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/11/25**
(c) Date of acknowledgement of notification 08/04/2011
(d) Title of the project
The project is a clinical trial entitled "Phase I-II therapeutic vaccination clinical trial in patients with chronic hepatitis C by administration of autologous dendritic cells transduced with an adenoviral vector encoding NS3 protein"

…

(e) Proposed period of release From 2011until 2013

2. Notifier

Name of institution or company: Clínica Universidad de Navarra

3. GMO characterisation

(a) Indicate whether the GMO is a:

viroid	(.)
RNA virus	(.)
DNA virus	(x)
bacterium	(.)
fungus	(.)
animal	
- mammals	(.)
- insect	(.)
- fish	(.)
- other animal	(.)

specify phylum, class …

(b) Identity of the GMO (genus and species)
AdNS3 is a recombinant defective adenovirus derived form human adenovirus type 5 , Family *Adenoviridae*, Genus *Mastadenovirus*…

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

AdNS3 is an adenovirus with genetic stability. This is verified at different steps during the production process, and incluyes identity análisis (genomic sequencisng), purity, biological potency and safety (análisis of replicative competent adenovirus), both for inicial cell lysates and for vialed final product.

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.

…

There are no data concerning potential environmental impact of AdNS3, because this is the first time it is used.

There are some data concerning other defective recombinant adenoviruses used in clinical trials, both directly administered and in cell-therapy strategies (contained inside cell vehicles). However there is no scientific reason to suppose that the use of NS3 as transgene leads to changes in distribution, viral shedding, replicative ability compared to other transgenes used in the same viral vector, so that the observations done for the first generation adenoviral vectors should be considered applicable to AdNS3. Recombinant defective adeoviruses have been widely used in previous clinical trial, and they have been directly administred or used as vectors in cellular therapies. Viral shedding through urine and saliva has been documented, but it usually disappears few days after adenovirus administration. In some cases it has been detected two weeks after administration, mainly in those groups of patients receiving high viral doses which were partially immunocompromissed, as occurs in some cancer patients, where adaptive immunity (responsible for viral clearance) is impaired. As an example, an adenoviral vector encoding p53 used in phase I to III clinical trials in more than 200 patients, could be detected by ELISA against hexon antigen in urine and

saliva in four patients. Adenovirus with infectious capacity could only be confirmed in one of these patients. Of note, replicative adenovirus, indicative in vivo recombination events, were not detected (Grace, 2000). Similarly, in another experience with patients with lung cancer, infectious particles were not detected in body secretions beyond day 2, they were never detected in urine and no transmission to relatives or clinical staff was observed (Escudier, 2000). Previous clinical study carried out in CLinica Universidad of Navarra, have failed to detect viral shedding in biological sample (sputum, saliva, urine and stool) among patients treated with dendritic cells transduced with adenovirus codifying the IL-12 gene. In some cases a very low amount of viral particles have been detected in blood, but it has never been persisted for more than 120 minutes after administration.

Since humans are the only host for type 5 adenovirus and a high percentage of population has been in contact with this virus, developing antiviral immunity, a higher potential impact of AdNS3, as compared to wild type adenovirus, is not expected.

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)	Adenoviridae…
(ii)	genus	Mastadenovirus…
(iii)	species	Human adenovirus type 5…
(iv)	subspecies	Type C adenovirus…
(v)	strain	…
(vi)	pathovar (biotype, ecotype, race, etc.)	…
(vii)	common name	Human adenovirus type 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:

Atlantic	x..
Mediteranean	x..
Boreal	..
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 (.)

(d) Is it frequently kept in the country where the notification is made?
Yes (.) No (.)

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 type 5 adenovirus only replicates in human cells

(b) If the organism is an animal: natural habitat or usual agroecosystem:
…

5. (a) Detection techniques
…
Human type 5 adenovirus is detected by real-time PCR from tissue samples, cultures, fluids or any other biological samples, by using primers specific for E1 region. The sensitivity of this technique is of 100 DNA copies.

(b) Identification techniques
…
Human type 5 adenovirus is identified by using primers belonging to E1 region, which is absent in recombinant defective adenoviruses.

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

Human type 5 adenovirus is classified as biosafety level 2…

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

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

If yes:

(a) to which of the following organisms:

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

(b) give the relevant information specified under Annex III A, point II. (A)(11)(d) of Directive 2001/18/EC

…

Human Adenovirus type 5 is regarded as class II bio-safety level. Adenovirus infection could lead to acute upper respiratory syndrome of various degrees and pharyngoconjunctival fever. The most common symptoms are fever, rhinitis, faringitis, cough and conjuntivitis, including exudative no-streptococcal faringitis among children. Laringitis, croup, bronchiolitis and severe pneumoniae are regarded as the most serious complications. Human Adenovirus 5 has been shown to be endemic in the world with a maximum incidence between autumn and spring in temperate zones. Humans are the natural hosts of the infection. The most common transmission route is via inhalation or uptake in the eye of aerosols produced through couching/sneezing by infected individuals. Adenovirus transmission can also occur via the fecal-oral route, but this transmission route requires intimate contact and is unusual. Incubation is 1-10 days and the minimum infective dosis is 150 PFU.

8. Information concerning reproduction

(a) Generation time in natural ecosystems:
Not applicable, since it is not found in natural habitats…

(b) Generation time in the ecosystem where the release will take place:
Not applicable…

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

(c) Factors affecting reproduction:
…

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 …	

Not applicable…

(b) relevant factors affecting survivability:

Infectious capacity of adenovirus decays at room temperature, although outside of its natural host type 3 adenovirus can survive up to ten days in paper at room temperature, and type 2 survives around 3-8 weeks in surfaces at the same temperature. Adenoviruses are susceptible to different chemical agents, such as 1% sodium hypochloride and 2% glutaraldehide, commonly used as disinfectants. Also, they are sensitive to heat, as a physical inactivation method. Thus, a complete elimination is achieved by autoclaving samples at 121 ºC during 15 minutes.

…

10. (a) Ways of dissemination

Concerning dissemination, humans constitute the natural host. No other natural vectors or zoonosis have been described. Minimal infectious dose is of 150 plaque forming units by intranasal route. Transmission is via inhalation or uptake in the eye of aerosols produced through couching/sneezing by infected individuals. Adenovirus transmission can also occur via the fecal-oral route, but this transmission route requires intimate contact and is inusual.

(c) Factors affecting dissemination

Dissemination is dose-dependent, affected by aerosol formation and proximity of contact.

…

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)

A/ES/97/04, A/ES/99/04, A/ES/99/08, A/ES/02/08, A/ES/00/17, A/ES/01/17, A/ES/05/14

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

AdNS3 is a recombinant, defective, non-replicative adenovirus. E1 and E3 adenoviral genes have been deleted from the adenoviral genome to make AdNS3, resulting in an adenovirus which non-replicative, except in HEK293 cells, able to complement in trans the E1 deficiency. Also, exogenous sequences including a cassette for expression of hepatitis C virus (HCV) NS3 protein has been inserted in AdNS3 genome. This cassette contains the cytomegalovirus promoter, HCV NS3 region and a SV40 polyadenylation signal. All these modifications are intended to yield a non-replicative adenovirus able to express HCV NS3 protein. If, as described in the present document, AdNS3 is used to infect dendritic cells, these cells will present antigenic epitopes to lymphocytes to activate anti-HCV immune responses.

…

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

Vector used is plasmid pAdNS3, obtained through homologous recombination of the following plasmids: a) pShuttle/CMV/NS3, previously prepared by inserting HCV between nucleotides 4889 and 4923 of plasmid pShuttle/CMV (purchased from Agilent), and b) pAdEasy-1 (also purchased from Agilent). …

(d) Host range of the vector

pAdNS3 replicates in BJ5183 E. coli. Linearized version of pAdNS3, obtained after digestión with Pac I, originates a sequence that, when transfected into HEK293 cells leads to the production of AdNS3 adenoviral particles.

…

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

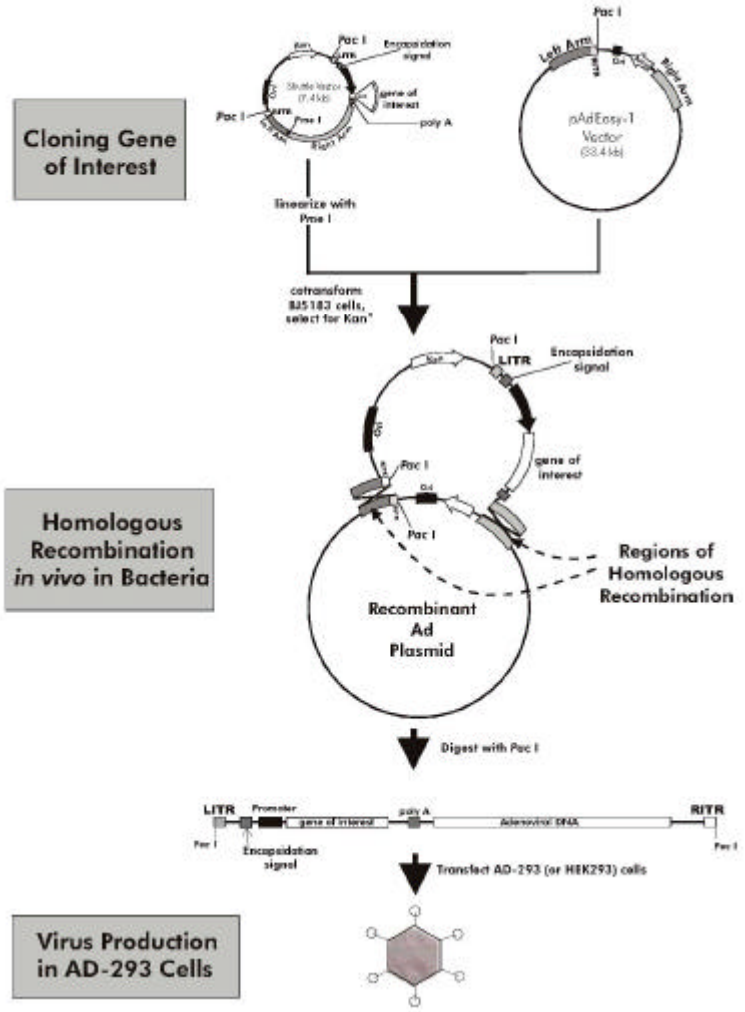
Yes (x) No (.)

antibiotic resistance (x)

other, specify …

Indication of which antibiotic resistance gene is inserted
Kanamycin. However, this resistance gene present in pAdNS3 is no longer present in AdNS3 genome
…

(e) Constituent fragments of the vector
pAdNS3, obtained by homologous recombination of: a) pShuttle/CMV/NS3, previamente made by inserting HCV NS3 between nucleotides 4889 and 4923 of plasmid pShuttle/CMV, and b) pAdEasy-1. pAdNS3 contains element shown in the following Figure, being NS3 the gene of interest.



…

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

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

Transfection of the digested, linearized version of pAdNS3 into HEK293 cells originates AdNS3 adenoviral particles
…

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

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

6. Composition of the insert

(a) Composition of the insert

The insert contains the elements from plasmid pShuttle/CMV/NS3 which recombine with plasmid pAdEasy: CMV promoter, HCV NS3 region and SV40 polyadenylation signal.…

(b) Source of each constituent part of the insert

Both CMV promoter and SV40 polyadenylation signal come from the commercial pShuttle/CMV plasmid from Agilent, subsequently used to make plasmids pShuttle/CMV/NS3 and pAdNS3. HCV NS3 encoding region corresponding to a genotype 1b Japanese isolate (HCV-J; GenBank D90208) was synthesized by Genscript (www.genscript.com), using sequence optimization to increase usage of codons more frequently used in human cells. Additional optimization was dealing with GC content, mRNA secondary structure, cryptic splicing sites, internal chi sites and ribosomal binding sites, RNA instability motifs, inhibition sites and repeat sequences. NS3opt coding sequence's ORF was placed to appropriate Kozak sequence. Accuracy of synthesis was verified at GenScript by partial sequencing of the insert.

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

Both CMV promoter and SV40 polyadenylation signal are responsible for expression of the gene of interest, in this case HCV NS3, allowing recognition by RNA polymerase for transcription and increasing stability of newly synthesized mRNA molecules. HCV NS3 is gene to be expressed by AdNS3-infected cells, an antigenic protein behaving as target for antiviral T cell responses.

(d) Location of the insert in the host organism

- on a free plasmid (.)
- integrated in the chromosome (.)
- other, specify …

Integrated in the genome of AdNS3

(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		(x)
DNA virus		(.)
bacterium		(.)
fungus		(.)
animal		
-	mammals	(.)
-	insect	(.)
-	fish	(.)
-	other animal	(.)
	(specify phylum, class)	…
other, specify		…

2. Complete name

(i)	order and/or higher taxon (for animals)	…
(ii)	family name for plants	Flaviviridae…
(iii)	genus	Hepacivirus…
(iv)	species	Hepatitis C virus…
(v)	subspecies	…
(vi)	strain	…
(vii)	cultivar/breeding line	…
(viii)	pathovar	…
(ix)	common name	Hepatitis C virus…

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

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

If yes, specify the following:

(b) to which of the following organisms:

humans	(x)
animals	(x)
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 (x) No (.)

If yes, specify

Hepatitis C virus is classified as biosafety level 3*. However, among those organisms belonging to level 3, those noted as 3* are certain biological agents which may present a limited risk of infection for workers because they are not normally infectious by air-borne route.…

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

Yes (.) No (x) 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 As AdNS3 can replicate only in HEK293 cells, the dissemination ability of AdNS3 is lower than that of human adenovirus type 5 , so that differently from the wild-type, AdNS3 infection cannot be transmitted to humans.

(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

Although wild type human adenovirus can reproduce in a wide variety of human cells, AdNS3 can only reproduce in HEK293 cell line.

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

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

Specify As AdNS3 can replicate only in HEK293, its dissemination ability is lower than wild type human adenovirus type 5.

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

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

Specify

AdNS3 in unable to replicate except in HEK293 cells. Thus, it can only cause primary infections, preventing the risk of transmission to secondary cells, which presumably results in lower or nil pathogenicity. For this reason, there are not reported cases of adenoviral illness among patients recruited in gene therapy clinical

trial that have used adenoviral vectors. The reported adverse effects are associated to the cytokine liberation as consequence of primary infection.

2. Genetic stability of the genetically modified organism
AdNS3 is an adenovirus with genetic stability.…

3. Is the GMO significantly pathogenic or harmful in any way (including its extracellular products), either living or dead?
Yes (x) No (.) Unknown (.)

(a) to which of the following organisms?

humans	(x)
animals	(.)
plants	(.)
other	…

(c) give the relevant information specified under Annex III A, point II(A)(11)(d) and II(C)(2)(i)

As previously mentioned, human adenovirus type 5 is regarded as class II bio-safety level. Acute infection is characterized by upper respiratory syndrome including pharyngoconjunctival fever. The most common symptoms are fever, rhinitis, faringitis, cough and conjuntivitis, including exudative no-streptococcal faringitis among children below 3-years.
The MGO AdNS3 is a recombinant defective adenovirus, it has infective ability but is not able to replicate because of the lack of E1gene. It means that AdNS3 could lead only to primary infection but, differently form the wild type , it cannot infect other cells, because of the lack of replicative ability. On the other hand, in this clinical trial AdNS3 will not be given directly to humans, but it will be used for *in vitro ex vivo* transduction of human dendritic cells, which will be washed before the human administration. In this way the amount of viral particle with infective capability will be minimal. For these reasons, the risk of any human illness related to adenovirus administration with this route, are at least lower than that derived from the direct administration. In our previous experience with dendritic cells transduced with IL-12encoding adenoviral vector, none adverse events potentially related to viral infection have been observed.

4. Description of identification and detection methods

(a) Techniques used to detect the GMO in the environment
Quantitative real-time PCR…

(b) Techniques used to identify the GMO
Quantitative real-time PCR using primers corresponding to CMV promoter and HCV NS3 region, which are contained only in AdNS3, but not in wild type adenovirus or HCV…

F. Information relating to the release

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

Purpose of the release is a clinical trial (phase I/II therapeutic vaccination clinical trial in patients with chronic hepatitis C with genotype 1b, non-responders to conventional therapy), who will receive monocyte-derived autologous dendritic cells transduced with AdNS3. The main purpose of the clinical trial is to asses the safety of that treatment.

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): Clínica Universidad de Navarra, Av. Pío XII 36, 31008, Pamplona (Navarra)…

(b) Size of the site (m^2): (day hospital room, Gene therapy Laboratory of GMP Cellular Therapy Laboratory) below 12m^2

(i) actual release site (m^2): … m^2

(ii) wider release site (m^2): … m^2

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

(g) 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:
AdNS3 is not going to be directly administered to patients. It is going to be used in a clinical trial using an autologous cell product (dendritic cells) previously transduced with AdNS3. Three patient groups will be included in the clinical trial, classified according to the dose, number of cells administered (5×10^6, 10^7 and 2×10^7). Since each cell is transduced with 30 viral infectious units, this corresponds to 1.5×10^8-3×10^8-6×10^8 infectious units in each administration. Patients will receive three injections of dendritic cells thus prepared. Before injection, the cell product will be thorougly washed in order to avoid any carry over of adenoviral particles, in such a way that the amount of free virus can be considered irrelevant. Previous studies in patients treated with dendritic cells transduced with an adenovirus encoding IL-12 have not detected viral release through feces, urine or saliva in any of treated patients. Only one or two hours after cell administration, low amounts of viral RNA were detected, but not 24 hours later. For these reasons, the estimated release risk can be considered minimum.

(b) Duration of the operation:
The study will take two years…

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

Staff envolved in the preparation of the cell product will proceed according to Good Clinical Practice and Good Manufacturing Practice rules. GMP laboratory where the cell product will be prepared is a level 2 biosafety laboratory. The cell product, destinated only for clinical used, will be vialed in sealed tubes and labelled appropriately.

Personnel responsible for cell product administration will wear disposable gloves, masks and lab coats. Areas of administration will be washed with 1% sodium hypochloride immediately after patient vaccination. Contaminated waste will be transported in sealed yellow containers or in red bags of a special thickness labelled with stickers indicating "Group III-sanitary residues"….

5. Short description of average environmental conditions (weather, temperature, etc.)
Not applicable…

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 clinical trial using AdNS3…

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	Hominidae…
(iii)	genus	Homo…
(iv)	species	Homo sapiens…
(v)	subspecies	…
(vi)	strain	…
(vii)	cultivar/breeding line	…
(viii)	pathovar	…
(ix)	common name	…

2. Anticipated mechanism and result of interaction between the released GMOs and the target organism (if applicable)
The purpose of the clinical trial is to analyze safety of administration of dendritic cells transduced with AdNS3 to induce immune responses against hepatitis C virus. Thus, as a result of the interaction between AdNS3 and autologous dendritic cells, NS3 will be expressed inside these cells, to be presented to T lymphocytes and induce the activation of anti-NS3 T cell responses. AdNS3 will be first used in vitro, to transduce dendritic cells, then cells will be washed to eliminate free adenoviral particles, and finally cells will be administered to the patients. This means that AdNS3 is inside dendritic cells, without replicative capacity, eliminating thus other potential interaction between the virus and the patient.

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

Since AdNS3 is a non replicative adenovirus, potential interactions with other organisms are not 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
…

5. Types of ecosystems to which the GMO could be disseminated from the site of release and in which it could become established
AdNS3 is a defective, non-replicative adenovirus. According to the intended mode of use in the clinical trial, it is not expected that AdNS3 can be released to the ecosystem. …

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

Not applicable.

(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:
Not applicable.…

(b) from other organisms to the GMO:
Not applicable.…

(d) likely consequences of gene transfer:
Not applicable.…

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.):
Not applicable …

9. Possible environmentally significant interactions with biogeochemical processes (if different from the recipient or parental organism)
Not applicable …

H. Information relating to monitoring

1. Methods for monitoring the GMOs
Previous studies by the same investigational group have failed to detect viral shedding in biological samples of patients treated with dendritic cells transduced by a IL-12-encoding adenoviral vector. The only exception was the detection of a very low amount of viral particles in serum samples, that has never persisted for more than 120 minutes. For this reason viral shedding analysis are not been planned in this clinical trial. The occurrence of any adverse event related to the treatment will be assessed by medical history, physical examination, blood and urine analysis and will be notified to the regulatory agency.

2. Methods for monitoring ecosystem effects
As the MGO will be used for ex-vivo transduction of dendritic cells and as it lacks of replicative ability, the risk for the ecosystem does not exists.

3. Methods for detecting transfer of the donated genetic material from the GMO to other organisms
Not applicable.

4. Size of the monitoring area (m^2)
Not applicable

5. Duration of the monitoring
See point 1
6. Frequency of the monitoring
See point 1

I. Information on post-release and waste treatment

1. Post-release treatment of the site
The site of release will be cleaned with hypoclorite 1% solution and with GMP-approved disinfectants immediately after the release.

2. Post-release treatment of the GMOs
Transportation of waste will be performed in a special sealed containers or bags identified with stickers indicating “Group III-sanitary residues”

3. (a) Type and amount of waste generated
Waste originated from the production or the administration of the MGO-containing products will be regarded and treated as type III infective sanitary waste.
Solids and sharping waste will be stored in different specific containers appropriately labeled.
The estimated total amount of waste is a 30L container for patients.

3. (b) Treatment of waste
Waste will be collected by trained staff with the appropriate safety measures.
The trasnsportation inside the hospital will be performed by special trolleys using specific circuits.
Waste will be stored in appropriate containers until the collection by an external company. The sites of storing will be maintained in the appropriate safety conditions.

J. Information on emergency response plans

1. Methods and procedures for controlling the dissemination of the GMO(s) in case of unexpected spread
   Accidental contaminations of personnel involved in the production or administration of the MGO will be notified to main investigator and to the Service for the Prevention of Occupational Risks as soon as possible. All of the people involved in the trial will be trained about the procedures and measures to be taken in case of accidental release.

2. Methods for removal of the GMO(s) of the areas potentially affected
   The site will be cleaned with hypoclorite 1% solutions.

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

In case of skin contamination: Wash energically and then disinfect with Iodine 4% solution.

In case of eyes contatmination: wash with steril NaCl 0.9% solution for at least 15 minutes. An ophthalmologist consultation will be performed as soon as possible.

In case of accidental injection: wash energically with water and detergent and then disinfect with Iodine 9-12% solution for at least 5 minutes or use hypoclorite solutions.

Patients will be monitored for the occurrence of serious adverse events (SAE) according to the clinical protocol and the GCP guidelines: each SAE will be registered and evaluated, and will be notified to main investigator and to health authorities when relevant.

Due to the administration modalities the risk of accidental release is considered minimal. Moreover , as the vector lacks of replicative ability, the environmental risk of an eventual accidental release are very low.