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	The Netherlands
(b)	Notification number	B/NL/18/003
(c)	Date of acknowledgement of notification	15/05/2018
(d)	Title of the project	”A Phase 1/2, Open-Label Safety and Dose-Finding Study of Adeno-Associated Virus (AAV) Serotype 8 (AAV8)-Mediated Gene Transfer of Glucose-6-Phosphatase (G6Pase) in Adults with Glycogen Storage Disease Type Ia (GSDIa)”. Protocol Number 401GSDIA01.
(e)	Proposed period of release	October 2018 – December 2019

2. Notifier

Name of institution or company:	Ultragenyx Pharmaceutical, Inc. 840 Memorial Drive Cambridge, MA 02139 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	

other, specify (kingdom, phylum and class)

(b) Identity of the GMO (genus and species)

Genus: Dependoparvovirus
Species: Adeno-associated virus serotype 8 (AAV8)

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

AAV is a single stranded DNA virus that demonstrates a high degree of genetic stability as evidenced by the close relationship of the rep and cap genes from multiple AAV serotypes and genomovars. In support of these sequence homology data is the fact that AAV uses a host DNA polymerase for viral replication which is not error prone when compared 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 sequence.

Based on these characteristics of wild-type AAV, DTX401 is also expected to be highly genetically stable. The DTX401 vector genome sequence is verified by direct sequencing.

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) ES

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 USA, Canada
- Notification number: NIH Protocol No. 1706-1617 (USA); NSN No. 19426 (Canada).

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

DTX401, the study product, is AAV8G6PC; a non-replicating recombinant adeno-associated virus vector serotype 8 (AAV8) encoding human glucose-6-phosphatase-α (G6Pase or G6PC) for the treatment of patients with GSDIa.

The release of DTX401 as described in this application is not expected to result in adverse environmental impact, for the following reasons:

- Lack of pathogenicity of the parental virus: Despite an estimated seroprevalence of up to 90% for some common human serotypes, no pathogenic effects of AAV have been identified.

- Replication-incompetent GMO: DTX401 is a non-infectious recombinant AAV vector that lacks all AAV viral genes and cannot replicate without AAV-specific helper functions and helper virus activities. DTX401 replication could only occur in the extremely unlikely event of a host cell being infected by three separate viruses (DTX401, wild-type AAV and a helper virus such as human adenovirus or herpes simplex virus). This risk of this occurring is negligible.

- Minimal risk of transmission by viral shedding: AAV vector DNA has been shown to be shed in the saliva, urine, and stool of nonhuman primates following systemic administration. The extent and duration of shedding of DTX401 will be monitored as part of the proposed clinical trial. However, as DTX401 is non-replicative, shed viral particles are unable to multiply and their spread is thus inherently limited. Furthermore, potential hazards of exposure to DTX401 to humans are predicated upon systemic administration of DTX401. Minimal exposure, such as environmental exposure, to persons other than the subjects receiving DTX401 as part of the study would not be of sufficient dose to represent significant gene expression nor safety levels in humans. The viral load in urine and stool is expected to be low. Other than potential human hosts, exposure to DTX401 is not expected to affect any non-target organisms, either directly or indirectly. The risk to humans and the environment associated with viral shedding of DTX401 is thus low to negligible.

- Minimal risk of insertional mutagenesis: The risks of insertional mutagenesis are considered to be low to negligible, as the vast majority of rAAV vector DNA persists as episomal (≥99.5%) rather than as integrated DNA.

- Liver-specific transgene expression: Adeno-associated virus serotype 8, as a clade E AAV, has strong tropism for the liver, and transduces the liver highly efficiently when administered intravenously. DTX401 is an AAV8 vector that encapsidates the G6PC gene with expression driven by the native human G6Pase promoter/enhancer (GPE), which has almost exclusive activity in the liver. Transgene expression in cells other than human hepatocytes is thus considered to be unlikely.

- Minimal risk associated with the transgene: The G6PC gene encodes for the human G6Pase (glucose-6-phosphatase). No genes for toxins, potential oncogenes, growth factors or other genes that could be potentially harmful have been inserted into the GMO.

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

1. Recipient or parental organism characterisation:

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

(select one only)

viroid	(.)
RNA virus	(.)
DNA virus	(X)
bacterium	(.)
fungus	(.)
animal	
- mammals	(.)
- insect	(.)
- fish	(.)
- other animal	(.)

(specify phylum, class)

other, specify

2. Name

(i)	order and/or higher taxon (for animals)	ssDNA virus
(ii)	genus	Dependoparvovirus
(iii)	species	Adeno-associated virus
(iv)	subspecies	N/A
(v)	strain	serotype 8
(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
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	In association with animals (primate hosts)

(b) If the organism is an animal: natural habitat or usual agroecosystem: Not applicable.

5. (a) Detection techniques

AAV can be detected by quantitative polymerase chain reaction (qPCR) using primers specific for the viral genome.

(b) Identification techniques

AAV can be identified by quantitative polymerase chain reaction (qPCR) using primers specific for the viral genome.

6. Is the recipient organism classified under existing Community rules relating to the protection of human health and/or the environment?

Yes (.) No (X)

If yes, specify

Wild-type AAV is 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 90%. Consequently, AAV fulfils the definition of a group 1 biological agent according to the Directive 2000/54/EC (a biological agent that is unlikely to cause human disease).

7. Is the recipient organism significantly pathogenic or harmful in any other way (including its extracellular products), either living or dead?

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

If yes:

(a) to which of the following organisms:

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

(b) give the relevant information specified under Annex III A, point II. (A)(11)(d) of Directive 2001/18/EC

8. Information concerning reproduction

(a) Generation time in natural ecosystems:

AAV is replication defective, thus the generation time is variable depending on the presence or absence of a helper virus.

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

AAV is replication defective, thus the generation time is variable depending on the presence or absence of a helper virus.

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

(d) Factors affecting reproduction:

The presence of a helper virus, such as adenovirus or herpes simplex virus, promotes AAV gene expression, genome replication and production of virions. In absence of a helper virus, wild-type AAV is replication-incompetent. Please note that the final GMO, DTX401, is replication-incompetent even in the presence of a helper virus due to the removal of the viral rep and cap genes.

9. Survivability

(a) ability to form structures enhancing survival or dormancy:

(i)	endospores	(.)
(ii)	cysts	(.)
(iii)	sclerotia	(.)
(iv)	asexual spores (fungi)	(.)
(v)	sexual spores (funghi)	(.)
(vi)	eggs	(.)
(vii)	pupae	(.)
(viii)	larvae	(.)
(ix)	other, specify	AAV does not form survival structures.

(b) relevant factors affecting survivability:

Parvoviruses such as AAV are stable viruses that can persist in the environment for extended periods of time (thought to be on the order of several weeks). AAV particles are resistant to a wide range of pH (pH 3-9) and can resist elevated temperatures (55°C for 1 hour). AAV does not form survival structures. However, as with all viruses, replication of AAV cannot occur outside of a host cell.

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).

The sponsor, Ultragenyx Pharmaceutical, Inc., (formerly Dimension Therapeutics, Inc.) has not notified any previous genetic modifications of the parental virus (AAV8) for release in the Netherlands.

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 containing a human G6PC expression cassette for the treatment of patients with Glycogen Storage Disease Type Ia. DTX401 is an AAV8 vector that encapsidates the G6PC gene with expression driven by GPE. AAV8, as a clade E AAV, has strong tropism for the liver, and transduces the liver highly efficiently when administered intravenously. It is thus expected that administration of DTX401 will result in the expression of the G6PC gene in the liver 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
pDTX.hG6PCco.401

(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

pDTX.hG6PCco.401 contains the G6PC expression cassette. The expression cassette consists of a liver-specific promoter and enhancer, a codon-optimized G6PC transgene and a polyadenylation signal, flanked by AAV inverted terminal repeats (ITRs). Only the G6PC expression cassette is present in the final GMO. In addition, the vector contains a bacterial origin of replication and the gene for kanamycin resistance to allow for propagation of the plasmid in *E.coli*.

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

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

Triple transfection of packaging cells with pDTX.hG6PCco.401 and two helper plasmids, resulting in production of recombinant AAV 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 insert consists of a liver-specific promoter and enhancer, a codon-optimized G6PC transgene and a polyadenylation signal, flanked by AAV inverted terminal repeats (ITRs).

(c) Source of each constituent part of the insert

- Liver-specific promoter and enhancer: *homo sapiens.*
- G6PC gene: *homo sapiens.*
- Polyadenylation signal: SV40.
- ITRs: AAV.

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

- Liver-specific promoter and enhancer: Intended to drive liver specific G6PC gene expression.
- G6PC gene: Gene transfer of G6PC is expected to be effective for the treatment of Glycogen Storage Disease Type Ia, given that the disease is caused by mutations within this gene that affect the expression or activity of the G6Pase enzyme.
- Polyadenylation signal: Intended to provide cis sequences for efficient polyadenylation of the G6PC mRNA. This element functions as a signal for a specific cleavage event at the 3' end of the nascent transcript and addition of a long polyadenyl tail.
- ITRs: Required for vector genome replication and packaging.

(e) Location of the insert in the host organism

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

(f) Does the insert contain parts whose product or function are not known?
Yes (.) No (X)
If yes, specify

D. Information on the organism(s) from which the insert is derived

1. Indicate whether it is a:

viroid (.)
RNA virus (.)
DNA virus (.)
bacterium (.)
fungus (.)

animal

- mammals (X)
- insect (.)
- fish (.)
- other animal (.)

(specify phylum, class)

other, specify

2. Complete name

(i)	order and/or higher taxon (for animals)	Primates
(ii)	family name for plants	N/A
(iii)	genus	*homo*
(iv)	species	*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):
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

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

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

E. Information relating to the genetically modified organism

1. Genetic traits and phenotypic characteristics of the recipient or parental organism which have been changed as a result of the genetic modification

(a) is the GMO different from the recipient as far as survivability is concerned?
Yes (.) No (X) Not known (.)
Specify

(b) is the GMO in any way different from the recipient as far as mode and/or rate of reproduction is concerned?
Yes (X) No (.) Unknown (.)
Specify

The DTX401 viral genome has been significantly modified compared to the parental virus in order to render it replication incompetent. The AAV rep and cap genes have been replaced with a eukaryotic expression cassette, and only the viral ITR sequences, which are non-coding DNA sequences (< 300 bp), have been retained. Thus, DTX401 contains no native viral genes.

Wild-type AAV requires the presence of a helper virus such as human adenovirus or herpes simplex virus to replicate. DTX401 replication would require the presence of wild-type AAV in addition to the presence of a helper virus. The likelihood of this occurring is extremely low.

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

As DTX401 replication could only occur in the extremely unlikely event of a host cell being infected by three separate viruses, the likelihood of dissemination is lower than that of wild-type AAV.

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

No pathogenic effects of wild-type AAV in humans are known. The introduction of the G6PCexpression cassette is not expected to result in development of pathogenicity. Thus, neither the wild-type AAV nor DTX401 are known or expected to be pathogenic.

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, DTX401 is also expected to be genetically stable. The integrity of the G6PC expression cassette will be confirmed by direct sequencing.

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)

4. Description of identification and detection methods

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

DTX401 can be detected by quantitative polymerase chain reaction (qPCR).

(b) Techniques used to identify the GMO

DTX401 can be identified by quantitative polymerase chain reaction (qPCR).

F. Information relating to the release

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

Study 401GSDIA01 is a Phase 1/2, open-label, single arm, multicenter, safety and dose-finding study of DTX401 in adults with GSDIa disease. The primary objective of the study is to determine the safety of single IV doses of DTX401. The secondary objective of the study is to identify the optimal biological dose (OBD) of DTX401.

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

The natural habitat of wild-type AAV8 is primate host cells. DTX401 will be administered to humans in the context of clinical study 401GSDIA01.

3. Information concerning the release and the surrounding area

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

Site 1: University Medical Center Groningen
Hanzeplein 1
9713 GZ Groningen, the Netherlands

(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 DTX401 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 DTX401 will be administered to patients as part of a clinical trial.

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

Not applicable. DTX401 will be administered by a one-time IV infusion in a hospital setting. It is thus not anticipated it will come into contact with any recognized biotopes or protected areas.

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

Administration of DTX401 will occur only within a controlled hospital setting; therefore, it is not anticipated that it will come into contact with plants, animals or soil.

4. Method and amount of release

(a) Quantities of GMOs to be released:

Dosing is based on the patient's body weight. It is estimated that a total amount of approximately 10^{14} – 10^{15} GC of DTX401 may be administered to patients in The Netherlands.

(b) Duration of the operation:

The duration of the study is defined for each subject as the date signed written informed consent is provided through the visit at Week 52.

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

DTX401 will be stored, prepared and administered by trained medical professionals, in a hospital setting only to patients that meet criteria for inclusion into clinical study, 401GSDIA01. Staff will follow the waste and disposal policies as per local site requirement to dispose of consumables used in the preparation and administration of the GMO. Where permitted, used and unused vials of DTX401 will be retained at the study site until study drug accountability has been performed by the Clinical Research

Associate (CRA). If destruction is not permitted onsite, unused vials will be returned to the manufacturing facility that released the product as per standard requirements for the IMP and as specified in the Pharmacy Manual.

DTX401 is an Investigational Medicinal Product (IMP) manufactured and released by a Qualified Person (QP) in Europe 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. The study 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 or return. 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. However, to prevent potential exposure to sexual partners, patients in clinical study 401GSDIA01 are required to use barrier contraceptives for a period of 52 weeks following administration of DTX401 in the clinical trial (see section 4.1 of the study protocol).

Patients will receive DTX401 by a one-time IV infusion in a clinical setting and only be discharged 24 hours after administration of DTX401, thus limiting the likelihood of family member exposure. Additionally, viral vector shedding will be assessed in this study. This will indicate when vector shedding in urine, saliva and stool has ceased. As DTX401 is non-replicative, shed viral particles are unable to multiply; the spread of the GMO is thus inherently limited.

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

Not applicable. Administration of DTX401 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.

DTX401 has not previously been released into the environment.

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	*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)

   DTX401 encodes the G6PC gene with expression driven by the liver-specific GPE, encapsidated within an AAV8 vector. AAV8, as a clade E AAV, has strong tropism for the liver, and transduces the liver highly efficiently when administered intravenously. It is thus expected that administration of DTX401 will result in the expression of the G6PCgene in the liver of study subjects. Gene transfer of G6PC is expected to be effective for the treatment of Glycogen Storage Disease Type Ia, given that the disease is caused by mutations within this gene that affect the expression of G6Pase.

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 DTX401 that could represent potential hazard. Potential hazards of exposure to DTX401 are predicated upon systemic administration of DTX401. Minimal exposure, such as environmental exposure, to organisms other than the subjects receiving DTX401 as part of the study would not be of sufficient dose to represent significant gene expression or potential safety risks in humans. As DTX401 is also replication-incompetent, it is expected that the vector would be rapidly cleared from any non-target organisms without causing any harmful effects. Furthermore, transgene expression is designed to only occur in hepatocytes. Other than potential human hosts, exposure to DTX401 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 DTX401 is unable to replicate, post-release selection cannot occur.

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

   As DTX401 is unable to replicate, it is not expected to spread to the environment to a significant degree, and it is not expected to become established in any ecosystems.

6. Complete name of non-target organisms which (taking into account the nature of the receiving environment) may be unintentionally significantly harmed by the release of the GMO

(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 DTX401 viral genome will be transferred into hepatocytes within the liver of patients enrolled in study 401GSDIA01. The vast majority of DTX401 vector genomes within subject cells are expected to be episomal, rather than integrated into the host cell DNA. As DTX401 is non-replicative, and is only expected to be shed in study subjects' bodily fluids to a limited extent, transmission and gene transfer to organisms other than the study subjects is considered unlikely.

(b) from other organisms to the GMO:

The elimination of 94% of the viral DNA reduces the probability of homologous recombination with related viruses that could lead to variants of the GMO.

(c) likely consequences of gene transfer:

While recombination between DTX401 and a wild-type AAV to generate a hybrid vector genome that contains both the G6PC expression cassette 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. It is known that AAV possesses a packaging limit of approximately 5 kb (Wu 2010), and a hybrid molecule of rep-cap genes plus the G6PC expression cassette would be predicted to be in excess of this limit. The risks associated with gene transfer from wild-type AAV to DTX401 are thus considered to be negligible.

8. Give references to relevant results (if available) from studies of the behaviour and characteristics of the GMO and its ecological impact carried out in stimulated natural environments (e.g. microcosms, etc.):

No such studies have been conducted with DTX401.

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

DTX401 is not known or predicted to have an impact on biogeochemical processes.

H. Information relating to monitoring

1. Methods for monitoring the GMOs

Viral shedding will be closely monitored in study 401GSDIA01. Other methods to monitor the effects of DTX401 include both safety and efficacy assessments.

2. Methods for monitoring ecosystem effects

The presence of DTX401 in bodily fluids following administration of DTX401 will be determined by quantitative polymerase chain reaction (qPCR).

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

Transfer of the G6PC expression cassette to study subjects will be detected by assessing G6Pase 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 viral shedding in patients' bodily fluids.

5. Duration of the monitoring

Viral shedding will be assessed until at least 3 consecutive negative results are obtained for each sample matrix. Safety and efficacy assessments will be conducted throughout the duration of the study as described in the study protocol.

6. Frequency of the monitoring

Viral shedding will be assessed until at least 3 consecutive negative results are obtained for each sample matrix. Safety and efficacy assessments will be conducted throughout the duration of the study as described in the study protocol.

I. Information on post-release and waste treatment

1. Post-release treatment of the site

Any surfaces contaminated with DTX401 will be disinfected in accordance with local laws and institutional procedures related to the management of biohazardous substances and using a disinfectant effective against AAV (e.g. 1% sodium hypochlorite, 2% glutaraldehyde, 0.25% sodium dodecyl sulphate).

2. Post-release treatment of the GMOs

All disposable materials that come into contact with the investigational product will be disposed of according to individual institutional practices and policies for the disposal and decontamination of biohazardous waste. In general, disposable materials will be disposed of in sharps containers or biohazard bags and decontaminated by autoclaving or incineration, or both. Non-disposable materials will be decontaminated according to institutional practices and procedures, e.g. by treatment with an appropriate disinfectant and/or autoclaving.

Where permitted, used and unused vials of DTX401 will be retained at the study site until study drug accountability has been performed by the CRA. If destruction is not permitted onsite, unused vials will be returned to the manufacturing facility that released the product as per standard requirements for the IMP and as specified in the Pharmacy Manual.

3. (a) Type and amount of waste generated

The following types of waste are anticipated:

- Glass vials containing DTX401. The number of vials of DTX401 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

All disposable materials that come into contact with the investigational medicinal product will be disposed of according to individual institutional practices and policies. For example, materials are disposed of in sharps containers or biohazard bags and decontaminated by autoclaving or incineration, or both. Liquid waste will be decontaminated and discarded as per institutional practice

J. Information on emergency response plans

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

In the event that the contents of the DTX401 vial/s or diluted product for infusion are accidentally released and come in contact with shipping materials, pharmacy/ hospital surfaces, the spillage will be decontaminated and removed according to institutional practice.

DTX401 is stored in glass 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.

In case of accidental contact of DTX401 with skin, eyes or clothing, staff will follow institutional procedures for the management of biohazardous material.

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

Any surface area exposed to the GMO will be disinfected using appropriate disinfectant as per local laws and institutional policies and procedures.

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

Administration of DTX401 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, DTX401 is not capable of infecting plants or microbes.

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

Staff will follow local law and institutional procedures for the handling and disposal of genetically modified organisms. Furthermore, safety recommendations and guidance on the management of incidents related to DTX401 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 (DMC) will be responsible for monitoring safety data from the study. The DMC may, at any time, recommend modifying or stopping the study early due to safety concerns based on review of the data.