PART 1 (COUNCIL DECISION 2002/813/EC)

SUMMARY NOTIFICATION INFORMATION FORMAT FOR THE RELEASE OF GENETICALLY MODIFIED ORGANISMS OTHER THAN HIGHER PLANTS IN ACCORDANCE WITH ARTICLE 11 OF DIRECTIVE 2001/18/EC

In order to tick one or several possibilities, please use crosses (meaning x or X) into the space provided as (.)

A. General information

1. Details of notification

(a)	Member State of notification	Belgium...
(b)	Notification number	...
(C)	Date of acknowledgement of notification	...
(d)	Title of the project	A Phase 3, Multinational, Randomized, Double-Blind, Placebo-Controlled Systemic Gene Transfer Therapy Study to Evaluate the Safety and Efficacy of SRP-9001 in Non-Ambulatory and Ambulatory Subjects With Duchenne Muscular Dystrophy (ENVISION)
(e)	Proposed period of release	Q2 2023 – Q2 2026

2. Notifier

Name of institution or company: Sarepta Therapeutics, Inc.

3. GMO characterisation

(a) Indicate whether the GMO is a:

viroid	(.)
RNA virus	(.)
DNA virus	(X)
bacterium	(.)
fungus	(.)
animal	
mammals	(.)
insect	(.)
fish	(.)
other animal	(.)

specify phylum, class

(b) Identity of the GMO (genus and species)
Genus: Dependoparvovirus
Species: Adeno Associated Virus (AAV) serotype rh74 (micro-dystrophin)

(c) Genetic stability – according to Annex IIIa, II, A(10)
...

AAV is a single stranded DNA virus that demonstrates a high degree of genetic stability as evidenced by the high degree of sequence conservation of the rep and cap genes from multiple AAV serotypes and genomovars. Furthermore, AAV uses host DNA polymerases for viral replication, which are characterised by high fidelity DNA polymerization and additional proofreading exonuclease activity leading to a very low error rate of DNA replication, when compared, for example, to RNA polymerases used by RNA viruses. It is known that wild type AAV DNA as well as that of AAV-based vectors persists in transduced cells as circular (extrachromosomal) episomal concatemers in human tissues (Chen 2005, Penaud-Budloo 2018, and Schnepp, 2005).

The genetic stability of SRP-9001 is expected to be equivalent to wild type AAV. However, due to the lack of viral Rep and Cap genes, SRP-9001 is expected to remain in the cells as episomes and will not replicate and produce viral particles.

4. Is the same GMO release planned elsewhere in the Community (in conformity with Article 6(1)), by the same notifier?

Yes (X) No ()

If yes, insert the country code(s)

BE
DE
ES
FR
IT
SE

5. Has the same GMO been notified for release elsewhere in the Community by the same notifier?

Yes (X) No ()

If yes:

- Member State of notification: BE; ES
- Notification number: B/BE/21/BVW5; B/ES/21/25

Please use the following country codes:
Austria AT; Belgium BE; Germany DE; Denmark DK; Spain ES; Finland FI; France FR; United Kingdom GB; Greece GR; Ireland IE; Iceland IS; Italy IT; Luxembourg LU; Netherlands NL; Norway NO; Portugal PT; Sweden SE

6. Has the same GMO been notified for release or placing on the market outside the Community by the same or other notifier?

Yes (X) No (.)

If yes:

- Member State of notification: United States; United Kingdom
- Notification number: IND 017763; CTA 43810/0008/001-0001

7. Summary of the potential environmental impact of the release of the GMOs.

SRP-9001 is a non-replicating AAV vector containing the SRP-9001-dystrophin gene for the treatment of patients with Duchenne Muscular Dystrophy.

The release of SRP-9001 as described in this application, is not expected to have an adverse environmental impact, including in the patient population, for the following reasons:

Pathology

Adeno-associated viruses (AAV) are single stranded DNA viruses that have not been found to cause pathogenic effects in humans. The modifications which have led to the generation of the GMO have not raised the pathogenicity of SRP-9001.

Productivity of Harmful Substances

The viral vector does not contain any viral sequences, except ITRs, which facilitate transgene expression and do not lead to production of viral proteins, particles or DNA replication.

SRP-9001 encodes for the SRP-9001-dystrophin protein, a shortened version of a naturally occurring protein, and is therefore unlikely to be toxic to humans or other organisms. Comprehensive toxicity studies failed to demonstrate any toxic effect of SRP-9001 at the intended dose.

No genes for oncogenes, toxins, or potentially harmful genes have been included into the GMO.

Property of Transmitting Nucleic Acid Horizontally

It has been shown that only a very small proportion of the total number of SRP-9001 viral genomes infused in the patient population are shed in a matrix of bodily fluids (whole blood, serum, urine, saliva). SRP-9001's ability to transmit nucleic acid horizontally is considered to be substantially reduced compared to wtAAV, which is the taxonomical species to which the altered organism belongs.

As viral shedding is limited and SRP-9001 is replication-incompetent, further distribution is not possible, and it cannot spread in the environment. In addition, minimal exposure of non-target individuals to SRP-9001 is unlikely to result in any effect. As a result, SRP-9001 would not be expected to pose any risk of transmitting nucleic acid horizontally.

Replication Incompetent and Insertional Mutagenesis

SRP-9001 is a non-pathogenic recombinant AAV vector that lacks all AAV viral genes and cannot replicate without AAV-specific helper functions and helper virus activities. SRP-9001 replication could only occur in the extremely unlikely event of a host cell being infected by wild-type AAV and a helper virus such as human adenovirus or herpes simplex virus. If replication occurred, the only expected products would be SRP-9001 and WT AAV, both intrinsically nonpathogenic viruses.

The rAAV vectors do not contain viral coding sequences and do not express Rep proteins which play a key role for DNA replication, site-specific integration into chromosomal DNA, and cellular growth inhibitory effects. Human gene therapy recombinant AAVs are used to deliver and ultimately express a therapeutic "transgene" in somatic cells for the purposes of treating genetically inherited diseases. Somatic cells contribute to the various tissues of the body but not to the germline. The effects of

changes made to somatic cells are limited to the treated individual and would not be inherited by future generations.

Tumorigenicity by insertional mutagenesis is a theoretical concern for any gene therapy vectors. It is generally hypothesized that viral ITR sequences may have a structure with the potential for recombination even in the absence of Rep proteins. Data from mice, dogs, NHPs and humans suggest that the integration of AAV vectors into the host genome is a rare event, with most of the vector assimilating into concatemeric episomes. Although integration of vector sequences into the cellular genome seems to occur preferentially into transcriptionally active regions in mice, tumor formation has not been observed after rAAV mediated therapy in NHPs, dogs, rats or in any patients in clinical trials to date, even after long-term follow up (Colella, 2018).

Immunogenicity

The transgene product and the virus capsid are the only sources of foreign antigen which are thought to have the possibility of eliciting an immune response. Additionally, pre-existing immunity to AAV in a large proportion of the human population could potentially complicate the use of rAAV vectors derived from serotypes isolated from human samples. Patients will be monitored for immune responses and treated prophylactically with corticosteroids during the first few weeks to months post gene transfer.

B. Information relating to the recipient or parental organism from which the GMO is derived

1. Recipient or parental organism characterization:

(a) Indicate whether the recipient or parental organism is a: (select one only)

viroid	(.)
RNA virus	(.)
DNA virus	(X)
bacterium	(.)
fungus	(.)
animal	
mammals	(.)
insect	(.)
fish	(.)
other animal	(.)
(specify phylum, class)	...
other, specify	...

2. Name

(i)	order and/or higher taxon (for animals)	... Parvoviridae Family
(ii)	genus	... Dependovirus
(iii)	species	... Adeno-associated virus
(iv)	subspecies	... N/A
(v)	strain	... N/A
(vi)	pathovar (biotype, ecotype, race, etc.)	... Serotype rh74
(vii)	common name	... AAV rh74

3. Geographical distribution of the organism

(a) Indigenous to, or otherwise established in, the country where the notification is made:
Yes (X) No (.) Not known (.)

(b) Indigenous to, or otherwise established in, other EC countries:
(i) Yes (X)

If yes, indicate the type of ecosystem in which it is found:

Atlantic	.. (X)
Mediteranean	.. (X)
Boreal	.. (X)
Alpine	.. (X)
Continental	.. (X)
Macaronesian	.. (X)

(ii) No (.)
(iii) Not known (.)

(c) Is it frequently used in the country where the notification is made?
Yes (.) No (X)

(d) Is it frequently kept in the country where the notification is made?
Yes (.) No (X)

4. Natural habitat of the organism

(a) If the organism is a microorganism

water (.)
soil, free-living (.)
soil in association with plant-root systems (.)
in association with plant leaf/stem systems (.)
other, specify ...

AAV rh74 has been isolated from non-human primates (*Rhesus macaque*), although other animals or humans can be hosts.

(b) If the organism is an animal: natural habitat or usual agroecosystem:
...
Not applicable

5. (a) Detection techniques

AAV can be detected by quantitative polymerase chain reaction (qPCR) using primers specific for the viral genome. (b) Identification techniques
...
Sanger sequencing is used as ID testing for all product lots

6. Is the recipient organism classified under existing Community rules relating to the protection of human health and/or the environment?
Yes (.) No (X)
If yes, specify
...
Wild type AAV is not classified in Risk Groups 2,3, or 4 in the European Union (EU) according to directive 2000/54/EC on protection of workers from risks related to exposure to biological agents at work (Appendix III). Consequently, AAV fulfills the definition of a Risk Group 1 biological agent, defined in the EU as 'one that is unlikely to cause human disease'.

7. Is the recipient organism significantly pathogenic or harmful in any other way (including its extracellular products), either living or dead?
Yes (.) No (X) Not known (.)

If yes:

a) to which of the following organisms:

humans (.)
animals (.)
plants (.)
other (.)

(b) give the relevant information specified under Annex III A, point II. (A)(11)(d) of Directive 2001/18/EC
Not applicable

8. Information concerning reproduction

a) Generation time in natural ecosystems:

The replication of recombinant AAV rh74 in an infected host cell is dependent on co-infection with a helper virus such as adenovirus. The generation time of wild-type AAV in a natural ecosystem will be significantly very high, depending on the timing of the co-infection. Replication competent AAV generation time of SRP-9001 is not relevant since it lacks the rep and cap genes that are required for the reproduction of rcAAV.

b) Generation time in the ecosystem where the release will take place:
...

(c) Way of reproduction: Sexual .. Asexual .. X

(d) factors affecting reproduction:
...
Triple infection is needed before horizontal infection to occur in patients who are administered with SRP-9001, and the possibility of this occurring is very low. Moreover, even when rcAAV forms, co-infection with a helper virus is needed for horizontal infection, so considering the formation rate of rcAAV, the possibility of horizontal infection is very low. Although it is known that wild-type AAV is inserted into the infected cell genome at a low probability, since SRP-9001 is deficient of the rep/cap gene, it is not proliferation competent. Moreover, even when horizontal infection occurs, it is very unlikely that SRP-9001 derived hMicro-Dys nucleic acid is incorporated into the infected cell genome.

9. Survivability

(a) ability to form structures enhancing survival or dormancy:

(i) endospores (.)
(ii) cysts (.)
(iii) sclerotia (.)
(iv) asexual spores (fungi) (.)
(v) sexual spores (funghi) (.)
(vi) eggs (.)
(vii) pupae (.)
(viii) larvae (.)
(ix) other, specify ...
Wild type and recombinant AAVs, remain episomal for extended periods of time by the formation of genome concatemerization.

(b) relevant factors affecting survivability:

...

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). Proper decontamination procedures such as 10% bleach, ionic detergents, or alkaline solutions (pH > 9.5) must be employed to ensure safety (Howard, 2017).

10. (a) Ways of dissemination

Wild type and recombinant AAV vectors are possibly transmitted by the ingestion, inhalation of aerosols or droplets, contact with mucous membranes, bodily fluids and fecal matter.

(b) Factors affecting dissemination

Replication of the virus is only possible in host cells that have been co-infected with a helper virus (e.g. adenovirus, herpes simplex virus).

11. Previous genetic modifications of the recipient or parental organism already notified for release in the country where the notification is made (give notification numbers)

...

None

C. Information relating to the genetic modification

1. Type of the genetic modification

(i)	insertion of genetic material	(X)
(ii)	deletion of genetic material	(X)
(iii)	base substitution	(.)
(iv)	cell fusion	(.)
(v)	others, specify ...	

2. Intended outcome of the genetic modification

...

The intended outcome of the genetic modification was to generate a recombinant AAV vector lacking viral genes so that the vector would be replication incompetent and serve only to introduce the transgene and to include the sequence coding for SRP-9001-dystrophin to cause replacement of the absent dystrophin and thus enable the treatment of patients with Duchenne Muscular Dystrophy. The goal of SRP-9001 therapy is to promote the expression level of the SRP-9001-dystrophin protein in skeletal and cardiac muscle to increase strength and protect them from contraction induced injury. The clinical studies conducted to evaluate safety and efficacy of SRP-9001 appear to have a favorable safety profile and to be generally well tolerated in clinical studies.

3. (a) Has a vector been used in the process of modification?

Yes (X) No (.)

If no, go straight to question 5.

(b) If yes, is the vector wholly or partially present in the modified organism?

Yes (X) No (.)

If no, go straight to question 5.

4. If the answer to 3(b) is yes, supply the following information

(a) Type of vector

plasmid	(X)
bacteriophage	(.)
virus	(.)
cosmid	(.)
transposable element	(.)
other, specify	...

(b) Identity of the vector

...

SRP-9001 is a non-replicating, recombinant adeno-associated virus containing a human SRP-9001-dystrophin gene under the control of the MHCK7 promotor/enhancer, has been optimized for driving expression in cardiac and skeletal muscle (Rodino-Klapac et al. 2013). The recombinant vector genome contains elements required for gene expression, including a MHCK7 promoter, a transgene encoding functional domains of the human dystrophin gene and a polyadenylation signal, flanked by AAV inverted terminal repeats (ITRs).

(c) Host range of the vector

...

E. coli (bacterial) cells.

(d) Presence in the vector of sequences giving a selectable or identifiable phenotype

Yes (X) No (.)

antibiotic resistance (X)
other, specify ...

Indication of which antibiotic resistance gene is inserted

...

The antibiotic resistance genes are only present in the plasmids used in the manufacturing of SRP-9001. The SRP-9001 viral vector does not contain any antibiotic resistant genes.

(e) Constituent fragments of the vector

...

The SRP-9001 vector is produced by a process known as “triple transfection”, which utilizes 3 different plasmid DNA constructs.

1. AAV Vector Plasmid
2. AAV RepCap plasmid
3. Ad Helper plasmid

(f) Method for introducing the vector into the recipient organism

(i) transformation (.)

(ii) electroporation (.)
(iii) macroinjection (.)
(iv) microinjection (.)
(v) infection (.)
(vi) other, specify ... **Transfection**

5. If the answer to question B.3(a) and (b) is no, what was the method used in the process of modification?

(i) transformation (.)
(ii) microinjection (.)
(iii) microencapsulation (.)
(iv) macroinjection (.)
(v) other, specify ...

6. Composition of the insert

(a) Composition of the insert

The packaged rAAV vector genome comprises of a promoter, a transgene encoding functional domains of the human dystrophin gene and a polyadenylation signal, flanked by AAV inverted terminal repeats (ITRs).

(b) Source of each constituent part of the insert:
- MHCK7 Promoter: *Mus musculus*
- SRP-9001-dystrophin transgene: *Homo sapiens*
- Polyadenylation signal: Synthetic PolyA
- AAV Inverted Terminal Repeats (ITRs): Wild-type AAV2

(c) Intended function of each constituent part of the insert in the GMO

- *Promoter***:** Intended to drive skeletal and heart muscle specific gene expression.
- *Functional domains of the human dystrophin gene retained in SRP-9001 dystrophin*:

  Gene transfer may be effective for the treatment of patients with Duchenne Muscular Dystrophy, given that the disease is caused by mutations within the DMD gene that affect the expression or activity of dystrophin.
- *Polyadenylation signal*: Terminate transcription of the SRP-9001-dystrophin gene.
- *AAV ITRs*: Inverted Terminal Repeat (ITR) sequences required for second strand DNA synthesis to facilitate gene expression

(d) Location of the insert in the host organism
- on a free plasmid (.)
- integrated in the chromosome (.)
- other, specify ...

The vector is located in the form of episomal concatemers as extrachromosomal bodies.

(e) Does the insert contain parts whose product or function are not known?

Yes (.) No (X)

If yes, specify ...

D. Information on the organism(s) from which the insert is derived

1. Indicate whether it is a:

- viroid (.)
- RNA virus (.)
- DNA virus (.)
- bacterium (.)
- fungus (.)
- animal (.)
- mammals (X)
- insect (.)
- fish (.)
- other animal (.)

(specify phylum, class) ...

other, specify ...

2. Complete name

(i)	order and/or higher taxon (for animals)	... N/A
(ii)	family name for plants	... N/A
(iii)	genus	... *Homo*
(iv)	species	... *sapiens*
(v)	subspecies	... N/A
(vi)	strain	... N/A
(vii)	cultivar/breeding line	... N/A
(viii)	pathovar	... N/A
(ix)	common name	... Human

3. Is the organism significantly pathogenic or harmful in any other way (including its extracellular products), either living or dead?

Yes (.) No (X) Not known (.)

If yes, specify the following:

(a) to which of the following organisms:

humans (.)
animals (.)
plants (.)
other ...

(b) are the donated sequences involved in any way to the pathogenic or harmful properties of the organism

Yes (.) No (X) Not known (.)

If yes, give the relevant information under Annex III A, point II(A)(11)(d): ...

4. Is the donor organism classified under existing Community rules relating to the protection of human health and the environment, such as Directive 90/679/EEC on the protection of workers from risks to exposure to biological agents at work?
Yes (.) No (X)
If yes, specify ...

5. Do the donor and recipient organism exchange genetic material naturally?
Yes (.) No (.) Not known (.)
Not applicable

E. Information relating to the genetically modified organism

1. Genetic traits and phenotypic characteristics of the recipient or parental organism which have been changed as a result of the genetic modification

(a) is the GMO different from the recipient as far as survivability is concerned?
Yes (.) No (X) Not known (.)
Specify ...
Since capsid particle of SRP-9001 is similar to that of wild type AAV rh74, *ex vivo* survival characteristics are identical for both recombinant and wild type serotype.

(b) is the GMO in any way different from the recipient as far as mode and/or rate of reproduction is concerned?
Yes (X) No (.) Unknown (.)
Specify ...
Due to the removal of the rep and cap genes SRP-9001 is unable to replicate even in the presence of wild-type AAV helper virus.

(c) is the GMO in any way different from the recipient as far as dissemination is concerned?
Yes (.) No (X) Not known (.)
Specify ...
The viral capsid proteins have the same dissemination/tropism as the parent AAV rh74 virus. However, since SRP-9001 replication deficient, the dissemination is limited to the administration of the SRP-9001 to the patient.

(d) is the GMO in any way different from the recipient as far as pathogenicity is concerned?
Yes (.) No (X) Not known (.)
Specify ...
No pathogenic effects of wild-type AAV in humans are known. The introduction of the expression cassette, encoding SRP-9001-dystrophin, is not expected to result in development of pathogenicity. Thus, neither the wild-type AAV nor SRP-9001 are known or expected to be pathogenic. Removal of viral genes in making the vector would be expected to further reduce any risk of pathogenesis.

2. Genetic stability of the genetically modified organism

...

The stability of the recombinant SRP-9001 is confirmed by characterizing the identity, purity, and quality. The administration of SRP-9001 to DMD subjects infects target cells by forming multiple SRP-9001 genomes assembled to form larger double stranded DNA concatemers. However, no new virus particles are being formed in the subjects. These concatemers persist in the cell as stable episomal structures and are transcriptionally active. Based on the known genetic stability of the wild type AAV, and the absence of an intrinsic mechanism for the genetic variation or instability, the genetic traits of SRP-9001 are expected to be stable.

3. Is the GMO significantly pathogenic or harmful in any way (including its extracellular products), either living or dead?

Yes (.) No (X) Unknown (.)

(a) to which of the following organisms?

humans	(.)
animals	(.)
plants	(.)
other	...

(b) give the relevant information specified under Annex III A, point II(A)(11)(d) and II(C)(2)(i)

...

Annex III A, point II(A)(11)(d): Recombinant AAVs engineered for gene therapy clinical trials, do not incorporate into the host cell genome and instead form episomal concatemers in the host cell nucleus (Kimura, et al., 2019). Preclinical data also indicate that AAV vectors predominantly persists as extrachromosomal elements (episomes) rather than integrating into host cell genomes (McCarty, et al., 2004). Based on the available clinical and non-clinical data, it is concluded that SRP-9001 does not integrate into the host cell genome. However, long-term consequences of administering AAV viral vectors to humans are yet to be studied. Since SRP-9001 uses AAV rh74 with all the wild-type DNA removed, except for the Inverted Terminal Repeats, the potential risk of incorporation of SRP-9001 into the patient chromosomal DNA is thought to be significantly reduced.

The recombinant SRP-9001 vector 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 et al., 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, 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 and adenovirus.

II(C)(2)(i): In general, the viral shedding is observed for a short period after the administration of non-replicating SRP-9001 with very limited exposure to the environment. Thus, exposure of plants or animals is not expected. SRP-9001 is non-pathogenic and the human dystrophin protein is not known to have toxic effects. No

side-effects have been reported for the environment or human health after the release of similar GMOs (adeno-associated virus from serotypes 2 and 9).

SRP-9001 is replication-incompetent and is not expected to survive, multiply, or disperse if it were to be eliminated intact from the treated patient. AAV-based gene therapies are known to shed via bodily fluids. It has been shown consistently that vectors are shed for a short period of time, but then become undetectable in bodily fluids. The viral load shed in bodily fluids is expected to be low, compared to the necessary dose required to achieve detectable gene expression in humans.

Regardless, instructions should be provided to patient families and care givers regarding use of protective gloves if/when coming into direct contact with patient bodily fluids and/or waste as well as good hand-hygiene for 4 weeks after the injection. Additionally, patients are prohibited from donating blood for 6 months following the vector injection.

4. Description of identification and detection methods

(a) Techniques used to detect the GMO in the environment

...

SRP-9001 can be detected by qPCR/ddPCR assay using primers and probe specific to the MHCK7 promoter.

(b) Techniques used to identify the GMO

...

SRP-9001 can be monitored by qPCR/ddPCR assay using primers and probe specific to the MHCK7 promoter.

F. Information relating to the release

1. Purpose of the release (including any significant potential environmental benefits that may be expected)

...

Phase III, pediatric gene therapy study with SRP-9001 in subjects with Duchenne Muscular Dystrophy

2. Is the site of the release different from the natural habitat or from the ecosystem in which the recipient or parental organism is regularly used, kept or found?

Yes (X) No (.)

If yes, specify ...

SRP-9001 is administered intravenously to Duchenne Muscular Dystrophy patients.

3. Information concerning the release and the surrounding area

(a) Geographical location (administrative region and where appropriate grid reference):

... UZ Gent Neuromuscular reference center
C. Heymanslaan 10, 9000 Gent – Belgium

(b) Size of the site (m^2): Not Applicable
(i) actual release site (m^2): ... m2
(ii) wider release site (m^2): ... m2

(c) Proximity to internationally recognised biotopes or protected areas (including drinking water reservoirs), which could be affected:

...

Not applicable.

SRP-9001 will be administered by a one-time single intravenous infusion in a hospital setting. Thus, it is not anticipated to come into contact with any recognised biotopes or protected areas.

(d) Flora and fauna including crops, livestock and migratory species which may potentially interact with the GMO

...

Administration of SRP-9001 will occur only within a controlled hospital setting; therefore, it is not anticipated that it will come into contact with plants, animals or soil.

4. Method and amount of release

(a) Quantities of GMOs to be released:

...

SRP-9001 will be administered in patients with a one-time dose for the treatment of Duchenne Muscular Dystrophy. Approximately 116 subjects will be dosed globally with SRP-9001 in this study globally across 2 cohorts:

- Cohort 1 includes male DMD non-ambulatory subjects (no age limit)
- Cohort 2 includes male DMD ambulatory subjects who are ≥ 8 to < 18 years of age.

10 patients are anticipated to be administered with SRP-9001 in Belgium.

The quantities that will be released into the environment by shedding will be a very small proportion of the total number of viral genomes. SRP-9001 is detectable by qPCR/ddPCR in the shed samples from day 1 post injection.

(b) Duration of the operation:

...

The administration procedure including preparation of the infusion system is expected to take approximately 2 hours.

(c) Methods and procedures to avoid and/or minimise the spread of the GMOs beyond the site of the release

...

Healthcare professionals handling SRP-9001 will be trained in biosafety practices to use during the preparation of SRP-9001 in the pharmacy, transport to the administration room, precautions during administration. Staff will follow the waste and disposal policies as per local site requirement to dispose of consumables used in the preparation and administration of SRP-9001.

A Pharmacy Manual and training material located at sites provides pharmacy personnel and clinical medical staff directions on use, storage and destruction of the IMP.

Healthcare professionals handling SRP-9001 will wear Personal Protective Equipment (PPE) including:

- Gloves (double gloving)
- Safety goggles
- Disposable isolation gown
- Appropriate PPE should also be used for lower arms such as sleeve covers.
- Personnel with open sores or cut should be restricted.

The administration room will be cleaned according to local standard institutional procedures after the administration of SRP-9001 to the patient. It is not expected that SRP-9001 will be deliberately released into the environment outside the administration site.

The risks related to the release into the environment of the GMO or risks to personnel in the event there is a breach in container integrity and/or storage or accidental spillage at the site or during shipping/storage, is considered to be negligible. In the event that a spillage did occur, the product is non-pathogenic and non-replicative, limiting spread and risks to the environment or personnel.

5. Short description of average environmental conditions (weather, temperature, etc.)

Not applicable. Administration of SRP-9001 will occur only within a controlled hospital environment and conducted in the administration room at room temperature.

...

6. Relevant data regarding previous releases carried out with the same GMO, if any, specially related to the potential environmental and human health impacts from the release.

…

SRP-9001 administration increases the dystrophin expression *in vivo* which may potentially improve subject's muscle function and, importantly, may preserve diaphragm and cardiac muscle. These improvements would increase patient quality of life and may prolong survival based on the Phase 1 and Phase 2 studies conducted in the US.

G. Interactions of the GMO with the environment and potential impact on the environment, if significantly different from the recipient or parent organism

1. Name of target organism (if applicable)

(i)	order and/or higher taxon (for animals)	**…** Primate
(ii)	family name for plants	**…** N/A
(iii)	genus	**…** *Homo*
(iv)	species	**…** *sapiens*
(v)	subspecies	**…** N/A
(vi)	strain	**…** N/A
(vii)	cultivar/breeding line	**…** N/A
(viii)	pathovar	**…** N/A
(ix)	common name	**…** Human

2. Anticipated mechanism and result of interaction between the released GMOs and the target organism (if applicable)

…

Duchenne muscular dystrophy affects all skeletal muscle in the body, in addition to the diaphragm and heart. As such, a systemic approach is necessary in order to provide the best possible prospect of direct benefit to patients. Utilizing the rAAV rh74 serotype allows for efficient transduction of cardiac, skeletal and diaphragm muscle.

3. Any other potentially significant interactions with other organisms in the environment

…

Persons other than the human subjects receiving the medicinal product will not be exposed to levels of SRP-9001 that could represent potential hazard. Minimal exposure, such as environmental exposure, to organisms other than the subjects receiving SRP-9001 as part of the study would not be of sufficient dose to represent significant gene expression or potential safety risks. As SRP-9001 is also replication-incompetent, it is expected that the vector would be rapidly cleared from any non-target organisms without causing any harmful effects. Other than potential human hosts, exposure to SRP-9001 is not expected to affect any non-target organisms, either directly or indirectly.

4. Is post-release selection such as increased competitiveness, increased invasiveness for the GMO likely to occur?

Yes (.) No (X) Not known (.)

Give details

…

Since SRP-9001 is replication deficient, increased competitiveness, increased invasiveness is not expected.

5. Types of ecosystems to which the GMO could be disseminated from the site of release and in which it could become established

...

As SRP-9001 is unable to replicate, it is not expected to spread to the environment to a significant degree and is not expected to become established in any ecosystems.

6. Complete name of non-target organisms which (taking into account the nature of the receiving environment) may be unintentionally significantly harmed by the release of the GMO.

Not applicable.

(i)	order and/or higher taxon (for animals)	...
(ii)	family name for plants	...
(iii)	genus	...
(iv)	species	...
(v)	subspecies	...
(vi)	strain	...
(vii)	cultivar/breeding line	...
(viii)	pathovar	...
(ix)	common name	...

7. Likelihood of genetic exchange in vivo

(a) from the GMO to other organisms in the release ecosystem:

There is no effect or danger to the biodiversity with horizontal transmission by diffusion of the genetic material. Even if horizontal gene transfer occurred, the sequences would not confer a selective advantage to other organisms such as bacteria since SRP-9001 does not contain any prokaryotic promoters, resistance genes or any genes, which would enhance their growth. Therefore, it is unlikely that SRP-9001 would influence the natural dynamics of microbial populations or the biogeochemical cycles at any given site in the environment.

(b) from other organisms to the GMO:

...

Since SRP-9001 contains ITR-sequences, there is a very low possibility of homologous recombination of the vector with wild type AAV in case of a co-infection in exposed persons. The result of such a recombination would be that SRP-9001 would gain functional genes of the AAV required for replication and encapsidation. Hence, recombination would lead to the formation of viruses that are identical to the recombinant strain that is replication incompetent.

(c) likely consequences of gene transfer:

...

Expression of human SRP-9001-dystrophin) protein.

8. Give references to relevant results (if available) from studies of the behaviour and characteristics of the GMO and its ecological impact carried out in stimulated natural environments (e.g. microcosms, etc.):

...

No such studies have been conducted with SRP-9001.

9. Possible environmentally significant interactions with biogeochemical processes (if different from the recipient or parental organism)
...

SRP-9001 is not known or predicted to have an impact on biogeochemical processes.

H. Information relating to monitoring

1. Methods for monitoring the GMOs
...
qPCR/ddPCR

2. Methods for monitoring ecosystem effects
...
None.

3. Methods for detecting transfer of the donated genetic material from the GMO to other organisms
...
Transfer of genetic material from the SRP-9001 to other organisms is negligible. qPCR/ddPCR can be used for detecting transfer of the donated genetic material from the GMO to other organisms

4. Size of the monitoring area (m^2)
...
Not applicable.

5. Duration of the monitoring
...
Not applicable.

6. Frequency of the monitoring
...
Not applicable.

I. Information on post-release and waste treatment

1. Post-release treatment of the site
...

After administration of the SRP-9001 to the patients, the procedure room will be disinfected using an appropriate disinfectant as per the pharmacy and dose administration manuals and local guidelines for handling of biological waste.

2. Post-release treatment of the GMOs
...

Any open vials or unused material must be sealed in leak-proof containers. The Sponsor will determine whether unused vials are to be destroyed or returned. Empty vials and used

vials and the product contact delivery system components (cannula, injection needles and syringes), gauzes, personal protective equipment and components used for collecting body fluid samples after administration will be disinfected or incinerated as per end-user medical waste treatment regulations.

3. (a) Type and amount of waste generated

...

After administration of SRP-9001, the following waste is generated; empty vials, used vials, guide tube, cannula, injection needles and syringes, gauzes, gloves, and components used for collecting body fluids samples.

4. (b) Treatment of waste

...

All materials that may have come in contact with SRP-9001 must be disposed of in accordance with local guidelines on handling of biological waste. Patient families will be advised to follow instructions for the proper handling of patient stools, good hand-hygiene when coming into direct contact with patient bodily waste for a minimum of 4 weeks after the treatment with SRP-9001. Diapers should be sealed in plastic bags and then double-bagged and disposed of in household waste.

J. Information on emergency response plans

1. Methods and procedures for controlling the dissemination of the GMO(s) in case of unexpected spread

…

In case of accidental spillage of SRP-9001 during the dose preparation and administration to the patient, instructions provided in the Sponsor´s pharmacy and dose administration manuals will be followed to contain and immediately disinfect the spill and 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 Pharmacy and Dose Administration Manuals.

- Evacuate area, remove contaminated PPE and allow agents to settle for a minimum of 30 minutes. Initiate spill response procedure.
- Cover the spill with absorbent material. Starting at the edges and work towards the center.
- 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.
- Allow sufficient contact period to inactivate the material in the spill. Non-viscous spills require 15-20 minutes: viscous spills require 30 minutes.
- Use paper towels to wipe up the spill, working from the edge to center. Use tongs or forceps to pick up broken plastics, glass or other sharps that could puncture gloves.
- Discard absorbent material in biological waste bags.
- Clean the spill area with fresh paper towels soaked in disinfectant. Thoroughly wet the spill area, allow to disinfect for 15-20 minutes longer, and wipe with

towels. Discard all cleanup materials (soaked with disinfectant) in a Chemical bag/container, and any contaminated PPE in a biohazard bag. Close and secure the bags.

- Place bag in a second biohazard bag, secure and dispose as per institutional guidelines for biohazardous waste.

2. Methods for removal of the GMO(s) of the areas potentially affected

   Any surface area exposed to the GMO will be disinfected using appropriate disinfectant as per local guidelines and institutional policies and procedures. All materials used in the clean-up will be discarded as clinical waste and will be incinerated.

3. Methods for disposal or sanitation of plants, animals, soils, etc. that could be exposed during or after the spread

   ...

   Administration of SRP-9001 will occur only within a controlled hospital setting; therefore, it is not anticipated that it will come into contact with plants, animals or soil. Furthermore, SRP-9001 is not capable of infecting plants or microbes.

4. Plans for protecting human health and the environment in the event of an undesirable effect ...

   Healthcare professionals will follow local laws and institutional procedures for the handling and disposal of genetically modified organisms. Furthermore, safety recommendations and guidance on the management of incidents related to SRP-9001 are provided in the Pharmacy and Dose Administration manuals for investigators and staff included in this submission. All patients will be carefully monitored for any adverse reactions during this study. An external data monitoring committee (DMC) will be responsible for monitoring safety data from the study.