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 BE; DE; IT; ES.
(b) Notification number …/../../….
(c) Date of acknowledgement of notification ../../….
(d) Title of the project
A Phase 3 Multinational, Open-label, Systemic Gene Delivery Study to Evaluate the Safety and Efficacy of SRP-9003 in Subjects with Limb Girdle Muscular Dystrophy 2E/R4.

Proposed period of release: Start: May 2024
End: January 2031

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 (β-sarcoglycan).

(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 YW, Nagaraju K, Bakay M, McIntyre O, Rawat R, Shi R, Hoffman EP. Early onset of inflammation and later involvement of TGFbeta in Duchenne muscular dystrophy. Neurology. 2005 Sep 27;65(6):826-34. doi: 10.1212/01.wnl.0000173836.09176.c4. Epub 2005 Aug 10. PMID: 16093456., Penaud-Budloo M, François A, Clément N, Ayuso E. Pharmacology of Recombinant Adeno-associated Virus Production. Mol Ther Methods Clin Dev. 2018 Jan 8;8:166-180. doi:10.1016/j.omtm.2018.01.002. PMID: 29687035; PMCID: PMC5908265., Schnepp BC, Jensen RL, Chen CL, Johnson PR, Clark KR. Characterization of adeno-associated virus genomes isolated from human tissues. J Virol. 2005 Dec;79(23):14793-803. doi:10.1128/JVI.79.23.14793-14803.2005. PMID: 16282479; PMCID: PMC1287572.).

The genetic stability of SRP-9003 is expected to be equivalent to wild type AAV. However, due to the lack of viral Rep and Cap genes, SRP-9003 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; IT; ES.

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

Yes (.) No (X)

If yes:

- Member State of notification N/A
- Notification number N/A

Please use the following country codes:
Austria AT; Belgium BE; Germany DE; Denmark DK; Spain ES; Finland FI; France FR; 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
- Notification number IND 017060

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

SRP-9003 is a self-complementary, non-replicating, recombinant AAVrh74 vector containing full-length hSGCB cDNA for the treatment of patients with limb-girdle muscular dystrophy (LGMD) 2E/R4 (LGMD2E/R4s).
The release of SRP-9003 as described in this application is not expected to result in adverse environmental impact, including the human population, for the following reasons:

1. **Lack of pathogenicity of the parental virus and the GMO:**
   AAVs are non-enveloped, single stranded DNA (ssDNA) viruses that have not been found to cause pathology in humans. The modifications which have led to the generation of the SRP-9003 GMO have not raised the pathogenicity.

2. **Replication-incompetent GMO:**
   SRP-9003 is a recombinant AAV vector that lacks all AAV viral genes and cannot replicate without AAV-specific helper functions and helper virus activities. SRP-9003 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-9003 and WT AAV, both intrinsically non-pathogenic viruses.

3. **Property of Transmitting Nucleic Acid Horizontally:**
   It has been shown that only a very small proportion of the total number of SRP-9003 viral genomes infused in the patient population are shed in a matrix of bodily fluids (whole blood, serum, urine, saliva). SRP-9003'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.
   The risk of transmission by viral shedding is minimal as it is replication-incompetent and is not expected to survive, multiply or disperse if it were to be eliminated intact from the treated subject. In addition, minimal exposure of non-target individuals to SRP-9003 is unlikely to result in any effect. As a result, SRP-9003 would not be expected to pose any risk of transmitting nucleic acid horizontally. Vector shedding will continue to be monitored in the ongoing SRP-9003-101 and SRP-9003-102 clinical studies, as well as in future clinical studies.
4. **Minimal risk of insertional mutagenesis:**
   SRP-9003 does not contain viral coding sequences, except the inverted terminal repeat (ITRs) and do not express Rep proteins which play a key role not only for DNA replication but also for site-specific integration and cellular growth inhibitory effects. Human gene therapy recombinant products 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, Non-Human Primates (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, tumour 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 P, Ronzitti G, Mingozzi F. Emerging Issues in AAV-Mediated In Vivo Gene Therapy. Mol Ther Methods Clin Dev. 2017 Dec 1;8:87-104. doi: 10.1016/j.omtm.2017.11.007. PMID: 29326962; PMCID: PMC5758940.).

5. **Productivity of Harmful Substances:**
   SRP-9003 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-9003 encodes for the naturally occurring human β-sarcoglycan (hSGCB) protein and is therefore unlikely to be toxic to humans or other organisms. Comprehensive toxicity studies failed to demonstrate any toxic effect of SRP-9003 at the intended dose.
   No genes for oncogenes, toxins, or potentially harmful genes have been included into the GMO.

6. **Minimal risk associated with immune responses in patients:**
   Clinical experience to date, suggests an acceptable risk profile of monitorable, manageable, and reversible safety concerns associated with SRP-9003. SRP-9003 did not elicit any concerning immune responses. As expected, AAVrh74 antibodies were detected. No antibodies to the transgene were detected, and no significant T cell responses were observed to either the transgene or AAVrh74; therefore, the risk that immune-mediated decrease of expression would occur remains extremely low.
   Patients will receive glucocorticoid (prednisone or equivalent) oral dose treatment to minimize the host immune response to AAV therapy. Patients will be monitored closely, particularly in the first few weeks after treatment, when the risk of an immune response is greatest.

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

1. Recipient or parental organism characterisation:

(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)
Mediterranean	(X)
Boreal	(X)
Alpine	(X)
Continental	(X)
Micronesian	(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: Serological testing

(b) Identification techniques: Serotype specific antibodies with DNA sequencing.

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

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 (.)

Additional information:
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 fulfils the definition of a Risk Group 1 biological agent, defined in the EU as 'one that is unlikely to cause human disease'.

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-9003 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:
See response to 8a above.

(c) Way of reproduction: Sexual (N/A) Asexual (N/A)

(d) Factors affecting reproduction:

Reproduction of wild-type AAV in an infected host is dependent on co-infection with helper virus (such as adenovirus or herpesvirus). However, SRP-9003 is replication-incompetent even in the presence of a helper virus due to the removal of the viral *rep* and *cap* genes.

9. Survivability

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

(i)	endospores	(.)
(ii)	cysts	(.)
(iii)	sclerotia	(.)
(iv)	asexual spores (fungi)	(.)
(v)	sexual spores (fungi)	(.)
(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 L, Lehtonen E, Monahan PE. Evaluation of risks related to the use of adeno-associated virus-based vectors. Curr Gene Ther. 2003 Dec;3(6):545-65. Doi 10.2174/1566523034578131.PMID: 14683451.). Proper decontamination procedures such as 10% bleach, ionic detergents, or alkaline solutions (pH > 9.5) must be employed to ensure safety (Howard, D. and Harvey, B., 2017. Assaying the Stability and Inactivation of AAV Serotype 1 Vectors. Human Gene Therapy Methods, 28(1), pp.39-48.).

10. (a) Ways of dissemination

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

(c) 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)

Release for SRP-9003 was notified in the United States (IND 017060).

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 β-sarcoglycan (β-SG) to cause replacement of the absent protein for the treatment of patients with LGMD2E/R4.

The goal of SRP-9003 gene therapy is to induce the expression of β-SG protein in muscles to correct the underlying genetic defect, thus improving the clinical trajectory of the patients as demonstrated by clinical outcomes such as stabilization and/or slowed progression of neuromuscular weakness and, ideally, improve muscle strength over time. The goal of this clinical study is to evaluate the safety and efficacy of SRP-9003 in subjects with LGMD2E/R4.

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?

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

(b)Identity of the vector

SRP-9003 is a non-replicating, recombinant adeno-associated virus containing full length β-SG (*SGCB*) gene under the control of the MHCK7 promotor/enhancer and has been optimized for driving expression in cardiac and skeletal muscle (Rodino-Klapac L, Pozsgai ER, Lewis S, et al. Systemic Gene Transfer with AAVrh74.MHCK7.SGCB Increased β-sarcoglycan Expression in Subjects with Limb Girdle Muscular Dystrophy Type 2E. Presented at: 2019 Muscular Dystrophy Association Clinical and Scientific Conference; April 13-17, 2019; Orlando, FL.). 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

Bacteria, mammalian 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-9003. The SRP-9003 viral vector does not contain any antibiotic resistant genes.

(e) Constituent fragments of the vector

The vector genome contains the minimal elements required for gene expression, including AAV2 inverted terminal repeats (ITR), the full human beta sarcoglycan gene, DNA, SV40 intron (SD/SA), and synthetic polyadenylation (Poly A) signal. The aforementioned elements are under the control of the α-myosin heavy-chain creatine kinase 7 (MHCK7) promoter/enhancer to restrict expression to skeletal and cardiac muscle. All of the DNA from the wild-type (WT) AAVrh74 has been removed.

The SRP-9003 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 is comprised of a MHCK7 promoter, chimeric intron (SV40 Intron), hSGCB transgene, and a polyadenylation signal; flanked by AAV inverted terminal repeats (ITRs).

(b) Source of each constituent part of the insert

- MHCK7 Promoter: *Mus musculus*, modified and chemically synthesized.
- Chimeric intron: SV40 Intron
- hSGCB: *Homo sapiens*, human codon optimized and chemically synthesized.
- 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.
- *Chimeric Intron:* Facilitate mRNA nuclear export and cytoplasmic accumulation for enhanced gene expression and mRNA translation.
- *Functional domains of the human dystrophin gene retained in SRP-9003 dystrophin*:

Gene transfer may be effective for the treatment of patients with LGMD, given that the disease is caused by mutations within the BSG gene that affect the expression or activity of BSG protein.

- *Polyadenylation signal*: Terminate transcription of the BSG 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 location of the insert in the host organism will be mainly extrachromosomal by formation of episomal concatemers.

(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	(X)
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): N/A

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, due to transfer of SRP-9003 hSGCB to humans.

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 SRP-9003 capsid proteins are 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-9003 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-9003 is replication deficient, the dissemination is limited to the administration of SRP-9003 to the patient.

(c) 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 beta-sarcoglycan, is not expected to result in the development of pathogenicity. Thus, neither the wild-type AAV nor SRP-9003 are known or expected to be pathogenic. Removal of viral genes during the construction of 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-9003 is confirmed by characterizing the identity, purity, and quality. The administration of SRP-9003 to LGMD subjects infects target cells by forming multiple SRP-9003 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 SRP9003 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, T., Ferran, B., Tsukahara, Y., Shang, Q., Desai, S., Fedoce, A., Pimentel, D., Luptak, I., Adachi, T., Ido, Y., Matsui, R. and Bachschmid, M., 2019. Production of adeno-associated virus vectors for in vitro and in vivo applications. Scientific Reports, 9(1).). Preclinical data also indicate that AAV vectors predominantly persists as extrachromosomal elements (episomes) rather than integrating into host cell genomes (McCarty DM, Young SM Jr, Samulski RJ. Integration of adeno-associated virus (AAV) and recombinant AAV vectors. Annu Rev Genet. 2004;38:819-45. doi:10.1146/annurev.genet.37.110801.143717. PMID: 15568995.). Based on the available clinical and non-clinical data, it is concluded that SRP-9003 does not integrate into the host cell genome. Since SRP-9003 uses AAV rh74 with all the wild-type DNA removed, except for the Inverted Terminal Repeats, the potential risk of incorporation of SRP-9003 into the patient chromosomal DNA is thought to be significantly reduced.

AAV is non-pathogenic and has not been classified under Directive 2000/54/EC of the European Parliament and of the Council of 18 September 2000 on the protection of workers from risks related to exposure to biological agents at work. AAV has no known pathogenic effects, even though the estimated seroprevalence of some common human serotypes is 80%. Consequently, AAV fulfils the definition of a risk group 1 biological agent according to the Directive 2000/54/EC (a biological agent that is unlikely to cause human disease).

II(C)(2)(i): In general, the viral vector shedding is observed for a short period after the administration of non-replicating SRP-9003 with very limited exposure to the environment. Thus, exposure of plants or animals is not expected. SRP-9003 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-9003 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 vector 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 a minimum of 4 weeks after the infusion. Patients are prohibited from donating blood, organs, tissues, and cells for 2 years following SRP-9003 administration.

4. Description of identification and detection methods

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

SRP-9003 can be detected by qPCR/ddPCR assay using primers and probe specific to the MHCK7 promoter.

(b) Techniques used to identify the GMO

SRP-9003 can be identified by DNA sequencing or 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) A Phase III Study to Evaluate the Safety and Efficacy of SRP-9003 in Subjects with Limb Girdle Muscular Dystrophy 2E/R4.

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 (.)

SRP-9003 is administered by intravenous infusion to eligible patients with Limb-girdle muscular dystrophy type 2E/R4.

3. Information concerning the release and the surrounding area

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

Site 1	UZ Leuven Herestraat 49 3000 Leuven Belgium
Site 2	UZ Gent Corneel Heymanslaan 10 9000 Gent Belgium

(b) Size of the site (m^2):

(i) actual release site (m^2): Not applicable. A specific size for the site of release cannot be defined as SRP-9003 will be administered to patients as part of a clinical trial.

(ii) wider release site (m^2): Not applicable. A specific size for the site of release cannot be defined as SRP-9003 will be administered to patients as part of a clinical trial.

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

Not applicable. SRP-9003 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-9003 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-9003 is administered as a single IV infusion through peripheral vein at a dose of 7.41×10^{13} vg/kg, infused over approximately 1 to 2 hours. Approximately 15 patients will be enrolled globally into the SRP-9003-301 clinical study at approximately 12 to 14 sites.

4 of patients are anticipated to be administered with SRP-9003 in Belgium

(b) Duration of the operation:
The duration of the clinical study is up to 66 months. SRP-9003 is a single-dose therapy administered via a 1-to-2-hour systemic infusion through a peripheral vein.

(d) Methods and procedures to avoid and/or minimise the spread of the GMOs beyond the site of the release:
Healthcare providers and onsite personnel will be trained in best biosafety practices to be applied during preparation of SRP-9003 in the pharmacy, transport to the administration room, precautions during administration and disposal of product contact bio-logical waste and leftover drug product.
The training also involves, wearing adapted protective clothing, gloves and goggles, the constant presence of a spill kit and the decontamination of waste prior to disposal.
Personal Protective Equipment (PPE) used for the procedure include:

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

The SRP-9003 gene vector will be prepared for dosing by the study site research pharmacist according to the Pharmacy Manual.

SRP-9003 will be shipped to study sites in line with standard recommendations for the transport of biohazardous materials.

Only subjects enrolled in the clinical study may receive study drug and only authorized personnel may supply or administer study drug. All study drugs must be stored in a secure, environmentally controlled, and monitored (manual or automated) area in accordance with the labelled storage conditions with access limited to the Investigator and authorized site staff.

The clinical investigator, institution, or the head of the medical institution (where applicable) is responsible for study drug accountability, reconciliation, and record maintenance (i.e., receipt, reconciliation, and final disposition records). It is not expected that SRP-9003 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.

Instructions will be provided to subjects' families and caregivers regarding use of protective gloves if/when coming into direct contact with subject's bodily fluids and/or waste, as well as utilizing good hand hygiene for a minimum of 4 weeks after the injection of SRP-9003.

Additionally, subjects are prohibited from donating blood for 2 years following the vector injection.

5. Short description of average environmental conditions (weather, temperature, etc.)
Not applicable. Administration of SRP-9003 will occur only within a controlled hospital setting.

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-9003 has been administered to wild-type (WT) C57Bl/6J mice and/or in the β-SG deficient mice (B6.129Sgcb).

SRP-9003 is currently being evaluated in 2 clinical studies being conducted at investigational sites located in the USA. SRP-9003-101 is a single-centre, open-label, Phase 1/2a, dose escalation study to evaluate the safety and efficacy of SRP-9003 in 6 patients with LGMD2E/R4. Clinical study SRP-9003-102 is being conducted at 2 investigational sites in the USA in older ambulatory and non-ambulatory LGMD2E/R4 patients.

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) Primates
(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)
SRP-9003 contains a codon-optimized complementary deoxyribonucleic acid (cDNA) sequence coding a human β-sarcoglycan (hSGCB) protein for the treatment of patients with limb-girdle muscular dystrophy, type 2E/R4 (LGMD2E/R4). SRP-9003 is an scAAVrh74 vector carrying the hSGCB gene under the control of the alpha myosin heavy-chain creatine kinase 7 (MHCK7) promoter.

Clinical results from the ongoing clinical study SRP-9003-101 demonstrate that systemic administration of SRP-9003 drives full-length β-SG transgene expression restoring components of the DAPC, improves muscle pathology, and is associated with improved clinical functional outcomes measured post-treatment.

The goal of SRP-9003 gene therapy is to induce the expression of β-SG protein in muscles to correct the underlying genetic defect; therefore, improving the clinical trajectory of the patients as demonstrated by stabilization and/or slowed progression of neuromuscular weakness and, ideally, improve muscle strength over time.

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-9003 that could represent a potential hazard. Minimal exposure, such as environmental exposure, to organisms other than the patients receiving SRP-9003 as part of the clinical study would not be of sufficient dose to represent significant gene expression or potential safety risks. As SRP-9003 is also replication-incompetent, it is expected that the vector would be rapidly cleared from any non-target organisms without causing any harmful effects. Furthermore, transgene expression is designed to occur only in skeletal and cardiac muscle tissue. Other than potential human hosts, exposure to SRP-9003 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:
As SRP-9003 is unable to replicate, post-release selection cannot occur.

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-9003 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) N/A
(ii) family name for plants N/A
(iii) genus N/A
(iv) species N/A
(v) subspecies N/A
(vi) strain N/A
(vii) cultivar/breeding line N/A
(viii) pathovar N/A
(ix) common name N/A

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-9003 does not contain any prokaryotic promoters, resistance genes or any genes, which would enhance their growth. Therefore, it is unlikely that SRP-9003 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:
Insignificant. Since SRP-9003 contains the 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-9003 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:
While recombination between SRP-9003 and a wild-type AAV to generate a hybrid vector genome that contains both the transgene and the AAV rep and cap genes remains a theoretical possibility, such a hybrid genome, even if generated in a cell, would not replicate unless a helper adenovirus/herpes virus was also present. The risks associated with gene transfer from wild-type AAV to SRP-9003 are thus considered to be negligible.

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.):
Literature references are not available. No such studies have been conducted with SRP-9003.

9. Possible environmentally significant interactions with biogeochemical processes (if different from the recipient or parental organism)
Environmental interactions with biogeochemical processes are not available.

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-9003 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 SRP-9003 to the patients, the procedure room will be disinfected as per the instructions provided in the Pharmacy Manual and standard institutional regulations.

2. Post-release treatment of the GMOs
All materials used for preparation and administration of the IMP injection, including sterile drapes and needles in contact with SRP-9003, must be sealed in leak-proof primary and secondary containers. All waste must be double bagged in bags bearing the biohazard symbol and sealed with tape. The bag must then be disposed of in a biohazard waste container.
The infusion set and dosing syringe used for delivery of SRP- 9003 should be placed in a biohazard bag and destroyed in accordance with the site's pharmacy and institutional policy.

Any open vials or unused material must be sealed in leak-proof containers. 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 the SRP-9003, 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.

3. (b) Treatment of waste
Immediately after dose preparation, partially used and used vials will be placed back inside the shipping box in which it was received and then inside a sealed and labelled biohazard bag

and then returned to storage at ≤ -60°C in a freezer by the site pharmacist. SRP-9003 vials will be destroyed as per the site's drug destruction procedure/policy.

All supply waste from inside the biosafety cabinet is placed inside a sealed biohazard bag and place in biohazard waste containers for disposal as per pharmacy and institutional policy.

Sites that are not authorized to destroy IMP on site, may return the SRP-9003 vials to a distribution depot for destruction.

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-9003 during the dose preparation and administration to the patient, instructions provided by the Sponsor´s pharmacy manual will be followed to contain and immediately disinfect the spill to prevent further spread. All contaminated materials will be disposed of locally by incineration or autoclaving. All other places will be cleaned, according to normal decontamination procedures as per the EU Directive 2000/54/EC guidance for handling of biological agents at work and the Pharmacy Manual.

Handling Spills:

- Evacuate the area, remove contaminated PPE such as lab coats, shoes, and other clothing 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 centre.
- Carefully pour disinfectant (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 centre. Use tongs or forceps to pick up broken plastics, glass or other sharps that could puncture gloves.
- Discard absorbent material in chemical 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 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 of in a biohazardous waste container.

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

Any material used in the cleanup (soaked with disinfectant) is placed in a chemical bag/ container, and any contaminated PPE placed into a biohazard bag. The biohazard bag is closed and secured.

The biohazard bag id placed into a second biohazard bag, secured and disposed of in a biohazardous waste container for incineration.

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

   Administration of SRP-9003 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. Plans for protecting human health and the environment in the event of an undesirable effect

   Independent Review Board (IRB)/ Independent Ethics Committee (IEC) and Local Health Authority approval must be obtained in compliance with local laws and regulations.

   Staff will follow local law and institutional procedures for the handling and disposal of GMOs. Furthermore, safety recommendations and guidance on the management of incidents related to SRP-9003 are provided in the safety instructions for investigators and staff included in this submission. All patients will be carefully monitored for any adverse reactions during this clinical study. A study-specific independent data monitoring committee (DMC) will be formed to assist in the periodic monitoring of safety, efficacy, data quality, and integrity of the clinical study.

References:

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