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/16/002
(c)	Date of acknowledgement of notification	28/4/2016
(d)	Title of the project: *A single dose clinical trial to study the safety of ART-I02 in patients with Rheumatoid Arthritis*	
(e)	Proposed period of release:	From Q3/2016 until Q4 2025

2. Notifier

Name of institution or company: *Leiden University Medical Center*
Albinusdreef 2
2333 ZA Leiden

The Netherlands

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 Adeno-associated virus

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

Genus: Dependovirus
Species: Adeno-associated virus

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

ART-I02 is a replication-deficient viral vector. It is produced by transient transfection of a vector plasmid containing the ART-I02 vector and helper plasmid providing the AAV5 cap and AAV2 rep genes as well as helper sequences E2A, E4, en VA from adenovirus type 5, which are essential for production, in trans. The genetic stability of the ART-I02 vector during production was confirmed by sequencing of the genome of ART-I02 clinical material and comparing it with the corresponding sequence in pART-I02 sequence. Furthermore, the clinical batch of ART-I02 tested negative for replication competent AAV2 and AAV5 (no detectable replication competent AAV2 and AAV5 at the limit of detection of 10 infectious units into 2x10e10 vg/mL of ART-I02). In addition, ART-I02 drug substance is tested for viral genome titer and identity confirming the stability of ART-I02.

Upon administration to human subjects, ART-I02 infects target cells, but no new virus particles are being formed. In the cell, multiple ART-I02 genomes assemble to form larger double stranded DNA concatemers. These concatemers persist in the cell as stable episomal structures and are transcriptionally active.

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 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 (X)

If yes:

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

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

rAAV vectors have a very good safety profile. Their parent virus, wild-type AAV is not associated with any disease pathology in humans and can only infect humans and other primates. rAAV vectors are completely devoid of viral genes. Therefore the induction of an immunological response to the transduced cells is minimized, and this allows for long term

expression of the transgene in AAV transduced cells. Wild type AAV2 and AAV5 virus are unable to replicate unless in the presence of a helper virus, such as adenovirus or herpes virus. As ART-I02 does not contain any of the viral genes necessary for replication (rep, cap), it is replication defective even in the presence of a helper virus. Only in the hypothetical situation that a cell is co-infected with ART-I02, wild type AAV, and helper virus, replication of (disseminated) ART-I02 will occur. Thus, the pathogenicity of ART-I02 is expected to be even less than that of its parental AAV5 or AAV2 viruses, which are already considered non-pathogenic.

The effects of unintended exposure of human beings to ART-I02 are the same as those from intended exposure to subjects (patients): effects related to the expression of hIFN-β protein, induction of anti-AAV5 immune responses, and potential consequences of insertional mutagenesis and vertical transmission. The likelihood that these effects occur and/or cause harmful effects are negligible, because unintended exposure of human beings to (infectious) ART-I02 can only be many orders of magnitude lower than the subjects' exposure due to the replication incompetence of ART-I02 and the limited amount and duration (if any) of infectious ART-I02 shedding from subjects.

In conclusion, taking into consideration the low risk ART-I02 presents to the environment and due to the preventative and protective measures in compliance will all applicable GMO and institutional regulations to reduce the exposure to the vector, it is justified to classify the overall risk for the environment as negligible.

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

1. Recipient or parental organism characterization:

(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)	*Family of Parvoviridae*
(ii)	genus	*Dependovirus*
(iii)	species	*Adeno-associated virus*

(iv)	subspecies	….
(v)	strain	*serotype 2 and 5*
(vi)	pathovar (biotype, ecotype, race, etc.)	…
(vii)	common name	*AAV2/5*

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:
Approximately 50 to 80% of the European human population is seropositive to at least one AAV serotype

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	human

Humans are the natural hosts

If the organism is an animal: natural habitat or usual agroecosystem:
Not applicable

5. (a) Detection techniques
Q-PCR with primers specific to detect virus DNA

(b) Identification techniques
Q-PCR with primers specific to detect virus DNA, DNA sequencing

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
Wild type AAV2 and AAV5 have been classified as pathogenicity class 2 according to the Dutch regulations "Regeling GGO 2013 Appendix 4.1"

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

AAV2 and AAV5 are not pathogenic, toxigenic, virulent, allergenic or a carrier (vector) of a pathogen. These are replication-defective viruses and require the presence of a helper virus (such as adenovirus or herpes) for replication. They are known only to infect humans and other primates in the environment. AAVs do not activate latent virus and are not able to colonize other organisms.

8. Information concerning reproduction

(a) Generation time in natural ecosystems:
Because replication of AAV2 and AAV5 in an infected host is dependent on co-infection with a helper virus such as adenovirus, the generation time of wild-type AAV in a natural ecosystem will vary from weeks to years, depending on the timing of the co-infection.

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

(c) Way of reproduction: Sexual .. Asexual X

(c) Factors affecting reproduction:
Permissive host cell, reproduction of wild-type AAV is dependent on co-infection with helper virus (such as adenovirus or herpesvirus)

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
Upon infection of cells by AAVs, their genomes form concatemers that remain episomal for extended periods of time.

(b) relevant factors affecting survivability:
AAV is a non-enveloped virus that is relatively stable in the environment and stable to desiccation. AAV is sensitive to appropriate virucidal disinfectants, such as 1000 PPM chlorine solution. Replication of wild-type AAV is dependent on co-infection of helper viruses such as adenovirus or herpes-simplex virus. In presence of helper virus, AAV undergoes productive infection characterized by genome replication, viral gene expression and virion production. In absence of a helper virus co-infection, the virus DNA it will persist within infected cells in episomal form or may integrate into the host cell genome. In both cases the virus remains latent.

10. (a) Ways of dissemination
Dissemination may occur by inhalation (aerosolized droplets), contact with mucous membranes (eyes, nose and mouth) fecal-oral transmission.

(b) Factors affecting dissemination
Co-infection with a helper 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)
None

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
ART-I02 is a recombinant pseudotyped AAV2/5 vector with an inserted human interferon (hIFN-β) gene. The genetic modification involves removal of wild type AAV2 rep and cap genes, and replacement with an expression cassette encoding hIFN-β in between the inverted

terminal repeats (ITRs) of AAV2. This recombinant genome is packaged in the capsid proteins of AAV5.
As such, all of the viral gene-encoding sequences have been removed leaving only the two small AAV2 ITR sequences at the 5' and 5' ends flanking the expression cassette consisting of a NF-kB responsive promoter, hIFN-β gene, and human growth hormone polyA sequence. The intention of the modifications is to render the virus replication-incompetent, to maintain the tropism of AAV5, and to allow expression of the therapeutic hIFN-β gene during inflammation.

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; pART-I02 and pDP5-kan3.
pART-I02 contains the recombinant ART-I02 genome and pDP5-kan3 contains the AAV5 cap and AAV2 rep genes as well as helper sequences E2A, E4, en VA from adenovirus type 5 (pDP5-kan3 sequences are not part of the ART-I02 viral genome).

(c) Host range of the vector
Bacterial 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
There are no antibioticresistance genes in the ART-I02 rAAV vector. However, kanamycin resistant genes are present in the two plasmids used in the manufacturing process of ART-I02.

(e) Constituent fragments of the vector

pART-I02:

- *The ART-I02 vector, consisting of the AAV2 inverted terminal repeats (ITRs) and the expression cassette, consisting of the hIFN-β gene under the transcriptional control of the nuclear factor kappa B (NF-κB) responsive promoter and a human growth hormone polyadenylation signal (hGHpA)*
- *The aminoglycoside 3'-phosphotransferase gene, conferring kanamycin resistance*
- *A bacterial origin of replication, a M13/F1 origin of replication,*
- *A 4572 bp fragment of bacteriophage lambda DNA (backbone stuffer). The lambda DNA increases the size of the plasmid backbone to 8484 bp, which reduces backbone packaging during AAV vector production*

pDP5-kan3:

- *The aminoglycoside 3'-phosphotransferase gene, conferring kanamycin resistance*
- *AAV2 rep gene and AAV5 cap gene under control of the Mouse mammary tumor virus long terminal repeat promoter*
- *Adenovirus E2 and E4 genes and Adenovirus VA RNAs required for helper function*
- *L4 100K, L4 pVIII, and fiber genes from adenovirus*
- *pUC origin of replication*

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

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

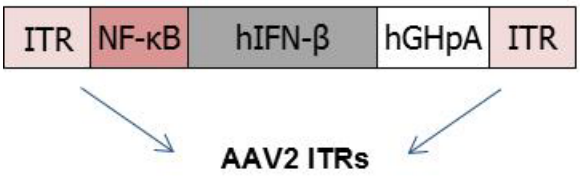
5. If the answer to question B.3(a) and (b) is no, what was the method used in the process of modification?

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

6. Composition of the insert

(a) Composition of the insert

The ART-I02 vector, consisting of the AAV2 inverted terminal repeats (ITRs) and between the ITRs, the expression cassette, consisting of a nuclear factor kappa B (NF-κB) responsive promoter, the hIFN-β gene and a human growth hormone polyadenylation signal (hGHpA)



(b) Source of each constituent part of the insert

Part of insert	Source	Intended function
Human Interferon β	*Human fibroblast*	*cDNA encoding the therapeutic protein, i.e. the hIFN-β protein*
NF-kB promoter	*Motif derived from HIV LTR fused to minimal CMV promoter*	*Promotes and enhance transcription during inflammation*
Poly adenylation signal	*Human growth hormone*	*Effective translation/stabilization of hIFN-β mRNA*
Inverted terminal repeat	*AAV2*	*Enable packaging into AAV capsids*

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

(a) Location of the insert in the host organism

- on a free plasmid (.)
- integrated in the chromosome (.)
- other, specify *The insert described is the entire ART-I02 vector genome and encodes a human protein.*

(b) 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)	*primate*
(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 (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 (.) No (.) Not known (.)
Not applicable as the transfer is a human protein (hIFN-β protein) to humans

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

ART-I02 virus particles have similar ex vivo survival characteristics in the open environment as wild type AAV5, as the ART-I02 capsid particle is identical to that of AAV5.

(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

Due to the removal of the rep and cap genes ART-I02 is unable to reproduce/replicate even in the presence of wild-type AAV helper virus (Adenovirus)

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

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

Specify

The viral capsid proteins have the same dissemination/tropism as the parent AAV5 virus.

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

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

Specify: *Neither wild type AAV nor ART-I02 are pathogenic to humans or the environment.*

2. Genetic stability of the genetically modified organism

All tests for identity, purity, and quality have confirmed the stability of ART-I02. Upon administration to human subjects, ART-I02 infects target cells, but no new virus particles are being formed. In the cell, multiple ART-I02 genomes assemble to form larger double stranded DNA concatemers. These concatemers persist in the cell as stable episomal structures and are transcriptionally active.

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)

ART-I02 is considered non-pathogenic and not harmful to human, animal and plant health for the following reasons:

- *ART-I02 is replication-defective, by deletion of the rep and cap genes.*
- *Similar to its parent viruses, host tropism of ART-I02 is restricted to humans/primates.*
- *Immune responses raised against the AAV5 viral capsid are asymptomatic.*
- *Significant levels of adaptive immunity exist in the human population.*
- *Adaptive immunity in humans naïf to AAV will reduce the likelihood of further dissemination.*
- *ART-I02 expresses a human protein, the human interferon β protein, upon infection of cells.*
- *In animal studies (rats and monkeys), injection of high levels of ART-I02 were well tolerated.*
- *Shedding of ART-I02-derived DNA is expected but shedding of infectious vector particles is considered highly unlikely.*
- *The recombinant AAV5 vector does not have the capacity for colonization, although it will persist within infected cells in episomal form.*

4. Description of identification and detection methods

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

ART-I02 can be detected and quantified using a Q-PCR method with specific primers for ART-I02

(b) Techniques used to identify the GMO

ART-I02 can be identified by Q-PCR with primers specific for vector (most sensitive method). In addition, enzyme restriction analysis and sequencing of the vector can be performed.

F. Information relating to the release

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

The purpose of the release is a first in human clinical trial to investigate the safety of ART-I02 after an intra-articular injection in one inflamed joint of human subjects with rheumatoid arthritis. There are no environmental benefits.

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 ART-I02 vector will be administered by intra-articular injection in the joint. Shedding of vector DNA in urine, saliva, feces and semen may occur during the first weeks after injection, however shedding of infectious ART-I02 is limited, if any.

3. Information concerning the release and the surrounding area

(a) Geographical location (administrative region and where appropriate grid reference):
University Medical Center Leiden (LUMC)
Albinusdreef 2, 2333 ZA,
Leiden,
The Netherlands

(b) Not applicable

Size of the site (m^2): … m^2
(i) actual release site (m^2): … m^2
(ii) wider release site (m^2): … m^2

Within the LUMC only storage, dose preparation of ART-I02 and diagnostic testing on biological samples from patients treated with ART-I02 will be performed The administration of the vector is at a different location..

(a) Proximity to internationally recognised biotopes or protected areas (including drinking water reservoirs), which could be affected:
Not applicable

(b) Flora and fauna including crops, livestock and migratory species which may potentially interact with the GMO
Not applicable

4. Method and amount of release

(a) Quantities of GMOs to be released:
At maximum 50 patients will be enrolled in the proposed clinical study. The total dose ranges between 1 x10^{11} vector genomes (vg, determined by Quantitative PCR) and 5 x10^{13} vg per subject. The quantities that will be released into the environment by shedding will be a very small proportion of the total number of viral genomes injected, of which the majority, if not all, is not infectious.

(b) Duration of the operation:
Each subject will receive one single injection in the joint, this will take less than 30 minutes. Based on preclinical biodistribution and shedding data with ART-I02, the maximum DNA shedding duration after treatment will not exceed 12 weeks.

(c) Methods and procedures to avoid and/or minimise the spread of the GMOs beyond the site of the release
The GMO ART-I02 will be supplied to LUMC Pharmacy Department. All personnel involved in handling of ART-I02 will be trained in best biosafety practices, in compliance with all applicable GMO and institutional regulations.
The dose preparation will be performed in a cleanroom according to local Biosafety regulations and procedures. All handlings will be performed in a Class II biological safety cabinet and personnel will wear protective clothing and gloves during the entire procedure. The syringe with ART-I02 will be disinfected before packaging, and after disinfection the syringe will be packed in a secondary closed, fracture and leak proof container for

transportation to the administration room. Before the container leaves the containment laboratory, the container will be decontaminated.

The intra-articular injections will be performed by trained medical professionals according to standard aseptic procedures. Personnel performing the preparation and administration will wear adapted protective clothing (gown, gloves, mask and goggles). Collection and disposal of biologically hazardous material and cleaning procedures for residual (unused) ART-I02, sharps, waste and other materials that (potentially) have been into contact with ART-I02 will be in compliance with applicable GMO and institutional regulations.

5. Short description of average environmental conditions (weather, temperature, etc.)
The clinical trial will be performed at a single investigational site in the Netherlands. The risk of release of ART-I02 into the environment is unrelated to climate characteristics. The preparation and injection of ART-I02 will take place in environmentally controlled hospital rooms (ambient indoor conditions for administration). ART-I02 is stored ≤-65°C prior to administration.

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.
This is the first study where ART-I02 will be used, therefore no data on potential environmental and human health impacts are available.

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)	*Primate*
(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)
The target cells for transduction are human synovial fibroblasts present in the (inflamed) joint. This should result in transgene expression and synthesis and secretion of human interferon β from the fibroblasts into the synovial tissue and joint space. It is expected that expression of the therapeutic protein (hIFNβ) will reduce inflammation and bone and cartilage destruction. ART-I02 genomes persists in the transduced joint cells at the site of injection in an episomal form.

3. Any other potentially significant interactions with other organisms in the environment
No such interaction is expected.

4. Is post-release selection such as increased competitiveness, increased invasiveness for the GMO likely to occur?

Yes (.) No (X) Not known (.)
Give details
ART-I02 is replication incompetent, unable to form new vector particles, and is therefore not subject to selective pressure

5. Types of ecosystems to which the GMO could be disseminated from the site of release and in which it could become established
Although ART-I02 could theoretically infect the cells of other mammals, it cannot initiate a productive replication cycle because it is replication incompetent and therefore does not establish in other 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
None

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:
Negligible.

(b) from other organisms to the GMO:
Negligible

(a) likely consequences of gene transfer:
Expression of hIFNβ

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 references available

9. Possible environmentally significant interactions with biogeochemical processes (if different from the recipient or parental organism)
None known or predictable since wild type AAV is not known to be involved in any biogeochemical process

H. Information relating to monitoring

1. Methods for monitoring the GMOs

In order to investigate ART-I02 pharmacokinetics in humans, urine, blood, saliva, feces and semen will be analyzed. The amount of vector DNA will be detected with a sensitive qualified Quantitative PCR method.

2. Methods for monitoring ecosystem effects
Not required

3. Methods for detecting transfer of the donated genetic material from the GMO to other organisms
The method for detecting transfer of the donated genetic material to other organisms will be Quantitative PCR. Since vector DNA is expected in urine, saliva, feces, blood, and semen, for a number of weeks after administration of the GMO ART-I02 to the test subject, samples from the treated patients will be collected to determine presence of vector DNA. However, transfer of donated genetic material from the patient to other organisms is not expected.

4. Size of the monitoring area (m^2)
… m^2
Not applicable. Only subject's body fluids will be monitored after administration.

5. Duration of the monitoring
The biological samples from each patient who has received ART-I02 will be monitored until three consecutive samples have been tested negative for the presence of vector DNA.

6. Frequency of the monitoring
At regular visits between day 1 and week 24 after administration according to the clinical study protocol blood, saliva and urine sample will be taken.

I. Information on post-release and waste treatment

1. Post-release treatment of the site
After administration of ART-I02 to the subject, non-disposable materials (tools, devices) are cleaned with 1000 ppm chlorine after usage and then autoclaved, if possible. Contact surfaces are disinfected with 1000 ppm chlorine solution. In case of a spillage all potentially contaminated surfaces will be disinfected preferably with 1000 ppm chlorine solution. In case of spillage on the floor, the floor will be disinfected using 1000 ppm chlorine solution.

2. Post-release treatment of the GMOs
Any unused ART-I02 will be stored in accordance to local GMO containment procedures, until in a dedicated ≤-65°C freezer labelled with a biohazard sign until shipment for further analysis at external laboratories.

3. (a) Type and amount of waste generated
The following items will be discarded in a UN3291 certified container as clinical waste:
- *Syringe and needle*
- *Sterile cover of the ultrasound probe*
- *Used gloves*
- *Used FFP2 mask*
- *Disposable gown*
- *Used gauzes*

- *Plasters*
- *Tubes and other disposables used for collection of biological samples*

3. (b) Treatment of waste
All waste that has potentially been exposed to ART-I02 during all procedures will be collected as clinical waste and will be discarded in a UN3291certified container to an incinerator for immediate incineration. The packaging cannot be opened and is leak-proof. Before the containers are transported the outside will be decontaminated.

J. Information on emergency response plans

1. Methods and procedures for controlling the dissemination of the GMO(s) in case of unexpected spread.
In case of accidental spillage of ART-I02 (e.g. on the workbench or on the floor), local procedures will be followed to contain and immediately disinfect the spill to prevent further spread. In brief:
- *Put on appropriate protective clothing and equipment*
- *Lay absorbing material around the spill to prevent further spreading*
- *Soak absorbent material with 1000 ppm chlorine solution and leave for at least 5 min.*
- *Wipe up the spill with absorbent material, starting from the outside and moving*
- *Inwards.*
- *Remove the absorbing material*
- *Soak absorbent material with 1000 ppm chlorine solution*
- *Disinfect the contaminated area with the absorbent material soaked in 1000 ppm*
- *chlorine solution*
- *Stainless steel surfaces will be rinsed with water regarding corrosion*
- *Discard all materials used in the spill clean-up as clinical waste, UN3291.*

Accidental exposure of health care professionals to ART-I02 should be treated according to the following measures:
- *Needle stick injury: Encourage bleeding of the wound. Wash injection area well with water and physiological salt solution. Desinfect the wound subsequently with 70% alcohol.*
- *Eye contact: Immediately flush eyes with water. Assure adequate flushing by separating the eyelids with fingers. Flush from the nose to the outer side to prevent contamination of the other eye.*
- *Mucous membrane contact: Immediately flush with water.*
- *Skin contact: Wash-off with a gauze soaked in a 0.5% chlorohexidine in 70% alcohol solution and subsequently wash with water.*

2. Methods for removal of the GMO(s) of the areas potentially affected
See 1 above; All materials used in the spill clean up will be discarded as clinical waste, UN3291, and will be incinerated

3. Methods for disposal or sanitation of plants, animals, soils, etc. that could be exposed during or after the spread
Not applicable

4. Plans for protecting human health and the environment in the event of an undesirable effect
Not applicable