

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/17/16
(c) Date of acknowledgement of notification 04/09/2017
(d) Title of the project:
A Global, Open-Label, Multicenter, Phase 1/2 Study of the Safety and Dose Escalation of BAX 888, an Adeno-Associated Virus Serotype 8 (AAV8) Vector Expressing B-Domain Deleted Factor VIII (BDD-FVIII) in Severe Hemophilia A Subjects Administered a Single Intravenous Infusion

Proposed period of release From 30/Dec/2017 until 30/Jun/2022

2. Notifier

Name of institution or company: Baxalta Innovations GmbH

3. GMO characterisation

BAX 888 is an AAV vector containing single-stranded DNA carrying the B-domain deleted (BDD) human coagulation F8 (BDD-FVIII) cDNA. BAX 888 has an INFORMATION DELETED FOR CONFIDENTIALITY PURPOSES AAV capsid composed of proteins belonging to the AAV serotype 8 (AAV8).

(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: Dependovirus

Species: Adeno-associated virus

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

In contrast to wild-type AAV, the BAX 888 vector AAV8.BDD-FVIIIopt has the entire encoding sequences for virus structural proteins deleted and instead a gene cassette inserted which comprises three elements: i) a promoter/enhancer sequence, ii) a B domain deleted codon-optimized FVIII sequence for the expression of a truncated human FVIII protein and iii) a synthetic poly A sequence. The only virus genome sequences from wild-type AAV in vector AAV8.BDD-FVIIIopt are the genome flanking ITR sequences, derived from AAV2. These modifications render vector AAV8.BDD-FVIIIopt entirely replication- incompetent. The overall stability of these modifications is considered high, as at least in the context of mouse and human liver cells, the FVIII expression can be maintained over a longer period of time. In-vivo studies demonstrated stable expression of human FVIII in mice over more than 24 weeks.

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

Yes No

If yes, insert the country code(s) AT, IT, DE, HU, FR

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

Yes No

If yes:

- Member State of notification AT, IT, DE, HU, FR
- Notification number B/././...

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 No

If yes:

- Member State of notification
- Notification number B/././...

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

The Environmental Risk Assessment carried out according to the scientific principles and methodology as laid down in the EMEA/CHMP/GTWP/125491/2006 identified only four adverse effects that could occur directly or indirectly due to the deliberate release of BAX 888 in the proposed clinical trial. These potential adverse effects are associated with (i) thrombotic events, (ii) immune responses, (iii) insertional mutagenesis, and (iv) residual rcAAV particles in BAX 888 from manufacturing. The consequences of these potential adverse effects are considered of low magnitude and the likelihood of these adverse effects occurring is considered negligible due to a variety of factors, such as pre-existing

neutralizing antibodies against the capsid in general population, the non-pathogenicity of the host strain (AAV), the non-pathogenicity of the genetically modified sequences, replication deficiency of the modified vector, and the very low concentrations to which a non-target organism may potentially be exposed to in the worst case. Furthermore, the likelihood of direct and indirect exposure to the drug product or modified gene-sequences per se is very low due to appropriate handling procedures in the clinic, due to needleless preparation of the dose and administration via safe catheters, and due to the small number of subjects to be enrolled in the clinical trial.

Even if the modified vector or modified gene-sequences were transferred to other human and non-human species in the environment:

- this would not result in an increased growth rate compared to wild-type organisms, and therefore would not adversely affect the dynamics of population or the genetic diversity of these populations
- this would not alter the susceptibility to pathogens that facilitate the dissemination of infectious diseases or create new vectors
- the overall risk of causing disease in humans, animals, or plants is therefore considered negligible.

In conclusion, the applicant considers the environmental risks associated with the deliberate release of the BAX 888 drug product to the environment by the proposed clinical trial (first in human, phase 1/2 study, protocol number 201501) as negligible.

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

1. Recipient or parental organism characterisation:

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

(select one only)

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

2. Name

- | | |
|--|-------------------------------|
| (i) order and/or higher taxon (for animals) | <i>Parvoviridae</i> |
| (ii) genus | <i>Dependovirus</i> |
| (iii) species | <i>Adeno-associated virus</i> |
| (iv) subspecies | - |
| (v) strain | - |
| (vi) pathovar (biotype, ecotype, race, etc.) | - |
| (vii) common name | <i>AAV 8</i> |

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.

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

5. (a) Detection techniques

Quantitative Polymerase Chain Reaction (qPCR) can be used to detect viral genome using specific primers in both qualitative and quantitative manner.
 Viral vector proteins can be detected using Enzyme-Linked Immunosorbent Assay (ELISA) methods.

(b) Identification techniques

Quantitative Polymerase Chain Reaction (qPCR) can be used to identify viral genome using specific primers in both qualitative and quantitative manner.
 Viral vector proteins can be identified using Enzyme-Linked Immunosorbent Assay (ELISA) methods.

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

Yes (X) No (.)

If yes, specify

AAV is not known to be a pathogenic virus in humans. Literature search indicates that AAV is also not pathogenic to the non-human environment. The AAV-derived vectors for gene therapy are classified as Risk Group 1 agents regarding both biosafety and GMO considerations.

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:

N/A – BAX 888 has been rendered entirely replication-incompetent.

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

N/A

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

(d) Factors affecting reproduction:

Wild type AAV is a non-autonomous virus. It cannot replicate without a helper-virus present in the same cell. Helper viruses shown to promote AAV replication include adenovirus, herpes simplex virus, human papillomavirus and vaccinia virus. BAX 888 is a recombinant AAV-based virus (rAAV) in which the entire viral genome (*rep* and *cap* genes) is replaced by the transgene expression cassette. The only viral elements remaining are the ITR sequences flanking the transgene. Such a virion is unable to replicate and proliferate under any circumstance.

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 structures to enhance its survival.

(b) relevant factors affecting survivability:

AAV belong to the genus Dependovirus within the family Parvoviridae. The stability of parvoviruses against physico-chemical stress is considered to be high. Parvoviruses are stable in the presence of lipid solvents, upon exposure to pH 3-9 or incubation at

56°C for 60 minutes. In dried condition, infectivity of parvovirus particles can be maintained over several weeks.

The modification of the vector AAV8.BDD-FVIIIopt does not alter the physical stability of virus particles in comparison to wild type AAV, as the capsid structure of the recombinant particles is identical to that of the wild type AAV virus.

10. (a) **Ways of dissemination**

AAV may be transmitted by inhalation of aerosolized droplets, by contact with mucosa, by parenteral injection and by ingestion.

(b) **Factors affecting dissemination**

AAV is a non-autonomous virus. It cannot replicate without a helper-virus present in the same cell. Helper functions can be supplied either by co-infecting helper viruses or by DNA damaging agents. Helper viruses shown to promote AAV replication include Adenovirus, herpes simplex virus, human Papillomavirus and vaccinia 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 has not notified any previous genetic modifications for release in any country.

C. Information relating to the genetic modification

1. Type of the genetic modification

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

2. Intended outcome of the genetic modification

During the manufacture of recombinant AAV gene delivery vectors, all viral genes are removed such that the only protein-coding DNA sequence that is delivered in the BAX 888 vector is the FVIII gene along with DNA elements required for expression of the potentially therapeutic *F8* gene. The rationale for the choice of the serotype 8 for the BAX 888 capsid is that, among the naturally occurring AAV serotypes, AAV8 is particularly efficient at infecting and directing gene expression in the human liver, with minimal “off-target” gene expression in other tissues (e.g. in antigen presenting cells). In addition, BAX 888 incorporates a hepatocyte specific promoter to further restrict FVIII expression to the target liver tissue.

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

If no, go straight to question 5.

- (b) If yes, is the vector wholly or partially present in the modified organism?
Yes 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
- bacteriophage
- virus
- cosmid
- transposable element
- other, specify ...

(b) Identity of the vector

[pAAV8.BBD-FVIIIopt](#)

(c) Host range of the vector

[Mammalian and other animal cells, bacterial cells.](#)

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

- Yes No

antibiotic resistance

other, specify ...

Indication of which antibiotic resistance gene is inserted

Ampicillin

Note, that the ampicillin resistance gene is carried by the plasmids used in the manufacturing process, but not by the final drug product pAAV8.BBD-FVIIIopt.

(e) Constituent fragments of the vector

The AAV clinical vector genome consists of a INFORMATION DELETED FOR CONFIDENTIALITY PURPOSES transthyretin (pre-albumin, TTR)

promoter/enhancer driving expression of the human codon optimized BDD-FVIII- INFORMATION DELETED FOR CONFIDENTIALITY PURPOSES transgene. The gene insert has a synthetic polyadenylation (polyA) sequence. The expression cassette is bound by the AAV2 inverted terminal repeats (ITRs) having the 145 nucleotide wild-type sequence (see figure below).



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

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

During the manufacturing process of pAAV8.BBD-FVIIIopt three different plasmids are transfected into HEK293 cells: i) the plasmid encoding the corresponding FVIII vector, ii) the helper plasmid INFORMATION DELETED FOR CONFIDENTIALITY PURPOSES carrying adenoviral helper genes and iii) the packaging plasmid AAV8, delivering the *rep* and *cap* genes.

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

BAX 888 is a rAAV virus in which the entire viral genome (*rep* and *cap* genes) is replaced by the FVIII transgene expression cassette. The only viral elements remaining are the ITR sequences flanking the transgene. The FVIII transgene expression cassette consists of

- a transthyretin (TTR) promoter/enhancer
- the BDD-FVIIIopt (codon-optimized B-domain deleted human factor VIII gene) transgene,
- a polyadenylation (polyA) sequence, and
- inverted terminal repeats (ITRs)

(b) Source of each constituent part of the insert

- TTR promoter/enhancer: INFORMATION DELETED FOR CONFIDENTIALITY PURPOSES
- BDD-FVIII transgene: human, codon optimized
- polyA sequence: synthetic
- ITRs: wild-type AAV

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

- TTR promoter/enhancer: Recruitment of transcription factors for FVIII transcription
- BDD-FVIII transgene: Coagulation factor VIII
- polyA sequence: Provides stability of FVIII transcript
- ITRs: Encapsidation, self-priming

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

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

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

If yes, specify the following:

(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):

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 (.)

Human infections with AAV are common but AAV has never been reported as etiological agent for any disease either in human or animals. Serological studies in healthy humans showed that up of 70% of the worldwide population has been infected with AAV without correlation with any clinical disease.

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 ...

No alteration in physical stability is anticipated due to the modification of the vector.

(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

As previously mentioned, BAX 888 is a recombinant AAV-based virus (rAAV) in which the entire viral genome (*rep* and *cap* genes) is replaced by the transgene expression cassette. The only viral elements remaining are the ITR sequences flanking the transgene. Such a virion is unable to replicate and proliferate under any circumstance.

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

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

Specify

The dissemination of the GMO in the environment is strongly hampered in that the vector AAV8.BDD-FVIIIopt particles are not replication-competent due to deletion of the AAV structural gene sequences.

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

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

Specify

The gene sequences included in vector AAV8.BDD-FVIIIopt (ITRs from AAV2, INFORMATION DELETED FOR CONFIDENTIALITY PURPOSES TTR promoter, B-domain deleted FVIII sequence, and synthetic poly A sequence) are not known to have any harmful implications for other organisms. The ITRs from AAV2 and the INFORMATION DELETED FOR CONFIDENTIALITY PURPOSES TTR promoter do not present a novel sequence to the environment.

2. Genetic stability of the genetically modified organism

In contrast to wild-type AAV, the BAX 888 vector AAV8.BDD-FVIIIopt has the entire encoding sequences for virus structural proteins deleted and instead a gene cassette inserted as described above. The only virus genome sequences from wild-type AAV in vector AAV8.BDD-FVIIIopt are the genome flanking ITR sequences, derived from AAV2. The overall stability of these modifications is considered high, as at least in the context of mouse and human liver cells, the FVIII expression can be maintained over longer time of period. *In-vivo* studies demonstrated stable expression of human FVIII in mice over more than 24 weeks.

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)

N/A

4. Description of identification and detection methods

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

Polymerase Chain Reaction (PCR) can be used to detect viral genome using specific primers.

(b) Techniques used to identify the GMO

Polymerase Chain Reaction (PCR) can be used to identify viral genome using specific primers.

F. Information relating to the release

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

Study 201501 is a Global, Open-Label, Multicenter, Phase 1/2 Study of the Safety and Dose Escalation of BAX 888, an Adeno-Associated Virus Serotype 8 (AAV8) Vector Expressing B-Domain Deleted Factor VIII (BDD-FVIII) in Severe Hemophilia A Subjects Administered a Single Intravenous Infusion. The primary objective is to evaluate the safety of a single intravenous (IV) infusion of BAX 888 in 2 dose cohorts. The main secondary objective is to evaluate plasma FVIII levels pre- and post-BAX 888 infusion and investigate the relationship between FVIII activity and BAX 888 dose.

2. Is the site of the release different from the natural habitat or from the ecosystem in which the recipient or parental organism is regularly used, kept or found?

Yes (.) No (X)

If yes, specify

3. Information concerning the release and the surrounding area

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

| Site | Principal Investigator |
|-------------------------------|-------------------------------------|
| Hospital Universitario La Paz | M ^a Teresa Álvarez Román |

| | |
|---|--------------------------------------|
| Hospital Universitario de Salamanca | Jose Ramón González Porras |
| Hospital Regional Universitario de Málaga | M ^a Eva Mingot-Castellano |

- (b) Size of the site (m²):
- (i) actual release site (m²): N/A, will be administered to subjects.
 - (ii) wider release site (m²): N/A, will be administered to subjects.

- (a) Proximity to internationally recognised biotopes or protected areas (including drinking water reservoirs), which could be affected:
N/A, will be administered to subjects in medical centers.

- (b) Flora and fauna including crops, livestock and migratory species which may potentially interact with the GMO
N/A, will be administered to subjects in medical centers thus interaction with flora and fauna considered negligible.

4. Method and amount of release

- (a) Quantities of GMOs to be released:
A maximum amount of 8.0×10^{12} capsid particles (cp) per kg of patient's body weight will be administered to subjects. 6 subjects will take part in this study.
- (b) Duration of the operation:
The duration of the study is approximately 5 years, with up to 2 years' extension.
- (c) Methods and procedures to avoid and/or minimise the spread of the GMOs beyond the site of the release
Release takes place in a hospital by intravenous administration to patients. Usual hospital hygiene measures apply. Any unused product and used administration sets will be discarded according to hospital routine for infectious material.

5. Short description of average environmental conditions (weather, temperature, etc.)
N/A, will be administered to subjects in medical centers.

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.
Study 201501 is first in human study. BAX 888 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 -
 - (iii) genus *Homo*
 - (iv) species *Sapiens*
 - (v) subspecies -
 - (vi) strain -
 - (vii) cultivar/breeding line -
 - (viii) pathovar -
 - (ix) common name *Human*

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

Genetic transfer of *F8* to liver cells in recipient human subjects in the clinical trial for endogenous protein expression is the intended mechanism of action.

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

4. Is post-release selection such as increased competitiveness, increased invasiveness for the GMO likely to occur?
Yes (.) No (X) Not known (.)
Give details
Theoretically the modified gene sequences might be transferred into the environment by shedding, however the dissemination of these sequences in the environment is strongly hampered in that the vector AAV8.BDD-FVIIIopt particles are not replication-competent due to deletion of the AAV structural gene sequences. And even if the vector were integrated into other organisms in the environment (e.g. microbial populations in waste water treatment plants), the resulting organisms would not have an increased growth rate compared to the wild-type organisms. The sequences of AAV8.BDD-FVIIIopt do not generate a selective advantage, and therefore would not have an adverse effect on the population dynamics or the genetic diversity of the receiving environment.

5. Types of ecosystems to which the GMO could be disseminated from the site of release and in which it could become established
BAX 888 is replication defective, hence the dissemination from the site of release to ecosystems is considered negligible.

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:

Genetic transfer of *F8* to liver cells in recipient human subjects in the clinical trial for endogenous protein expression is the intended mechanism of action. BAX 888 is only administered intravenously to subjects in the clinical trial and is not release differently to the ecosystem.

(b) from other organisms to the GMO:

BAX 888 is a recombinant AAV virus in which the entire viral genome (*rep* and *cap* genes) is replaced by the FVIII transgene expression cassette. The only viral elements remaining are the ITR sequences flanking the transgene. Therefore, homologous recombination between BAX 888 and related viruses can be excluded.

However, it is theoretically possible that non-homologous recombination between BAX 888 and wild type AAV occurs if both are present in the same hepatocyte or other tissue/cell with AAV8 tropism. If such recombination takes place it could only result in the exchange of the FVIII cDNA present in the BAX 888 vector with the *rep* and *cap* genes of the wild type virus. It is not possible for the AAV genome to contain both *rep* and *cap* genes and the transgene, as this is beyond the packaging limit of the virion.

If a cell carrying AAV8.BDD-FVIIIopt vector is co-infected with a helper virus such as an adenovirus or hepev simplex, no harmful effects are expected to occur because AAV8.BDD-FVIIIopt is replication incompetent and replication of rcAAV particles that may contaminate BAX 888 will only resemble a natural AAV infection.

A recombination between AAV8.BDD-FVIIIopt and wild type AAV may occur in co-infected cells. However, due to the limited packaging capacity, generation of a replication competent AAV particle that additionally carries the BDD-FVIIIopt gene is not possible.

A triple-infection of a cell with BAX 888, a wild type AAV or (a replication competent AAV particle) and a helper virus is theoretically possible. This could result in the replication and amplification of wild type-like viruses as well as in an increased synthesis of AAV8.BDD-FVIIIopt vector. However, the probability of such an event is considered very low.

(a) likely consequences of gene transfer:

No harmful effects are expected.

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.

9. Possible environmentally significant interactions with biogeochemical processes (if different from the recipient or parental organism)
No such interactions are known.

H. Information relating to monitoring

1. Methods for monitoring the GMOs
Quantitative Polymerase Chain Reaction of vector genomes will be used to monitor the duration (shedding) of BAX 888 genomes in blood, saliva, urine, stool, and semen.
2. Methods for monitoring ecosystem effects
Quantitative Polymerase Chain Reaction of vector genomes will be used to monitor the duration (shedding) of BAX 888 genomes in blood, saliva, urine, stool, and semen.
3. Methods for detecting transfer of the donated genetic material from the GMO to other organisms
In the study subjects, transfer of vector genome will be detected by assessing circulating plasma FVIII activity and antigen levels, annualized bleed rate (ABR) in comparison to before gene transfer, and consumption of exogenous FVIII in comparison to before gene transfer.
4. Size of the monitoring area (m²)
Not applicable, the duration of BAX 888 genomes in blood, saliva, urine, stool, and semen will be monitored in study subjects.
5. Duration of the monitoring
The duration of BAX 888 genomes in blood, saliva, urine, stool, and semen will be monitored until the viral genome is undetectable on two consecutive occasions. In each occasion, new samples will be collected and analyzed for the presence of viral genome.
6. Frequency of the monitoring
Duration of vector genomes in blood, saliva, urine, stool, and semen will be checked first at the Screen 2 visit to get the baseline. After receiving BAX 888, samples will be collected at the following visits until 2 consecutive negative tests: Day 1, then weekly at the clinic visits between Weeks 1-15, and then at Months 4, 5, 6, 9, 12.

I. Information on post-release and waste treatment

1. Post-release treatment of the site
N/A. Release takes place in a hospital by intravenous administration to patients. Usual hospital hygiene measures apply.
2. Post-release treatment of the GMOs
 - (a) Type and amount of waste generated

Unused liquid product in product vials, used syringe and infusion set.

- (b) Treatment of waste
Waste will be treated in the hospital according to local hospital waste requirements for infectious material.

J. Information on emergency response plans

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

For protecting human health and the environment – amongst others – the following risk management strategies will be in place in addition to standard biosafety practices (such as secure storage or training of personnel):

- Handling and application of BAX 888 is restricted to trained healthcare professionals (pharmacists, doctors, nurses) which are able to adequately prevent and remediate incidents or accidents in order to minimize the likelihood of incidental exposure or self-inoculation.
- Restriction of using BAX 888 by trained personnel in qualified hospital centers also minimizes an accidental release of the product into the environment.
- Patients are instructed to apply basic hygiene measures like frequent hand-washing during the time of shedding in order to minimize the release of BAX 888 into the environment and exposure of care givers, family and friends. Furthermore patients are instructed to use physical contraception as long as BAX 888 is expected in seminal fluid and to avoid kissing and using same glasses as long as BAX 888 is expected in saliva.
- Baxalta and Quintiles have to be informed on any accidental human exposure such that the exposed person should be taken up into the accidental exposure database.

Detailed handling instructions including instructions on dealing with spills, breakages or accidental exposure are provided below:

- **Inhalation:** Move to fresh air. Call a physician if symptoms develop or persist.
- **Skin contact:** Wash off with soap and water. Get medical attention if irritation develops and persists.
- **Eye contact:** Rinse with water. Get medical attention if irritation develops and persists.
- **Ingestion:** Rinse mouth. Get medical attention if symptoms occur.

The following instructions will be followed in case of a spill:

For non-emergency Personnel: Unnecessary personnel away. Do not touch damaged containers or spilled material unless wearing appropriate protective clothing. Local authorities should be advised if significant spillages cannot be contained.

For emergency responders: Keep unnecessary personnel away. Wear appropriate protective equipment and clothing during clean-up.

Further instructions on cleaning up the spills are described in section J.2.

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

Spill: Contain spill and decontaminate the area using a fully virucidal disinfectant such as Klercide Sporocidal Active Chlorine (Shield Medicare) or chlorine bleach (10% f.c.) or 0.1 M sodium hydroxide for 10 minutes. Clean surface thoroughly to remove residual contamination. Following product recovery, flush area with water.

Waste Disposal: Dispose of viral stock by autoclaving at 121°C for at least 20 minutes. Dispose of infected liquid cultures by decontamination with chlorine bleach (10% f.c.) or 0.1 M sodium hydroxide for 10 minutes and then dispose of in sink. Dispose of infected tissues by incineration.

3. Methods for disposal or sanitation of plants, animals, soils, etc. that could be exposed during or after the spread
N/A. No exposure to plants, animals, soil etc. is anticipated, as GMO is only used in the hospital in human study subjects.
4. Plans for protecting human health and the environment in the event of an undesirable effect
Study subjects are monitored in the study.
For protecting the human health and the environment see answer to J.1 and J.2 above.