Adviesraad voor Bioveiligheid
Conseil consultatif de Biosécurité

Advice of the Belgian Biosafety Advisory Council on the notification B/BE/21/BVW5 of the company Sarepta Therapeutics, for deliberate release in the environment of genetically modified organisms other than higher plants for research and development

30/05/2022
Ref. SC/1510/BAC/2022_0677

Context

The notification B/BE/21/BVW5 has been submitted by Sarepta Therapeutics to the Belgian Competent Authority in October 2021 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 Phase 3 Multinational, Randomized, Double-Blind, Placebo-Controlled Systemic Gene Delivery Study to Evaluate the Safety and Efficacy of SRP-9001 in Subjects With Duchenne Muscular Dystrophy (EMBARK)".***

The purpose of this study is to assess the safety and the efficacy of the study treatment SRP-9001 in in male subjects ages ≥4 to <8 years, 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. It predominantly affects boys. The lack of functional dystrophin protein results in progressive muscle weakness and wasting. Ultimately heart and respiratory muscles are affected, causing premature death of DMD patients.

As a gene therapy product, 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) 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

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Overall, approximately one hundred twenty patients will be included in this Phase III study and six patients will be included in Belgium, each receiving a single dose intravenous infusion into a peripheral limb vein (arm or leg). This study will be conducted in one clinical site located in Flanders.

The dossier has been officially acknowledged by the Competent Authority on 12 January 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. Two 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 17 February 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 29 April 2022 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 12 May 2022. The answers of the notifier were received on 19 May 2022 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 three reactions from the public of which none were related to biosafety issues.

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 the 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, and upon request for additional information, the production system was found to be sufficiently described from an environmental risk point of view.
The strategy for demonstrating the absence of replication-competent virus in the clinical batch has been described in the common application form. Because the absence of replicating AAV is one of the key element to be assessed in the context of the environmental risk assessment, the notifier was asked to provide more information on the lower limit of detection of the assay. The demonstration of the absence of replication-competent virus was found to be adequately described in the notifiers answers.

3. The conditions of the release

The study consists of two parts. In Part 1, approximately 60 subjects will receive intravenous (IV) one-single dose of SRP-9001 and approximately 60 subjects will receive matching infusion volumes of placebo. In Part 2, subjects who received placebo in Part 1 will receive IV SRP-9001, and subjects who received SRP-9001 in Part 1 will receive placebo. All patients will stay for a minimum of 4-6h at the hospital and then leave the reference hospital without quarantine measures.

The germline transmission has been evaluated during a non-clinical study on DMD and wt male mice treated with a single IV dose of SRP-9001. Upon BAC's request, the notifier provided further information on the approach taken, the tissues examined, the study design and the numeric results.

No shedding analysis will be planned during this clinical trial because biological samples for saliva, urine and stool for the monitoring of the GMO is currently being collected in Sarepta study SRP-9001-103 which is using the same IMP at the same dose. Since shedding data collected from previous or ongoing trials can contribute to a proper environmental risk evaluation, the notifier provided, following BAC's request, a clear and detailed update on preliminary shedding data obtained so far during the clinical trial SRP-9001-103 on children treated with the same single dose of microdystrophin vector SRP-9001 as for the current study.

4. The risks for the environment or human health

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, 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 of transfer of vector to an unintended immune-competent human recipient, the risks are expected to be considerably reduced as compared to any potential risk for the participant, since the vector is not able to replicate and the 'dose' that may conceivably be transferred (from e.g. aerosol, splashing or fomites) will be orders of magnitude lower than that received by patients. Worst case, the receiver will develop an immune response to the AAVrh74 capsid.

After injection, patients, patient's family 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. Following BAC's request, the notifier clarified that bodily fluids, waste, materials and surfaces frequently and potentially contaminated, such as toys…, will be decontaminated with a bleach-based disinfectant. Protective gloves will be worn when handling bodily fluids/waste and when disposing of potentially contaminated materials. These gloves together with other potentially contaminated materials will be placed in a sealable bag before being placed in the household trash.

In order to reduce study burden while maintaining ongoing in-person surveillance by the study site, some visits may be performed remotely. Following BAC's request, study nurse, who will visit the patients at home, will receive clear instructions to avoid any potential dissemination of the recombinant virus in the environment during such visits.

The patients are prohibited from donating blood for a duration of two years following the vector injection which corresponds to the duration of the study.

All these instructions for the patients and patient's family with respect to good hygiene practices have been detailed in a short, readable format document that will be provided to each patient.

Since the notifier's environmental risk assessment (ERA) was not specific for the rAAV drug product that is used in the study, following BAC's request, the notifier provided an updated version of the ERA in accordance with the 'Good Practice on the assessment of GMO related aspects in the context of clinical trials with AAV clinical vectors[2]'.

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

Upon BAC's request, the notifier provided a 2-4 pages technical sheet 'Addendum to Pharmacy Manual_Belgium' including all relevant handling instructions, mandatory PPE (double gloves, personal lab coat), detailed instructions in case of accidental spill or breakage of a vial containing the GMO, procedure in case of inadvertent exposure of human to SRP-9001 product, clean-up procedure and waste management.

2. https://ec.europa.eu/health/system/files/2022-01/aavs_gp_en.pdf

Given the assessment of the likelihood of further propagation of 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 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 following updated documents:

- Addendum to Pharmacy Manual_Belgium_update_12May2022
- BEL Hygiene Guidance for participants - *to be updated in accordance with condition 1 here below*
- Confidential EU Common Application GMO_20220516

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

- 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 recommends, as a precautionary measure, to increase the period of time to practice appropriate hand hygiene for at least 60 days. As a consequence, the notifier is requested to adapt the document "BEL_Hygiene_Guidance" by specifying for each instruction the period during which the recommendation must be followed.

- The notifier and the investigators must strictly apply the clinical trial protocol version 3, and all the safety instructions as described in the dossier and the updated and new documents listed here above.

- 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 SRP-9001;
- The BAC would genuinely appreciate to receive an update of the shedding results upon completion of the SRP-9001-103 study.

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/21/BVW5 (ref. SC/1510/BAC/2022_0230 and SC/1510/BAC/2022_0594)

Compilation of comments of experts in charge of evaluating the dossier B/BE/21/BVW5 And comments submitted to the notifier

18 February 2022
Ref. SC/1510/BAC/2022_0230

Mandate for the Group of Experts: Mandate of the Biosafety Advisory Council (BAC) of 26 October 2021.
Coordinator: Anton Roebroek (KULeuven)
Experts: Rik Gijsbers (KULeuven), Willy Zorzi (ULiège)
SBB: Sheela Onnockx

INTRODUCTION

Dossier **B/BE/21/BVW5** concerns a notification from Prevail Therapeutics 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 12th of January 2022 and concerns a clinical trial entitled "Phase 3 Multinational, Randomized, Double-Blind, Placebo-Controlled Systemic Gene Delivery Study to Evaluate the Safety and Efficacy of SRP-9001 in Subjects With Duchenne Muscular Dystrophy (EMBARK)". The investigational medicinal product is a recombinant adeno-associated virus, serotype rh74, carrying the human micro-dystrophin (hMicro-Dys) 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 17-02-2022 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

In B_BE_21_BVW5_EU CAF SBB PLAN 219 20200912.pdf it is not clear what the production format is for the rAAV based vectors. What cell type is used? Transient transfection, baculo production?
Based on the info in other documents provided, it is stated that the drug product is generated in the absence of a helper virus, but I could not find detailed info on the production process and the type of cells used.

At p4/33 in B_BE_21_BVW5_Part 1B_Common Application GMO_Confidential.pdf the production system is described to employ three plasmids. The plasmids are discussed but not the production or purification method. In the testing a PCR is performed for rh74 CAP sequences to assess rcAAV. It is not clear whether other tests are performed to assess other rcAAV that may contaminate the prep (eg. wtAAV2), which may be copurified.

SBB Comment:

According to ERA, p18/29, the production of SRP-9001 is accomplished via the cellular transfection using three such expression cassettes (plasmids). These 3 plasmids are used for the manufacturing of SRP-9001 vector genome in HEK 293 cells (ERA, p12/29). The SRP-9001 vector is produced by a process known as “triple transfection”, which utilizes 3 different plasmid DNA constructs (ERA, p12/29).

In section 2.1 of Part 1B_CAF_confidential (4.2 EU Common Application GMO 20211012), the three plasmids are well described, however, the production system and the purification method have not been described in this section. The notifier could be requested to complement section 2.1 by elaborating on the production system (transient transfection? Cell type used?). Aspects related to the purification method per se do not belong to the data requirements for the biosafety dossier, except for the information on absence of replication-competent virus in the clinical batch. The strategy of the latter has been described in section 2.2 of the common application form (both in public and confidential part), however no numeric information is given on the lower limit of detection of the assay used. Because the demonstration of the absence of replicating AAV is one of the key elements to be assessed in the context of the environmental risk assessment, the notifier could be requested to indicate the lower limit of detection.

The likelihood of formation of wtAAV2 in the context of the current production system was further discussed and clarified with the expert : CAP gene from AAV2 is missing in the three plasmids used to transfect HEK293 cells and no reports could be found pointing to the contamination of wtAAV2 in cultured cells.

Coordinator comment:
Agreed to communicate these questions to the notifier. In addition, the notifier could be requested to indicate whether the clinical batch was tested for the presence of other rAAV or wtAAV viruses or other viruses (e.g. adenoviruses)?

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

The applicant specifically assess rcAAV with rh74 capsid. Are tests also set up to assess other rcAAV that may be present in the prep (eg rAAV2?) (see 2.1 comment).

SBB Comment:
See above SBB comment in section 2.1 for comment 2

Coordinator comment:
See comment by coordinator above with respect to presence of other rAAV or wtAAV viruses or other viruses (e.g. adenoviruses)?

2.3. Diagram (map) of the clinical vector

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

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

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

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

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

2.5. Description of the insert

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

Comment 1

Has evaluated this item and has no questions/comments.

Comment 2

Has evaluated this item and has no questions/comments.

2.6. Biodistribution and shedding

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

Comment 1

p12. in the B_BE_21_BVW5_Part 2_SNIF_SRP-9001 document:
The recombinant SRP-9001vector containing the DMD gene could interact with other viruses with which the patients come in contact and cause viremia. This unlikely scenario has been studied(Favre e tal., 2001)in cell culture. However, in vivo rescue experiments have failed to show rescue and replication, except in one case in which very large doses of wtAAV and adenovirus were administered in a particular setting (Afione et al., 1996). Therefore, AAVrh74interaction with other viruses to cause infection appears to be a minimal risk for SRP-9001
At the end of this paragraph, the consequence of the before last sentence to the next one is not clearly evident.
The interaction is possible in case of administration of large amounts of wtAAV and adenovirus concomitantly. But in absence of these, the interference is not relevant (and not because AAV rh74 is inactivated or totally safe for the patient) and a minimal risk for SRP-9001 can be claimed.

Therefore, it could to proposed to modify the last sentence by :
"Therefore, risk of infection due to AAV rh74 interaction with other viruses appears to be at a minimal risk level in the context of this clinical Phase 3 multinational trial, in regard to the exclusion of large scale presence of additional interfering elements such as wtAAV, adenovirus…

SBB comment:
This comment could be added in the Typos and other errors/omissions section

Coordinator comment:
Agreed to add in the Typos and other errors/omissions section

According to Good Practice on the assessment of GMO related aspects in the context of clinical trials with AAV clinical vectors, hazards associated to vertical/germline transmission: *Biodistribution studies in animals have shown that DNA from AAV clinical vectors can be detected in gonadal DNA for a variable duration. Accordingly, the presence of recombinant AAV in the gonads cannot be excluded*.
About reproductive risks (p21 in B_BE_21_BVW5_Main ICF BEL V1.0, the notifier precise that "Since the risk is unknown, if your child is sexually active he must agree to use a male condom for the entire length of the study. A female partner also is strongly encouraged to use a medically acceptable method of birth control (for example, oral contraceptives) during this time frame. "

Question: Is the time during the entire length of the study sufficient to eliminate all the eventual DNA traces from AAV rh74 in gonads? It could be required to analyse patient gonadal DNA samples at different times after the end of the trial. Therefore, it would be possible to confirm or infirm the "total disappearance" of AAV clinical vector traces in gonads and on the basis of this analysis, to decide on the adequate moment for the interruption of the recommended contraceptive measures.

SBB comment

The Investigators Brochure (p27/104) mentions the outcome of a study (SR-20-14) regarding the expression of AAVrh74 capsid and transgene in the reproductive organs of male mice treated with SRP-9001. In this regard, the notifier could be asked to give more information on the approach taken and the study design, (e.g. timepoint of samples taken, statistical power, etc).

Considering that the trial is planned as part of a Phase III programme, the generation of data documenting the likelihood of presence of viral DNA in seminal fluid from patients, along with a review of non-clinical data, could be recommended should the notifier envision a marketing authorisation application. However, in the context of the current clinical trial in which only 6 patients ≥ 4 to < 8 years of age at time of randomization are enrolled in Belgium, it could be questioned whether an analysis of the patients seminal fluid as part of the extension study, could generate a sufficient amount of data that are statistically powered and that would actually inform on the potential of germline transmission of SRP-9001.

Coordinator comment:

Given the age of the trial participants (only 6 patients ≥ 4 to < 8 years of age at time of randomization are enrolled in Belgium) it does not seem opportune to study the presence of viral DNA in seminal fluid from patients. With respect to the outcome of a study (SR-20-14) revealing that no expression of AAVrh74 capsid and transgene could be demonstrated via in situ hybridization in the reproductive organs of male mice treated with SRP-9001, however, the notifier could be asked to give more information on the approach taken and the study design, (e.g. timepoint of samples taken, statistical power, etc).

Comment 2

Of note, in 2.6.1 p13/49 in B_BE_21_BVW5_EU CAF SBB PLAN 219 20200912.pdf, the doses are indicated wrongly. (1014 should be 10^{14}).

SBB comment:

This comment could be added in the Typos and other errors/omissions section

Coordinator comment:

Agreed to add in the Typos and other errors/omissions section

The applicant indicates "Instructions should be provided to patient families and caregivers regarding use of protective gloves if/when coming into direct contact with patient bodily fluids and/or waste, as well as utilizing good hand-hygiene for a minimum of 4 weeks after the injection." at p99/104 in B_BE_21_BVW5_Investigator Brochure Version 6.0.pdf. What would be the instructions provided to the parents and caregivers? I reckon this should be stipulated in the leaflet of the study, that is provided with the product.

SBB comment:

According to SNIF, p13/19; ERA, p25/29 and the Investigator Brochure, p99/104, "instructions should be provided to the patient's family and care giver(s) regarding use of protective gloves if/when they come into direct contact with the patient's bodily fluids and/or waste, as well as good hand-hygiene for a minimum of four weeks after gene replacement therapy." Such instructions to the patients, patient's family and caregivers should indeed be provided and sufficiently detailed in order to avoid potential transmission of the virus to other people or to the environment when patients are leaving the hospital setting.

The notifier could be requested to detail the instructions that will be given to the patients, patient's family and caregivers. More particularly, in meeting this request, the notifier could also be asked:

-to detail and specify how bodily fluids and surfaces frequently contaminated with bodily fluids (e.g. handkerchiefs, toys that may be shared with brothers/sisters of the trial participant, etc) should be handled, especially during the first days post IV administration of SRP- 9001.

-If gloves are used, it should be specified how these are supposed to be disposed of.

- to give study nurse adequate instructions to avoid any potential dissemination of the recombinant virus in the environment during the remote visits. Which personnel protective equipment will be required? How will the waste be collected? How will the samples be transported back to the hospital?

- Since shedding was found in urine and stool, to ask parents to bring back to the hospital the diapers (at least at the beginning (peak at day 2)), whenever applicable ?

The notifier should provide a timeframe for the caregivers and family (point (f) on p18/49 in B_BE_21_BVW5_EU CAF SBB PLAN 219 20200912.pdf) to strictly adhere to the recommendations. In this regard, the notifier could be asked what is the rationale for asking patient families and caregivers to follow instructions for 1 month considering that the vector is primarily cleared from the body in urine, stool, and plasma at below the limit of quantitation by day 44 post-infusion (p12 SNIF).

Furthermore, in order for patients, patient's family and caregivers to adhere to and practice good hygiene, it is important to explain why measures are taken and what are the likely sources of contaminated material. A small take home summary (preferably one-page, plasticized document) could insure that patients, patient's family and caregivers easily can consult the information in a understandable format whenever needed.

According to the Main ICF_BEL, p19/42, the patient can refers to the Participant Study Guide. The notifier could be requested to provide this guideline.

Coordinator comment:

Agreed: to communicate these questions and concerns to the notifier. It should be noted, that at least for the participants and their parents the relevant documents, including the ICF, should be provided in the native language.

SBB comment:

Main ICF has been provided in French, in Dutch and in English.

Additionally, "subjects are prohibited from donating blood for 2 years following the vector injection." What is the rationale here? Prevent spreading to the environment? Is this an estimated time frame, or has this been tested for?

SBB comment:
Agrees with expert's comment, the notifier could be asked to give a scientific rationale for the proposed time frame for prohibition of donating blood.

Coordinator comment:
Agreed to communicate this question to the notifier

In B_BE_21_BVW5_Main ICF BEL V1.0.pdf, I reckon more info should be included for the parents/caregivers on how to take care of bodily fluids, especially in the first days post treatment (it is not clear whether patients remain hospitalised or not during this period). The current info refers to the Participant Study Guide, but I cannot check this info (not included?).

SBB comment :
According to the study visit schedule presented in the protocol and the Informed Consent form it is indicated that trial participants can be discharged from the study site after monitoring of vital signs for approximately 4-6 hours after SRP-9001 administration and if approved by study staff. Subjects will be followed for up to 52 weeks in Part 1 and Part 2 and will complete follow-up visits at the following time points: Day 2, Weeks 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 24, 36, and 52 (relative to the infusion on Day 1). Where applicable, Week 2, 3, 5, 6, 7, 9, 10, and 11 visits may be performed remotely. Some of these visits will be 1-day visits and others will require a study site for 2 days in a row.

Agreed with expert's comment : the notifier could be asked to detail and specify how bodily fluids and surfaces frequently contaminated with bodily fluids (handkerchiefs, toys that may be shared with brothers/sisters of the trial participant, etc) should be handled, especially during the first days post IV administration of SRP- 9001.

See also SBB comment in section 2.6. under comment 2 regarding the suggestion to ask the notifier to the Participant Study Guide.

Coordinator comment:
Agreed to communicate this question to the notifier

Additional question SBB
Shedding analysis and results have not been sufficiently detailed in the dossier. Only partial or approximate information is reported (e.g. '*a very small proportion of the total number*', '*up to a few weeks following injection*'.)
It is not clear whether shedding of viral vector has been observed in feces or not. According to SNIF, p3, no shedding in feces has been observed but according to some results from the Sarepta study SRP-9001-103 (see ERA section 6.1.4, p22), shedding of viral vectors in feces was observed. Do these results relate to the outcome of two separate studies?

It is also not clear form the dossier whether shedding analysis will be performed during the current proposed clinical trial.

Any shedding data collected from previous or ongoing trials will contribute to a proper environmental risk evaluation. It is noticed from ERA, section 6.1.4, p22/29 that biological samples for saliva, urine,

and stool are currently being collected in Sarepta study SRP-9001-103. Ideally these data will need to be evaluated in light of the observed quantity of shed viral material, the period of time during which shedding is observed. It will be important to answer the question whether the observed shed SRP-9001 is only vector DNA or a remnant thereof, whether or not present in shed cells, whether it reflects integrated vector DNA in shed cells or whether it consists out of remaining or rescued replication-deficient viral vector particles. On p23/29 of the ERA document, section 6.3, it is stated that '*The quantities that will be released into the environment by shedding will be a very small proportion of the total number of viral genomes injected, of which the majority, if not all, is not considered "infectious*". Are there any data available for SRP-9001 that can substantiate this statement?

It is likely that not all shedding data were available at the time of the current biosafety dossier submission. However, the notifier could be requested to provide a clear and detailed update of shedding data obtained so far, in particular such as for the shedding analysis from study SRP_9001_103.

Coordinator comment:

Agreed with the SBB comments: Available shedding data with greater details on quantities, nature etc. from ongoing studies should be provided. It is important to answer the question whether any observed shed SRP-9001 is only vector DNA or a remnant thereof, whether or not present in shed cells, whether it reflects integrated vector DNA in shed cells or whether it consists out of remaining or rescued replication-deficient viral vector particles. With respect to the answers to these questions the notifier should also be asked whether shedding analysis will be performed during the current proposed clinical trial.

3. INFORMATION RELATED TO THE CLINICAL TRIAL

3.3. Storage of the clinical vector at the clinical site

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

Comment 1

Has evaluated this item and has no questions/comments.

Comment 2

Has evaluated this item and has no questions/comments.

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

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

Comment 1

Has evaluated this item and has no questions/comments.

Comment 2

Has evaluated this item and has no questions/comments.

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

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

Comment 1

Has evaluated this item and has no questions/comments.

Comment 2

In B_BE_21_BVW5_Pharmacy Manual_V3.0.pdf (p18/28) the applicant describes the preparation of the drug product. It is not clear why in point 13 and 16, the outside of the bag and the syringe is to be cleaned. Is this to wipe of and inactivate possible drug product? If so, please state this, because rAAV is resistant to a lot of standard disinfectants and sporicidals.

SBB comment

During the validation period of the current dossier, the notifier was recommended to include a dedicated document for Study staff instructions, however the notifier did not met this recommendation when handing in its biosafety dossier at the time it was validated.

Hence, the notifier could again be requested to provide a 2-4 page **technical sheet** detailing all relevant handling instructions, detailed instructions in case of spill, waste management and other risk management measures. See text proposal under section 3.6.

Coordinator comment:

Agreed with the SBB suggestion.

3.6. Measures to prevent dissemination into the environment

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

Comment 1

In the B_BE_21_BVW5_Part 3_Environmental Risk Assessment_20210726 Clean document:
p 24 of section 6.4.1. Methods and Procedures for Controlling the Dissemination of the GMO(s) In Case of Unexpected Spread Carefully pour disinfectant (fresh 10% bleach solution followed by alcohol wipes) over the absorbed spill, again starting at the edges. Saturate the area with disinfectant.

1) 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?

2) 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…
Please complete by a list of decontamination / disinfection solutions with their protocols and application scope.

3) In point 6. of this section the notifier propose to discard absorbent material in biological waste bags: B1 or B2 bags? Or plastic bags discarded in B2 (UN3291) container? Could it be specified?

4) In points 8 and 9, could the notifier precise the type of bags they intend to use to discard biological waste (refer to comment 3).

SBB comment :
Remark 1 and 2 have been implemented in the text proposal hereunder (section 3.6 , under comment 2) on the suggestion to request the notifier to provide a 2-4 page **technical sheet** detailing all relevant handling instructions and implementing the experts remarks.
Regarding remark 3 and 4, it is remarked that these waste management aspects are evaluated in the context of the evaluation of biosafety dossiers 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 comment:
OK.

Comment 2
It is not clear why the site should be disinfected to minimize environmental spread of rAAV on p16/49 in B_BE_21_BVW5_EU CAF SBB PLAN 219 20200912.pdf.
The applicant indicates that all personnel will be trained. It would be advisable to provide minimal info to all institutions that will take part in the clinical trial, for example as integral part of the study information.

SBB comment :
See text proposal hereunder on the suggestion to request the notifier to provide a 2-4 page **technical sheet** detailing all relevant handling instructions and implementing the experts remarks

Coordinator comment:
OK.

The use of double gloves should be standard, and not only considered (p17/49 in B_BE_21_BVW5_EU CAF SBB PLAN 219 20200912.pdf and p16-17/33 + p28/33 B_BE_21_BVW5_Part 1B_Common Application GMO_Confidential.pdf), in my opinion. Lab coats should be personal, and dedicated for the specific room where the rAAV is applied to prevent spreading to the environment.
When considering a spill, I would not readily evacuate the area. Please check first where the materials have spread. If on labcoat, shoes, or others clothing, evacuating the area will result in more spreading of the material. Double gloves will be instrumental here as well. Ideally, personnel would work in duos, which would imply that there is always a free person that can coordinate the spill removal, while the person that might be contaminated remains in place (the same info is included in the public information files and should be updated in my opinion – eg p3/4 in B_BE_21_BVW5_Part 5_Public Information_EN.pdf).

SBB comment :

The notifier could be requested to provide a 2-4 page **technical sheet** detailing all relevant handling instructions including detailed instructions in case of spill, waste management and other risk management measures. In meeting this request, 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 as chloroform. The notifier may consider to include the procedure cited in the « NIH/CDC guidance for handling of biosafety level 1 agents » used as reference in the present section

- 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.
- risk management procedures used in case of needle-stick injury or the formation of aerosols in case of bag rupture
- 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 p17/49 in B_BE_21_BVW5_EU CAF SBB PLAN 219 20200912.pdf and p16-17/33 + p28/33 B_BE_21_BVW5_Part 1B_Common Application GMO_Confidential.pdf).

- 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 through a splash in the eyes or mucous membrane
- procedures for treatment of accidental spill (concentration of disinfectant, contact time). When considering a spill, and before readily evacuating the area, contaminated PPE like labcoat, shoes, or others clothing, should not leave the area. Personnel should work in duos, with one person coordinating the spill removal and the second that might be contaminated remaining in place. (the same info is included in the public information files and should be updated in my opinion – eg p3/4 in B_BE_21_BVW5_Part 5_Public Information_EN.pdf).
- 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 and align all relevant documents making part of the dossier in accordance with the requested study staff 'technical sheet'.

Coordinator comment:
OK. Agreed to communicate these questions and concerns to the notifier.

It would be best to provide a timeframe for the caregivers and family (point (f) on p18/49 in B_BE_21_BVW5_EU CAF SBB PLAN 219 20200912.pdf) to strictly adhere to the recommendations.

SBB comment:
This suggestion is implemented in the SBB comment and text proposal formulated under section 2.6 , comment 2

Coordinator comment:
OK.

Point 3.7 should also consider the presence of rAAV (even though this will be in the muscle fibers and in a dsDNA version (probably circular or as a concatemer). Samples should be considered 'contaminated' until neutralized with formalin or 4% formaldehyde, or until lysed for protein analysis or other routine testing procedures. Furthermore, when blood samples are taken for analysis (p20/49 point (b)) extra care should be taken, especially at early time-points. Viral vectors are administered to the venous blood, and will be circulating at fairly high concentration in the circulation (e.g. the day after dosing, and possibly the first few weeks post dosing). The protocol for processing these samples should be clearly stipulated in a specific document (which is currently lacking as far as I could find).
Storing of these samples should be in a dedicated freezer since these samples may contain substantial amounts of rAAV. When samples (especially blood) will be assessed in routine testing procedures, these procedures should be ran under the proper biosafety levels (to prevent to drug product to spread). Will this all be taken place under L2 as indicated in point 4 (p22/49 in B_BE_21_BVW5_EU CAF SBB PLAN 219 20200912.pdf).

SBB comment :
It is remarked that the evaluation of procedures for the safe handling of samples is also covered in the context of the evaluation of biosafety dossiers 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 comment:
It is not clear for the coordinator whether the SBB comment is suggestive for addressing this issue further via questions for the notifier or that implementing Directive 2009/41/EC on the contained use of GMOs and pathogens (contained use procedure) by the study sites is already sufficient.

SBB comment :
Procedures for the safe handling of samples will be carefully evaluated during the contained use procedure.

Considering working practices the applicant indicates that info on correct handling of the 'vaccine' will be available (point 5.3, p23/49 in B_BE_21_BVW5_EU CAF SBB PLAN 219 20200912.pdf). I guess this info is mistakenly entered here, or the txt was recycled. Please check whether the information itself indeed consider rAAV vectors and not a previous vaccine.

SBB comment:
This comment could be added in the Typos and other errors/omissions section

Coordinator comment:
Agreed to add in the Typos and other errors/omissions section

In the application 'bleach, or bleach 10%' in suggested as decontaminant. It would be better to be more specific. I assume house-hold bleach is meant. Still, to be an effective disinfectant, working bleach solutions must contain >0.5% but <2% sodium hypochlorite. Hypochlorite concentration in household bleach varies by manufacturer. Many household bleach solutions contain 5.25% sodium hypochlorite, and a 1:10 dilution (5,000 ppm Cl) will produce a 0.53% hypochlorite solution. Use of bleach solutions with lower hypochlorite concentrations might not provide the proper level of disinfection. Prepare a fresh

1:10 household bleach solution regularly. This information I retrieved from http://www.cdc.gov/mmwr/preview/mmwrhtml/su6101a1.htm.
In the B_BE_21_BVW5_Pharmacy Manual_V3.0.pdf the applicant suggest a solution (p18/29 - 1:10 dilution of 5.25% sodium hypochlorite solution for 30 minutes, followed by water, followed by isopropyl alcohol). It would be better to include this throughout the application.

SBB comment :
See text proposal here above (section 3.6 , under comment 2) on the suggestion to request the notifier to provide a 2-4 page **technical sheet** detailing all relevant handling instructions and implementing the experts remarks. The proposed text regarding the correct use of hypochlorite solution could be as follows :
Because hypochlorite concentration in household bleach solutions varies by manufacturer, all decontamination procedures involving the use of sodium hypochlorite solution should 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), special attention should be given to the use of freshly prepared hypochlorite solution.

Coordinator comment:
OK.

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
Please refer to the comment in the 2.6.Biodistribution and shedding section of this evaluation report

Comment 2
The environmental risk assessment provided in point (9) p32/49 and further in B_BE_21_BVW5_EU CAF SBB PLAN 219 20200912.pdf, is not specific for the rAAV drug product that is used in the study. Even though the information is interesting, most of the information is more a review of options, not specifically addressing the specific rAAV used here in this study. Same info in (p18/29) B_BE_21_BVW5_Part 3_Environmental Risk Assessment_20210726 Clean.pdf.

SBB comment

The SBB is also of the opinion that information in B_BE_21_BVW5_Part 3_Environmental Risk Assessment_20210726 Clean.pdf is not sufficiently specific to SRP-9001.
For example,

- ERA, section 1.4.6, p9/29: '*AAV is ultimately inactivated within a short period of time'* whereas in the SNIF, p 7/19, it is stated that : *AAV particles are stable outside host organisms for up to several weeks under normal environmental conditions at a wide pH and temperature ranges. Due to the high stability of the capsid, AAV can remain infectious for at least a month at room temperature (Tenenbaum, 2003).*

- ERA section 4.3, p16, states: *Persons with specialized knowledge and experience will continue to evaluate the possibility of Adverse Effects on Biological Diversity caused by the SRP-9001 in accordance with local regulations*. The notifier could be asked to explain what are the local regulation, what will be analysed and how it will be analysed.

- ERA, section 5.1, p18/29, mentions : *Efforts have been made to develop non-replicative vectors to lower the probability of contact between the viral vector and the wild-type parental virus, thereby reducing the probability of recombination*. The notifier should develop here which efforts have been made for the current viral vector SRP-9001.

- ERA section 5.4.1: Other properties: This section summarizes the potential of rAAV for gene therapy while no specific information is provided with respect to the environmental risk assessment of SRP-9001. The purpose of this section remains unclear.

- ERA section 6.5, p 25/29: Could the notifier clarify to which extent both detection assays (physical titer determination and infectious titer assay) will be performed on shedding samples that will be collected during the course of the proposed trial?

Furthermore, the information is not presented in accordance with the 'Good Practice on the assessment of GMO related aspects in the context of clinical trials with AAV clinical vectors'.

For example :

- For each hazard identified, the characterization of the hazard is missing. An estimate of the magnitude of each of the identified potential adverse effects should be provided. Where a quantitative estimation is not possible, a category ("high", "moderate", "low" or "negligible") should be assigned.

- Section 5.1.1: Potential hazard related to the insert, the human micro-dystrophin (hMicro-Dys) gene, has not been developed. Should the insert be considered as a hazardous insert?

- Section 5.1.3: The second point related to vertical transmission has been reported in this section "Tumorigenicity" whereas it could have been reported as a separate hazard.

- Section 5.1.4: It is not mentioned in which animals the biodistribution results have been obtained. A summary of the results obtained for SRP-9001 analysis could have been added in this section.

- Section 5.1.5: It is not clear whether one of the technique mentioned in the section "off-target expressions" has been developed for the SRP-9001 vector in order to reduce the off-target expressions or not?

- Section 5.2: The evaluation of the exposure pathways corresponds to the evaluation of pathways through which viral vectors and their inserted gene products may interact with human (other than patients receiving treatment with the viral vector) or the environment. These exposure pathways include the spreading occurring at the site of administration, the biodistribution and the shedding. The characteristics of the environment into which the viral vector is intended to be released and the modalities of the release are also important factors to be considered. It may be not possible to estimate the likelihood of exposure precisely for each

hazard that is identified and characterized. Therefore, the likelihood of exposure can be expressed qualitatively using a category ("high", "moderate", "low" or "negligible").

The conclusion could also be improved by developing whether the overall risk is acceptable and if the clinical trial can be carried out. This final evaluation should be expressed as a summary of the overall risk related to the specific application.

Because all of the above remarks show how the evaluation of the notifier's environmental risk assessment was hampered by the poor quality of the document, the notifier could be asked to give it due consideration (2 options are possible)

- by asking a an amended ERA (as part of question addressed to the notifier). In meeting this request to notifier could be referred to the 'Good Practice on the assessment of GMO related aspects in the context of clinical trials with AAV clinical vectors'.
- whenever the notifier foresees any other clinical trial application involving the use of a GMO (in this case the notifier will not be asked to rewrite the ERA in the context of the handling of the current biosafety dossier).

Coordinator comment:

Agreed to communicate the remarks as described in detail above to the notifier. With respect to the two options, the rationale behind these two options is presently not clear to the coordinator.

SBB comment:

The ERA should be completely rewritten as it contains general information that is not specific to SRP-9001. There are two options:

- Either the notifier could be asked to completely rewrite it for this dossier, taking into account the comments mentioned here above
- Or the notifier should not rewrite the ERA for dossier 21-BVW5 and all our comments will be provided to help them in the future when submitting a new biosafety dossier.

Coordinator comment:

Because the ERA is actually the most essential document for the evaluation of such a dossier by the BAC, the coordinator prefers the first option. In the ERA, the notifier has to support the conclusions regarding SRP-9001 with scientific data and arguments. These can of course usually be found in the other documents of the global dossier, but the ERA as a stand-alone document must also contain a sound substantiation of the conclusions.

Discussion on the biodistribution is not correct (p24/33 in B_BE_21_BVW5_Part 1B_Common Application GMO_Confidential.pdf). The distribution of the drug product is defined by the capsid rh74, not the promoter driving the expression. The promoter will only define whether the transgene encoded is expressed in the cells transduced. A promoter does not show a tropism, only the capsid does. The muscle specific promoter will limit 'off-target' expression (in non-muscle cells).

SBB comment:

This comment could be added in the Typos and other errors/omissions section

Coordinator comment:

Agreed to add in the Typos and other errors/omissions section

6. OTHER INFORMATION

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

Comment 1
None.

Comment 2
None.

Typos and other errors/omissions

CAF, p9/32: Animals were dosed once by intravenous (bolus) injection via a tail vein at a volume of 50.6 L/g (Groups 1, 3, 4, and 6) or 16.8 L/g (Groups 2 and 5). Volume unit should be corrected.

CAF, section 3.6, p16/32; CAF, section 5.3.1, p27/32: Please make sure the first point reported for the control measures during reconstitution, handling and administration, "the transportation is performed" is complete.

CAF, section 3.7, p19/32: Does this manual for the surgical personnel and lab personnel correspond to the pharmacy manual? If not, Sarepta could be requested to provide this manual as details of the sample handling, storage and transportation are included in the manual.

According to the Main ICF, p14/42, "some of these visits will be 1-day visits and others will require you to come to the study site for 2 days in a row". This information cannot be found back in the protocol. Since information reported in the protocol and the Main ICF should be consistent, the notifier could be requested to update the appropriate document.

Coordinator comment:
Agreed to add in the Typos and other errors/omissions section

Adviesraad voor Bioveiligheid
Conseil consultatif de Biosécurité

Compilation of the expert's evaluations of the answers of Sarepta Therapeutics on the list of questions for dossier B/BE/21/BVW5

12 may 2022
Ref. SC/1510/BAC/2022_0594

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

INTRODUCTION

Dossier **B/BE/21/BVW5** concerns a notification from Sarepta Therapeutics for a clinical trial entitled "A Phase 3 Multinational, Randomized, Double-Blind, Placebo-Controlled Systemic Gene Delivery Study to Evaluate the Safety and Efficacy of SRP-9001 in Subjects With Duchenne Muscular Dystrophy (EMBARK)".

On 17 February 2022, based on a list of questions prepared by the BAC (SC/1510/BAC/2022_0203), 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 29 April 2022. This complementary information was reviewed by the coordinator and the experts in charge of the evaluation of this notification.

Evaluation Expert 1

In the «1 - 2019-003374-91_ 9001-301 BE BAC RFI RCVD (17-FEB-2022) RESPONSE DOCUMENT» p16/22- Concerning the response to « 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 », the notifier claims that :

« *The SRP-9001 study agent is designed to be replication-defective and not able to replicate inside of transduced cells. Therefore, it is very unlikely that an exposure to the study agent would occur from exposure to transduced cells.*
Hazards from an exposure to blood, urine, vomit or other bodily fluids from study participants is expected to be the same as a similar exposure from a non-study participant. Therefore, personnel should follow existing site procedures to manage an exposure to blood, urine, vomit or other bodily fluids. »

-However, in the response to the question 4, the notifier precised that :
« *For SRP-9001-103 samples for cohort I we have demonstrated that the vector shed is for a transient period and cleared as early as 4 weeks for saliva and urine. For stool we see the clearance observed by 12 weeks… »* and *« SRP-9001-301 does not have vector shedding analysis.* »

-On the basis of the principal shedding data obtained (shedding analysis from study SRP_9001_103), it could be estimated that the hazards study from an exposure to blood, urine, vomit or other bodily fluids from study participants could be evaluated at this stage as not sufficient enough to well identify and characterize each hazard. Thereby, in the current stage of knowledge, the extrapolation of the expected hazards with a similar exposure from a non-study participant must be nuanced. »

Question : could the notifier explain the following line :
« *Therefore, personnel should follow existing site procedures to manage an exposure to blood, urine, vomit or other bodily fluids*. ».
Is it an allusion, in case of spill, to generic clinical clean up procedures or to the specific one included in the instructions dossier for study staff personal ? »

SBB's Comment:
Risk assessment is based on the principle: Risk = hazard x exposure.
This means that if there is no hazard, there is no risk despite a real possibility of exposure. Conversely, if there is no exposure, there will be no risk even if a hazard is identified.
In the context of this dossier, there are no data pointing to danger or adverse effects for the non-patient as the parental virus from which the viral vector is derived is replicative-defective and not pathogenic and the transgene is a truncated human gene which does not exhibit toxic properties. Even if exposure of close contacts to shedding by patients cannot be excluded, the measures that are put in place will help to reduce exposure to the viral vector as much as possible as a preventive measure. Shedding analysis are currently ongoing during the clinical study SRP-9001-103 which is using the same IMP at the same dosis.
The applicant refers to the general SOP for clinical personnel available at the hospital.

Coordinator's comment:
Agreed with the opinion of SBB

Evaluation Expert 2

Document: 1 - 2019-003374-91_ 9001-301 BE BAC RFI RCVD (17-FEB-2022) RESPONSE DOCUMENT.pdf

Q1

The applicant provides the production procedure for the plasmids, where is mentioned "The final clone was selected based on acceptable ITR retention". Does the applicant indicate here that the integrity of the ITR or the retention is not ensured in all plasmids? Please clarify. If ITR is not present or parts are deleted what is considered 'acceptable'.

Coordinator's comment:
This needs to be addressed further via the second list of questions.

Q2

The answer to Q2 is a bit strange. Do I interpret correctly that the applicant indicates producer cells here as possible adventitious agents?

The applicant indicates that the working cell bank is tested for bovine virus (even though the viruses screened are not only bovine). Does this imply that the final drug product is not tested, but only the producer cells are?

SBB's Comment:

According to the SRP-9001_IMPD, three bacterial cell banks of E. coli Endura strain were used to produce the three plasmids needed for HEK293 cell transfection and SRP-9001 production. The detection of viral contaminants is conducted by direct inoculation of the drug product into MRC-5, Vero and HEK293 cell lines followed by an extended incubation. Viral adventitious agents that have been tested include bovine viruses, porcine viruses and human viruses including AAV and Adenovirus. To assure product safety from adventitious agents, both the master and working cell banks have been tested. At appropriate stages in the production process, tests have been performed to ensure that in-process intermediates remain free of adventitious agents. They have also evaluate the purification process for its ability to inactivate and remove viruses.

Q3

Putting aside that animal experiments are not allow extrapolation to human settings, mice were IV injected with 1e12 vg rh74 AAV with specific muscle tropism (assuming 25gr weight). No rAAV could not be shown to be present in testes by ISH. In a second exp negligible to very low levels of vector genome and transgene mRNA were detected in both ovaries and testes, but also here data are not shown. What is meant with very low levels? Also, the timepoints when samples were collected are not indicated.

Does the applicant refer here to data already provided in the original application?

SBB's Comment:

In the study SR-20-14, male mice treated with SRP-9001 were euthanized on Week 12 +/- 15 days. The definitive GLP study (SR-20-015) with male and female mice evaluated the biodistribution, immunogenicity, and toxicity of SRP-9001 and assessed neurobehavioral function and reversibility of any effects after a 13-week post-dosing phase.

Study SR-20-015 shows that vector genome and transgene mRNA were detected at negligible to very low levels in both ovaries and testes with a low number of positive samples. However, no numerical results have been provided. What were the levels of mRNA detected both in male and female reproductive organs? How many positive samples from male and from female mice were observed? The applicant is requested to give further information on the results obtained from study SR-20-15.

Coordinator's comment:

Agreed with the opinion of SBB

Q4

Stool, saliva and urine are assessed, and shedding is shown. I agree levels will be low, but should awareness be raised towards avoiding contact with immune compromised persons or elderly people (eg grand-parents) for that 4 weeks period? The patients here are 4-8 years old.

SBB's Comment:
Such recommendation has never been asked in the past for dossiers AAV viral vectors. Although, exposure of close contacts to shedding by patients cannot be excluded, parental virus from which the viral vector is derived is replicative-defective and not pathogenic and amounts shedded are significantly lower than therapeutic dose.

Coordinator's comment:
Agreed with the opinion of SBB

Q5

I presume, conservative is meant here.

Is there any regulation as to blood donation in general for patients included in gene therapy trials? I understand that young children may not be relevant here anyway. On the other hand, this advice may be important when also elder patients would be included.

SBB's Comment:
Restriction on blood donation for participants to a clinical trial involving a GM medical product is determined on a case by case basis depending on the viral vector used and the nature of the insert gene.

Eligibility criteria for donors of whole blood and blood components are laid down in Annex III to Commission Directive 2004/33/EC of 22 March 2004 implementing Directive 2002/98/EC of the European Parliament and of the Council as regards certain technical requirements for blood and blood components (OJ L91, 30.3.2004, p. 25).

Coordinator's comment:
The answer of the applicant is OK

Q6-8

Q6-8 were answered, but Document 2 - 2019-003374-91_244701 BEL Hygiene Guidance 20220404 0.1 English DRAFT needs additional updating: it states "contain a modified virus. The virus is delivered into cells by a vector. Studies have shown that some vectors can be passed through bodily fluids for several weeks after an infusion; this is called vector shedding."

The product does not contain a virus, but a viral vector (single round, not replicating). The sentence 'The virus is delivered into cells by a vector.' Is confusing for the patients and caregivers and will result in unnecessary issues. Please replace the wording 'vector' with 'viral vector'.

Coordinator's comment:
This needs to be addressed further via the second list of questions.

Q7 was answered adequately.

Q9

In Document 3 - 2019-003374-91_Addendum to Pharmacy Manual_Belgium_FINAL 04Apr2022 (1).pdf the following sentence should be removed: "The use of double gloves should be standard, and not only considered as it is currently stated on p17/49 in EU CAF SBB PLAN 219 20200912.pdf and p16-17/33 + p28/33 of the Common Application GMO_Confidential.pdf." since this is part of the question put forward by the SBB and should not be included in the addendum.

Coordinator's comment:
Use of double gloves needs to be standard. Should it not be indicated as such in all relevant documents?

Q10

The introduction of section 5.1.6 in 4 - 2019-003374-91 V-4.2 CONFIDENTIAL EU Common Application GMO_REDLINE-mb (003)_EG_REDLINE.pdf is not relevant here in my opinion. I assume the applicant refers here to the data in the paper: these relate to other serotypes than the one used here. Different serotypes will demonstrate different tropisms, and thus the conclusion of the Tenenbaum paper cannot readily be extrapolated. One could assume that the rh74 serotype behaves the same, but this has not been tested.

SBB's Comment:

In her paper written in 2003, Tenenbaum *et al.* evaluated the risk related to the use of recombinant AAV2- based vector. Whereas, the AAV serotype used in the currently clinical trial corresponds to serotype rh74, which has been isolated from non-human primates (Rhesus macaque). Serotype and route of administration are important factors impacting the biodistribution pattern of AAV-based gene therapies, meaning that different serotypes will demonstrate different tropisms. Therefore, the conclusion observed in the Tenenbaum's paper cannot readily be extrapolated. One could assume that the rh74 serotype behaves the same, but this has not been tested.

Coordinator's comment:
Agreed with the opinion of SBB

Q11 was answered adequately.

Reference:

L. Tenenbaum *et al*. Evaluation of Risks Related to the Use of Adeno-Associated Virus-Based Vectors. Current Gene Therapy, 2003, 3, 545-565