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
   GB

   (b) Notification number

   (c) Date of acknowledgement of notification

   (d) Title of the project
   A Phase I/II, Open label, Multicentre, Ascending Single Dose, Safety Study of a Novel Adeno-associated Viral Vector (FLT180a) in Patients With Haemophilia B

   (e) Proposed period of release
   It is anticipated that the trial will be open for enrolment from November 2017 for approximately 1 year.

2. Notifier

   Name of institution or company:

   University College London
   Joint Research Office, UCL
   Gower Street
   London WC1E 6BT

3. GMO characterisation

   (a) Indicate whether the GMO is a:

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

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fungus
animal
    - mammals
    - insect
    - fish
    - other animal

other, specify (kingdom, phylum and class)

(b) Identity of the GMO (genus and species)
Family: Parvoviridae,
Genus: Dependovirus,
Species: Adeno-associated virus dependoparvovirus

(c) Genetic stability – according to Annex IIIa, II, A(10)
In general, DNA viruses have greater genetic stability than RNA viruses. Firstly, DNA is more thermodynamically stable than RNA; secondly, replication of DNA is a much less error-prone process than the replication of RNA; and thirdly, more mechanisms exist in the host cell for repairing errors in DNA than in RNA. Gao and colleagues have found that AAV in NHPs behaves more like an RNA virus with a diversity of sequences within variable regions which are located on the capsid surface (Gao et al., 2003). This would suggest that there is evolution within infected cells, by homologous recombination of positive and negative strands from different serotypes during replication. Even minor changes in the capsid can alter tropism and immune reactivity to neutralising antibodies, and therefore a selective pressure to drive the recombination and generation of diverse capsid structures.

4. Is the same GMO release planned elsewhere in the Community (in conformity with Article 6(1)), by the same notifier?
   Yes (.)  No (X)
   If yes, insert the country code(s)

5. Has the same GMO been notified for release elsewhere in the Community by the same notifier?
   Yes (.)  No (X)
   If yes:
   - Member State of notification
   - Notification number

Please use the following country codes:
Austria AT; Belgium BE; Germany DE; Denmark DK; Spain ES; Finland FI; France FR; United Kingdom GB; Greece GR; Ireland IE; Iceland IS; Italy IT; Luxembourg LU; Netherlands NL; Norway NO; Portugal PT; Sweden SE

6. Has the same GMO been notified for release or placing on the market outside the Community by the same or other notifier?
   Yes (.)  No (X)
   If yes:
   - Member State of notification
   - Notification number
7. Summary of the potential environmental impact of the release of the GMOs.

FLT180a, the study product, is a recombinant adeno associated virus which has been designed for the treatment of haemophilia B. The viral rep and cap genes have been removed from the expression cassette and replaced with a minimal liver specific promoter and a codon optimized Factor IX (FIX) transgene with a bovine polyadenylation signal. The cassette is packaged within a novel capsid. The capsid has been designed to preferentially target human hepatocytes.

All AAVs require a helper virus such as adenovirus or herpes simplex virus to provide the necessary helper function. However, recombinant AAV such as FLT180a do not contain the Rep and Cap genes of wild-type AAV which is necessary for the virus to replicate. Therefore FLT180a cannot cause a replicative infection. FLT180a will only transduce cells of the study recipient, the FLT180a will enter the hepatocytes, the expression cassette will then enable generation of the therapeutic FIX protein.

Due to the extremely low numbers of FLT180a particles potentially released into the environment during the study, either by accident or through shedding, horizontal gene transfer is unlikely. Even if horizontal gene transfer occurred, FLT180a sequences would not confer a selective advantage to bacteria: FLT180a does not contain any prokaryotic promoters, any antibiotic- or other types of resistance genes or any genes, which would enhance or constrain their growth. Therefore, it is unlikely that the vector would interfere with the control of pathogenic microorganisms or that it would have an effect on the natural dynamics of microbial populations or the biogeochemical cycles at any given site in the environment.

Any FLT180a that is released outside of the study participant will have minimal environmental impact as FLT180a cannot cause a replicative infection even in the presence of a helper virus.

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 (.)

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2. Name
(i) order and/or higher taxon (for animals) Parvoviridae
(ii) genus Dependovirus
(iii) species Adeno-associated dependoparvovirus A
(iv) subspecies
(v) strain
(vi) pathovar (biotype, ecotype, race, etc.) AAV2
(vii) common name Adeno associated virus (AAV) type 2

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 No (.)

   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, X hosts are humans and non-human primates

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

5. (a) Detection techniques
Quantitative polymerase chain reaction (qPCR). qPCR can be used to detect vector genome sequences in a quantitative manner using primers specific either to wildtype sequences such as the rep and cap genes. This detection can be serotype selective depending on the choice of primer sequence. The presence of viral genomes, does not necessarily imply that the virus particle is infectious.

Enzyme Linked Immunosorbent Assay (ELISA) method. ELISA based methods can detected intact capsids, based on monoclonal antibodies to the viral particle capsid. However, this technique is dependent on a monoclonal antibody recognising the serotypes. There are commercial ELISA kits available, but currently they cover only a few of the AAV serotypes.

(b) Identification techniques
Polymerase chain reaction (PCR). PCR of a target region of the virus can be used to identify the species with primers unique to the serotype. In addition, sequencing of the PCR product will confirm identity.

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
AAVs are not known to be associated with a pathogenic effect and are not assigned an Advisory Committee on Dangerous Pathogens (ADCP) category, or 2,3 or 4. Wild type AAV is therefore designated a risk group 1 biological agent, defined as “one that is unlikely to cause human disease”. Recombinant AAV-based vectors are also usually classified as Biosafety Class 1.

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 as the vector is replication incompetent

(b) Generation time in the ecosystem where the release will take place:
N/A as the vector is replication incompetent

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

(d) Factors affecting reproduction: Reproduction of wild-type virus are dependent on helper viruses eg Adenovirus or Herpesvirus. Recombinant AAV is devoid of the Rep and Cap genes which are required to produce AAV.

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 can persist in the host cells as episomal concatemers, or integrated into the host cell DNA.

(b) relevant factors affecting survivability:
Parvoviruses are stable viruses that can persist in the environment for extended periods of time.

10. (a) Ways of dissemination
AAV may be transmitted through direct or indirect contact with bodily fluids from an affected individual.

(b) Factors affecting dissemination
Replication of AAV is only possible in cells that have been coinfected with a helper virus eg Adenovirus or Herpes simplex virus.
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 modifications was to remove the Rep and Cap genes from the wild type genome. The only remaining viral elements are the inverted terminal repeats (ITRs) which are necessary for production of FLT180a. Between the ITRs, sequences have been inserted to include a liver specific promoter, a partially codon optimized Factor IX (FIX) gene containing a gain of function mutation and a polyadenylation signal. This cassette is packaged into a novel capsid which has been generated by domain swapping and has been engineered for enhanced transduction of human tissue over other serotypes. FLT180a will therefore result in expression of the coagulation FIX protein, from the liver of treated subjects. The FIX protein will be secreted into the circulation and will correct the bleeding diathesis in patients with severe haemophilia B.

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
Two plasmids are used to supply all the necessary components to produce FLT180a. These were constructed using synthetic DNA and standard molecular biology techniques to form the final plasmid constructs.

(c) Host range of the vector
Plasmids have been propagated in bacteria, and selected on antibiotic resistance.

(d) Presence in the vector of sequences giving a selectable or identifiable phenotype
   Yes (X)   No (.)
   antibiotic resistance (X)
   other, specify

Indication of which antibiotic resistance gene is inserted
Kanamycin

(e) Constituent fragments of the vector
The necessary components to make FLT180a will be provided by plasmids. These plasmids will contain the transgene cassette flanked by ITRs, the AAV-2 Rep genes (for replication and packaging of the transgene cassette), the cap gene (required to make the capsid), and adenoviral helper genes (E4 ORF 6, E2a and VA RNA).

(f) Method for introducing the vector into the recipient organism
   (i) transformation (.)
   (ii) electroporation (.)
   (iii) macroinjection (.)
   (iv) microinjection (.)
   (v) infection (.)
   (vi) other, specify Transfection

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 contains a liver specific promoter, a codon optimized FIX transgene and a polyadenylation signal. The cassette is flanked by the AAV inverted terminal repeats.

   (b) Source of each constituent part of the insert.
All of the vector components have been synthesised. The FIX DNA is human in origin, but with a codon optimized sequence to enhance expression. The liver specific promoter was designed by combining minimal elements from two human liver promoters. The polyadenylation sequence is mammalian, the insert also contains ITRs which are the only viral DNA sequences in the vector. The viral ITRs are the same as AAV serotype 2.

(c) Intended function of each constituent part of the insert in the GMO
ITRs- to facilitate replication and packaging of the cassette within the capsid
Promoter- to drive and restrict expression to the liver
FIX- to replace the defective gene in haemophilia B
Polyadenylation- to add the Poly A tail to mRNA and maintain stability of mRNA.

(d) Location of the insert in the host organism
- on a free plasmid (.)
- integrated in the chromosome (.)
- other, specify Episomal concatemers in the host cell.

(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 ...

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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  (X)  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  (. )

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
       FLT180a has been modified to the original AAV by removing all viral genes, and replacing them with an expression cassette. The only viral sequences remaining are the ITRs which are non-coding sequences. Therefore even in the presence of a helper virus FLT180a cannot replicate.
(c) is the GMO in any way different from the recipient as far as dissemination is concerned?
Yes (X)  No (.)  Not known (.)
Specify
Due to the removal of viral genes FLT180a is unable to replicate even in the presence of a helper virus. Dissemination is therefore unlikely.

(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 in human are known. The expression cassette is not expected to result in any pathological effects.

2. Genetic stability of the genetically modified organism
FLT180a is a single stranded AAV vector, and as such demonstrates a high degree of DNA stability in the predominant form of episomal concatemers.

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
FLT180a can be detected by quantitative PCR
(b) Techniques used to identify the GMO
FLT180a can be detected by quantitative PCR

F. Information relating to the release

1. Purpose of the release (including any significant potential environmental benefits that may be expected)
FLT180a is to be used in a clinical trial. The study is a phase I/II open label safety and dose finding study in adults with haemophilia B. The primary objective of the study is to
determine the safety of a single IV infusion of FLT180a, the secondary objective is to the study the efficacy and optimal dose of FLT180a.

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 1:
       Royal Free Hospital
       Pond Street
       London
       NW3 2QG

   (b) Size of the site (m²):
       (i) actual release site (m²): N/A. A specific size of release cannot be defined as FLT180a will be administered to patients as part of a clinical trial
       (ii) wider release site (m²): N/A. A specific size of release cannot be defined as FLT180a 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:
       N/A as FLT180a will be administered in a hospital setting.

   (d) Flora and fauna including crops, livestock and migratory species which may potentially interact with the GMO.
       N/A as FLT180a will be administered in a hospital setting.

4. Method and amount of release
   (a) Quantities of GMOs to be released:
       Dosing is based on the body weight of the patient. It is estimated that the total amount of FLT180a would be $3 \times 10^{13} - 6 \times 10^{14}$ vector genomes depending on body weight and dose level.

   (b) Duration of the operation:
       FLT180a will be administered as an infusion over a one hour period. Shedding studies from non-human primates indicate that vector sequences clear within a few weeks. Patients will be monitored for 6 months.
(c) Methods and procedures to avoid and/or minimise the spread of the GMOs beyond the site of the release. FLT180a will be prepared and administered by trained medical professionals to patients that have met the acceptance criteria and have been enrolled into the study. All clinical waste from the procedure will be placed into yellow clinical waste bags, sealed and sent for destruction offsite by incineration.

5. Short description of average environmental conditions (weather, temperature, etc.)
N/A administration of FLT180a will occur in a 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. FLT180a 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) …
   (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)
   FLT180a will enter the patient by direct administration via an intravenous infusion. FLT180a will circulate within the patient. FLT80a will enter cells of the recipient through receptor mediated binding. FLT180a will be uncoated within the cell and the genome transported to the nucleus. FLT180a is a single stranded DNA virus and as such for functional expression the single stranded copies need to be converted into a double strand from by either second strand synthesis or annealing of positive and negative DNA strands. Translation of the viral genome will be predominately restricted to hepatocytes by the liver specific promoter. FIX protein will be translated and secreted from the cell and into the circulation. The expression of FIX protein will ameliorate the bleeding phenotype in patients with a phenotype of severe haemophilia B, which is characterized by spontaneous bleeding events.

3. Any other potentially significant interactions with other organisms in the environment.
   NO
4. Is post-release selection such as increased competitiveness, increased invasiveness for the GMO likely to occur?
Yes (.)  No (X)  Not known (.)

Give details
FLT180a is a replication defective AAV and contains no selection markers, therefore 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
FLT180a is a replication defective AAV and is not expected to spread to the environment in any significant quantities

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
None
(i) order and/or higher taxon (for animals) ...
(ii) family name for plants ...
(iii) genus ...
(iv) species ...
(v) subspecies ...
(vi) strain ...
(vii) cultivar/breeding line ...
(viii) pathovar ...
(ix) common name ...

7. Likelihood of genetic exchange in vivo

(a) from the GMO to other organisms in the release ecosystem:
FLT180a will be administered to the patients enrolled in the clinical trial by intravenous infusion. The vast majority of the FLT180a will be taken into the cells of the patient, with hepatocytes a dominant target. The FLT180a expression cassette will be maintained predominantly as episomal concatamers. Factor IX expression will be restricted to expression in hepatocytes by the promoter. FLT180a will be shed in the study patients for a limited duration of time and will be monitored until samples are negative.

(b) from other organisms to the GMO:
FLT180a does not contain the Rep and Cap genes that are required for replication, therefore impact on other organisms are minimal.

(c) likely consequences of gene transfer:
The gene transfer will cause FIX protein to be expressed from the hepatocytes of clinical trial subject. This will correct the bleeding diathesis allowing the patients to discontinue their existing protein replacement therapy, or reduce the interval between prophylactic administrations.
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 studies conducted with FLT180a

9. Possible environmentally significant interactions with biogeochemical processes (if different from the recipient or parental organism)
   FLT180a is not known to have any impact on biogeochemical processes.

H. Information relating to monitoring

1. Methods for monitoring the GMOs
   Viral shedding will be closely monitored during the clinical trial until negative samples are obtained. Patients will also undergo routine monitoring for safety and efficacy.

2. Methods for monitoring ecosystem effects
   FLT180a will be monitored by viral shedding with quantitative PCR.

3. Methods for detecting transfer of the donated genetic material from the GMO to other organisms.
   The transfer of the genetic material of FLT180a will be assessed by quantitation of the levels of FIX expression.

4. Size of the monitoring area (m²)
   N/A as will be administered to patients in a clinical trial.

5. Duration of the monitoring
   Viral shedding will be assessed for up to 26 weeks.

6. Frequency of the monitoring
   3 times in the first 10 days and then weekly until vector sequences are negative on two consecutive occasions.

I. Information on post-release and waste treatment

1. Post-release treatment of the site
   Any surfaces contaminated with FLT180a will be disinfected according to local rules relating to biohazardous substances.

2. Post-release treatment of the GMOs
   All materials that have been in contact with FLT180a will be disposed of via the sharp bins or yellow clinical waste bags according to the local rules. Surfaces will be disinfected following local rules.

3. (a) Type and amount of waste generated
Glass vials containing FLT180a. Number of vials is dependent on the dose and weight of the study participant. Materials used for the administration, infusion sets and personal protective equipment as worn by the clinical staff.

3. (b) Treatment of waste
   Sharp materials will be placed into yellow sharp bins and will be disposed of according to the local rules. All other materials will be placed into yellow clinical waste bags which will also be disposed of according to local rules for biohazardous substances.

J. Information on emergency response plans

1. Methods and procedures for controlling the dissemination of the GMO(s) in case of unexpected spread.
   If there is an accidental spillage of FLT180a the spill will be treated by following local rules for a biological hazardous spill. Spill kits will be located in all areas where the vector is handled.

2. Methods for removal of the GMO(s) of the areas potentially affected.
   The surfaces will be disinfected with an appropriate disinfectant according to local rules.

3. Methods for disposal or sanitation of plants, animals, soils, etc. that could be exposed during or after the spread
   N/A administration of FLT180a will occur in a controlled hospital setting with trained staff.

4. Plans for protecting human health and the environment in the event of an undesirable effect
   Staff will follow local rules for handling and disposal of genetically modified organisms and biological hazards. All patients will be monitored for adverse events as detailed in the clinical trial protocol.