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, Multicenter, Randomized, Double-Blind, Placebo-Controlled Study to Evaluate the Safety and Efficacy of PF-06939926 for the Treatment of Duchenne Muscular Dystrophy

Proposed period of release: Q3 2020 to end of treatment period ~Q4 2021, depending on timepoint of patient recruitment

2. Notifier

Name of institution or company:
Pfizer, Inc. 235 East 42nd Street, New York, NY 10017, USA.

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: Recombinant adeno-associated viral vector derived from naturally occurring AAV9 serotype

(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. Sequence homologies often are >90% and >80% for the rep and cap genes, respectively. Furthermore, AAV uses host DNA polymerases for viral replication, which are characterised by high fidelity DNA polymerization and additional proofreading exonuclease activity leading to very low error rate of DNA replication, when compared, for example, to RNA polymerases used by RNA viruses. In support of genetic stability is the observation that AAV proviral DNA episomes, isolated from multiple human tissue samples, consistently have the expected canonical AAV2 rep and cap sequences.

Homologous recombination is thought to have occurred between serotypes AAV2 and AAV3 based on phylogenic analysis of the AAV2/3 hybrid virus, but has not been observed for other serotypes, supporting that only under the presumably rare circumstance where a cell is infected simultaneously by two different serotypes of AAV and a helper virus (triple infection) would conditions be appropriate for such recombination to occur.

PF-06939926 is expected to be highly genetically stable. Production of the vector in the manufacturing process and second strand synthesis of the vector genome rely on the host DNA polymerase, leading to very low error rate of DNA replication. The Be vector genome will be assayed by specific qPCR before release. Exemplary batches will also be sequenced to confirm the absence of any changes.

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) DE; ES; FR; IT;

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

Yes (X) No (X)

If yes:

- Member State of notification FR, ES, DE, GB
- Notification number B/ES/20/08

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 USA
- Notification number NIH Protocol 1704-160;

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

PF-06939926 is a non-replicating recombinant vector derived from adeno-associated virus containing a miniaturized version of the human dystrophin gene (mini-dystrophin), that may be effective for the treatment of patients with Duchenne Muscular Dystrophy.

The release of PF-06939926 as described in this application is not expected to result in adverse environmental impact, including the human patient population, for the following reasons:

1. Lack of pathogenicity of the parental virus and the GMO: Despite an estimated seroprevalence of up to 80% for some common human serotypes, no pathogenic effects of AAV have been identified. The modifications which have led to the generation of the GMO have not raised the pathogenicity (see point 6. below).

2. Replication-incompetent GMO: PF-06939926 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. PF-06939926 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 PF-06939926 and WT AAV, both intrinsically non-pathogenic viruses.

3. Minimal risk of transmission by viral shedding: PF-06939926 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. The subjects treated in study C3391003 will be aged 4 to ~9 when receiving PF-06939926. Therefore, they are not sexually matured and it can be safely assumed that any traces of vector will have disappeared from semen, when the subjects reach sexual maturity. Vector shedding will be assessed for a maximum of 6 years or until 2 consecutive negative readings (at or below the limit of detection of the assay) for an individual are obtained for a given sample matrix (saliva, whole blood, urine).
   Minimal exposure, such as environmental exposure, of persons other than study participants would not be of sufficient dose to result in significant gene expression in humans. Other than potential human hosts, exposure to PF-06939926 is not expected to affect any non-target organisms, either directly or indirectly. The risk to humans and the environment associated with viral shedding of PF-06939926 is thus negligible.

4. Minimal risk of insertional mutagenesis: 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. Unlike retroviral vectors, which encode viral proteins to create double-stranded breaks, when AAV integration does occur, it does so at pre-existing chromosomal breaks. The results of integration are deletions in the AAV ITRs and duplications of host sequences. Given the tissue tropism of AAV9 and the results of non-clinical studies, the greatest potential for integration is within hepatocytes, skeletal and cardiac myocytes. No clinical trials to date with AAV have reported incidences of insertional mutagenesis.

5. Tissue-specific transgene expression: PF-06939926 shows a strong tropism for liver, skeletal and cardiac muscles following IV administration. PF-06939926 transgene expression is driven by a muscle-specific promoter. Transduction of non-muscle cells should not result in transgene expression.

6. Minimal risk associated with the transgene: 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. A comprehensive toxicity study (single-dose in rats) failed to demonstrate any toxic effect of the PF-06939926 at the intended dose. The protein encoded by the transgene is a shortened version of a naturally occurring protein and is therefore unlikely to be toxic to humans or other organisms. No genes for toxins, potential oncogenes, growth factors or other genes that could be potentially harmful have been inserted into the GMO. With administration of PF-06939926 to humans, the only foreign proteins that the immune system will be exposed to are the viral capsid proteins.

7. Minimal risk associated to immune responses in patients: Patients will receive corticosteroids in order to minimize the immune response to the viral capsid proteins. 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)	ssDNA virus
(ii)	genus	Dependoparvovirus
(iii)	species	Adeno-associated virus
(iv)	subspecies	N/A

(v) strain AAV9
(vi) pathovar (biotype, ecotype, race, etc.) N/A
(vii) common name N/A

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	In association with animals (primate 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

AAV can be identified by qPCR using primers specific for the viral genome. It can also be identified by 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 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% (European Parliament and of the Council 2000). Consequently, AAV fulfils the definition of a group 1 biological agent according to the Directive 2000/54/EC (a biological agent 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:
rAAV is replication defective; thus, the generation time is variable depending on the presence or absence of a helper virus.

(b) Generation time in the ecosystem where the release will take place:
rAAV is replication defective; thus, the generation time is variable depending on the presence or absence of a helper virus.

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

(d) Factors affecting reproduction:
The presence of a helper virus, such as adenovirus or herpes simplex virus, promotes AAV gene expression, genome replication and production of viral particles. In absence of a helper virus, wild-type AAV is replication-incompetent. Please note that

the final GMO, PF-06939926, 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	AAV does not form survival structures

(b) relevant factors affecting survivability:
Members of the parvovirus family such as AAV are stable viruses that can persist in the environment for extended periods of time (thought to be on the order of several weeks). AAV particles are resistant to a wide range of pH (pH 3-9) and can resist elevated temperatures (55°C for 1 hour). AAV does not form survival structures. However, as with all viruses, replication of AAV cannot occur outside of a host cell. Treatment with substances such as 10% bleach will destroy viral particles within 20 minutes.

10. (a) Ways of dissemination
AAV may be transmitted through direct or indirect contact. AAV may be transmitted through inhalation, ingestion and possibly sexual transmission.

(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). Please note that the final GMO, PF-06939926, is replication-incompetent even in the presence of a helper virus due to the removal of the viral *rep* and *cap* genes.

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)
No release for PF-06939926 was notified in Belgium.

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 mini-dystrophin to cause replacement of the absent dystrophin and thus enable the treatment of patients with Duchenne Muscular Dystrophy.

PF-06939926 contains a gene encoding a shortened, but functional variant of the human dystrophin gene. Expression is driven by a skeletal- and cardiac muscle-specific promoter. Biodistribution in animal studies of PF-06939926 demonstrated predominant gene transfer to skeletal muscle, heart and liver tissue.

It is expected that administration of PF-06939926 will result in the expression of the mini-dystrophin transgene and improve the condition of study subjects.

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

(b) Identity of the vector

Plasmid comprising vector genome (pAAV-OptiDys)

(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

Kanamycin

(e) Constituent fragments of the vector

The vector genome comprises a synthetic skeletal muscle and cardiac muscle specific promoter, a transgene encoding the essential functional domains of the human dystrophin gene and a polyadenylation signal, flanked by AAV inverted terminal repeats (ITRs). Only the vector genome is present in the final GMO. In addition, the plasmid vector contains a bacterial origin of replication and the gene for kanamycin resistance to allow for propagation of the plasmid in *E.coli*. These two elements are not transferred to the final GMO.

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

(i)	transformation	(.)
(ii)	electroporation	(.)
(iii)	macroinjection	(.)
(iv)	microinjection	(.)
(v)	infection	(.)
(vi)	other, specify …	Transfection of mammalian cells with vector genome plasmid, a packaging plasmid and a helper plasmid, resulting in production of recombinant vector particles.

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 vector genome comprises a synthetic promoter, a transgene encoding the essential 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

- Synthetic promoter: *Mus musculus,* modified and chemically synthesised
- Gene encoding functional domains of the human dystrophin gene: *Homo sapiens*
- Polyadenylation signal: *Bos Taurus*
- ITRs: AAV2

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

- Synthetic promoter: Intended to drive skeletal and heart muscle specific gene expression.
- Essential functional domains of the human dystrophin gene (mini-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 mini-dystrophin gene.

- AAV ITRs: Inverted Terminal Repeat (ITR) sequences required for second strand DNA synthesis required for gene expression.

(e) Location of the insert in the host organism

-	on a free plasmid	(.)
-	integrated in the chromosome	(.)
-	other, specify	ssDNA viral genome

(f) 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)	Primates
(ii)	family name for plants	N/A
(iii)	genus	Homo
(iv)	species	*Homo 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:

(b) 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 (.) 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 (X) No (.) Not known (.)

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 …

(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:

The PF-06939926 viral genome has been significantly modified compared to the parental virus in order to render it replication incompetent. The AAV rep and cap genes have been replaced with a eukaryotic expression cassette, and only the viral ITR sequences, which are non-coding DNA sequences (<300 bp), have been retained. Thus, PF-06939926 contains no native viral coding genes.

Wild-type AAV requires the presence of a helper virus such as human adenovirus or herpes simplex virus to replicate. PF-06939926 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.

(c) is the GMO in any way different from the recipient as far as dissemination is concerned?
Yes (X) No (.) Not known (.)

Specify

As PF-06939926 replication could only occur in the extremely unlikely event of a host cell being infected by two separate viruses, a wild type AAV and a helper virus such as human adenovirus or herpes simplex virus, the likelihood of dissemination is lower than that of wild-type AAV.

(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 mini-dystrophin, is not expected to result in development of pathogenicity. Thus, neither the wild-type AAV nor PF-06939926 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

AAV is a single stranded DNA virus that demonstrates a high degree of genetic stability; based on this, PF-06939926 is also expected to be genetically stable. The integrity of the vector genome has been confirmed.

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)

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).

A large body of data generated over the past ~20 years in more than 2000 patients (clinicaltrials.gov) suggests that the safety risks associated with AAV gene transfer are negligible.

4. Description of identification and detection methods

(a) Techniques used to detect the GMO in the environment
PF-06939926 can be detected by qPCR.

(b) Techniques used to identify the GMO
PF-06939926 can be identified by qPCR and sequencing.

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 PF-06939926 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 (.) No (X)

3. Information concerning the release and the surrounding area

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

Site 1	Dr. Deconinck, UZ Gent, Corneel Heymanslaan 10, 9000 Gent
Site 2	Dr. De Waele, UZ Leuven, Herestraat 49, 3000 Leuven
Site 3	Dr. Daron, CHR de la Citadelle, Boulevard du XIIeme de Ligne 1, 4000 Liège

(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 PF-06939926 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 PF-06939926 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. PF-06939926 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 PF-06939926 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:
Dosing of PF-06939926 will be weight based and is anticipated to be 2E+14 vg/kg. Approximately 99 patients overall and 3 patients in Belgium are foreseen.

(b) Duration of the operation:
The duration of the study is defined for each subject as the date signed written informed consent is provided over 5, respectively 6 years. The study is expected to be active for 5 to 6 years; however, the treatment is administered once by IV infusion and the remainder of the study is for observation of the treatment effects.

(c) Methods and procedures to avoid and/or minimise the spread of the GMOs beyond the site of the release
PF-06939926 will be shipped to study sites in line with standard recommendations for the transport of biohazardous materials. PF-06939926 will be stored, prepared and administered by trained medical professionals, in a hospital setting only, to patients that meet criteria for inclusion into the clinical study C3391003. Staff will follow the waste and disposal policies as per local site requirement to dispose of consumables used in the preparation and administration of the GMO. The use of needles will be kept to a minimum.

PF-06939926 is an Investigational Medicinal Product (IMP) released by a Qualified Person (QP) located in a European Union Member State for clinical trial use after meeting defined specifications in terms of quality and safety of the product for administration to human subjects in accordance with the clinical study protocol. In addition, it is used and approved as per the clinical study protocol by both regulatory agencies and Ethics Committees in the country where the study is to be conducted. For this reason, the supply chain of the IMP and its management at site is governed in the context of clinical trial regulations, local law, and relevant guidelines for receiving, storing, handling, dispensing, accounting, and returning IMP. 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. It also includes directions for documenting the control of the IMP from the time of receipt at the trial site until final accountability and destruction. In addition, it describes the required processes for managing and documenting any issues, such as shipment or storage, temperature excursions and reporting of technical product complaints. 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. The GMO will only be handled by delegated, trained personnel and 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.

Patients will receive PF-06939926 by a one-time IV infusion in a clinical setting, will remain at the infusion center or study site for at least 24 hours after being dosed to have their vital signs assessed. Additionally, viral vector shedding will be assessed in this study. This will indicate when vector shedding in saliva, urine and serum has ceased. As PF-06939926 is non-replicative, shed viral particles are unable to multiply and thus, the spread of the GMO is inherently limited.

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

Not applicable. Administration of PF-06939926 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.

PF-06939926 has been administered to mice, rats (WT and DMD knock-out) and dogs.

PF-06939926 is currently being investigated in a Phase I FIH study (C3391001) in up to 12 patients. Two serious adverse events have been reported after receipt of the drug but could be resolved.

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	*Homo 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)

PF-06939926 contains a gene encoding a shortened human dystrophin protein. AAV9 has a strong tropism for skeletal and heart muscle, and other tissues. Expression is driven by a muscle-specific promoter, encapsidated within an AAV9 capsid. It is expected that administration of PF-06939926 will result in the expression of the transgene primarily in cardiac and skeletal tissues.

Gene transfer of the essential functional domains of the human dystrophin 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.

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 PF-06939926 that could represent potential hazard. Minimal exposure, such as environmental exposure, to organisms other than the subjects receiving PF-06939926 as part of the study would not be of sufficient dose to represent significant gene expression or potential safety risks. As PF-06939926 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 PF-06939926 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 PF-06939926 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 PF-06939926 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

N/A

(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:

It is expected that the PF-06939926 vector genome will be transferred into tissues within the body of patients. The vast majority of PF-06939926 vector genomes within subject cells are expected to be episomal, rather than integrated into the host cell DNA. As PF-06939926 is non-replicative and is only expected to be shed in study subjects' bodily fluids to a limited extent, transmission and gene transfer to organisms other than the study subjects is considered unlikely.

(b) from other organisms to the GMO:

The probability of homologous recombination with related viruses that could lead to variants of the GMO is strongly reduced with the ITRs being the only viral sequences remaining in the vector, making up only 6.5% of the final vector sequence. It is not expected that any organism's DNA could be transferred to the viral episomes and be incorporated into the PF-06939926 genome.

(c) likely consequences of gene transfer:

While recombination between PF-06939926 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 molecule, even if generated in a cell, would not replicate unless a helper adenovirus/herpes virus was also present. Moreover, such a hybrid genome would be too large to package the hybrid DNA into an AAV particle. AAV possesses a packaging limit of approximately 5 kb (Wu,

Yang, and Colosi 2010), and a hybrid molecule of rep-cap genes plus the mini-dystrophin expression cassette would be predicted to be far in excess of this limit with ~9 kb. The risks associated with gene transfer from wild-type AAV to PF-06939926 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.):
No such studies have been conducted with PF-06939926.

9. Possible environmentally significant interactions with biogeochemical processes (if different from the recipient or parental organism)
PF-06939926 is not known or predicted to have an impact on biogeochemical processes.

H. Information relating to monitoring

1. Methods for monitoring the GMOs
Vector shedding will be closely monitored. Other methods to monitor the effects of PF-06939926 include both safety and efficacy assessments.

2. Methods for monitoring ecosystem effects
The presence of PF-06939926 in bodily fluids following administration of PF-06939926 will be determined by qPCR. No other methods are foreseen.

3. Methods for detecting transfer of the donated genetic material from the GMO to other organisms
Transfer of vector genome to study subjects will be detected by qPCR.

4. Size of the monitoring area (m^2)
Not applicable; monitoring techniques will only be used with regards to vector shedding in patients' bodily fluids.

5. Duration of the monitoring
Shedding data will be collected with the Phase 3 study (C3391003) of PF-06939926 in DMD, which is anticipated to provide definitive characterization of the viral shedding profile. In this study samples will be collected from 3 matrices (whole blood, saliva, and urine) from approximately the first 45 randomized participants.
Safety and efficacy assessments will be conducted throughout the duration of the study.

6. Frequency of the monitoring
See section H.5.

I. Information on post-release and waste treatment

1. Post-release treatment of the site
Any surfaces contaminated with PF-06939926 will be disinfected decontaminated using an appropriate disinfectant such as 10% chlorine bleach or detergent-based disinfectant. The required minimum contact time with PF-06939926 is 20 minutes for 10% bleach or as otherwise stated in the label information of an alternative equivalent decontamination solution. Upon completion of this contact time, the area may be cleaned according to

standard local procedures. This process should be discussed with the local environmental health and safety officer and/or biosafety committee before receipt of any PF-06939926 product on site so that an appropriate plan and supplies are in place.

2. Post-release treatment of the GMOs

All unused vials need to be kept in the required storage conditions (-90°C to -60°C); Used/partly used vials can be discarded at the site following local requirements. Consumables used in the preparation and administration of the GMO that may have come into contact with PF-06939926 will be decontaminated prior to disposal (either by autoclaving or by treatment with an appropriate chemical disinfectant with effectiveness against AAV), and/or incinerated. Liquid waste will be decontaminated using an appropriate chemical disinfectant or autoclaved. Disinfectants that are effective against AAV include 10% chlorine bleach or detergent-based disinfectant.

3. (a) Type and amount of waste generated

- Clear 10 mL closed vial containing PF-06939926 residuals. The number of vials of PF-06939926 required per patient is dependent on the body weight of the patient.
- Materials used for the preparation and administration of the study product, e.g. saline bag, IV administration set, syringes, needles
- Personal protective equipment, e.g. gloves

3. (b) Treatment of waste

Refer to post-release treatment I.2.

J. Information on emergency response plans

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

Procedures for use of all batches of PF-06939926 are described in the component-specific Material Safety Datasheet (MSDS). In addition, the IP manual will also be provided to staff at the site, for the management and disposal of PF-06939926, which should be followed by all personnel responsible for transporting, preparing, administering, disposing of PF-06939926 medicinal product or equipment/consumables that have come into contact with the product designated for use in clinical study. **Table 1** summarises the procedures that will be used by staff to manage incidents related to PF-06939926

Table 1: Management of incidents related to PF-06939926 product

Incident	Procedure
Accidental spillage	In the event that the contents of the PF-06939926 vial/s or diluted product for infusion are accidentally released and come in contact with shipping materials, pharmacy/ hospital surfaces, the spillage should be decontaminated and removed according to institutional practice.
Sharps injury	The use of needles is to be kept to a minimum. In the event of injury, follow local institutional procedures and report to Principle Investigator (PI). PI to notify CRA.
Contact with skin and clothing	Remove contaminated clothing. Flush area with large amounts of water. Use soap. Seek medical attention
Contact with eyes.	Flush with water while holding eyelids open for at least 15 minutes. Seek medical attention immediately.

PF-06939926 is stored in clear 10 mL closed vials. Staff will be advised that care must be taken when manipulating vials and that the use of needles should be kept to a minimum. In the event of injury, staff will follow local institutional procedures.

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. Disinfectants effective against AAV include 10% chlorine bleach or detergent-based disinfectant. Equivalent disinfectants available at the investigational site may be used if effectiveness against AAV has been demonstrated.

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

Administration of PF-06939926 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, PF-06939926 is not capable of infecting plants or microbes.

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

Staff will follow local law and institutional procedures for the handling and disposal of genetically modified organisms. Furthermore, safety recommendations and guidance on the management of incidents related to PF-06939926 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 study. An external data monitoring committee (eDMC) will be responsible for monitoring safety data from the study.

References:

European Parliament and of the Council. 2000. '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,'

Wu, Z., H. Yang, and P. Colosi. 2010. 'Effect of genome size on AAV vector packaging', *Mol Ther*, 18: 80-6.