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/18/017
(c)	Date of acknowledgement of notification	14 december 2018
(d)	Title of the project	Clinical Study 19429, "A Phase 1/2 Open-Label Safety and Dose-Finding Study of BAY 2599023 (DTX201), an Adeno-Associated Virus (AAV) hu37-Mediated Gene Transfer of B-Domain Deleted Human Factor VIII, in Adults with Severe Hemophilia A"
(e)	Proposed period of release	July 2018 – December 2030

2. Notifier

Name of institution or company:	Bayer AG D-51368 Leverkusen Germany

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 human serotype 37 (AAVhu37)

(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, BAY 2599023 (DTX201) is also expected to be highly genetically stable. The BAY 2599023 (DTX201) 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) NL

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 placed on the market outside the Community by the same or other notifier?
   Yes (X) No (.)
   If yes:
   - Member State of notification USA
   - Notification number: NIH Protocol # 1707-1646

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

BAY 2599023 (DTX201), the study product is AAVhu37FVIII; is a non-replicating recombinant AAVhu37 vector containing a single stranded DNA genome encoding a form of

human FVIII under control of the liver-specific transthyretin (TTR) promoter for the treatment of patients with Haemophilia A.
The release of BAY 2599023 (DTX201) 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: BAY 2599023 (DTX201) 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. BAY 2599023 (DTX201) replication could only occur in the extremely unlikely event of a host cell being infected by three separate viruses (BAY 2599023 (DTX201), wild-type AAV and a helper virus such as human adenovirus or herpes simplex virus). The 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, stool and semen of patients following systemic administration. In general, shedding was observed in patients receiving a single dose of 2×10^{11} to 2×10^{12} GC/kg of a recombinant AAV vector in stool, urine, saliva and semen for up to 10 to 50 days after administration of the vector, with peak levels around 2 days after administration (Nathwani 2011, Nathwani 2012, Nathwani 2014). The extent and duration of shedding of BAY 2599023 (DTX201) will be monitored as part of the proposed clinical trial. However, as BAY 2599023 (DTX201) is non-replicative, shed viral particles are unable to multiply and their spread is thus inherently limited. Furthermore, potential hazards of exposure to BAY 2599023 (DTX201) to humans are predicated upon systemic administration of BAY 2599023 (DTX201). Minimal exposure, such as environmental exposure, to persons other than the subjects receiving BAY 2599023 (DTX201) 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 BAY 2599023 (DTX201) 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 BAY 2599023 (DTX201) 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 ($\geq$ 99.5%) rather than as integrated DNA. No clinical trials to date with AAV have reported incidences of insertional mutagenesis.
- Liver-specific transgene expression: Adeno-associated virus human serotype 37, as a clade E AAV, has strong tropism for the liver, and transduces the liver highly efficiently when administered intravenously. BAY 2599023 (DTX201) encodes the B Domain Deleted (BDD) Factor VIII gene with expression driven by both a liver-specific enhancer and liver-specific promoter encapsidated within an AAVhu37 vector. Transgene expression in cells other than human hepatocytes is thus considered to be unlikely.
- Minimal risk associated with the transgene: The BDD Factor VIII gene encodes for the human BDD Factor VIII gene. Genes encoding 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 hu37
(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

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 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, BAY 2599023 (DTX201), is replication-incompetent even in the presence of a helper virus due to the removal of the viral rep and cap genes

9. Survivability

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

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

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

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, Bayer AG has not notified any previous genetic modifications of the parental virus (AAVhu37) 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 BDD FVIII expression cassette for the treatment of patients with Haemophilia A. BAY 2599023 (DTX201) contains a codon-optimized cDNA that encodes for the human FVIII protein lacking the B-domain (BDD hFVIII). Expression is driven by both a liver specific enhancer element and a liver specific promoter encapsulated within a non-replicating AAVhu37 vector. AAV hu 37 serotype, 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 BAY 2599023 (DTX201), will result in the expression of the BDD hFVIII 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.hFVIIIco SQ.201

(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.hFVIIIco-SQ.201 contains the BDD hFVIII expression cassette. The expression cassette consists of a liver-specific promoter and enhancer, a codon-optimized BDD hFVIII transgene and a polyadenylation signal, flanked by AAV inverted terminal repeats (ITRs). Only the BDD hFVIII expression cassette is present in the final GMO. In addition, the vector contains a bacterial origin of replication, the gene for Kanamycin resistance to allow for propagation of the plasmid in *E. coli,* and the AAV rep and cap genes required for vector DNA amplification and packaging.

(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 stable HeLa S3-derived producer cell line with pDTX.hFVIIIco-SQ.201 and infection with wild-type adenovirus type 5 (Ad5) to induce 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 BDD hFVIII transgene and a polyadenylation signal, flanked by AAV inverted terminal repeats (ITRs)

(b) Source of each constituent part of the insert

- Liver-specific promoter and enhancer: *homo sapiens*
- Human Factor VIII gene: *homo sapiens*
- pA75 Polyadenylation signal: human alpha-2 globin gene
- enTTR/TTR liver specific promoter/enhancer: murine (enTTR)/ human (TTR)
- ITRs: AAV

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

- Liver-specific promoter and enhancer: Intended to drive high-level, liver specific BDD hFVIII gene expression.
- BDD hFVIII gene: Gene transfer of BDD hFVIII is expected to be effective for the treatment of haemophilia A, given that the disease is caused by mutations within this gene that affect the expression or activity of the Factor VIII protein.
- Polyadenylation signal: Intended to provide cis sequences for efficient polyadenylation of the hFVIII 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 in the presence of rep and cap.

(d) Location of the insert in the host organism

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

(e) Does the insert contain parts whose product or function are not known?
Yes (.) No (X)

If yes, specify

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

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:

(a) 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 BAY 2599023 (DTX201) 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, BAY 2599023 (DTX201) 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. BAY 2599023 (DTX201) replication would require the presence of wild-type AAV in addition to the presence of a helper virus. This likelihood of this occurring is extremely low.

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

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

Specify

As BAY 2599023 (DTX201) replication could only occur in the extremely unlikely event of a host cell being infected by three separate viruses (BAY 2599023, wild-type AAV and a helper virus such as 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 BDD hFVIII expression cassette is not expected to result in development of pathogenicity. Thus, neither the wild-type AAV nor BAY 2599023 (DTX201) 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, BAY 2599023 (DTX201) is also expected to be genetically stable. The integrity of the BDD hFVIII 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
BAY 2599023 (DTX201) can be detected by quantitative polymerase chain reaction (qPCR).

(b) Techniques used to identify the GMO
BAY 2599023 (DTX201) can be identified by qPCR.

F. Information relating to the release

1. Purpose of the release (including any significant potential environmental benefits that may be expected)
Study #19429 is a Phase 1/2 Open-Label Safety and Dose-Finding Study of BAY 2599023 (DTX201), an adeno-associated Virus (AAV) hu37-mediated gene transfer of B-domain deleted human Factor VIII, in adults with severe Hemophilia A.

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

The natural habitat of wild-type AAV8 is primate host cells. BAY 2599023 (DTX201) will be administered to humans in the context of clinical study 19429.

3. Information concerning the release and the surrounding area

(a) Geographical location (administrative region and where appropriate grid reference):
Site 1:
Departement van Creveld kliniek,
UMC Utrecht
Heidelberglaan 100
3584 CX Utrecht.

Site 2:

Site 3:

Site 4:

(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 BAY 2599023 (DTX201) 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 BAY 2599023 (DTX201) 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. BAY 2599023 (DTX201) will be administered by a one-time single intravenous infusion in a hospital setting. It is thus not anticipated to come into contact with any recognized biotopes or protected areas.

(d) Flora and fauna including crops, livestock and migratory species which may potentially interact with the GMO
Administration of BAY 2599023 (DTX201) 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 BAY 2599023 (DTX201) 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 minimize the spread of the GMOs beyond the site of the release
BAY 2599023 (DTX201) 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 19429. Once study drug accountability has been completed, vials are to be decontaminated per institutional practice. Used and unused vials of BAY 2599023 (DTX201) should be retained at the study site until study drug accountability has been performed by the Clinical Research Associate (CRA) (PPD). All unused vials need to be kept in the required storage conditions (≤ -60°C); used/partly used vials can be stored at room temperature. Unused and used/partly used vials can be discarded at the site following local requirements only after the CRA completes accountability and gives approval.

BAY 2599023 (DTX201) is an Investigational Medicinal Product (IMP) manufactured and released by a Qualified Person (QP) in Europe, for clinical trial use after meeting defined specification 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 Committee 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 destruction of 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. In addition, it describes the required processes for managing and documenting deviations, such as temperature excursions, and reported technical product complaints will be transferred to sites by personnel trained in the shipment of medicinal products. 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, patient in clinical study 19429 are required to use barrier contraceptives from the time of the study drug administration until the follow-up visit 12 weeks after study drug administration.

Semen samples will be collected as detailed in the clinical protocol, and patients will be required to use double-barrier contraception methods until 3 negative sample results have occurred during the safety follow up period in all sample matrices and as advised by the lead Investigators.

Patients will receive BAY 2599023 (DTX201) by a one-time IV infusion in an outpatient setting and will be discharged at least 8 hours after administration of BAY 2599023 (DTX201), 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, stool and semen has ceased. As BAY 2599023 (DTX201) 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 BAY 2599023 (DTX201) 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. BAY 2599023 (DTX201) 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)

BAY 2599023 (DTX201) encodes the BDD Factor VIII gene with expression driven by both a liver-specific enhancer and liver-specific promoter, encapsidated within an AAVhu37vector. AAVhu37, 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 BAY 2599023 (DTX201) will result in the expression of the BDD hFVIII gene in the liver of study subjects. Gene transfer of BDD hFVIII gene is expected to be effective for the treatment of Haemophilia A, given that the disease is caused by mutations within this gene that affect the expression or activity of the Factor VIII protein.

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 BAY 2599023 (DTX201) that could represent a potential hazard. Potential hazards of exposure to BAY 2599023 (DTX201) are predicated upon systemic administration of BAY 2599023 (DTX201). Minimal exposure, such as environmental exposure, to organisms other than the subjects receiving BAY 2599023 (DTX201) as part of the study would not be of sufficient dose to represent significant gene expression or potential safety risks in humans. As BAY 2599023 (DTX201) 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 BAY 2599023 (DTX201) 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 BAY 2599023 (DTX201) 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 BAY 2599023 (DTX201) 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

(i)	order and/or higher taxon (for animals)	Primates
(ii)	family name for plants	Hominidae
(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

7. Likelihood of genetic exchange in vivo

(a) from the GMO to other organisms in the release ecosystem:
It is expected that the BAY 2599023 (DTX201) viral genome will be transferred into hepatocytes within the liver of patients enrolled in study 19429. The vast majority of BAY 2599023 (DTX201) vector genomes within subject cells are expected to be episomal, rather than integrated into the host cell DNA. As BAY 2599023 (DTX201) 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 BAY 2599023 (DTX201) and a wild-type AAV to generate a hybrid vector genome that contains both the BDD hFVIII 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 5kb (Wu 2010), and a hybrid molecule of rep-cap genes plus the BDD hFVIII expression cassette would be predicted to be in excess of this limit. The risks associated with gene transfer from wild-type AAV to BAY 2599023 (DTX201) are thus considered to be negligible.

8. Give references to relevant results (if available) from studies of the behavior 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 BAY 2599023 (DTX201).

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

   BAY 2599023 (DTX201) 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 19429. Other methods to monitor the effects of BAY 2599023 (DTX201) include both safety and efficacy assessments.

2. Methods for monitoring ecosystem effects
   The presence of BAY 2599023 (DTX201) in bodily fluids following administration of BAY 2599023 (DTX201) 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 BDD Factor VIII expression cassette to study subjects will be detected by assessing Factor VIII 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 negative on at least three consecutive occasions for two separate sample matrices. 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 negative on at least three consecutive occasions for two separate sample matrices. 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 BAY 2599023 (DTX201) will be disinfected in accordance with local guidelines 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.

Used and unused vials of BAY 2599023 (DTX201) should be retained at the study site until study drug accountability has been performed by the CRA. 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 and used/partly used vials can be discarded at the site following local requirements only after the CRA completes accountability and gives approval.

3. (a) Type and amount of waste generated

The following types of waste are anticipated:

- Glass vials containing BAY 2599023 (DTX201). The number of vials of BAY 2599023 (DTX201) 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

(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 BAY 2599023 (DTX201) 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. Disinfectants effective against AAV include 1% sodium hypochlorite, 2% glutaraldehyde and 0.25% sodium dodecyl sulphate.

BAY 2599023 (DTX201) 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 BAY 2599023 (DTX201) with skin, eyes or clothing, the affected area will be washed with copious amounts of water and 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 guidelines and institutional policies and procedures. Disinfectants effective against

AAV include 1% sodium hypochlorite, 2% glutaraldehyde and 0.25% sodium dodecyl sulphate.

3. Methods for disposal or sanitation of plants, animals, soils, etc. that could be exposed during or after the spread
   Administration of BAY 2599023 (DTX201) 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, BAY 2599023 (DTX201) 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 guidelines and institutional procedures for the handling and disposal of genetically modified organisms. Furthermore, safety recommendations and guidance on the management of incidents related to BAY 2599023 (DTX201) 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.

References

Directive 2001/18/EC of the European Parliament and of the Council on the deliberate release into the environment of genetically modified organisms.

Council Directive 90/679/EEC of 26 November 1990 on the protection of workers from risks related to exposure to biological agents at work.

Nathwani AC, Rosales C, McIntosh J, et al. 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. 2011;19(5):876-85.

Nathwani AC, Tuddenham EG, Ranqarajan S, et al. Adenovirus-associated virus vector mediated gene transfer in hemophilia B. N Engl J Med. 2012;365(25):2357-65.

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Wu Z, Yang H, Colosi P. Effect of genome size on AAV vector packaging. Mol Ther. 2010 Jan;18(1):80-6.