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 two-part, open-label systemic gene delivery study to evaluate the safety and expression of RO7494222 (SRP-9001) in subjects under the age of four with Duchenne Muscular Dystrophy
(e)	Proposed period of release	Start (Belgium): Q4 2022; End (Globally): Q4 2027* **Final study dose delivered in Q4 2027. Q4 2027 to Q4 2032 (study end) is safety follow up with no IMP administered.*

2. Notifier

Name of institution or company: F. Hoffmann-La Roche Ltd

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 (replication-deficient viral vector containing human *hMicro-Dys* cDNA)
(c) Genetic stability – according to Annex IIIa, II, A(10)
…

The genetic stability of RO7494222 (SRP-9001) is expected to be equivalent to wild type AAV. It is also 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). However, due to the lack of viral Rep and Cap genes, RO7494222 (SRP-9001) is expected to remain in the cells as episomes and will not replicate and produce viral particles. The expression cassette will be transcribed and translated by host cell enzymes leading to expression of micro-dystrophin. The stability of RO7494222 (SRP-9001) is deemed comparable to wtAAV.

There is a possibility of spontaneous homologous genomic recombination in nature between the viral genomes of AAV strains if the host organism is infected simultaneously by two different strains of AAV and a helper virus.

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

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

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.

RO7494222 (SRP-9001) is a non-replicating AAV vector containing the micro-dystrophin gene for treatment in patients with Duchenne Muscular Dystrophy. RO7494222 (SRP-9001) is not expected to have an environmental impact, including in the patient population, for several reasons listed below.

Pathology

Adeno-associated viruses (AAV) are single stranded DNA viruses that have not been found to cause pathology in humans.

Productivity of Harmful Substances

The recombinant virus expresses micro-dystrophin protein and does not produce any harmful substances. No genes for oncogenes, toxins, or potentially harmful genes have been inserted into the GMO.

Property of Transmitting Nucleic Acid Horizontally

Genetically altered AAV used to deliver RO7494222 (SRP-9001) has been shown to shed a very small proportion of the total number of viral genomes injected in the patient population in a matrix of fluids (whole blood, serum, urine, saliva), and its ability to transmit nucleic acid horizontally is considered to be substantially degraded compared to wtAAV which is the taxonomical species to which the altered organism belongs. Additionally, there are no expected exposures to non-target organisms or environmental exposures.

As a result, RO7494222 (SRP-9001) would not be expected to pose any of transmitting nucleic acid horizontally.

Replication Incompetent and Insertional Mutagenesis

Recombinant AAV technology is distinct from that of wild type (wt) AAV. The rAAV vectors do not contain viral coding sequences 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. 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 NHP's, 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) (select one only)	Indicate whether the recipient or parental organism is a:
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
…
Serological testing

(b) Identification techniques
…
Serotype specific antibodies and 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
…
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). It is most appropriately designated as Risk Group 1 biological agent, defined in the EU as ‘one that is unlikely to cause human disease’. The only viral sequences included in the vector construct for RO7494222 (SRP-9001) are the ITRs of AAV2, which are required for both viral DNA replication and the packaging of the rAAV vector genome.

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
N/A

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 RO7494222 (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

(c) Factors affecting reproduction:
…
Triple infection is needed before horizontal infection to occur in patients who are administered with RO7494222 (SRP-9001) , and a possibility 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 RO7494222 (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 RO7494222 (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

Co-infection with a helper virus is needed for the infection.

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

…

After the administration of RO7494222 (SRP-9001), the vector translocates into the nucleus and is converted into double-stranded DNA and exist independently of the chromosome. The persistence of gene expression is very high in non-dividing cells. The goal of RO7494222 (SRP-9001) therapy is to increase the expression level of the micro-dystrophin protein in skeletal and cardiac muscle to increase strength and protect from contraction induced injury. RO7494222 (SRP-9001) appears to have an acceptable safety profile and be generally well tolerated in all ongoing 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
…
RO7494222 (SRP-9001) is a non-replicating, recombinant adeno-associated virus (rAAV) containing a human micro-dystrophin gene under the control of the MHCK7 promotor/enhancer, has been optimized for driving expression in cardiac and skeletal muscle (Rodino-Klapac et al. 2013). The recombinant vector genome contains the minimal elements required for gene expression, including AAV2 inverted terminal repeats (ITR), the MHCK7 promoter/enhancer, the micro-dystrophin gene, an SV40 intron (SD/SA), and a synthetic polyadenylation (Poly A) signal.

(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 plasmid. The RO7494222 (SRP-9001) viral vector does not contain any antibiotic resistant genes.

(e) Constituent fragments of the vector
…
The RO7494222 (SRP-9001) vector is produced by a process known as “triple transfection”, which utilizes 3 different plasmid DNA constructs.

1. AAV Vector Plasmid; Gene of Interest; pAAV.MHCK7.Micro-Dystrophin; Vector plasmid encoding a human micro-dystrophin 137 kDa protein and regulatory elements flanked by AAV2 derived inverted terminal repeats (ITRs).

2. AAV Helper plasmid; pNLREP2-Caprh74; Packaging plasmid containing the AAV *rep* gene for coding non-structural and *cap* gene for coding structural proteins.

3. Ad Helper plasmid; pHELP; Adenovirus helper plasmid encoding the type 2 genes *E2A*, *E4* and *VA* RNAs required for AAV replication in HEK 293 cells.

(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 an MHCK7 promoter, micro-dystrophin 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
Micro-dystrophin transgene: *Homo sapiens*, human codon optimized and chemically synthesized
Polyadenylations signal: Synthetic PolyA
AAV Inverted Terminal Repeats (ITRs): Wild-type AAV2

(c) Intended function of each constituent part of the insert in the GMO
MHCK7 Promoter: Drive skeletal and cardiac specific gene expression
Micro-dystrophin transgene: Codes for the parts of dystrophin gene, which are critical for muscle function
Polyadenylation signal (PolyA): Specifies transcriptional termination and also important for mRNA stability and nuclear export.
AAV Inverted Terminal Repeats (ITRs): Functions as both the origin of vector DNA replication and the packaging signal of the rAAV vector genome, when AAV and adenovirus helper functions are provided in trans.

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

The transgene (hMicro-Dys) structure including the ITR is provided. 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:

(p) 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 RO7494222 (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 RO7494222 (SRP-9001) is unable to reproduce even in the presence of wild-type AAV helper virus.

(q)
(r) 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 RO7494222 (SRP-9001) replication deficient, the dissemination is limited to the administration of the RO7494222 (SRP-9001) to the patient.

(s) is the GMO in any way different from the recipient as far as pathogenicity is concerned?
Yes (.) No (X) Not known (.)
Specify …
Both wild type AAV rh74 and RO7494222 (SRP-9001) are non-pathogenic to humans and animals in the environment. Since recombinant RO7494222 (SRP-9001) is replication deficient, it cannot enter an infectious cycle even in the presence of helper functions.

2. Genetic stability of the genetically modified organism
…
The stability of the recombinant RO7494222 (SRP-9001) is confirmed by characterizing the identity, purity, and quality. The administration of RO7494222 (SRP-9001) to DMD subjects, it infects target cells by forming multiple RO7494222 (SRP-9001) genomes assemble 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 RO7494222 (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 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 RO7494222 (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 the RO7494222 (SRP-9001) product uses AAV rh74 with all the wild-type DNA removed, except for the Inverted Terminal Repeats, the potential risk of incorporation of RO7494222 (SRP-9001) into the patient chromosomal DNA is thought to be significantly reduced.

The recombinant RO7494222 (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 short period after the administration of non-replicating RO7494222 (SRP-9001) with very limited exposure to the environment. Thus, exposure of plants or animals is not expected. RO7494222 (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).

The viral shedding analysis of RO7494222 (SRP-9001) found peak levels at Day 2 in both C57BL/6J and DMD^{MDX} animals at both dose levels. The levels of RO7494222 (SRP-9001) progressively fell in line with time from dose. The vector is primarily cleared from the body in urine, stool, and plasma at below the limit of quantitation by day 44 post-infusion. The risks associated

with the shed vector are not known at this time; however, it is unlikely as the vector is non-infectious and cannot replicate. 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 few weeks after the injection. Additionally, patients are prohibited from donating blood for two years following the vector injection. Also see viral shedding and biodistribution study results described under General Information Section A response to question 7 above.

4.Description of identification and detection methods

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

…

The recombinant micro-dystrophin vector (RO7494222 (SRP-9001)) is monitored by qPCR/ddPCR assay using primers and probe specific to the MHCK7 promoter.

(b) Techniques used to identify the GMO

…

The recombinant micro-dystrophin vector (RO7494222 (SRP-9001)) is 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)

…

For the treatment of Duchenne Muscular Dystrophy. No potential benefit is expected.

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 …

RO7494222 (SRP-9001) is administered intravenously to the Duchenne Muscular Dystrophy patients.

3. Information concerning the release and the surrounding area

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

…

Patient eligibility will be determined based on genetic analysis of DMD gene. The specialized treatment centers will be identified to administer the RO7494222 (SRP-9001) in pediatric subjects.

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(b) Size of the site (m^2): **Not Applicable**

(i) actual release site (m^2): … m^2

(ii) wider release site (m^2): … m^2

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

…
Not applicable considering that shed material, if any at all, is non-infectious.

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

…
None.

4. Method and amount of release

(a) Quantities of GMOs to be released:

…
RO7494222 (SRP-9001) will be administered in patients with a one-time dose at 1.33 x 10^{14} vg/kg for the treatment of Duchenne Muscular Dystrophy. Approximately 21 patients will be globally recruited for pivotal clinical studies. The quantities that will be released into the environment by shedding will be a very small proportion of the total number of viral genomes. RO7494222 (SRP-9001) is detectable by qPCR/ddPCR in the shed samples from day 1 post injection.

(b) Duration of the operation:

…
The vials must thaw prior to administration which will take approximately 90 minutes to 2 hours as per the Pharmacy Manual. RO7494222 (SRP-9001) will be administered over approximately 1-2 hours through a peripheral limb vein according to the procedures described in the Administration Instructions (or Dose Administration Manual).

(c) 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 on the handling of AAV agents to be applied during preparation of RO7494222 (SRP-9001) in the pharmacy. The training will also include indications on transport to the administration room, precautions during administration and disposal of product contact biological 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
- Disposable isolation gown
- Appropriate PPE should also be used for lower arms such as sleeve covers or securing gloves over the sleeves of the laboratory coat.
- Personnel should not work with AAV if skin is cut or scratched.

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

…

RO7494222 (SRP-9001) will be administered in patients with a one-time dose at 1.33 x 10^{14} vg/kg for the treatment of Duchenne Muscular Dystrophy. Approximately 21 patients will be globally recruited for pivotal clinical studies.

RO7494222 (SRP-9001) vials will be shipped frozen and stored at ≤ -60 °C. RO7494222 (SRP-9001) will be removed from ≤ -60°C storage ONLY when ready to use.The vials are thawed at the environmentally controlled hospital pharmacy prior to administration. Once the dose volume is drawn into the syringe it must be administered within 12 hours at 2-8°C (36-46°F). Within this 12-hour storage period, the dose solution may be temporarily held at 9-25°C (47-77°F) up to a maximum of 4 hours.

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.

…

RO7494222 (SRP-9001) administration increases the dystrophin expression *in vivo* 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 in USA.

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

…

None.

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 RO7494222 (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

…

Theoretically RO7494222 (SRP-9001) can not infect the other mammalian cells in the ecosystem because it is replication incompetent.

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.

( )	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 RO7494222 (SRP-9001) does not contain any prokaryotic promoters, resistance genes or any genes, which would enhance their growth. Therefore, it is unlikely that RO7494222 (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:

…

Insignificant. Since RO7494222 (SRP-9001) 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 RO7494222 (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 micro-dystrophin (hMicro-Dys) 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.):

…

Literature references are not available.

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 RO7494222 (SRP-9001) to other organisms is negligible. qPCR/ddPCR can be used to detect the genetic material.

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 RO7494222 (SRP-9001) to the patients, the procedure room will be disinfected as per pharmacy manual and standard institutional regulations. All materials used for preparation that come in contact with RO7494222 (SRP-9001) will be sealed in leak-proof primary and secondary containers. These containers will then be placed in red biohazard waste for incineration.

2. Post-release treatment of the GMOs

…

All vials, both used and unused, must be sealed in leak-proof containers and should be retained until the monitor has completed drug accountability and the sponsor has provided permission for

destruction either at site or returned to depot for destruction. If the local Standard Operating Procedure (SOP) does not allow the used IMP to be maintained for accountability, the site needs to file a copy of the SOP in the pharmacy manual and document destruction of the study drug after infusion.

After administration the product contact delivery system components (inline filters, injection needles and syringes), gauzes and personal protective equipment will either be disinfected or incinerated as per end-user medical waste treatment regulations.

3. (a) Type and amount of waste generated

…

After administration of the RO7494222 (SRP-9001), the following waste is generated; empty vials, used vials, administration set with in-line filter, syringe closing cap, injection needles and syringes, gauzes, gloves.

3. (b) Treatment of waste

…

Patient families will be advised to follow instructions for the proper handling of patient stools, wearing of gloves and good hand-hygiene when coming into direct contact with patient bodily waste for a minimum of one month after the treatment with RO7494222 (SRP-9001). Diapers should be sealed in double bags and can be 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 RO7494222 (SRP-9001) during the dose preparation and administration to the patient at the health-care provider, instructions provided by the Sponsor´s pharmacy manual will be followed to contain and immediately disinfect the spill to prevent further spread. All contaminated materials will be disposed of locally by incineration or autoclaving. All other places or surfaces will be cleaned, according to normal decontamination procedures as per the NIH/CDC guidance for handling of biosafety level 1 agents and the Pharmacy Manual.

- 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 (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 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

…

All materials used in the clean-up will be discarded as clinical biohazard 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

…

Not applicable.

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 must be obtained in compliance with local laws and regulations.