn opgenomen en geven de start voor vector DNA replicatie evenals het signaal voor het inkapselen van het vectorgenoom. Zonder de *rep* en *cap* sequenties zijn de virale vectordeeltjes echter replicatie-defectief en zijn potentiële risico's sterk beperkt.
Voor de productie van de virale vector wordt gebruikt gemaakt van de plasmide pAAV.MHCK7.microdystrophin die de met AAV2 ITR geflankeerde expressiecassette draagt.

Vraag 3: Zorgt de promotor ervoor dat er geen expressie optreedt buiten de bedoelde weefsels of dat de expressie daar zeer beperkt zal zijn?

Antwoord :
De micro-dystrofine coderende sequentie is onder de controle van het MHCK7 promoter geplaatst. Hoewel, de expressie hiervan buiten de bedoelde weefsels niet met zekerheid volledig kan worden uitgesloten, hebben Salva *et al.,* aangetoond dat de MHCK7-promoter wordt geassocieerd met hoge waarden van expressie in skeletspieren, inclusief het middenrif, en ook in de hartspier, terwijl de expressie in niet-doelwit weefsels zoals de lever, de longen en de milt minimaal is.
De biodistributie van SRP-9001 werd bestudeerd bij niet humane primaten en toonde een robuuste expressie aan van het micro-dystrofine in alle spierstalen.

Vraag 4: Andere dossiers vermelden vaak een zeker quarantaineperiode in het ziekenhuis om de shedding (= uitscheiding) op te volgen. Waar zit het verschil tussen die dossiers en dit geval waar enkel voorzichtigheid wordt aangeraden bij inwonende familie van de patiënt?

Antwoord :
Onder quarantaineperiode wordt de periode verstaan waarbij de patiënt nog in het ziekenhuis verblijft na de toediening van de klinische vector. Het zijn doorgaans in de eerste plaats klinische overwegingen die bepalen of de patiënt onmiddellijk na de toediening al dan niet het ziekenhuis kan verlaten. Wanneer een quarantaineperiode wordt voorgesteld, komen deze ten goede aan de bioveiligheid. Er zijn echter

ook andere mogelijkheden om de bioveiligheid te garanderen en die in verhouding staan met de potentiële risico's die gepaard gaan met deze klinische proef. In dit dossier worden namelijk eveneens bijkomende maatregelen voorgesteld die de patiënt dient na te leven wanneer hij/zij het ziekenhuis verlaat. Deze maatregelen houden rekening met de wijze waarop de virale vectoren worden toegediend (in dit geval intraveneus), de concentratie van het toegediende product en de mogelijkheid dat er nog virale vectordeeltjes of hiervan resterend vector DNA, al dan niet intact, kan worden uitgescheiden gedurende de weken na de toediening. Deze maatregelen hebben als doel om de blootstelling van derden en het leefmilieu aan de mogelijks door de patiënten uitgescheiden virale vectordeeltjes of hiervan resterend al dan niet intact vector DNA te verkleinen. Er werd bovendien aangetoond dat de uitscheiding van vector DNA (al dan niet intact of onder de vorm van virale vectordeeltjes) in de eerste dagen na de toediening plaatsvindt en daarna sterk afneemt, wat een verder gereduceerd potentieel risico betekent voor derden en het leefmilieu.
In het kader van dit dossier zijn er geen data die wijzen op gevaar of nefaste gevolgen voor de niet-patiënt gezien de parentale virus waarvan de virale vector afgeleid is, niet pathogeen is en het transgen geen toxische eigenschappen vertoont. Zelfs al kan blootstelling van nauwe contacten aan shedding door patiënten niet worden uitgesloten zijn er geen data die wijzen op een reëel risico voor de nauwe contacten. Daarom worden de voorgestelde maatregelen in dit dossier voldoende geacht.

Vraag 5:

- Vraag 5.1: Wat is de kans in de realiteit van een recombinatie met een wtAAV en wat zijn de mogelijke gevolgen en risico's wanneer dit gebeurt?
- Vraag 5.2: Om bovenstaande vraag te specifiëren, bij een theoretische opname van het micro-dystrofine coderende sequentie zal er inderdaad geen fitness-voordeel optreden, maar ook geen fitness-nadeel. Is er een mogelijkheid dat deze coderende sequentie een stabiel onderdeel wordt van het genoom na recombinatie met bv. een bacterie?
- Vraag 5.3: In een vorig dossier werd de recombinatie van het transgen in een wt-AAV uitgesloten aangezien het aantal baseparen niet paste in het kapsel van een wt-AAV. Zijn zulke maatregelen ook mogelijk in deze proef om deze mogelijkheid helemaal weg te nemen (rekening houdend met de proportionaliteit van zo een maatregel)?

Antwoord :
5.1. : Recombinatie van de toegediende virale vector met een wilde type AAV vergt een simultane infectie in één cel. Deze recombinatie kan in principe niet leiden tot de generatie van replicatie competente AAV zonder de bijkomende aanwezigheid van helpervirussen. Om nieuwe virale vectordeeltjes te verkrijgen, moet dezelfde cel een drievoudige infectie ondergaan door de virale vector, het wildtype AAV-virus en een helpervirus. Het risico op een drievoudige infectie is verwaarloosbaar. Bovendien, bij de beoordeling van mogelijke gevolgen dient te worden rekening gehouden met ontbreken van de genetische sequenties *rep* en *cap* in de toegediende virale vector. Zonder de *rep* en *cap* sequenties blijven de nieuwe virale vectordeeltjes replicatie-defectief. In het geval van een simultane triple infectie, zou enkel een recombinatie met een uitwisseling van de micro-dystrofine coderende sequentie onder de controle van het MHCK7 promoter door de rep en cap sequenties kunnen resulteren in een replicatie competent AAV, dat in principe nagenoeg gelijk is aan het parentale AAV virus, dat niet pathogeen is (zie ook antwoord 5.3). Een recombinatie waarbij de micro-dystrofine coderende sequentie onder de controle van het MHCK7 promoter of een deel hiervan uitgewisseld wordt voor ofwel rep ofwel cap sequenties resulteert per definitie in een replicatie-defectief virusdeeltje, omdat ofwel rep ofwel cap ontbreekt.

5.2. Het is waarschijnlijk dat de micro-dystrofine coderende sequentie onder de vorm van episomale concatameren langere tijd aanwezig blijft in de door de virale partikels getransduceerde cellen. Het kan

anderzijds ook aanwezig zijn onder de vorm van virale vector DNA dat door de patiënt wordt uitgescheiden en op die manier in contact komen met bacteriën in het milieu.
De waarschijnlijkheid (en efficiëntie) van recombinatie berust op de mate van homologie tussen sequenties. Een mogelijke recombinatie van genetische informatie behorende tot de toegediende virale vector met het genetisch materiaal van een bacterie is ons niet gekend. Zoals aangegeven in de vraag zou een bacterie geen fitness-voordeel ondervinden bij de opname van het micro-dystrofine coderende sequentie. Wel integendeel, het zou de bacterie een fitness-nadeel kunnen geven aangezien de expressie en replicatie ervan meer energie vergt zonder dat het een duidelijk fitness-voordeel oplevert.

5.3. AAVs behoren tot de familie van de *Parvoviridae*, bestaan uit een éénstrengig DNA en hebben een inkapselvermogen van ~ 4.7 kilobasen (kb). Het inkapselvermogen is relatief klein vergeleken met andere virale vectoren.
Op basis van de gegevens in het huidige dossier over de expressiecassette van virale vector SRP-9001 en de lengte van de AAV2 rep en de caprh74 sequenties, kan worden gesteld dat het inkapselen van rep en cap en micro-dystrofine coderende sequenties het inkapselvermogen van een AAV vector zou overstijgen.
Met andere woorden, een homologe recombinatie van SRP-9001 met wilde type AAV zou kunnen leiden tot het winnen van functionele genen van AAV2 (*rep* en *cap*) maar zou tegelijkertijd in dit geval gepaard gaan met het verlies van de coderende sequentie voor het micro-dystrofine.

References:

Nicolas *et al*. Factors influencing helper-independent adeno-associated virus replication. Virology 432 (2012) 1–9.
Yalkinoglu *et al*. DNA Amplification of Adeno-associated Virus as a Response to Cellular Genotoxic Stress. Cancer Research 48 (1988) , 3123-3129, June 1.
Salva *et al*. Design of tissue-specific regulatory cassettes for high-level rAAV-mediated expression in skeletal and cardiac muscle. Mol Ther. 2007 Feb;15(2):320-9 (2007).

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/22/BVW5 de F. Hoffmann-La Roche Ltd pour l'introduction volontaire dans l'environnement, à des fins de recherche et développement, d'organismes génétiquement modifiés autres que les plantes supérieures

05/04/2023
Ref. SC/1510/BAC/2023_0310

Contexte

La notification B/BE/22/BVW5 a été soumise en octobre 2022 par F. Hoffmann-La Roche Ltd 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 21 octobre 2022, 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_0693). 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: Le dossier n'évoque pas directement la possibilité de réplication en cas de réponse cellulaire génotoxique à un stress (UV, cancérigènes, …) ou en cas de surinfection. Est-ce une possibilité sérieuse dans ce dossier, avec les gènes présents et les conditions de l'essai ?

Réponse :

En 1988, Yalkinogglu a montré que la réplication du virus adéno-associé sauvage (wt AAV) pouvait se produire en l'absence de virus auxiliaire dans les lignées cellulaires exposées à une variété d'agents génotoxiques tels que les cancérigènes chimiques, les UV ou les chocs thermiques. Cependant, Nicolas *et al.*, 2012 ont montré que la réplication de AAV wt indépendante d'un virus auxilliaire ne se produit que dans une mesure très limitée et dépend de la contribution synergique de divers facteurs extrinsèques tels que la présence du SV40LTag ou la présence de contaminants dérivés d'adénovirus.

Les AAV sont considérés comme non pathogènes pour l'homme. De plus, dans les AAV recombinants, tels que ceux qui seront administrés avec la demande, les séquences génétiques *rep* et *cap* ne sont plus présentes, ce qui réduit également les chances de réplications dans des conditions similaires (voir également la réponse à la question 5.1).

L'exposition d'une tierce personne (c'est-à-dire une personne autre que le patient), par exemple par l'exposition accidentelle de travailleurs de la santé sur le site de l'essai clinique ou l'exposition de personnes proches du patient en raison de l'excrétion de particules de vecteur viral par le patient traité, ne peut être exclue. Mais ces tierces personnes seront exposées à des quantités beaucoup plus faibles comparées à la dose clinique. De plus, les patients et les fournisseurs de soins de santé recevront des instructions pour limiter l'exposition des personnes non traitées et de l'environnement.

Question 2: A la page 4 du dossier technique, on peut lire sur la structure du vecteur: '*These are the only adeno-associated viral sequences included in this vector, which are required for viral DNA replication and packaging of the recombinant AAV vector genome'*, dans le document 4.2, il est précisé qu'il s'agit des AAV Inverted Terminal Repeats. Y a-t-il un risque associé à cette partie du plasmide ou quelle est sa fonction concrète dans le traitement ?

Réponse :

Le vecteur viral contient la séquence codante pour une dystrophine fonctionelle tronquée (le micro-dystrophine) qui a été inséré à côté du promoteur MHCK7. Ensemble, ils forment une cassette d'expression flanquée de deux Inverted Terminal Repeats (ITR) dérivés du virus AAV2. Ces deux ITR du AAV2 présents dans le génome du vecteur viral servent d'une part comme origine pour la réplication de l'ADN du vecteur et d'autre part comme signal pour l'encapsulation du génome du vecteur. Cependant, comme le vecteur viral ne contient pas les séquences *rep* et *cap*, les particules de vecteur viral sont incapables de se répliquer, ce qui limite fortement tout risque potentiel.
La production du vecteur viral utilise le plasmide pAAV.MHCK7.microdystrophine portant la cassette d'expression flanquée par deux ITR du AAV2.

Question 3: Le promoteur garantit-il que l'expression ne se produise pas en dehors des tissus ciblés ou que l'expression y sera très limitée ?

Réponse :

La séquence codante pour le micro-dystrophine est sous le contrôle du promoteur MHCK7. Bien que l'expression du gène de la micro-dystrophine en dehors des tissus ciblés ne peut être totalement exclue avec certitude, Salva *et al*., ont démontré que ce promoteur MHCK7 est associé à des niveaux élevés d'expression dans le muscle squelettique, y compris le diaphragme, ainsi que dans le muscle cardiaque, tandis que l'expression dans les tissus non cibles tels que le foie, les poumons et la rate est minime.
La biodistribution de SRP-9901 a été étudiée chez des primates non humains et a montré une forte expression de la micro-dystrophine dans tous les échantillons musculaires.

Question 4: D'autres dossiers mentionnent souvent une certaine période de quarantaine à l'hôpital pour faire le suivi du shedding (= excrétion). Quelle est la différence entre ces dossiers et ce cas où seule la prudence est de mise avec la famille du patient ?

Réponse :

La période de quarantaine est comprise comme étant la période pendant laquelle le patient reste à l'hôpital après l'administration du vecteur viral. Ce sont généralement des considérations cliniques qui déterminent si le patient peut ou non quitter l'hôpital immédiatement après l'administration. Lorsque une période de quarantaine est proposée, elle profite aussi à la biosécurité. Cependant il y a également autres possibilités de garantir la biosécurité et qui sont proportionnées au risque potential associé à l'essai clinique. En effet, dans ce dossier, des mesures supplémentaires doivent également être respectées par le patient à sa sortie de l'hôpital. Ces mesures tiennent compte du mode d'administration des vecteurs viraux (dans ce cas-ci par voie intraveineuse), de la concentration de la dose injectée et de la possibilité que des particules de vecteur viral ou de l'ADN du vecteur résiduel, intact ou non, soit encore excrété plusieurs jours voire plusieurs semaines après l'administration. Ces mesures visent à réduire l'exposition des non-patients et de l'environnement aux particules virales ou de l'ADN vecteur résiduel, intact ou non, pouvant être excrétées par les patients. Il a également été montré que l'excrétion d'ADN du vecteur (intact ou non ou sous forme de particules de vecteur viral) a lieu dans les premiers jours suivant l'administration puis diminue fortement, ce qui signifie un risque potentiel d'avantage réduit pour les non-patients et de l'environnement.
Dans le cadre de ce dossier, il n'existe pas de données indiquant un risque ou des conséquences néfastes pour le non-patient puisque le virus parental dont est issu le vecteur viral n'est pas pathogène et que le transgène ne présente aucune propriété toxique. Même si l'exposition des contacts étroits à l'excrétion par les patients ne peut être exclue, il n'existe aucune donnée indiquant un risque réel pour les contacts étroits. C'est pourquoi, les mesures imposées dans ce dossiers sont considérés suffisantes.

Question 5:

- question 5.1: Quelle est la probabilité en réalité d'une recombinaison avec un AAV wt et quelles sont les conséquences et les risques possibles si cela se produit ?
- question 5.2: Pour préciser la question ci-dessus, avec une inclusion théorique de la séquence codante pour la micro-dystrophine il n'y aura en effet pas d'avantage (fitness), mais pas de désavantage de la fitness non plus. Est-il possible que cette séquence codante devienne une partie stable du génome après recombinaison avec, par exemple, une bactérie?
- question 5.3: Dans un dossier précédent, la recombinaison du transgène dans un AAV wt était exclue car le nombre de bases ne rentrait pas dans la capsule d'un AAV sauvage. De telles mesures sont-elles également possibles dans ce procès pour éliminer complètement cette possibilité (en tenant compte de la proportionnalité d'une telle mesure) ?

Réponse :

5.1. La recombinaison du vecteur viral administré avec un AAV de type sauvage nécessite une infection simultanée dans une cellule. Cette recombinaison ne peut en principe pas conduire à la génération d'AAV compétents pour la réplication sans la présence supplémentaire de virus auxiliaires. Pour obtenir de nouvelles particules de vecteur viral, il faut qu'une même cellule subisse une triple infection par le vecteur viral, le virus AAV sauvage et un virus auxiliaire. Le risque d'une triple infection est négligeable. De plus, l'évaluation des conséquences possibles doit tenir compte de l'absence des séquences génétiques *rep* et *cap* dans le vecteur viral administré. Sans les séquences rep et cap, les particules de vecteur viral restes défectueuses pour la réplication. Dans le cas d'une triple infection, seule une recombinaison aboutissant à un échange de la séquence codante de la micro-dystrophine sous le contrôle du promoteur MHCK7 par les séquences rep et/ou cap pourrait aboutir à un AAV parental qui n'est pas pathogène pour l'homme (voir aussi réppnse 5.3). Une recombinaison dans laquelle la séquence codant pour la micro-dystrophine sous le contrôle du promoteur MMHCK7 ou d'une partie de

celle-ci est échangée contre des séquences rep ou cap par définition aboutit à une particule virale défectueuse pour la réplication, car rep ou cap est manquant.

5.2. Il est probable que la séquence codante pour la micro-dystrophine reste plus longtemps présent dans les cellules transduites par les particules virales sous forme de concatémères épisomiques. D'autre part, elle peut également être présente sous forme d'ADN vecteur viral qui est sécrété par le patient pour entrer ensuite en contact avec des bactéries de l'environnement.
La probabilité (et l'efficacité) de la recombinaison dépend du degré d'homologie entre les séquences. Nous n'avons pas connaissance d'une éventuelle recombinaison de l'information génétique d'une bactérie. Comme indiqué dans la question, une bactérie ne bénéficierait d'aucun avantage de fitness due à l'inclusion de la séquence codante pour la micro-dystrophine. Au contraire, cela pourrait donner à la bactérie un désavantage de fitness car son expression et sa réplication nécessitent plus d'énergie sans conférer un net avantage de fitness.

5.3. Les AAV font partie de la famille des *Parvoviridae* et consistent en un ADN simple brin ayant une capacité d'encapsulation d'environ 4,7 kilobases (kb). La capacité d'encapsulation est relativement faible par rapport aux autres vecteurs viraux.
Selon les données du dossier actuel sur la casette d'expression dans le vecteur viral SRP-9001 et la longeur des séquences AAV2 rep et cap-rh.74, l'encapsulation simultanée des gènes *rep*, *cap* et de la séquence codante pour la micro-dystrophine dépasserait la capacité d'encapsulation d'un vecteur AAV. En d'autres termes, une recombinaison homologue de SRP-9001 avec un AAV sauvage pourrait conduire à l'introduction des gènes fonctionnels de l'AAV2 (*rep* et *cap*) mais s'accompagnerait alors de la perte du gène de la micro-dystrophine.

References:

Nicolas *et al*. Factors influencing helper-independent adeno-associated virus replication. Virology 432 (2012) 1–9.
Yalkinoglu *et al*. DNA Amplification of Adeno-associated Virus as a Response to Cellular Genotoxic Stress. Cancer Research 48 (1988) , 3123-3129, June 1.
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