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	Phase 3, Open-Label, Single-Arm Study to Evaluate the Efficacy and Safety of PF-07055480 (Recombinant AAV2/6 Human Factor VIII Gene Therapy) in Adult Male Participants with Moderately Severe to Severe Hemophilia A (FVIII:C≤1%)

Proposed period of release: Q3 2020 to Q3 2021

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 AAV6 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-07055480 (international non-proprietary name: giroctocogene fitelparvovec) 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 PF-07055480 vector genome will be assayed by specific qPCR before release. Each clinical batch of PF-07055480 will be sequenced as a non-registered characterization test.

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; GR, SE

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

Yes (X) No ()

If yes:

- Member State of notification Spain, France
- Notification number B/ES/20/22, DUO#7902

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 USA
- Notification number NIH Protocol 1703-1588;

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

PF-07055480 is a non-replicating recombinant vector derived from adeno-associated virus containing a codon-optimised version of the human Factor VIII gene, that may be effective for the treatment of patients with hemophilia A.

The release of PF-07055480 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 ~80% for some common human serotypes, no pathogenic effects of AAV have been identified (Calcedo et al., 2009). 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-07055480 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-07055480 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-07055480 and WT AAV, both intrinsically non-pathogenic viruses.

3. Minimal risk of transmission by viral shedding: PF-07055480 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. Vector shedding will be assessed in Plasma, PBMC, saliva, semen, and urine. Specimens will be collected at baseline and every week after study intervention until 3 consecutive specimens test negative for the given specimen type. Participants are eligible to participate if they agree to the following during the intervention period and for at least the time required for 3 consecutive ejaculate samples to test negative for vector shedding: -Refrain from donating sperm. -Be abstinent from heterosexual or homosexual intercourse or must agree to use contraception/barrier.
   Minimal exposure to the PF-07055480, 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-07055480 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-07055480 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. No clinical trials to date with AAV have reported incidences of insertional mutagenesis.

5. Tissue-specific transgene expression: PF-07055480 shows a strong tropism for the liver, following IV administration. PF-07055480 transgene expression is driven by a liver-specific promoter. Therefore, transduction of non-hepatocyte 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. Comprehensive toxicity studies failed to demonstrate any toxic effect of PF-07055480 at the intended dose. The protein encoded by the transgene is 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-07055480 to humans, the only foreign proteins that the immune system will be exposed to are the viral capsid proteins.

7. Minimal risk associated with immune responses to the viral vector in patients: Patients will be monitored closely, particularly in the first 20 weeks after treatment, when the risk of an immune response is greatest. If such an immune response is suspected, patients will receive corticosteroids.

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)	Piccovirales (ssDNA virus)
(ii)	genus	Dependoparvovirus
(iii)	species	Adeno-associated virus
(iv)	subspecies	N/A
(v)	strain	AAV6
(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:

AAV 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:

AAV 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-07055480, 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-07055480, 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 related submission has been performed by the sponsor 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 which includes the sequence coding for the B-domain deleted hFVIII to treat hemophilia A.

PF-07055480 contains a gene encoding a codon-optimised B-domain deleted human Factor VIII. Expression is driven by a liver-specific promoter.

It is expected that administration of PF-07055480 will result in sustained hepatic production of FVIII in hemophilia A participants to reduce or eliminate the need for FVIII replacement therapy.

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
Recombinant Baculovirus (rBV) comprising vector genome

(c) Host range of the vector
Insect 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
Gentamycin

(e) Constituent fragments of the vector
The vector genome comprises a liver-specific promoter module, a transgene encoding the B-domain deleted human Factor VIII and a polyadenylation signal, flanked by AAV inverted terminal repeats (ITRs). Only the vector genome is present in the final

GMO. In addition, the rBV contains the Baculovirus backbone, including the Gentamycin resistance gene. These 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	(x)
(vi)	other, specify …	

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 promoter, a transgene encoding the B-domain deleted hFVIII and a polyadenylation signal, flanked by AAV inverted terminal repeats (ITRs).

(b) Source of each constituent part of the insert

- Promoter: *Homo sapiens* and modified minute virus of mouse
- (B-domain deleted) hFVIII gene: *Homo sapiens*
- Polyadenylation signal: synthetic
- ITRs: AAV2

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

- Promoter: Intended to drive liver-specific gene expression.
- (B-domain deleted) hFVIII gene: Therapeutic clotting factor.
- Polyadenylation signal: Terminate transcription of hFVIII.
- AAV ITRs: Required for vector genome replication and packaging.

(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-07055480 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-07055480 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-07055480 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-07055480 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 significantly 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 B-domain deleted hFVIII, is not expected to result in development of pathogenicity. Thus, neither the wild-type AAV nor PF-07055480 are known or expected to be pathogenic. Removal of viral genes in PF-07055480 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-07055480 is also expected to be genetically stable. The integrity of the vector genome has been confirmed by sequencing. Each clinical batch of PF-07055480 is sequenced as a non-registered characterization test.

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 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 expected to be manageable.

4. Description of identification and detection methods

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

PF-07055480 can be detected by qPCR.

(b) Techniques used to identify the GMO

PF-07055480 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, gene therapy study with PF-07055480 in subjects with Moderately Severe to Severe Hemophilia A.

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	Cliniques Universitaires Saint-Luc Avenue Hippocrate 10 1200 Brussels 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 PF-07055480 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-07055480 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-07055480 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-07055480 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-07055480 will be weight based and is anticipated to be $3.0x10^{13}$ vg per kg of body weight. Approximately 63 patients globally and 1 patient in Belgium are foreseen.

(b) Duration of the operation:

Each participant is expected to be in the study for approximately 5 years. The study is expected to be active for approximately 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-07055480 will be shipped to study sites in line with standard recommendations for the transport of biohazardous materials. PF-07055480 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 C3731003. 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-07055480 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. An IMP 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-07055480 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. Additionally, viral vector shedding will be assessed in this study. This will indicate when vector shedding in plasma, saliva, peripheral blood mononuclear cells (PBMCs), urine and semen has ceased (negativity has to be confirmed at 3 consecutive occasions). As PF-07055480 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-07055480 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-07055480 has been administered previously to mice and Cynomolgus monkeys.

PF-07055480 is being explored in an ongoing Phase 1/2 trial (Study SB-525-1603) in patients with severe hemophilia A (Factor VIII activity <1%). As of the last examination of data (31 July 2020) PF-07055480 had been administered to 11 patients with severe hemophilia A.

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-07055480 contains a gene encoding hFVIII. AAV6 has a strong tropism for the liver. Expression is driven by a liver-specific promoter. It is expected that administration of PF-07055480 will result in the expression of the transgene in the liver of patients.

Gene transfer of hFVIII gene is expected to be effective for the treatment of patients with Hemophilia A, given that the disease is caused by mutations within the FVIII gene.

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-07055480 that could represent potential hazard. As PF-07055480 is also replication-incompetent, it is expected that the vector would be rapidly cleared from any non-target organisms without causing any harmful effects. Other than potential human hosts, exposure to PF-07055480 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-07055480 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-07055480 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-07055480 vector genome will be transferred into tissues within the body of patients. The vast majority of PF-07055480 vector genomes within subject cells are expected to be episomal, rather than integrated into the host cell DNA. As PF-07055480 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 ~4.6% 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-07055480 genome.

(c) likely consequences of gene transfer:
While recombination between PF-07055480 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 - expression cassette would be predicted to be far in excess of this limit. The risks associated with gene transfer from wild-type AAV to PF-07055480 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-07055480.

9. Possible environmentally significant interactions with biogeochemical processes (if different from the recipient or parental organism)
PF-07055480 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-07055480 include both safety and efficacy assessments.

2. Methods for monitoring ecosystem effects
The presence of PF-07055480 in bodily fluids following administration of PF-07055480 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
In this study samples for shedding assessments will be collected from 5 matrices (plasma, saliva, PBMCs, urine, semen).
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-07055480 will be disinfected decontaminated using an appropriate disinfectant such as 10% chlorine bleach or detergent-based disinfectant. The required minimum contact time with PF-07055480 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-07055480 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-07055480 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-07055480 residuals. The number of vials of PF-07055480 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-07055480 are described in the component-specific Material Safety Datasheet (MSDS). In addition, unless stated in the IP manual that will also be provided to staff at the site, local procedures and guidelines for the management and disposal of a RG1 product should be followed by all personnel responsible for transporting, preparing, administering, disposing of PF-07055480 IMP 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-07055480.

Table 1: Management of incidents related to PF-07055480 product

Incident	Procedure
Accidental spillage	In the event that the contents of the PF-07055480 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-07055480 is stored in clear 10 mL closed vials, each containing 6 mL. 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-07055480 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-07055480 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-07055480 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:

Calcedo, R., Vandenberghe, L.H., Gao, G., Lin, J., and Wilson, J.M. (2009). Worldwide epidemiology of neutralizing antibodies to adeno-associated viruses. J. Infect. Dis. 199, 381–390.

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.