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	SPAIN
(b)	Notification number	B/ES/21/16
(c)	Date of acknowledgement of notification	09/Jun/2021
(d)	Title of the project	A Randomized, Double-Blind, Placebo-Controlled, Multicenter, Seamless, Adaptive, Safety, Dose-Finding, and Phase 3 Clinical Study of UX701 AAV-Mediated Gene Transfer for the Treatment of Wilson Disease

Proposed period of release:
To start up the study on Mar 2022 to May 2026, depending on timepoint of patient recruitment

2. Notifier

Ultragenyx Pharmaceutical Inc.
60 Leveroni Ct ,
Novato CA94949
USA

Name of institution or company:

Ultragenyx Pharmaceutical Inc.
60 Leveroni Ct
Novato CA94949
USA

3. GMO characterisation

(a) Indicate whether the GMO is a:

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

animal

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

specify phylum, class …

(b) Identity of the GMO (genus and species)
Genus: Dependoparvovirus
Species: Recombinant adeno-associated viral vector derived from naturally occurring AAV9 serotype

(c) Genetic stability – according to Annex IIIa, II, A(10)
AAV is a single-stranded DNA virus that demonstrates a high degree of genetic stability as evidenced by the high degree of sequence conservation of the rep and cap genes from multiple AAV serotypes. Sequence homologies often are >90% and >80% for the rep and cap genes, respectively. Furthermore, AAV uses host DNA polymerases for viral replication, which are characterised by high fidelity DNA polymerization and additional proofreading exonuclease activity facilitating a very low error rate of DNA replication, when compared, for example, to RNA polymerases used by RNA viruses. Genetic stability is supported by 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 the assertion that only under the presumably rare circumstance where a single 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.

UX701 is expected to be highly genetically stable. Production of the vector in the manufacturing process and second strand synthesis of the vector genome rely on the host DNA polymerase δ, leading to very low error rate of DNA replication. The UX701 vector genome will be assayed by ATPB specific ddPCR before release. Exemplary batches will also be sequenced to confirm the absence of any changes.

4. Is the same GMO release planned elsewhere in the Community (in conformity with Article 6(1)), by the same notifier?
Yes (X) No (.)
If yes, insert the country code(s) AT; BE; DE; DK; FI; FR; IT; PT.

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; 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 (.) No (X)

If yes:
- Member State of notification
- Notification number

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

UX701 is a non-replicating, recombinant adeno-associated virus (AAV) serotype 9 (AAV9) gene transfer vector that contains the human ATP7B coding sequence modified to contain only the last 3 of the 6 metal-binding domains (MBDs). Expression is driven by a liver-specific enhancer element and a liver-specific promoter. The mechanism of action of UX701 is to deliver the transgene (ATP7B-MBD456) to hepatocytes where it is translated into a functional ATP7B protein, thereby enabling restoration of normal copper metabolism. Following transduction, UX701 vector genomes mainly persist as episomal concatemers. UX701 may be effective for the treatment of patients with Wilson disease.

The release of UX701 as described in this application is not expected to result in adverse environmental impact, including the human patient population, for the following reasons:

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

2. Replication-incompetent GMO: UX701 is a non-pathogenic recombinant AAV vector that lacks all AAV viral genes and cannot replicate without AAV-specific helper functions and helper virus activities. UX701 replication could only occur in the extremely unlikely event of a transduced host cell being co-infected by wild-type AAV and a helper virus such as human adenovirus or herpes simplex virus. If replication occurred, the only expected products would be UX701 and WT AAV, both intrinsically non-pathogenic viruses.

3. Minimal risk of transmission by viral shedding: UX701 is replication-incompetent and is not expected to survive, multiply or disperse if it were to be eliminated intact from the treated patient. AAV-based gene therapies are known to shed via bodily fluids. It has been shown consistently that vectors are shed for a short period of time, but then become undetectable in bodily fluids. The viral load shed in bodily fluids is expected to be low, compared to the necessary dose required to achieve detectable gene expression in humans.

4. Vector shedding in saliva, urine, and stool will be assessed during the UX701-CL301 clinical study. vector shedding will be assessed for a maximum of 2 years or until 3 consecutive negative readings (at or below the limit of detection of the assay) for an individual are obtained for a given sample type (saliva, urine and stool).

Clinical studies with other AAV gene therapies have shown that transient vector shedding occurs following IV administration of recombinant AAV vectors; however, risks associated with vector shedding are expected to be low. Wild-type AAV has no known associated pathology and cannot replicate without a helper virus. UX701 is a non-replicating, recombinant AAV9 vector with high liver tropism and a liver-specific promoter, from which the native AAV genes required for replication have been removed and replaced with a transgene cassette. The UX701 product release process includes testing to confirm that UX701 does not include replication competent AAV9 particles.

Importantly, the vector shedding assay detects genome copies whether or not they are encapsidated in viral particles, and it is not known if the vector DNA in the biological matrix represents infectious material. It is not expected that exposure to this amount of vector even if it was infectious could result in any biologically significant level of transduction, particularly via transient contact, or immune response.

Minimal exposure, such as environmental exposure, of persons other than study participants would not be of sufficient dose to result in significant gene expression in humans. Other than potential human hosts, exposure to UX701 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 UX701 is thus negligible.

5. Minimal risk of insertional mutagenesis: Data from mice, dogs, non-human primates and humans suggest that the integration of AAV vectors into the host genome is a rare event, with most of the vector assimilating into concatemeric episomes. Unlike retroviral vectors, which encode viral proteins to create double-stranded breaks, when AAV integration does occur, it does so at pre-existing chromosomal breaks. The results of integration are deletions in the AAV ITRs and duplications of host sequences. In animal studies and human samples, no clear correlation has been established between recombinant AAV genomic integration and the development of HCC. Additionally, UX701 transgene protein (ATP7B-MBD456) is not a growth factor that can promote tumor progression, and it is not involved in biological pathways that can initiate tumorigenesis, such as oncogenes.

6. Tissue-specific transgene expression: UX701 shows a strong tropism for liver following IV administration. UX701 transgene expression is driven by a liver-specific promoter. As observed in the mouse GLP toxicity study, transduction of non-liver cells results in no expression or low levels of expression that decreases over time.

7. Minimal risk associated with the transgene: The viral vector does not contain any viral sequences, except ITRs, which facilitate transgene expression and do not lead to production of viral proteins, particles or DNA replication. A GLP toxicity and biodistribution study (single-dose in mice at low, mid and high dose) failed to demonstrate any adverse effect of UX701 at the proposed clinical dose range. The protein encoded by the transgene is a shortened version of a naturally occurring protein and is therefore unlikely to be toxic to humans or other organisms. No genes for toxins, potential oncogenes, growth factors or other genes that could be potentially harmful have been inserted into the GMO. With administration of UX701 to humans, the only non-human foreign proteins that the immune system will be exposed to are the AAV capsid proteins.

8. Minimal risk associated to immune responses in patients: Patients will receive corticosteroids in order to minimize the immune response to the viral capsid proteins.
The murine GLP toxicity study findings associated with UX701 administration were consistent with immune responses expected following administration of a gene therapy product or exposure of mice to a foreign protein, and included development of anti-AAV9 and anti-ATP7B antibodies, mild increases in some cytokines, and gross and microscopic findings in spleen. The minor transient hematology changes and the gross and microscopic findings in spleen on Day 4 recovered by Day 29 and were not considered to be adverse. Development of anti-ATP7B antibodies was not considered adverse based on the lack of observed toxicity and sustained transgene mRNA levels and did not affect the interpretability of the safety data from the GLP toxicity and biodistribution study. Animals would be expected to mount an immune response to a foreign (human) protein, and this is not necessarily predictive of responses in humans. However, there is a low potential risk of anti-ATP7B antibody development and subsequent loss of hepatocytes and decline in transgene expression following UX701 administration.
Patients will be monitored closely, particularly in the first few weeks after treatment, when the risk of an immune response is greatest.

9. T-cell response: T cell mediated response and transient rise in liver aminotransferase, the most common product-related AE observed in clinical studies with AAV-mediated gene transfer has been an asymptomatic transient rise in liver transaminases and concurrent decline in transgene expression approximately 7 to 10 weeks following vector administration (Manno 2006, Nathwani 2011a, Nathwani 2014). In all cases, the transient rise in liver transaminases resolved without clinical sequelae. It has been hypothesized that this vector-induced viral hepatitis is due to the activation of capsid specific cytotoxic T lymphocytes (CTLs) and destruction of transduced liver cells (Mingozzi 2007). However, in mice, T-cells activated against AAV capsid were not able to target and eliminate transduced hepatocytes (Wang 2007, Li 2007, Siders 2009) unless the wild-type AAV genome was present (Li 2007). To ensure that potential elevations in liver transaminases are closely monitored, appropriate safety measures have been incorporated into the study. Liver function tests will be assessed as part of the clinical chemistry allowing rapid detection of any elevations following administration of DTX701; a treatment plan to minimize this potential immune response, if it occurs, will be available on site.

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

3. Geographical distribution of the organism

(a) Indigenous to, or otherwise established in, the country where the notification is made:
Yes (X) No (.) Not known (.)

(b) Indigenous to, or otherwise established in, other EC countries:
(i) Yes (X)

If yes, indicate the type of ecosystem in which it is found:

Atlantic	(X)
Mediterranean	(X)
Boreal	(X)
Alpine	(X)
Continental	(X)
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 qPCR using primers specific for the viral genome. It can also be identified by DNA sequencing.

6. Is the recipient organism classified under existing Community rules relating to the protection of human health and/or the environment?
Yes (.) No (X)
If yes, specify

7. Is the recipient organism significantly pathogenic or harmful in any other way (including its extracellular products), either living or dead?
Yes (.) No (X) Not known (.)

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

If yes:

(a) to which of the following organisms:

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

(b) give the relevant information specified under Annex III A, point II. (A)(11)(d) of Directive 2001/18/EC
Not applicable

8. Information concerning reproduction

(a) Generation time in natural ecosystems:

WT AAV9 is replication defective and requires the presence of a helper virus to facilitate replication. Average WT AAV9 generation time is expected to be relative to helper virus generation time, which is typically 24-120 hours (Bloom and Kerr, 2006; Jogler et al, 2006; Timpe et al, 2006).

(b) Generation time in the ecosystem where the release will take place:
UX701 is replication defective; thus, the generation time is variable depending on the presence or absence of wild type AAV and a helper virus. Replication of UX701 would require simultaneous co-infection of a single cell with UX701, WT AAV and a helper virus. Average WT AAV9 or UX701 generation time is expected to be relative to helper virus generation time, which is typically 24-120 hours (Bloom and Kerr, 2006; Jogler et al, 2006; Timpe et al, 2006).

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

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

9. Survivability

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

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

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

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 WT AAV9 requires the presence of a helper virus (e.g. adenovirus, herpes simplex virus). A highly productive co-infection is required to generate enough WT AAV9 particles for high levels of WT AAV9 viral shedding.

11. Previous genetic modifications of the recipient or parental organism already notified for release in the country where the notification is made (give notification numbers)
No release for UX701 was notified in SPAIN .

C. Information relating to the genetic modification

1. Type of the genetic modification

(i)	insertion of genetic material	(X)
(ii)	deletion of genetic material	(X)
(iii)	base substitution	(.)
(iv)	cell fusion	(.)
(v)	others, specify …	

2. Intended outcome of the genetic modification
The intended outcome of the genetic modification was to generate a recombinant AAV vector lacking viral genes so that the vector would be replication incompetent and serve only to introduce the transgene and to include the sequence coding for modified ATP7B to cause replacement of the absent protein and thus enable the treatment of patients with Wilson disease. UX701 contains a gene encoding a truncated, but functional variant of the human ATP7B gene (ATP7B-MBD456). Expression is driven by a liver-specific promoter. Biodistribution in animal studies of UX701 demonstrated predominant gene transfer to liver tissue. It is expected that administration of UX701 will result in the expression of the modified ATP7B transgene into functional ATP7B protein and improve the condition of study participants.

3. (a) Has a vector been used in the process of modification?
Yes (X) No (.)

If no, go straight to question 5.

(b) If yes, is the vector wholly or partially present in the modified organism?
Partially Yes (X) No (.)

If no, go straight to question 5.

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

(a) Type of vector

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

(b) Identity of the vector

Plasmid comprising vector genome region as well as the AAV serotype 2 (AAV2) *rep* and AAV serotype 9 (AAV9) *cap* genes required for vector DNA amplification and packaging (pDTX.hATP7B_MBD-456.701)

(c) Host range of the vector
Mammalian cells (PCLC-0047 HeLa producer cell line, containing a stably integrated pDTX.hATP7B_MBD-456.701 plasmid). NEB Stable *Escherichia coli* cells were utilized to propagate the pDTX.hATP7B_MBD-456.701 plasmid.

(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, Puromycin

(e) Constituent fragments of the vector
The 4.5 kb nucleotide AAV vector genome contains the complementary DNA (cDNA) of the ATP7B-MBD456 transgene, a liver-specific transthyretin (TTR) promoter and enhancer, a simian virus 40 (SV40) intron, and a SV40 polyadenylation (polyA) sequence. The entire expression cassette is flanked by AAV serotype 2 (AAV2) inverted terminal repeat sequences (ITRs). Only the vector genome is present in the final GMO. In addition, the pDTX.hATP7B_MBD-456.701 plasmid used to originate the virions contains the AAV2 *rep* gene and the AAV9 *cap* gene, the gene for puromycin resistance - included in the plasmid for selection of stable integrants in HeLa cells post-transfection, while the gene for kanamycin resistance and an origin of replication are included for plasmid production in *Escherichia coli*. The additional plasmid elements are not transferred to the final GMO genome.

(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 (including rep and cap genes), and a helper virus, resulting in production of recombinant vector particles.

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

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

6. Composition of the insert

(a) Composition of the insert

The vector genome comprises a liver-specific transthyretin (TTR) enhancer and promoter, a simian virus 40 (SV40) small T intron, the cDNA of ATP7B-MBD456 transgene and a SV40 polyadenylation (polyA) sequence. The entire expression cassette is flanked by AAV serotype 2 (AAV2) inverted terminal repeat sequences (ITRs).

(b) Source of each constituent part of the insert

- Liver-specific TTR enhancer: *Mus musculus*
- Liver-specific TTR promoter: *Homo sapiens*
- SV40 small T intron: *Macaca mulatta* polyomavirus 1
- cDNA of ATP7B-MBD456 transgene: *Homo sapiens*
- SV40 poly(A) signal: *Macaca mulatta* polyomavirus 1
- ITRs: AAV2

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

- Liver-specific TTR enhancer: Intended to enhance liver specific transgene expression
- Liver-specific TTR sponsor: Intended to drive liver specific transgene expression
- SV40 small T intron: Intended to aid transgene expression
- cDNA of ATP7B-MBD456 transgene: gene transfer may be effective for the treatment of patients with Wilson disease, given that the disease is caused by mutations within the ATP7B gene that affect the expression or activity of the ATP7B protein with related impaired copper
- SV40 poly(A) signal: terminate transcription of transgene and efficient polyadenylation of the ATP7B-MBD456 mRNA
- ITRs: required for replication of the rAAV DNA and packaging into virions

(e) Location of the insert in the host organism

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

(f) Does the insert contain parts whose product or function are not known?

Yes (.) No (X)

If yes, specify …

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

1. Indicate whether it is a:

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

- mammals (X)
- insect (.)
- fish (.)
- other animal (.)
(specify phylum, class) …

other, specify …

2. Complete name

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

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

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

If yes, specify the following:

(b) to which of the following organisms:

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

(b) are the donated sequences involved in any way to the pathogenic or harmful properties of the organism

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

If yes, give the relevant information under Annex III A, point II(A)(11)(d):
N/A

4. Is the donor organism classified under existing Community rules relating to the protection of human health and the environment, such as Directive 90/679/EEC on the protection of workers from risks to exposure to biological agents at work?

Yes (.) No (X)

If yes, specify …

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

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

E. Information relating to the genetically modified organism

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

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

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

Specify:

The UX701 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, UX701 contains no native viral coding genes.

Wild-type AAV requires the presence of a helper virus such as human adenovirus or herpes simplex virus to replicate. UX701 replication could only occur in the extremely unlikely event of a host cell being co-infected by wild-type AAV and a helper virus such as human adenovirus or herpes simplex virus.

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

Specify

As UX701 replication could only occur in the extremely unlikely event of a host cell being infected by two separate viruses, a wild type AAV and a helper virus such as human adenovirus or herpes simplex virus, the likelihood of dissemination is lower than that of wild-type AAV.

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

No pathogenic effects of wild-type AAV in humans are known. The introduction of the expression cassette, encoding the modified ATP7B, is not expected to result in development of pathogenicity. Thus, neither the wild-type AAV nor UX701 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, UX701 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)

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 up to 80% (European Parliament and of the Council 2000). Consequently, AAV fulfils the definition of a Risk Group 1 biological agent according to Directive 2000/54/EC (a biological agent that is unlikely to cause human disease).

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

4. Description of identification and detection methods

(a) Techniques used to detect the GMO in the environment
UX701 can be detected by qPCR and ddPCR.

(b) Techniques used to identify the GMO
UX701 can be identified by qPCR, ddPCR and sequencing.

F. Information relating to the release

1. Purpose of the release (including any significant potential environmental benefits that may be expected)
Phase 1/2/3, adult gene therapy study with UX701 in subjects with Wilson disease.

2. Is the site of the release different from the natural habitat or from the ecosystem in which the recipient or parental organism is regularly used, kept or found?
Yes (.) No (X)

3. Information concerning the release and the surrounding area

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

Site 1	Hospital Vall de Hebrón_ Dr. Jesús Quintero Passeig Vall d'Hebrón 119-129 Barcelona 08035 Spain
Site 2	Hospital Universitari i Politecnic La Fe de Valencia_ Dr. Marina Berenguer Avda. Fernando Abril Martorell 106 Valencia 46026 Spain

(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 UX701 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 UX701 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. UX701 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 UX701 will occur only within a controlled hospital setting; therefore, it is not anticipated that it will come into contact with plants, animals or soil.

4. Method and amount of release

(a) Quantities of GMOs to be released:
Dosing of UX701 will be based on the clinical trial participant weight. Participants randomized to IMP will receive one of the following doses:
- Dose 1: 5.0×10^{12} GC/kg

- Dose 2: 1.0×10^{13} GC/kg
- Dose 3: 2.0×10^{13} GC/kg

It is expected to recruit approximately 90 patients globally, of which 3 patients in SPAIN .

(b) Duration of the operation:

The planned duration of subject participation, defined as the date the subject provides written informed consent through completion of the End of Study visit, includes a screening period of up to 12 weeks and a total follow-up period of at least 104 weeks; however, UX701 is administered only once by IV infusion and the remainder of the study is for observation of the treatment effects.

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

UX701 will be shipped to study sites in line with standard recommendations for the transport of biohazardous materials. UX701 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 UX701-CL301.

Staff will follow the biohazard's waste and disposal policies as per local site requirement to dispose of consumables used in the preparation and administration of the GMO. The use of sharps will be kept to a minimum.

UX701 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 as well as per Sponsor's guidelines provided to the site staff. A UX701-CL301 Pharmacy Manual and training material located at sites provides pharmacy personnel and clinical medical staff directions on use, storage and destruction of the IMP. It also includes directions for documenting the control of the IMP from the time of receipt at the trial site until final accountability and destruction. In addition, it describes the required processes for managing and documenting any issues, such as shipment or storage, temperature excursions and reporting of technical product complaints.

The risks related to the release into the environment of the GMO or risks to personnel in the event there is a breach in container integrity and/or storage or accidental spillage at the site or during shipping/storage, is considered to be negligible. The GMO will only be handled by delegated, trained personnel and in the event that a spillage did occur, the product is non-pathogenic and non-replicative, limiting spread and risks to the environment or personnel.

Patients will receive UX701 by a one-time IV infusion in a clinical setting and will be monitored for at least 6 hours following IP administration and until the Investigator

determines that the subject is clinically stable and safe to be discharged. Vital signs will be measured Pre Dose, approximately 1 hour (± 5 minutes) and 4 hours (± 15 minutes) after the start of the IP infusion, and prior to discharge

Additionally, UX701 shedding will be assessed in throughout subject study participation. This will indicate when vector shedding in saliva, urine and stool has ceased. As UX701 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 UX701 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.
UX701 has been administered to mice (wild-type C57BL/6J mice and toxic milk mouse model of Wilson disease (tx-J mice)) and normal cynomolgus monkeys.

UX701 will be administered to humans for the first time under the controlled UX701-CL301 clinical trial conditions.

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)
UX701 contains the cDNA encoding for a shortened human ATP7B protein. AAV9 has a strong tropism for liver and other tissues. The transgene expression in UX701 is driven by a liver specific promoter and encapsidated within an AAV9 capsid. It is expected that administration of UX701 will result in the expression of the transgene primarily in liver tissues.

Gene transfer of the cDNA of ATP7B-MBD456 transgene may be effective for the treatment of patients with Wilson disease, given that the disease is caused by mutations within the ATP7B gene that affect the protein expression or activity leading to impaired copper metabolism in Wilson disease patients.

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 UX701 that could represent potential hazard. Minimal exposure, such as environmental exposure, to organisms other than the subjects receiving UX701 as part of the study would not be of sufficient dose to represent significant gene expression or potential safety risks. As UX701 is also replication-incompetent, it is expected that the vector would be rapidly cleared from any non-target organisms without causing any harmful effects.

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 UX701 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 UX701 is unable to replicate, it is not expected to spread to the environment to a significant degree and is not expected to become established in any ecosystems.

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

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

7. Likelihood of genetic exchange in vivo

(a) from the GMO to other organisms in the release ecosystem:
It is expected that the UX701 vector genome will be transferred into tissues within the body of patients. Following transduction, UX701 vector genomes mainly persist as episomal concatemers. As UX701 is non-replicative and is only expected to be shed in study subjects' bodily fluids to a limited extent, transmission and gene transfer to organisms other than the study subjects is considered unlikely.

(b) from other organisms to the GMO:
The probability of homologous recombination with related viruses that could lead to variants of the GMO is strongly reduced with the ITRs being the only viral sequences remaining in the vector, making up only 6.5% of the final vector sequence. It is not expected that any organism's DNA could be transferred to the viral episomes and be incorporated into UX701 genome.

(c) likely consequences of gene transfer:
While recombination between UX701 and a wild-type AAV to generate a hybrid vector genome that contains both the transgene and the AAV *rep* and *cap* genes remains a theoretical possibility, such a molecule, even if generated in a cell, would not replicate unless a helper adenovirus/herpes virus was also present. AAV possesses a packaging limit of approximately 5 kb (Wu, Yang, and Colosi 2010), and a hybrid molecule of *rep-cap* genes plus the ATP7B-MBD456 expression cassette would be predicted to be far in excess of this limit with ~9 kb. The risks associated with gene transfer from wild-type AAV to UX701 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 UX701.

9. Possible environmentally significant interactions with biogeochemical processes (if different from the recipient or parental organism)
UX701 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 UX701 include both safety and efficacy assessments.

2. Methods for monitoring ecosystem effects
The presence of UX701 in bodily fluids following administration of UX701 to participants enrolled in the UX701-CL301 clinical study will be determined by qPCR. No other methods are foreseen.

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

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

5. Duration of the monitoring
Shedding data will be collected with the UX701-CL301 clinical study, which is anticipated to provide definitive characterization of the viral shedding profile in patients with Wilson disease. In this study, it is expected to collect samples from saliva, urine and stool from approximately 90 randomized participants. This includes enrolled subjects who will be randomized to the placebo cohort, considering they will be offered the possibility to receive UX701 administration as per UX701-CL301 Protocol. According to the protocol, the expected minimum number of subjects planned to receive UX701 is 48 subjects (i.e. IMP dose cohorts). Safety and efficacy assessments will be conducted throughout the duration of the UX701-CL301 study.

Furthermore, after completion of UX701-CL301, all subjects who received a full or partial dose of UX701 will be enrolled into a Disease Monitoring Program (DMP) that will evaluate the long-term safety and efficacy of UX701 for a total of approximately 4 years (i.e., a minimum of 5 years total follow-up from the time of UX701 administration in the UX701-CL301 study).

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

I. Information on post-release and waste treatment

1. Post-release treatment of the site
In the event that the contents of the UX701 vial(s) or diluted product for infusion are accidentally released and come in contact with shipping materials or pharmacy/hospital surfaces, the spillage should be decontaminated using an appropriate disinfectant, such as 1% sodium hypochlorite and 2% glutaraldehyde, for 10 minutes; ; equivalent disinfectants available at the investigational site may be used if effective against AAV

Consumables (including but not limited to gloves, masks, syringes, needles and tubing) and all disposable materials that come into contact with the investigational product should be must be disposed of as biohazard waste in accordance with the practices or procedures of each site. For example, materials are disposed of in sharps containers or biohazard bags and decontaminated by autoclave or incineration, or both.

Non-disposable materials should be decontaminated per local institutional requirements.

2. Post-release treatment of the GMOs

Return and Destruction of used and unused vials of UX701 should be retained at the study site until study drug accountability has been performed by the unblinded Pharmacy Monitor—unless this practice is not aligned with the institutions' policies and procedures. If the site pharmacy internal procedures do not allow for used, partially used, or wasted vials to be retained for study drug accountability, at a minimum, the original vial carton and labels must be retained for later accountability by the Sponsor or authorized representative. All unused vials need to be kept in the required storage conditions (≤ -60°C [-76°F]); used/partly used vials can be stored at room temperature. Unused vials will be returned to a designated depot, as needed. Used/partly used vials must be disposed of as biohazard waste, after the unblinded Pharmacy Monitor completes accountability and gives approval.

3. (a) Type and amount of waste generated
- Closed 6R vials containing UX701 residuals. The number of UX701 vials shipped to the site is variable depending on the subject's weight and may be between 3 to 24 vials.
- 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, masks

3. (b) Treatment of waste
Refer to post-release treatment I.2.

J. Information on emergency response plans

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

Procedures for use of all batches of UX701 are described in the component-specific Material Safety Datasheet (MSDS), appended to the UX701-CL301 Pharmacy manual. In addition, prior to receiving any IMP, a Pharmacy Binder will be sent to sites, which will include, at a minimum, the UX701-CL301 Pharmacy Manual and all Appendices, including instructions for the management and disposal of UX701, which should be followed by all personnel responsible for transporting, preparing, administering, disposing of UX701 medicinal product or equipment/consumables that have come into contact with the product designated for use in clinical study. In the event of injury, staff will follow local institutional procedures. **Table 1** summarizes the additional procedures that should be used by staff to manage incidents related to UX701.

Table 1: Management of Incidents Involving UX701

Incident	Procedure
Accidental spillage	In the event that the contents of the UX701 vial(s) or diluted product for infusion are accidentally released and come in contact with shipping materials or pharmacy/hospital surfaces, the spillage should be decontaminated using an appropriate disinfectant, such as 1% sodium hypochlorite and 2% glutaraldehyde, for 10 minutes; equivalent disinfectants available at the investigational site may be used if effective against AAV.
Sharps injury	UX701 is stored in glass vials. Care must be taken when manipulating vials. The use of needles is to be kept to a minimum. In the event of injury, follow local institutional procedures and report to the Unblinded Pharmacy Monitor for escalation.
Contact with skin, eyes and clothing.	Follow local Institutional procedures pertaining to the use of biohazardous material.
Visible particles	Contact your unblinded Pharmacy Monitor immediately
Vial(s) break	Contact your unblinded Pharmacy Monitor immediately. Do not dose the subject. An evaluation of the event will occur, and a determination will be made on the number of vials that require replacement, depending on the point in the process at which the incident occurred.

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

Any surface area accidentally exposed to the contents of the UX701 vial(s) or diluted product for infusion should be decontaminated and spillage removed according to institutional practices.

Consumables (including but not limited to gloves, masks, syringes, needles and tubing) and all disposable materials that come into contact with the investigational product must be disposed of as biohazard waste according to individual institutional practices and policies. For example, materials are disposed of in sharps containers or biohazard bags and decontaminated by autoclave or incineration, or both.

Non-disposable materials should be decontaminated using an appropriate disinfectant, such as 1% sodium hypochlorite and 2% glutaraldehyde, for 10 minutes; equivalent disinfectants available at the investigational site may be used if effective against AAV.

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

Administration of UX701 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, UX701 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 UX701 are provided in the safety instructions for investigators and staff included in this submission. All patients will be carefully monitored for any adverse reactions during this study. An external data monitoring committee (DMC) will be responsible for monitoring safety data throughout from the UX701-CL301 study.

References:

Bloom and Kerr. Parvoviruses (Part 2; Ch 22). Edited by Kerr et al. 2006

Jogler C, Hoffmann D, Theegarten D, Grunwald T, Uberla K, Wildner O. Replication properties of human adenovirus in vivo and in cultures of primary cells from different animal species. J Virol. 2006;80(7):3549-3558. doi:10.1128/JVI.80.7.3549-3558.2006

European Parliament and of the Council. 2000. 'Directive 2000/54/EC of the European Parliament and of the Council of 18 September 2000 on the protection of workers from risks related to exposure to biological agents at work,'

Manno CS, Pierce GF, Arruda VR, et al. (2006) Successful transduction of liver in hemophilia by AAV-Factor IX and limitations imposed by the host immune response. Nat Med 12: 342-347.

Mingozzi, F. (2007) CD8+ T-cell responses to adeno-associated virus capsid in humans. Nature Medicine Volume 13, No. 4, 419-422

Nathwani AC, Rosales C, McIntosh J, et al. (2011a) Long-term safety and efficacy following systemic administration of a self-complementary AAV vector encoding human FIX pseudotyped with serotype 5 and 8 capsid proteins. Mol Ther 19: 876-885.

Nathwani AC, Reiss UM, Tuddenham EG, et al. (2014) Long-term safety and efficacy of factor IX gene therapy in hemophilia B. N Engl J Med 371: 1994-2004.

Timpe JM, Verrill KC, Trempe JP. Effects of adeno-associated virus on adenovirus replication and gene expression during coinfection. J Virol. 2006;80(16):7807-7815. doi:10.1128/JVI.00198-06

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