Center for Cellular and Molecular Therapeutics at
The Children's Hospital of Philadelphia (CCMT/CHOP)

AAV8-hFIX19

Summary Information Format

relating to the placing on the market of genetically modified organisms as or in products in accordance with Part 1 of Council Decision (2002/813/EC) of 3 October 2002, pursuant to Directive 2001/18/EC of the European Parliament and of the Council.

Final – January 2013

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: Ireland
(b) Notification number: B/IE/13/01
(c) Date of acknowledgement of notification: 13 February 2013
(d) Title of the project:

A Phase 1 safety study in subjects with severe Hemophilia B (Factor IX deficiency) using a single-stranded, adeno-associated pseudotype 8 viral vector to deliver the gene for human Factor IX [Protocol # AAV8-hFIX19-101].

(e) Proposed period of release:

It is anticipated that the trial will start in Ireland in Q2 2013 and the last patient (worldwide) is expected to be treated by Q3 2015. This estimate is based on the global trial start date of Q3 2012 with an estimated 4 year duration for recruitment and completion of 1 year 'active phase' for the final patient (12 months after treatment).

2. Notifier

Center for Cellular and Molecular Therapeutics at The Children's Hospital of Philadelphia
5th Floor Colket Translational Research Building
3501 Civic Center Boulevard
Philadelphia, PA 19104-4319
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)

AAV8-hFIX19 is pseudotyped with AAV8 capsid proteins, containing a single-stranded DNA genome of 4275 nucleotides consisting of an hFIX expression cassette flanked by the 145 nucleotide inverted terminal repeats derived from AAV type 2. All of the viral coding sequences have been removed and replaced with the hFIX expression cassette.

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

In general, DNA viruses have greater genetic stability than RNA viruses. The GMO is unable to replicate, even in the presence of a helper virus, since the genes essential for replication (*rep* and *cap*) are deleted.

Homologous genomic recombination may occur spontaneously in nature between the viral genomes of AAV strains only under circumstances where a cell of the host organism is infected simultaneously by two different strains of AAV and a helper virus (triple-infection).

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

Yes (.) No (X)

If yes, insert the country code(s) …

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

Yes (.) No (X)

If yes:
- Member State of notification
- Notification number

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

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

Yes (X) No (.)

If yes:
- Member State of notification: United States of America

- Notification number IND 15149

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

AAV8-hFIX19 employs an adeno-associated virus as a delivery vehicle for the human factor IX gene; the recombinant vector is a non-enveloped icosahedral virion of approximately 25 nm in diameter.

AAV8-hFIX19 is pseudotyped with AAV8 capsid proteins, containing a single-stranded DNA genome of 4275 nucleotides consisting of an hFIX expression cassette flanked by the 145 nucleotide inverted terminal repeats derived from AAV type 2. All of the viral coding sequences have been removed and replaced with the hFIX expression cassette.

AAV8-hFIX19 is unable to replicate independently, even in the presence of a helper virus, since it lacks the *rep* and *cap* genes required for rescue/packaging.

Although human infections are common, wild type AAV is not known to be a pathogenic virus in humans. 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 the protection of workers from risks related to exposure to biological agents at work (Appendix III). It is most appropriately designated a Risk Group 1 biological agent, defined in the EU as 'one that is unlikely to cause human disease'.

There are no known natural predators, preys, parasites, competitors or symbionts associated with AAV (although it does require helper functions of co-infecting viruses for replication in nature as described above). Primate (human) AAV serotypes are not known to actively transfer genetic material to organisms other than primates under natural conditions, although an absence of zoonosis is not documented.

Wild type AAV is not known to be involved in environmental processes. It does not respire and does not contribute to primary production or decomposition processes. In its virion form, it does not display any metabolic activity.

None of the genetic modifications made to wild type AAV during construction of AAV8-hFIX19 would be expected to alter its effect on environmental processes. As such, there is no expected impact to the environment as a whole following the release of AAV8-hFIX19.

Based on the nature of the GMO, the parental organism and the receiving environment, the deliberate release of AAV8-hFIX19 is not anticipated to have any direct effects on the environment (other than humans).

The potential direct effects in humans are limited to the transmission of AAV8-hFIX19 to an unintended human recipient. These potential adverse effects are expected to be the same as those which may be anticipated in patients receiving the treatment (immune response, potential for insertional mutagenesis and potential for germline transmission).

Indirect effects of the release are limited to the consequences of the release of wild type AAV (through contamination of the medicinal product during manufacture or following recombination in the recipient's cells followed by shedding into the environment) **and the possible fate of contaminating DNA sequences derived from the manufacturing process**.

The potential magnitude of unintended spread within the human population is considered low. Cases of the transmission of AAV8-hFIX19 to an unintended human recipient are likely to be isolated, and transmission of AAV vectors to any unintended human recipient has not been reported. The medicinal product will be administered to (and handled by) a limited number of individuals. It is estimated that 4-8 patients will receive the treatment at the medical facility in Ireland. Any inadvertent exposure will be self-limiting, since AAV8-hFIX19 is unable to replicate independently, even in the presence of a helper virus, as it lacks the *rep* and *cap* genes required for rescue/packaging.

For those unintended individuals that may be exposed to AAV8-hFIX19, the potential adverse effects are expected to be of a lower severity than those expected in patients receiving considerably higher doses, and of no greater severity than wild type AAV, which is not known to be pathogenic.

The presence of wild-type AAV, either as a contaminant of AAV8-hFIX19 derived during manufacture, or as an indirect consequence of homologous recombination with an existing wild type AAV in an intended human recipient, is not considered to constitute an increased risk to the environment, since it is already globally endemic and is not associated with pathogenesis.

The consequences of dissemination of DNA derived from the plasmids used to manufacture AAV8-hFIX19 are theoretically uptake and integration into microbial genomes, although infection of microbes by AAV is not described to our knowledge and considered unlikely. These DNA sequences are not capable of replication independently. Therefore the likely route of contamination of the environment would be through exposure of the product itself to microorganisms. Such an event could result in uptake and transient expression of a plasmid gene, but the genes present are unlikely to confer any selective advantage and consequently be quickly lost. The possible exception is the transfer of the gene for Kanamycin resistance, which would confer a selective advantage in certain environments, though likely not in sewerage and water systems.

Therefore, the potential consequences in the case of exposure of AAV8-hFIX19 (or a contaminating or derived wild type AAV) to an unintended individual, or the dissemination of contaminating residual plasmid DNA to the environment, are expected to be LOW LEVEL and ISOLATED.

The survival and route of transmission of AAV8-hFIX19 is not expected to be different from that of wild type AAV.

The route of exposure will therefore most likely be through direct contact with the investigational product, as opposed from potential secondary exposure resulting from shedding of the vector from treated patients.

The potential for direct exposure to AAV8-hFIX19 will be limited to those healthcare professionals trained in the Clinical Trial Protocol (ie. those involved in dose preparation and administration). Procedures are in place to minimise this risk, including information provided to healthcare personnel relating to dose preparation, administration, disposal of potentially contaminated items. Dose preparation is performed in a BioSafety cabinet.

The likelihood of secondary exposure to close contacts of patients who receive the treatment is also considered very low. AAV8-hFIX19 cannot replicate in the human body, and shedding through saliva, urine, faeces and semen is expected to be low level and transient. The Clinical Trial Protocol specifies that patients are required to use an effective barrier contraceptive until at least two consecutive semen samples after vector administration are negative for vector sequences (see Section 8).

The likelihood of transmission of wild type AAV is considered even lower than that of transmission of AAV8-hFIX19. The potential routes of exposure will be the same as described for the AAV8-hFIX19 vector itself. However, each batch of the experimental product is tested for the absence of replication competent AAV to assure extremely low levels of contamination. Thus the quantity of wild type AAV which may be present in the product is extremely low. The likelihood of a homologous recombination event with wild type AAV in the patient's cells, giving rise to replication competent (wild type) AAV derived from the co-infecting viruses is also very low in itself.

Given the small scale of the release and the handling and disposal procedures in place, it is considered that the likelihood of exposure of the environment to residual plasmid DNA sequences is very low.

In conclusion, the likelihood of exposure of AAV8-hFIX19 (or a contaminating or derived wild type AAV) to an unintended individual, or the dissemination of contaminating residual plasmid DNA to the environment, is LOW.

The risk posed by AAV8-hFIX19 on human health (specifically an unintended recipient) and the environment is considered by combining the estimated consequences of the effect with the estimated likelihood of effect (in accordance with 2001/18/EC and 2002/623/EC). This estimation is made with reference to the risk attributed to the parental organism (wild type AAV) for context and 'Table 2: Guideline on Environmental Risk Assessments for Medicinal Products consisting of, or containing, genetically modified organisms (GMOs)'.

Thus, through a combination of the low level consequences of transmission and the low likelihood of this occurring, **the overall risk posed by AAV8-hFIX19 to the unintended recipient is considered LOW.**

Similarly, through a combination of the low level consequences of transmission and the low likelihood of this occurring, **the overall risk posed by wild type AAV (mediated by the experimental vector) to the unintended recipient is considered LOW.**

Finally, through a combination of the low level consequences of exposure and the low likelihood of this occurring, **the overall risk posed by residual plasmid DNA sequences to the environment is considered LOW.**

Appropriate risk management strategies are in place to minimise the risks of exposure to unintended individuals or the environment. Appropriate monitoring strategies are proposed to gather further information on safety, persistence and shedding prior to further (wider-scale) development.

In conclusion, overall the environmental risks associated with the deliberate release of AAV8-hFIX19 under the conditions of release proposed, and with the precautions and monitoring activities proposed, is considered acceptable.

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

1. Recipient or parental organism characterisation:

The parental virus concerned in this application is a primate (human) AAV. There are several naturally occurring serotypes of human or non-human primate adeno-associated virus (denoted AAV1 to AAV11) and further variants yet to be fully characterised. The serotype of AAV is determined by the capsid of the virion, which is integral to the species / tissue tropism and infection efficiency of AAV.

AAV8-hFIX19 is pseudotyped with AAV8 capsid proteins, containing a single-stranded DNA genome consisting of an hFIX expression cassette flanked by the 145 nucleotide inverted terminal repeats derived from (human) wild type AAV2. All of the viral coding sequences have been removed and replaced with the hFIX expression cassette. The capsid proteins are derived from (primate) wild type AAV8.

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

(select one only)

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

other, specify …

2. Name

(i)	order and/or higher taxon (for animals)	*Parvoviridae*
(ii)	genus	*Dependovirus*
(iii)	species	Adeno-associated virus
(iv)	subspecies	
(v)	strain	
(vi)	pathovar (biotype, ecotype, race, etc.)	AAV2/AAV8
(vii)	common name	Adeno-associated virus

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 …	

Wild type AAV survives in the environment as a persistent infection in the host vertebrate species or as a latent infection in the nucleus of some infected cells, where it may remain inactive indefinitely, or be reactivated giving rise to secretion of virus.

(b) If the organism is an animal: natural habitat or usual agroecosystem:

Not applicable.

5.

(a) Detection techniques

The presence of AAV may be detected and identified in clinical samples in three ways:

1. Polymerase Chain Reaction (PCR). PCR can be used to detect vector genome sequences associated with AAV in a qualitative or quantitative manner, using primers specific for the *rep* or *cap* genes. Detection of a specific serotype, or any AAV-like sequence, as well as distinction between wild type AAV and recombinant AAV is possible, depending on the choice of primers. Note that the presence of vector genomes does not necessarily imply infectious virus particles.

2. Viral culture: Samples containing suspected infectious AAV particles may be cultured in vitro on a permissive cell line, in the presence of a helper virus.

3. AAV vector particles may be detected using Enzyme-Linked Immunosorbent Assay (ELISA) methods. These methods rely on the generation of specific antibodies to the vector capsid proteins, and can therefore be specific to an individual serotype, or cross-react with several AAV serotypes. Detection of vector capsid particles does not necessarily imply infectious virus particles.

(b) Identification techniques

See above.

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 (.)

If yes: Not applicable.

(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

8. Information concerning reproduction

(a) Generation time in natural ecosystems:

AAV enters cells by interaction of specific viral capsid epitopes with cell surface receptors, influencing the infection efficiency, host range and specific tissue tropism of a virus.

After entry into the host cell nucleus, wild type AAV can follow either one of two distinct and interchangeable pathways of its life cycle: the lytic or the lysogenic. The former develops in cells infected with a helper virus whereas the latter is established in host cells in the absence of a helper virus.

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

The GMO is unable to replicate, even in the presence of a helper virus, since the genes essential for replication (*rep* and *cap*) are deleted.

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

(d) Factors affecting reproduction:

The GMO is unable to replicate, even in the presence of a helper virus, since the genes essential for replication (*rep* and *cap*) are deleted.

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 Not applicable.	

(b) relevant factors affecting survivability:

Wild type AAV survives in the environment as a persistent infection in the host vertebrate species or as a latent infection in the nucleus of some infected cells, where it may remain inactive indefinitely, or be reactivated giving rise to secretion of virus.

Outside of the host, non-lipid enveloped viruses such as AAV are resistant to low level disinfectants, survive well outside of the laboratory environment and can be easily transmitted via fomites. AAV particles are resistant to a wide pH range (pH 3-9) and can resist heating at 56^{o}C for 1 hour (Berns and Bohenzky, 1987). AAV does not form survival structures but can remain infectious for at least a month at room temperature following simple desiccation or lyophilization.

10. (a) Ways of dissemination

Dispersal (dissemination) of AAV is not documented definitively, but is likely through inhalation of aerosolized droplets, mucous membrane contact, parenteral injection, or ingestion.

(b) Factors affecting dissemination

AAV8-hFIX19 is a replication-incompetent virus derived from AAV2/8. The genetic modifications do not affect its survival outside the host or probable mode of dissemination. However, the lack of replicative ability prevents multiplication and therefore severely limits its ability to disseminate.

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)

The experimental strain of AAV (AAV8-hFIX19) is ultimately derived from wild type DNA sequences from AAV2 (ITR and *rep* functions) and AAV8 (cap proteins).

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 experimental strain of AAV (AAV8-hFIX19) is limited to the required elements designed to optimise expression of functional human coagulation Factor IX in the liver:

The expression cassette comprises:

- Human α1-antitrypsin (hAAT) liver-specific promoter coupled to the human apolipoprotein E (ApoE) enhancer/hepatocyte control region (Okuyama *et al.*, 1996; Nakai *et al.*, 1999; Miao *et al.*, 2000);
- Exon 1 from the human factor IX gene;
- A portion of the human factor IX intron 1;
- Exons 2-8 of the human factor IX gene; and
- Bovine polyadenylation signal sequence.

Small intervening DNA sequences are also present, derived from the process of assembling the genetic elements through recombinant DNA techniques. These sequences are non-functional and inactive.

The expression cassette is flanked by the 145 nucleotide inverted terminal repeats derived from AAV2.

The entire vector genome is encapsulated in cap proteins derived from AAV8.

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

The following plasmid stocks are used to manufacture AAV8-hFIX19:

1) Vector plasmid pAAV2-hFIX19, Lot C03SEP09A-6, containing the hFIX expression cassette flanked by AAV2 ITRs;

2) AAV packaging plasmid pAAV8PKv3, Lot C14OCT11H-2, containing the AAV2 *rep* and AAV8 *cap* genes coding for non-structural and structural proteins, respectively;

3) Adenovirus helper plasmid pCCVC-AD2HPv2, Lot 21973, encoding the adenovirus type 2 genes E2A, E4 and VA RNAs required for AAV replication in HEK293 cells.

(c) Host range of the vector

All the plasmids contain bacterial origins of replication, and are therefore able to replicate n 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 functional aminoglycoside 3'-phosphotransferase (APH) gene is present on all the plasmids. This gene confers Kanamycin resistance to bacterial cells used for plasmid production.

(e) Constituent fragments of the vector

The vector plasmid pAAV2-hFIX19 is a 11,207 bp plasmid encoding an hFIX expression cassette inserted between two AAV2 inverted terminal repeats (ITRs). The plasmid backbone contains a kanamycin resistance (KanR) gene, a bacterial origin of replication, a M13/F1 origin of replication, and a fragment of bacteriophage lambda DNA.

Packaging plasmid pAAV8PKv3 is a 7,518 bp plasmid construct that carries AAV2 Rep and AAV8 Cap genes under the control of AAV2 p5 promoter, bacterial origin of replication and a gene conferring resistance to Kanamycin in bacterial cells.

Plasmid pCCVC-AD2HPv2 is a 11,832 bp plasmid that carries three adenovirus genes, E2A, E4 and the VA RNAs that provide the 'helper' functions necessary for replication and encapsidation of AAV vectors. The construct also contains a bacterial origin of replication and a gene conferring resistance to Kanamycin in bacterial cells.

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

(i)	transformation	(.)
(ii)	electroporation	(.)
(iii)	macroinjection	(.)
(iv)	microinjection	(.)
(v)	infection	(.)
(vi)	other, specify: .	

The final vector, AAV8-hFIX19 is constructed from the plasmid stocks on a batch-by-batch basis by co-transfecting a Master Cell Bank (MCB) of Human Embryo Kidney (HEK) 293 cells with the three plasmid stocks:

1) Vector plasmid pAAV2-hFIX19 containing the hFIX expression cassette flanked by AAV2 ITRs;

2) AAV packaging plasmid pAAV8PKv3, containing the AAV2 *rep* and AAV8 *cap* genes coding for non-structural and structural proteins, respectively;

3) Adenovirus helper plasmid pCCVC-AD2HPv2, encoding the adenovirus type 2 genes E2A, E4 and VA RNAs required for AAV replication in HEK293 cells.

Note that the required helper functions are provided as a plasmid, NOT a viable adenovirus.

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 …	Not applicable.

6. Composition of the insert

(a) Composition of the insert

The expression cassette is limited to the required elements designed to optimise expression of functional human coagulation Factor IX in the liver:

The expression cassette comprises:

- Human α1-antitrypsin (hAAT) liver-specific promoter coupled to the human apolipoprotein E (ApoE) enhancer/hepatocyte control region (Okuyama *et al.*, 1996; Nakai *et al.*, 1999; Miao *et al.*, 2000);
- Exon 1 from the human factor IX gene;
- A portion of the human factor IX intron 1;
- Exons 2-8 of the human factor IX gene; and
- Bovine polyadenylation signal sequence.

Small intervening DNA sequences are also present, derived from the process of assembling the genetic elements through recombinant DNA techniques. These sequences are non-functional and inactive.

(b) Source of each constituent part of the insert

The constituent parts were derived from commercially or academically available plasmids, manipulated as required using standard molecular biological techniques.

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

In order to potentially improve both the safety and potency of the vector, a codon-optimized hFIX gene construct was used; the hFIX cDNA was modified at the nucleotide sequence level, but NOT at the protein sequence level, to potentially achieve higher levels of expression at the same vector dose. The modifications of the hFIX cDNA sequence include amino acid codon usage optimization for expression in *Homo sapiens* and removal of alternate open reading frames (ARF) in the hFIX sequence.

The Human α1-antitrypsin (hAAT) liver-specific promoter coupled to the human apolipoprotein E (ApoE) enhancer/hepatocyte control region drives liver-preferential expression of the hFIX gene and the bovine polyadenylation signal sequence functions to provide effective termination of transcription and stabilise transcribed mRNA.

(d) Location of the insert in the host organism

- on a free plasmid (.)
- integrated in the chromosome (X)
- other, specify: Though integration into the host cell chromosome is possible, the vector genome is predominately maintained as an episome in the nucleus of the host cell.

(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

The following information relates to the organism from which the inserted gene (hFIX) 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	
(iii)	genus	Homo
(iv)	species	*sapiens*
(v)	subspecies	*sapiens*
(vi)	strain	
(vii)	cultivar/breeding line	
(viii)	pathovar	
(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 Not applicable.

(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):

Not applicable.

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: Not applicable.

5. Do the donor and recipient organism exchange genetic material naturally?

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

At high multiplicity of infection, wild type AAV integrates into human chromosome 19 in ~60% of latently infected cell lines. However, it has been recently demonstrated that only approximately 1 out of 1000 infectious units can integrate (Tenenbaum *et al.*, 2003). Schnepp *et al.*, 2005 have provided evidence that following naturally acquired infection, wild type AAV DNA may persist mainly as circular double stranded episomes in human tissues.

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 (.) Not known (.)

Specify:

AAV8-hFIX19 is unable to replicate independently, even in the presence of a helper virus, since it lacks the *rep* and *cap* genes required for rescue/packaging.

(c) is the GMO in any way different from the recipient as far as dissemination is concerned?

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

Specify:

AAV8-hFIX19 is unable to replicate independently, even in the presence of a helper virus, since it lacks the *rep* and *cap* genes required for rescue/packaging. Therefore, though it has the capacity to infect cells, the lack of replicative capacity will severely restrict dissemination.

(d) is the GMO in any way different from the recipient as far as pathogenicity is concerned?

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

Specify …

2. Genetic stability of the genetically modified organism

Based on the fact that long term therapeutic activity of the investigational drug is not dependent on replication of the recombinant AAV, and the known genetic stability of the parent wild type AAV, the genetic traits of the organism 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 … Not applicable.

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

Neither wild type AAV, nor the experimental vector AAV8-hFIX19 is known to be pathogenic to humans.

4. Description of identification and detection methods

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

In the proposed clinical trial, subjects will be monitored for the presence of the vector in blood, urine, saliva and semen according to the Clinical Trial Protocol.

DNA will be extracted from the samples obtained during the clinical trial and the presence of AAV vector genomes in the sample quantitated using real-time polymerase chain reaction with reference to a standard curve of linearised plasmid DNA containing the target sequences. Spiked positive controls are included in the test procedure to detect sample matrix interference. A negative control (genomic DNA not exposed to AAV) is also included. All analyses are done in duplicate except semen which is performed in triplicate.

Transgene antigen/ activity tests will also be performed on blood samples as specified in the Clinical Trial Protocol.

Factor IX protein will be detected and quantified by analysis of plasma samples using enzyme-linked immunosorbant assay (ELISA). A microtiter plate is coated with an antibody to human Factor IX, and plasma samples are loaded into the wells. If hFIX is present in the sample, it is recognized by a peroxidase-labeled anti-human FIX antibody. Detection is subsequently performed by an enzymatic reaction and quantified with reference to a standard curve. Factor IX activity is determined by a standard coagulation assay, in which formation of a fibrin clot is the endpoint. Activity is quantified by reference to a standard curve. Positive and negative plasma controls are included in all test procedures.

(b) Techniques used to identify the GMO

The GMO may be identified by PCR as described above.

F. Information relating to the release

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

Following the approval of the Clinical Trial Authorisation for AAV8-hFIX19, it is planned to make the product available to clinical trial site(s) in Ireland for use in accordance with a Clinical Trial Protocol entitled:

'A Phase 1 safety study in subjects with severe Hemophilia B (Factor IX deficiency) using a single-stranded, adeno-associated pseudotype 8 viral vector to deliver the gene for human Factor IX' [Protocol # AAV8-hFIX19-101].

This is a Phase 1/2 safety and dose escalation study of intravenous administration of the AAV8-hFIX19 vector to subjects with severe Haemophilia B. The primary objectives are to evaluate the safety and tolerability of the treatment. A secondary objective is to measure biologic and physiologic activity of the transgene product. It is not known if the doses used in this study will increase the level of FIX in subjects, though based on prior clinical experience it is suspected that all doses tested will result in detectable factor IX levels. The information obtained in this study will guide the design of future studies in which a benefit to subjects may be anticipated.

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)

If yes, specify. Not applicable.

3. Information concerning the release and the surrounding area

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

The clinical trial is expected to be performed at a single site in Ireland:

National Centre for Hereditary Coagulation Disorders
St. James's Hospital,
James's Street,
Dublin 8,
Ireland

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

(i) actual release site (m^2): Not applicable

(ii) wider release site (m^2): Not applicable

Eligible subjects will be admitted to the hospital on the day of AAV8-hFIX19 infusion. After vector administration, the catheter will be removed approximately 20 ± 4 hours after the infusion. Haemostasis at the venipuncture site will be secured by applying pressure according to standard protocol for infusing FIX concentrates. Patients will remain in-hospital for approximately 24 hours of observation to monitor for adverse effects related to the procedure. Patients will then return to their normal daily routine.

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

Given the nature of the product administration, scale of release and procedures for waste treatment, the exposure to significant biotopes, protected areas and drinking water supplies is expected to be negligible.

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

AAV8-hFIX19 is a replication-incompetent virus derived from AAV2/8. The genetic modifications do not affect its natural host and tissue tropism. No transfer of genetic material between the GMO and other organisms is predicted.

Given the nature of the product administration (intravenous) and the transient/ low levels of shedding expected, the risk of unintended exposure of flora and fauna to AAV8-hFIX19 is minimal.

4. Method and amount of release

(a) Quantities of GMOs to be released:

Doses to be administered for the proposed study in an inter-subject group dose escalation study design are 1 x 10^{12} vg/kg, 2 x 10^{12} vg/kg, and 5 x 10^{12} vg/kg. Pharmacy instructions will be updated to ensure the correct dose is given, depending on the concentration of vector in the batch supplied.

The maximum total dose for an 80 kg individual patient is therefore approximately 22 mL as a single treatment (approximately 40 x 10^{13} vg).

The proposed clinical trial aims to recruit 15 evaluable patients across 4 sites in the US, Australia and Ireland. It is therefore reasonable to assume that approximately 4-8 patients will be treated in Ireland.

(b) Duration of the operation:

The use of AAV8-hFIX19 in Ireland will commence following approval of the Clinical Trial Authorisation by the Irish Medicines Board (IMB) and positive opinions from the Ethics Committee and Institutional Biosafety Committee. It is anticipated that the trial will start in Ireland in Q2 2013 and the last patient (worldwide) is expected to be treated by Q3 2015. This

estimate is based on the global trial start date of Q3 2012 with an estimated 4 year duration for recruitment and completion of 1 year 'active phase' for the final patient (12 months after treatment).

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

Following the approval of the Clinical Trial Authorisation for AAV8-hFIX19, it is planned to make the product available to clinical trial site(s) in Ireland for use in accordance with the Clinical Trial Protocol [Protocol # AAV8-hFIX19-101]:

AAV8-hFIX19 vector is supplied as a frozen liquid at a volume of 1 mL in a 1.5 mL polypropylene sterile cryogenic screw cap vial. AAV8-hFIX19 is to be stored securely, frozen at less than -60°C.

The investigational product will only be provided to sites on a subject-by-subject basis, following confirmation of subject eligibility and a review of registration documents/essential documents.

A qualified pharmacist with specific training on the protocol will be responsible for gene transfer material receipt from the Sponsor, storage, documentation of traceability of product at the investigational site, preparation (dilution and combination of components) on the day of administration and disposal.

Adequate records of study drug receipt and disposition will be maintained by the study site Investigational Pharmacy, and records of receipts, investigational drug orders, dispensing records, and disposition forms will be examined during the course of the study. The purpose of these records is to ensure that the investigational new drug will not be distributed to any person outside the terms and conditions set forth in the Clinical Trial Protocol. The study medication is to be prescribed by the Investigator or designee and may not be used for any purpose other than that described in the Clinical Trial Protocol.

The Investigational Drug Data Sheet (IDDS) provided with the Pharmacy Instructions instruct those involved with dose preparation to use universal precautions and appropriate Personal Protective Equipment. Dose preparation is to be performed in a BioSafety Cabinet (BSC) to reduce the risks posed by the possibility of generation and inhalation of aerosols. Once prepared, the dose (infusion bag) is labelled according to protocol (including a biohazard symbol), double bagged and taken to the administration area in a designated container.

AAV8-hFIX19 will be administered to the patient by a medical professional in a medical facility.

Due to the minimal manipulations involved in dose administration it is not considered necessary to require further precautions at the point of administration, beyond the use of disposable gloves (as specified in the Handling Instructions).

AAV8-hFIX19 is sensitive to inactivation by a variety of commonly available physical and chemical methods.

Following administration of AAV8-hFIX19 at a medical facility, used (or partially used) vials of vector/ empty capsids, syringes used in dose preparation, infusion bags and infusion sets are retained and stored in a labeled biohazard bag in the pharmacy at below -60^{o}C. These will then be returned to the sponsor or destroyed according to sponsor instructions (see the Investigational Drug Data Sheet (IDDS)/ Pharmacy manual and Handling Instructions for AAV8-hFIX19).

Any other disposable instruments or other materials used during the dose preparation procedure will be disposed of in a manner consistent with the standard practice of the institution for potentially biohazardous materials.

Instructions for dealing with spills/breakages and accidental exposure are provided in the Handling Instructions.

The worker protection measures proposed for the preparation and administration of AAV8-hFIX19 are therefore in line with those recommended globally for the handling of BSL-1/2 organisms in a research setting. An additional precaution of dose preparation in a BioSafety Cabinet (BSC) is also specified.

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

The clinical trial of AAV8-hFIX19 will occur in Ireland which has a temperate climate. The risk of release of AAV8-hFIX19 in to the environment is unrelated to climatic characteristics.

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.

No data available.

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	
(iii)	genus	Homo
(iv)	species	*sapiens*
(v)	subspecies	*sapiens*
(vi)	strain	
(vii)	cultivar/breeding line	
(viii)	pathovar	
(ix)	common name	Human

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

In treated subjects, AAV8-hFIX19 is expected to preferentially localise to the liver (dictated by the tissue tropism of the capsid, derived from AAV8). Following infection of hepatocytes, the vector is expected to persist for months or years, primarily as an episome but potentially by integration into the host cell genome.

The presence of the codon-optimised human coagulation factor IX gene under the transcriptional control of the liver-specific human alpha 1 antitrypsin (hAAT) promoter, Apolipoprotein E (ApoE) Enhancer and Hepatic Control Region and the bovine growth hormone polyadenylation sequence is expected to result in expression of functional hFIX and its excretion into the circulation at levels which result in clinically meaningful increases in clotting function.

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

AAV8-hFIX19 is a replication-incompetent virus derived from AAV2/8. The genetic modifications do not affect its natural host and tissue tropism. No potentially significant interactions with other organisms in the environment are predicted.

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

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

Give details: Not applicable.

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

Dissemination of AAV8-hFIX19 would most likely only occur between human beings, since it is derived from AAV2/8. However no replication is expected in normal cells of treated individuals

exposed to the replication-deficient virus, or from exposure of uninfected people to treated individuals.

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

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

Not applicable.

7. Likelihood of genetic exchange in vivo

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

AAV8-hFIX19 is unable to replicate independently, even in the presence of a helper virus, since it lacks the *rep* and *cap* genes required for rescue/packaging.

In its latent state, AAV8-hFIX19 viral DNA is maintained either as a stable episome or by integration into the host cell (human) DNA.

Homologous genomic recombination may occur spontaneously in nature between the viral genomes of AAV strains only under circumstances where a cell of the host organism is infected simultaneously by two different strains of AAV and a helper virus which is permissive in that species (triple-infection). In the case of AAV8-hFIX19, such recombination could only result in the exchange of the hFIX expression cassette with the *rep* and *cap* genes of the wild type virus. It is not possible for the AAV genome to contain both *rep/cap* genes and the transgene, as this is beyond the packaging limit of the virion.

Therefore the only mechanism by which the transgene could be mobilised is through a triple infection of the same cell by AAV8-hFIX19 (containing the transgene), wild type AAV (providing the rep and cap functions) and a helper virus. This scenario is expected to be a rare event, especially since the vector target cells (liver) are not the natural target cells of helper viruses. If it did occur, it would only result in the production of more wild type AAV and more AAV8-hFIX19 vector particles (which would still lack *rep* and *cap* genes and consequently could not be self-sustaining).

No genetic exchange to or from organisms other than humans or wild type AAV is expected.

(b) from other organisms to the GMO:

As above.

(c) likely consequences of gene transfer:

The scenario described above would only result in the production of more wild type AAV and more AAV8-hFIX19 vector particles (which would still lack *rep* and *cap* genes and consequently could not be self-sustaining). It is not possible for the AAV genome to contain both *rep/cap* genes and the transgene, as this is beyond the packaging limit of the virion.

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.):

AAV8-hFIX19 is a replication-incompetent virus derived from AAV2/8. The genetic modifications do not affect its natural host and tissue tropism.

No specific studies have been conducted regarding transmission of AAV8-hFIX19 between humans or animals.

Shedding has however been monitored in both humans and animals following administration of similar vectors to AAV8-hFIX19.

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

None known or predicted. AAV is not known to be involved in environmental processes. It does not respire and does not contribute to primary production or decomposition processes. In its virion form, it does not display any metabolic activity.

H. Information relating to monitoring

1. Methods for monitoring the GMOs

Monitoring during and after the clinical trial is restricted to patients who receive the AAV8-hFIX19 product.

Patients will be monitored throughout treatment by the Principal Investigator and delegates. An independent Data Safety Monitoring Board (DSMB) will oversee data and safety monitoring, with interval meetings based on DSMB guidelines. Independent Contract Research Organisations (CROs) will be used for study monitoring and data management activities. Any serious adverse event will be reported in the appropriate time-frame to the Sponsor, and as required to each of the national regulatory agencies according to pharmaceutical legislation.

After vector administration, vital signs will be monitored hourly for 6 hours and then every 2 hours for 6 hours and then at 4-hour intervals. Patients will remain in-hospital for approximately 24 hours of observation to monitor for adverse effects related to the procedure.

A comprehensive battery of laboratory evaluations, comprising primarily of blood tests, will be conducted at regular intervals to assess safety throughout the first year following administration. Safety tests will include PCR testing for vector shedding in saliva, blood and urine at each weekly visit until week 12 (or until 2 consecutive samples are negative). PCR testing of semen will be performed at each monthly visit until 2 consecutive samples are negative.

Factor IX activity/antigen will be monitored weekly until 12 weeks, and monthly thereafter until Month 12.

Patients will be subject to long term follow-up as described below:

In Years 2 to 5 following vector administration, an annual physical exam will be performed with complete history and a subset of laboratory evaluations for safety and Factor IX activity (as a surrogate test to indicate vector persistence).

The subject's body fluids will be monitored only during the first year of the study for persistent vector sequences. It is not anticipated, based on non-clinical studies and prior clinical experience, that testing for vector sequences will be necessary in the long-term follow-up period.

Subjects will be encouraged to monitor themselves and to assist in reporting adverse events; they will be provided with laminated wallet-sized cards with investigator contact information. Additionally, health care professionals from the subject's home haemophilia centre, who are not otherwise associated with the clinical trial, will be notified to provide prompt reports of adverse events to the investigators.

Investigators will maintain in the case history records of exposures to mutagenic agents and other medicinal products along with subjects' adverse event profiles.

Clinical information will focus on information pertaining to new malignancies, new incidence or exacerbation of a pre-existing neurological disorder, new incidence or exacerbation of a prior rheumatologic or other autoimmune disorder, and new incidence of a hematologic disorder.

For the subsequent ten years, subjects will be contacted at a minimum of once per year. A clinical questionnaire, administered by telephone call or at the subject's home haemophilia treatment centre, will focus on information pertaining to new malignancies, new incidence or exacerbation of a pre-existing neurological disorder, new incidence or exacerbation of a prior rheumatologic or other autoimmune disorder, and new incidence of a hematologic disorder. It will ask subjects to describe any adverse events, including any unexpected illness and/or hospitalization, and provide a description of exposures to mutagenic agents and other medicinal products. Additionally, ultrasound of the target organ, the liver, will be performed every three years for the fifteen year long-term follow-up period.

2. Methods for monitoring ecosystem effects

No monitoring of the environment or unintended recipients is planned or considered necessary.

3. Methods for detecting transfer of the donated genetic material from the GMO to other organisms

See Section E.4.

4. Size of the monitoring area (m^2)

Not applicable.

5. Duration of the monitoring

See Section H.1.

6. Frequency of the monitoring

See Section H.1.

I. Information on post-release and waste treatment

1. Post-release treatment of the site

Post-release treatment of the site will not be necessary, provided the precautions outlined in the Investigational Drug Data Sheet (IDDS)/ Pharmacy Instructions and Handling Instructions are adhered to when preparing or administering the product or when dealing with accidental spillages and breakages.

2. Post-release treatment of the GMOs

Following administration of AAV8-hFIX19 at a medical facility, used (or partially used) vials of vector/ empty capsids, syringes used in dose preparation, infusion bags and infusion sets are retained and stored in a labeled biohazard bag in the pharmacy at below -60^{o}C for a period of no less than two months, per manufacturer's retention instructions (as specified in the Investigational Drug Data Sheet (IDDS) and Handling Instructions for AAV8-hFIX19). These items will then be returned to the sponsor or destroyed according to sponsor instructions.

Any other disposable instruments or other materials used during the dose preparation procedure will be disposed of in a manner consistent with the standard practice of the institution for potentially biohazardous materials.

In the medical facility, this will involve temporary containment in sharps bins or clearly marked bags (e.g. biohazard, medical waste) prior to autoclaving and/or incineration either on or off site as per local institutional guidelines for handling potentially infectious materials.

All non-disposable equipment and other materials used during the procedure will be cleaned using a chemical disinfectant capable of virucidal activity for the required duration of contact, or sterilized by autoclaving consistent with local institutional guidelines for handling potentially infectious materials.

Typically, standard operating procedures for disposal within medical facilities (where the potential for contamination from other agents is potentially much more hazardous than that presented by AAV8-hFIX19) will be consistent with the guidance given in the WHO Laboratory Biosafety Manual, 3rd Ed (2004) for BSL1/2 as outlined below:

Contaminated (infectious) "sharps":

Hypodermic needles, scalpels, knives and broken glass; should always be collected in puncture-proof containers fitted with covers and treated as infectious.

After use, hypodermic needles should not be recapped, clipped or removed from disposable syringes. The complete assembly should be placed in a sharps disposal container. Disposable syringes, used alone or with needles, should be placed in sharps disposal containers and incinerated, with prior autoclaving if required. Sharps disposal containers must be puncture-proof/-resistant and must not be filled to capacity. When they are three-quarters full they should

be placed in "infectious waste" containers and incinerated, with prior autoclaving if laboratory practice requires it. Sharps disposal containers must not be discarded in landfills.

Contaminated (potentially infectious) materials for autoclaving and reuse:

No pre-cleaning should be attempted of any contaminated (potentially infectious) materials to be autoclaved and reused. Any necessary cleaning or repair must be done only after autoclaving or disinfection.

Contaminated (potentially infectious) materials for disposal:

Apart from sharps, which are dealt with above, all contaminated (potentially infectious) materials should be autoclaved in leak-proof containers (e.g. autoclavable, color-coded plastic bags), before disposal. After autoclaving, the material may be placed in transfer containers for transport to the incinerator. If possible, materials deriving from healthcare activities should not be discarded in landfills even after decontamination. If an incinerator is available on the laboratory site, autoclaving may be omitted; the contaminated waste should be placed in designated containers (e.g. color-coded bags) and transported directly to the incinerator. Reusable transfer containers should be leakproof and have tight-fitting covers. They should be disinfected and cleaned before they are returned to the laboratory for further use.

3. (a) Type and amount of waste generated

AAV8-hFIX19 will be administered by a single peripheral intravenous infusion into eligible, consenting adult males with X-linked severe Haemophilia B.

Waste generated from the preparation and infusion of AAV8-hFIX19 will be limited to:

- Used vials of the Investigational Medicinal Product
- Used vials of empty capsid (containing no DNA)
- Used preparation equipment in the pharmacy; syringes, needles, vials
- Used Infusion bags and infusion kits
- Bags used to transport potentially contaminated equipment to and from the pharmacy
- Used swabs and items used to clean injected area
- Personal Protective Equipment used during dose preparation and administration

AAV8-hFIX19 vector is supplied as a frozen liquid containing $>0.5 \times 10^{13}$ viral genomes (vg) in a volume of 1 mL in a 1.5 mL polypropylene sterile cryogenic screw cap vial. Doses to be administered for the proposed study in an inter-subject group dose escalation study design are 1×10^{12} vg/kg, 2×10^{12} vg/kg, and 5×10^{12} vg/kg. The maximum total dose for an 80 kg individual patient is therefore approximately 22 mL as a single treatment (approximately 40×10^{13} vg).

The proposed clinical trial aims to recruit 15 evaluable patients across 4 sites in the US, Australia and Ireland. It is therefore reasonable to assume that approximately 4-8 patients will be treated in Ireland.

Each administration will result in the waste identified above.

3. (b) Treatment of waste

AAV8-hFIX19 is a replication-deficient non-pathogenic virus which is considered to present a much lower hazard to human health than other human biological waste which is frequently disposed of in medical facilities.

AAV8-hFIX19 is sensitive to inactivation by a variety of commonly available physical and chemical methods.

Following administration of AAV8-hFIX19 at a medical facility, used (or partially used) vials of vector/ empty capsids, syringes used in dose preparation, infusion bags and infusion sets are retained and stored in a labeled biohazard bag in the pharmacy at below -60^{o}C. These will then be returned to the sponsor or destroyed according to sponsor instructions (see the Investigational Drug Data Sheet (IDDS)/ Pharmacy manual and Handling Instructions for AAV8-hFIX19).

Any other disposable instruments or other materials used during the dose preparation procedure will be disposed of in a manner consistent with the standard practice of the institution for potentially biohazardous materials (see above).

J. Information on emergency response plans

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

There are no specific procedures planned for controlling the GMO in the case of unexpected spread.

Wild type AAV is a non-pathogenic single-stranded DNA *Dependovirus*, requiring helper DNA virus for replication. AAV8-hFIX19 is derived from wild type AAV, but encodes no replication genes in the expression cassette and is incapable of independently replicating its genome.

The potential for unexpected spread of AAV8-hFIX19 in the environment is extremely low, due to:

- Attenuation of the GMO rendering it even less replication competent than the parental virus (AAV2/8), by deletion of the replication genes.
- Intravenous administration to eligible patients by medical professionals in a medical facility.
- Limited host and tissue tropism (human/primate) of the parental virus (AAV2/8).
- Low and transient incidence of shedding of infective virus from treated individuals.
- High levels of existing adaptive immunity in the human population.

Any spread of AAV8-hFIX19 to unintended human recipients is therefore highly unlikely, and would be isolated to single cases in discrete geographical locations. The risk of widespread infection is considered negligible.

In the theoretical event that wild type AAV, supplying the requisite replication gene products, were to co-infect a hepatocyte, along with a helper DNA virus such as adenovirus or herpes simplex virus and the AAV8-hFIX19 vector (a triple co-infection), it is possible that vector replication could occur.

However, even if this rare event were to occur, the resulting virologic outcome would be increased synthesis of vector and wild type AAV, both intrinsically non-pathogenic viruses. It is therefore unlikely that such an event would present clinical symptoms and is therefore unlikely to become apparent.

If such spread were detected, the individual could be isolated pending further investigation, and consultation with IMB and the Environmental Protection Agency in Ireland.

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

There are no specific procedures planned for decontaminating areas in the case of unexpected spread, since the risk of spread is considered negligible.

In the unlikely event that transmission to an unintended human recipient occurred, this would likely be a local occurrence affecting a healthcare professional or close contact of a treated individual.

Decontamination of areas in which a recently treated patient had frequented (their home and or examination room at a medical facility) could be implemented by applying standard detergents to areas of likely contact (for example, frequent touch-points such as handles, door knobs, hard surfaces, railings and hand-holds, washing facilities and lavatories). Fomites could be autoclaved or incinerated.

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

The predicted habitat of AAV8-hFIX19 is humans where it is expected to persist in a lysogenic state. AAV8-hFIX19 is a disabled version of a non-pathogenic wild-type primate (human) AAV, modified by deletion of the *rep* and *cap* genes rendering it unable to replicate, even in the presence of a helper virus.

Decontamination of plants, (non-human) animals and soils will not be required.

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

AAV8-hFIX19 will be regulated under medicines legislation in Ireland, requiring stringent pharmacovigilance overseen by the Competent Authority (Irish Medicines Board; IMB). Information will be collected regarding all individual adverse events and submitted to the IMB if they fulfil the criteria for a Serious Unexpected Suspected Adverse Reaction (SUSAR) as defined in the Clinical Trial Protocol. Development Safety Update Reports will be submitted to IMB on an annual basis while the trial is active. Procedures are in place at both IMB and CCMT/CHOP to monitor, review and act on urgent safety information relating to medicinal products so that human health is protected.

Information relating to trial-related monitoring activities is provided in Section H.

In the extremely unlikely event that spread of the vector to an unintended human recipient was detected, the individual could be isolated pending further investigation, and consultation with IMB and the Environmental Protection Agency in Ireland. Measures outlined above may be implemented to prevent further spread.