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 | tbd |
| (c) | Date of acknowledgement of notification | |
| (d) | Title of the project | |

Proposed period of release:

May 2024 – March 2030

2. Notifier

Name of institution or company:
Pfizer Inc., 66 Hudson Boulevard East, New York, NY 10001, United States

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 (kingdom, phylum, class) …

(b) Identity of the GMO (genus and species)
Genus: Dependoparvovirus

Species: AAVSpark100 - bioengineered adeno-associated viral vector derived from a naturally occurring AAV serotype (Rh74)

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

Adeno-associated virus is a single stranded DNA virus that demonstrates a high degree of genetic stability as evidenced by the close relationship of the rep and cap genes from multiple AAV serotypes and genomovars. Typically, homology is between 61 to 84% on the aminoacid sequence level, depending on the serotypes compared (Akache et al, 2006). Furthermore, AAV uses host DNA polymerases for viral replication, which are characterized 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 (Gao et al, 2004).

Fidanacogene elaparvovec is expected to be highly genetically stable. The fidanacogene elaparvovec vector genome is assayed by restriction map analysis for every batch.

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):

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: On study C0371002: FR, GR, SE, DE, (and UK while it was still part of EU), Spain and Italy have just been submitted on 23Jan2024 and Italy on 16Jan2024 (approvals not expected before April 2024). On study C0371006: DE, FR, ES, IT,
- Notification number: B/DE/20/PEI3860, B/ES/21/12, B/ES/20/24, 101996/2019 (Greece), Ref. 5.1-2019-66303 (Sweden).

**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, Australia, Canada, Brazil, Korea, Taiwan, Turkey

- Notification number: USA NIH OBA Protocol 1410-1355; AUS: RPAH IBC 16-009, CAN NSN 20125, ISR #201910662, BRA CTNBio Process #: 01250.053041/2019-38, TWN #: 1086027000, TUR # 19-PFI-01

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

Fidanacogene elaparvovec is replication-incompetent, even in the presence of a helper virus, and does not contain any harmful or toxic transgene. Furthermore, all viral genes have been removed from fidanacogene elaparvovec, leaving only the ITRs as remaining sequences of viral origin. The vector is shed from patients for some time after administration, however, released viral particles are subsequently quickly diluted in the environment to non-detectable levels and will ultimately be degraded by natural processes within a matter of hours (Fleischmann, 2023). The amount of vector shed from patients is expected to be too low to cause efficient transfection in any organism.
Fidanacogene elaparvovec is undergoing diligent testing during the complete manufacturing process, before being released for use.
According to international guidelines fidanacogene elaparvovec fulfils the requirements of a Risk Group 1 biological agent (COGEM report CGM/180316-01, NIH Guidelines For Research Involving Recombinant Or Synthetic Nucleic Acid Molecules April 2019 , Zentrale Kommission für die Biologische Sicherheit (ZKBS), Stellungnahme der ZKBS zur Risikobewertung, humaner Adeno-assoziierter Viren und AAV-abgeleiteter Vektoren) though applied biosafety measures may vary locally.
Medical staff will handle fidanacogene elaparvovec according to local guidelines for handling of biohazardous materials and will use appropriate PPE. Contaminated waste will be disposed of according to local guidelines for biohazardous waste. Workplaces and potentially contaminated areas will be disinfected appropriately in order to avoid unintended exposure to fidanacogene elaparvovec.
Taking into account the results of the environmental risk assessment and the applied mitigation measures, the overall environmental risk posed from use of fidanacogene elaparvovec is considered negligible.

## 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 | serotype AAV-Spark100 (derived from naturally occurring AAV serotype Rh74) |
| (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 |
| 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 — Rh74 is found in association with animals (primate hosts). AAV-Spark100 does not have a natural habitat.

(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, or 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: Wildtype 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 up to ~80% (European Parliament and of the Council 2000, Goncalves et al 2005, Calcedo et al 2009). Consequently, AAV fulfils the definition of a risk group 1 biological agent according to the Directive 2000/54/EC (a biological agent that is unlikely to cause human disease).

(a) 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:
Wildtype AAV is replication-dependent, thus, the generation time is variable depending on the presence or absence of a helper virus.

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

Not applicable.

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

(d) Factors affecting reproduction:

(e) Not applicable.

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:

Viral particles will not be able to remain intact and infectious for extended periods of time. AAV are effectively degraded in sewage water, most likely due to microbial activity, by hydrolysis, and also by UV radiation at the levels found in nature, and other degrading factors (Fleischmann, 2023). 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).

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)

Not applicable.

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 and containing the FIX-R338L variant of the human factor IX (hFIX) gene, for the potential treatment of patients with Haemophilia B.
The FIX-R338L has a higher specific activity than the regular FIX version. Expression is driven by a liver-specific enhancer and promoter. Biodistribution studies evaluating the tissue distribution of fidanacogene elaparvovec demonstrated predominant gene transfer to liver tissue.

It is expected that administration of fidanacogene elaparvovec will result in the expression of the hFIX transgene and raise circulating levels of endogenous FIX of study subjects.

3. (a) Has a vector been used in the process of modification?

Yes (X) No (.)

If no, go straight to question 5.

(b) If yes, is the vector wholly or partially present in the modified organism?

Yes (X) No (.)

If no, go straight to question 5.

4. If the answer to 3(b) is yes, supply the following information

(a) Type of vector

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

(b) Identity of the vector

Plasmid comprising vector genome (phFIX39v2)

(c) Host range of the vector

Bacteria, mammalian cells

(d) Presence in the vector of sequences giving a selectable or identifiable phenotype

Yes (X) No (.)

| | |
|---|---|
| antibiotic resistance | (X) |
| other, specify | … |

Indication of which antibiotic resistance gene is inserted

Kanamycin

(e) Constituent fragments of the vector

The vector genome comprises a modified liver-specific promoter and enhancer, gene encoding human FIX and a polyadenylation signal, flanked by AAV inverted terminal repeats (ITRs). Only the vector genome is present in the final GMO. In addition, the plasmid vector contains a bacterial origin of replication and the gene for kanamycin resistance to allow for propagation of the plasmid in *E. coli*. These two elements are not transferred to the final GMO.

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

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

5. If the answer to question B.3(a) and (b) is no, what was the method used in the process of modification?

(i) transformation (.)
(ii) microinjection (.)
(iii) microencapsulation (.)
(iv) macroinjection (.)
(v) other, specify …

6. Composition of the insert

(a) Composition of the insert
The expression cassette consists of a modified liver-specific promoter and enhancer, gene encoding human FIX and a polyadenylation signal, flanked by AAV ITRs.

(b) Source of each constituent part of the insert
- Liver-specific promoter and enhancer: *Homo sapiens.*
- Gene encoding factor IX expression cassette: *Homo sapiens*
- Polyadenylation signal: *Bos taurus*
- ITRs: AAV2

(c) Intended function of each constituent part of the insert in the GMO
- Liver-specific promoter and enhancer: Intended to drive liver-specific gene expression.
- FIX-R338L variant of the human factor IX (hFIX) gene: Gene transfer may be effective for the treatment of patients with Haemophilia B, given that the disease is caused by mutations within this gene that affect the expression or activity of the hFIX.
- Polyadenylation signal: terminate transcription of FIX gene.
- AAV ITRs: Inverted Terminal Repeat (ITR) sequences required for second strand DNA synthesis required for gene expression.

(f) Location of the insert in the host organism

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

(g) 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 (.)

(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 (.)

If yes, specify
The fidanacogene elaparvovec viral genome has been significantly modified compared to the original parental wildtype virus (Rh74) 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, fidanacogene elaparvovec contains no native viral coding genes.
Wildtype AAV requires the presence of a helper virus such as human adenovirus or herpes simplex virus to replicate. Fidanacogene elaparvovec replication could only occur in the unlikely event of a host cell being co-infected simultaneously by wtAAV 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 (.)

If yes, specify
As fidanacogene elaparvovec replication could only occur in the extremely unlikely event of a host cell being infected by two separate viruses, a wtAAV and a helper virus, the likelihood of dissemination is lower than that of wtAAV.

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

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

Additional Information:

No pathogenic effects of wtAAV or AAVSpark100 in humans are known. The introduction of the expression cassette, coding for FIX-R338L, is not expected to result in development of pathogenicity. Thus, neither the wtAAV nor fidanacogene elaparvovec are known or expected to be pathogenic. Removal of viral genes in making the vector would be expected to further reduce any risk of pathogenesis.

2. Genetic stability of the genetically modified organism

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

3. Is the GMO significantly pathogenic or harmful in any way (including its extracellular products), either living or dead?

Yes (.) No (X) Unknown (.)

(a) to which of the following organisms?

humans (.)
animals (.)
plants (.)
other …

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

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

Clinical studies using recombinant AAV vectors have been performed worldwide in more than 3000 patients (as of 2021/ Kuzmin et al 2021). The data generated suggests that the safety risks associated with AAV gene transfer are negligible. Immune responses to AAV, but not hFIX, have been observed and can be reduced or eliminated by (a) enrolling subjects with low anti-AAV nAbs and (b) administering immunosuppressor drugs if a T-cell response to AAV develops.

4. Description of identification and detection methods

(a) Techniques used to detect the GMO in the environment
Fidanacogene elaparvovec can be detected by qPCR.

(b) Techniques used to identify the GMO
Fidanacogene elaparvovec can be detected by qPCR or 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 fidanacogene elaparvovec in subjects with hemophilia B.

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

3. Information concerning the release and the surrounding area

(a) Geographical location (administrative region and where appropriate grid reference):
Cliniques Universitaires Saint-Luc, Haemostatis and Thrombosis Unit,
Hippokrateslaan 10, Batiment 54, 1200 Sint-Lambrechts-Woluwe, 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 Fidanacogene elaparvovec 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 fidanacogene elaparvovec 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. Fidanacogene elaparvovec 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 fidanacogene elaparvovec 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:

In Study C0371002, fidanacogene elaparvovec is administered as a single intravenous infusion at the dose of 5.0E11 vector genomes/kg body weight. Approximately 50 patients overall and 2 patients in Belgium are foreseen.

(b) Duration of the operation:

The duration of the study is defined for each subject as the date signed written informed consent is provided over 6 years. The study duration post-infusion for each patient in this study will be 312 weeks (i.e., 6 years).

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

Fidanacogene elaparvovec will be shipped to study sites in line with standard recommendations for the transport of biohazardous materials. Fidanacogene elaparvovec 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 C0371002. Consumables used in the preparation and administration of the GMO will be disposed as biohazard waste according to the procedures of the hospital sites.

Fidanacogene elaparvovec 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. In addition, the Sponsor will provide instructions on the biosafety requirements according to the GMO risk. A Pharmacy Manual and training material located at sites provides pharmacy personnel and clinical medical staff directions on use, storage and destruction of the IMP. It also includes directions for documenting the control of the IMP from the time of receipt at the trial site until final accountability and destruction. In addition, it describes the required processes for managing and documenting any issues, such as shipment or storage temperature excursions and reporting of technical product complaints. The risks related to the release into the environment of the GMO or risks to personnel in the event there is a breach in container integrity and/or storage or accidental spillage at the site or during shipping/storage, is considered to be negligible. The GMO will only be handled by delegated, trained personnel and in the event that a spillage did occur, the product is non-pathogenic and non-replicative, limiting spread and risks to the environment or personnel.

Recombinant AAV vectors are non-replicative and are not expected to pose a risk of transmission. Sexually active participants are expected to remain compliant with barrier contraceptive at least until 3 consecutive ejaculate samples containing sperm test negative for vector shedding.

Additionally, viral vector shedding will be assessed in this study. This will indicate when vector shedding has ceased. As fidanacogene elaparvovec 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 fidanacogene elaparvovec 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.
Fidanacogene elaparvovec has been well tolerated and no significant safety signals have emerged. Fidanacogene elaparvovec has demonstrated sustained efficacy in terms of FIX activity with associated decreases in bleeding episodes and FIX consumption and replacement infusions relative to prior to vector infusion. No emergence of FIX inhibitors or thrombosis or evidence of environmental impact was identified. The level of shedding in excreted fluids is low and was generally at the highest level in the first-time point sampled. Generally, PBMC took the longest time to clear (George et al., 2017).

## 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)
Fidanacogene elaparvovec contains a gene encoding a human factor IX protein variant, which has higher specific activity than wtFIX. Expression is driven by both a liver-specific enhancer and promoter encapsidated within a modified capsid derived from a naturally occurring AAV serotype, having strong tropism for the liver, which transduces the liver highly efficiently when administered intravenously.

It is expected that administration of fidanacogene elaparvovec will result in the expression of the FIX transgene and raise circulating levels of endogenous FIX in study subjects.

Gene transfer of the human factor FIX-Padua variant of the FIX gene, may be effective for the treatment of patients with Hemophilia B, given that the disease is caused by mutations within this gene that affect the expression or activity of the FIX.

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 fidanacogene elaparvovec that could represent potential hazard. Minimal exposure, such as environmental exposure, to organisms, human or other, other than the subjects receiving fidanacogene elaparvovec as part of the study would not be of sufficient dose to cause significant gene expression or potential safety risks. As fidanacogene elaparvovec 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 in the targeted population of human patients, exposure to fidanacogene elaparvovec 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 fidanacogene elaparvovec is unable to replicate, post-release selection cannot occur. Furthermore, the modifications introduced into fidanacogene elaparvovec, compared to wildtype AAV, have reduced the evolutionary competitiveness of the recombinant AAV.

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

As fidanacogene elaparvovec 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. Fidanacogene elaparvovec will be rapidly degraded when exposed to the natural environment.

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 fidanacogene elaparvovec vector genome will be transferred into hepatocytes within the patient's liver. The vast majority of fidanacogene elaparvovec vector genomes within subject cells are expected to be episomal, rather than integrated into the host cell DNA. As fidanacogene elaparvovec 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 highly unlikely.

(b) from other organisms to the GMO:

The probability of homologous recombination with related viruses that could lead to variants of the GMO is strongly reduced, with the ITRs being the only viral sequences remaining in the vector, making up only ~6% 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 fidanacogene elaparvovec genome.

(c) likely consequences of gene transfer:
While recombination between fidanacogene elaparvovec and a wildtype 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 hFIX expression cassette would be predicted to be in excess of this limit. The risks associated with gene transfer from wildtype AAV to fidanacogene elaparvovec 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.):
Related GMOs have been assessed in the context of a potential release into the sewage system. It was shown that rAAV are degraded within 24h to ~99% (Fleischmann, 2023).

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

2. Methods for monitoring ecosystem effects
The presence of fidanacogene elaparvovec in bodily fluids following administration of fidanacogene elaparvovec will be determined by qPCR.

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 assessing factor IX activity using appropriate clinical read-outs.

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
Vector shedding will be assessed until negative or below limit of quantification on at least three consecutive occasions. Safety and efficacy assessments will be conducted throughout the duration of the study.

6. Frequency of the monitoring
Vector shedding will be assessed prior fidanacogene elaparvovec infusion and at all visits after receiving fidanacogene elaparvovec until negative or below limit of quantification on at

least three consecutive occasions. Safety and efficacy assessments will be conducted throughout the duration.

## I. Information on post-release and waste treatment

1. Post-release treatment of the site

Any surfaces contaminated with fidanacogene elaparvovec will be disinfected decontaminated using an appropriate disinfectant such as 10% chlorine bleach, or other detergent-based disinfectant. This process should be discussed with the local environmental health and safety officer and/or biosafety committee before receipt of any fidanacogene elaparvovec 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 should be discarded at the site following requirements for biohazardous waste. Consumables used in the preparation and administration of the GMO that may have come into contact with fidanacogene elaparvovec will be decontaminated prior to disposal as biohazardous waste. Liquid waste will be decontaminated using an appropriate chemical disinfectant or autoclaved. Disinfectants that are effective against AAV include 10% chlorine bleach, or other detergent-based disinfectant.

3. (a) Type and amount of waste generated

- Emtpy/partly empty vials containing fidanacogene elaparvovec residue. The number of vials of fidanacogene elaparvovec required per patient is dependent on the dose cohort and 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

See 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

The IP manual will be provided to staff at the site, for the management and disposal of fidanacogene elaparvovec, which should be followed by all personnel responsible for transporting, preparing, administering, disposing of fidanacogene elaparvovec medicinal product or equipment/consumables that have come into contact with the product designated for use in clinical study, that must be disposed of as biohazardous waste.

**Table 1** summarises the procedures that will be used by staff to manage incidents related to fidanacogene elaparvovec.

**Table 1: Management of incidents related to fidanacogene elaparvovec:**

| Incident | Procedure |
|---|---|
| Accidental spillage | All spills of fidanacogene elaparvovec must be wiped with absorbent gauze pad and the spill area must be disinfected using a bleach solution followed by alcohol wipes. All clean up materials must be double bagged and disposed of per local guidelines for handling of biohazardous waste. |
| Sharps injury | The use of needles is to be kept to a minimum. In the event of injury, 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. |

Fidanacogene elaparvovec is stored in vials. Staff will be advised that care must be taken when manipulating vials and that the use of needles should be kept to a minimum. In the event of injury, staff will follow local institutional procedures as well as those listed in **Table 1**.

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

All spills of fidanacogene elaparvovec must be wiped with absorbent gauze pad and the spill area must be disinfected using a bleach solution followed by alcohol wipes. All clean up materials must be double bagged and disposed of per local guidelines for handling of biohazardous waste.

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

Administration of fidanacogene elaparvovec 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, fidanacogene elaparvovec is not capable of infecting plants or microbes.

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

The material that has been in contact with the GMO must be disposed of as biohazardous waste.

Furthermore, safety recommendations and guidance on the management of incidents related to fidanacogene elaparvovec 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 independent data monitoring committee (iDMC) will be responsible for monitoring safety data from the study.

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