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	Ireland
(b)	Notification number	B/../../….
(c)	Date of acknowledgement of notification	../../….
(d)	Title of the project	A Phase 1 study to assess the safety and immunogenicity of AdCh3NSmut1 and MVA-NSmut in HIV-1 seropositive HCV-uninfected adults on ART
(e)	Proposed period of release	From 01/07/2014 until 31/12/2015

2. Notifier

Name of institution or company: The GUIDE Department, St. James's Hospital, Dublin 8

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)
Chimpanzee Adenovirus 3 NSmut1

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

The clinical batches of AdCh3NSmut1 have undergone testing to demonstrate genetic stability of the virus. Endonuclease restriction analysis is used to demonstrate that the genetic structure of the vector is identical to the original pre-Adenovirus plasmid and replication competent adenovirus (RCA) detection testing is performed to exclude the presence or replication competent virus. Also, the NSmut region in the vectors is sequenced, to check if it had undergone any mutations or deletions.

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.

An Environmental Risk Assessment (ERA) has been carried out for AdCh3NSmut1 use in a clinical trial in Ireland. The overall risk was felt to be low / acceptable. AdCh3NSmut1 is a modified recombinant virus vaccine derived from an attenuated replication-incompetent adenovirus with a genetic modification to promote the expression of the HCV immunogen NSmut. There is much safety data from animals relating to this specific vaccine, and from humans relating to similar chimpanzee adenoviruses used in a similar situation. Direct and indirect risks were incorporated into the ERA. Direct risks included human toxicity, human immunoreactivity and recombination of AdCh3NSmut1 with other agents leading to reversion to replication competence. Indirect risks considered included attenuation of future treatment to Hepatitis C and was felt not to have any theoretical grounding.

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	Mastadeovirus
(iii)	species	…
(iv)	subspecies	Not applicable
(v)	strain	…
(vi)	pathovar (biotype, ecotype, race, etc.)	…
(vii)	common name	Chimpanzee adenovirus 3

3. Geographical distribution of the organism

(a) Indigenous to, or otherwise established in, the country where the notification is made:
Yes (.) No (X) Not known (.)

(b) Indigenous to, or otherwise established in, other EC countries:
(i) Yes No (X)

If yes, indicate the type of ecosystem in which it is found:

Atlantic	..
Mediteranean	..
Boreal	..
Alpine	..
Continental	..
Macaronesian	..

(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 …	

AdCh3NSmut1 is a replication-incompetent chimpanzee virus maintained in laboratories.

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

5. (a) Detection techniques
Indirect – Neutralizing antibodies
Direct – Polymerase chain reaction

(b) Identification techniques
Indirect – Neutralizing antibodies
Direct – Polymerase chain reaction

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
Adenovirus is classified as Class 2 under **Directive 93/88/EEC.** The unmodified Adenovirus is of limited pathogenicity but the virus we propose to release is replication incompetent and a chimpanzee species and therefore considered not to be pathogenic. Replication-deficient adenoviruses lacking E1 are classified as Biological Safety Level 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

Human adenovirus are a family of viruses that usually cause asymptomatic infection in humans but can also produce respiratory tract infections, gastrointestinal illness and ocular infections. They are most common in children. Incubation period is generally between 1 and 10 days. Most members of the human population are sero-positive against more than one subspecies of adenovirus and can produce neutralizing antibodies against adenovirus.

Most infections are minor and self-limiting. Adenovirus are usually not integrative and generally do not persist long-term in lymphoid tissue, although such cases are reported rarely.

The vaccine vector, AdCh3NSmut1 we propose to use is replication incompetent and a chimpanzee-specific strain, and therefore can be considered to be non-pathogenic.

8. Information concerning reproduction

(a) Generation time in natural ecosystems:
AdCh3NSmut1 does not generate in natural ecosystems

(b) Generation time in the ecosystem where the release will take place:
AdCh3NSmut1 will not generate effectively

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

(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 (fungi)	(.)
(vi)	eggs	(.)
(vii)	pupae	(.)
(viii)	larvae	(.)
(ix)	other, specify	…viral amplification

(b) relevant factors affecting survivability:
Adenoviruses lose bioactivity at room temperature with falls in levels on a logarithmic scale. Adenoviruses can survive for long periods on environmental surfaces; they are resistant to lipid disinfectants because they are non-enveloped, but are inactivated by heat, formaldehyde or bleach.

10. (a) Ways of dissemination
Transmission of adenoviruses can occur via aerosol droplets, the faecal-oral route and by contact with contaminated fomites.

However the adenovirus we propose to use is modified to be replication incompetent and therefore non-pathogenic.

(b) Factors affecting dissemination
Research protocol and safety measures. Research environment.
AdCh3NSmut1 is replication deficient in human since the E1 genes necessary for replication and *in vivo* propagation are deleted in this vector.

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)
…, B/../../…
Not applicable

C. **Information relating to the genetic modification**

1. Type of the genetic modification

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

2. Intended outcome of the genetic modification
Expression of the NSmut gene. The purpose of NSmut expressed by AdCh3 is to induce T cell immune responses to Hepatitis C antigens, which may confer protection against chronic infection.

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

pNEBCV32/NS MUT

(c) Host range of the vector
E.Coli

(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
Ampicillin resistance gene

(e) Constituent fragments of the vector

- E. Coli Amp^r (Ampicillin Resistance gene)
- *ori* site derived from pMB1 vector
- CV-32 viral DNA sequence: from 1 bp to 456 bp; from 3419 bp to 3811 bp; and the last 429 bp.
- human CMV promoter
- Kozac sequence
- NSmut transgene
- bgh polyadenylation signal sequence

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

(i) transformation (.)
(ii) electroporation (.)
(iii) macroinjection (.)
(iv) microinjection (.)
(v) infection (.)
(vi) other, specify: Homologous recombination in Bj5183 E.Coli cells

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
NSmut – The insert NSmut encodes for the 1985 amino acid-long HCV non structural region (NS), with genetically inactivated RNA-dependent RNA polymerase activity (NS5B). The NS fragment of the Hepatitis C genome is translated into a single polyprotein which is

then proteolytically cleaved into five mature products (NS3, NS4A, NS4B, NS5A and NS5B) by the encoded NS3 protease. The mutation in the active site of the HCV polymerase was introduced as a safety measure, to eliminate any potential replication capability of the vaccine.

(b) Source of each constituent part of the insert

NS region from HCV 1b genotype, BK strain isolate based on sequence accession number M58335

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

The purpose of NSmut expressed by AdCh3 is to induce T cell immune responses against Hepatitis C virus, which may confer protection against Hepatitis C chronic infection.

(d) Location of the insert in the host organism

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

NSmut expression cassette was inserted into AdCh3 backbone, in place of E1 region.

(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 …
(iii) genus Hepacivirus (Family Flaviviridae)
(iv) species …

(v)	subspecies	…
(vi)	strain	Genotype 1, subtype B, BK strain isolate
(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 (X) No () Not known (.)

If yes, give the relevant information under Annex III A, point II(A)(11)(d):

In the life cycle of Hepatitis C the non-structural (NS) proteins are involved in polyprotein processing and viral replication. The insert NSmut encodes for the 1985 amino acid-long HCV non structural region (NS), with genetically inactivated RNA-dependent RNA polymerase activity (NS5B). The NS fragment of the Hepatitis C genome is translated into a single polyprotein which is then proteolytically cleaved into five mature products (NS3, NS4A, NS4B, NS5A and NS5B) by the encoded NS3 protease. The mutation in the active site of the HCV polymerase was introduced as a safety measure, to eliminate any potential replication capability of the vaccine.

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 Classification 3*, Directive 93/88/EEC

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 …Unable to replicate, therefore non pathogenic

(b) is the GMO in any way different from the recipient as far as mode and/or rate of reproduction is concerned?

Yes (.) No (X) Unknown (.)

Specify …Unable to replicate, therefore non pathogenic

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

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

Specify …Unable to replicate, therefore unable to disseminate

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

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

Specify …Unable to replicate, therefore non-pathogenic

2. Genetic stability of the genetically modified organism

The clinical batches of AdCh3NSmut1 have undergone testing to demonstrate genetic stability of the virus. Endonuclease restriction analysis is used to demonstrate that the genetic structure of the vector is identical to the original pre-Adenovirus plasmid and replication competent adenovirus (RCA) detection testing is performed to exclude the presence of replication competent virus. Also, the NSmut region in the vectors is sequenced, to check if it had undergone any mutations or deletions.

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

Polymerase Chain Reaction (PCR testing)

(b) Techniques used to identify the GMO

Poymerase Chain Reaction (PCR testing)

F. Information relating to the release

1. Purpose of the release (including any significant potential environmental benefits that may be expected)
The vaccine AdCh3NSmut1 has been developed as a candidate vaccine for Hepatitis C virus infection. The release is necessary to conduct a phase 1 clinical trial to assess the safety and immunogenicity of this vaccine in HIV-1 seropositive individuals. If safety is found to be acceptable and immunogenicity encouraging, further trials would be planned.

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 (X) No (.)
If yes, specify …
Normally the GMO to be used is stored in laboratories and not found in the community at large

3. Information concerning the release and the surrounding area

(a) Geographical location (administrative region and where appropriate grid reference):
Release will occur at the Clinical Research Facility at St James's Hospital, Dublin 8

(b) Size of the site (m^2): … Approximately 30,000 m^2
(i) actual release site (m^2): …70 m^2
(ii) wider release site (m^2): … Dublin and surrounding areas 1 x 10^{10}m2

(c) Proximity to internationally recognised biotopes or protected areas (including drinking water reservoirs), which could be affected:
Nil. There are many such areas, but affecting them is not thought possible

(d) Flora and fauna including crops, livestock and migratory species which may potentially interact with the GMO
Nil. There are many flora and fauna but affecting them is not thought possible

4. Method and amount of release

(a) Quantities of GMOs to be released:
15 vials to be used in vaccination

(b) Duration of the operation:
Each vaccination takes only a few minutes. The vaccinations will be performed over a concentrated time period.

(c) Methods and procedures to avoid and/or minimise the spread of the GMOs beyond the site of the release
Inoculation of the vaccine will only be performed at the Clinical Research Facility in St James's Hospital. The vaccination site will be covered with a bandage. Materials used in the vaccination such as needles, syringes and swabs will be autoclaved prior to disposal.

5. Short description of average environmental conditions (weather, temperature, etc.)

The vaccination will be performed indoors at normal room temperature.

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.
Data regarding previous releases is listed in detail in our Environmental Risk Assessment. Previous testing has shown no evidence of viral shedding and that the candidate vaccines are well tolerated.

G. Interactions of the GMO with the environment and potential impact on the environment, if significantly different from the recipient or parent organism

1. Name of target organism (if applicable)

(i)	order and/or higher taxon (for animals)	Primates
(ii)	family name for plants	…
(iii)	genus	Homo
(iv)	species	Sapiens
(v)	subspecies	…
(vi)	strain	…
(vii)	cultivar/breeding line	…
(viii)	pathovar	…
(ix)	common name	Human

2. Anticipated mechanism and result of interaction between the released GMOs and the target organism (if applicable)
The development of humoral and cellular immune responses against the Hepatitis C immunogen NSmut that may confirm protection against future HCV exposure

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
…

5. Types of ecosystems to which the GMO could be disseminated from the site of release and in which it could become established
The GMO is replication incompetent and therefore dissemination is felt to be impossible

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:
Highly unlikely

(b) from other organisms to the GMO:
Highly unlikley

(c) likely consequences of gene transfer:
Nil

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 available

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

H. Information relating to monitoring

1. Methods for monitoring the GMOs
The GMO will not specifically be monitored but the patients in whom the GMO will be injected will be followed up for clinical review and will have blood draws to assess safety.

2. Methods for monitoring ecosystem effects
Clinical evaluation of patients, monitoring of health surveillance data

3. Methods for detecting transfer of the donated genetic material from the GMO to other organisms
PCR / Sequencing possible

4. Size of the monitoring area (m^2)
… 1 x 10^{10}m2

5. Duration of the monitoring
8 months

6. Frequency of the monitoring
Patients will have 12 clinical visits over the 8 months. They will also have contact numbers for the medical staff should they encounter any difficulties between these periods.

I. Information on post-release and waste treatment

1. Post-release treatment of the site
The injection site will be covered with a plaster / bandage. All materials used will be disposable and waste will be autoclaved. All surfaces that have been in contact with the GMO will be chemically disinfected.

2. Post-release treatment of the GMOs
Vials to be disposed of must be placed within a sharps box which will be autoclaved the same day

All materials that have been in contact with the vaccine or vaccination site (eg. dressings) must be punctiliously disposed of into an autoclave bag or suitable container for autoclaving. All sharps must be disposed of immediately after use into a sharps bin for autoclaving. Waste bags and sharps boxes must be placed in a robust container suitable for transport to the autoclave. The trial physician will be responsible for ensuring that this waste is autoclaved in a designated autoclave. A record of autoclaved waste will be kept local to the autoclave, by filing autoclave printouts in a designated log book with the date, time, name and signature of the person responsible for autoclaving the waste

3. (a) Type and amount of waste generated
Vaccine vials, used needles, gloves and syringes

3. (b) Treatment of waste
All materials that have been in contact with the vaccine or vaccination site (e.g. dressings) must be punctiliously disposed of into an autoclave bag or suitable container for autoclaving. All sharps must be disposed of immediately after use into a sharps bin for autoclaving. Waste bags and sharps boxes must be placed in a robust container suitable for transport to the autoclave. The trial physician will be responsible for ensuring that this waste is autoclaved in a designated autoclave.. A record of autoclaved waste will be kept local to the autoclave, by filing autoclave printouts in a designated log book with the date, time, name and signature of the person responsible for autoclaving the waste. The waste will then be disposed of with an approved waste contractor.

J. Information on emergency response plans

1. Methods and procedures for controlling the dissemination of the GMO(s) in case of unexpected spread
Standard national procedures for outbreak management. However, as stated before this is a replication incompetent virus. If there is spillage of the GMO the affected area would be chemically disinfected. Standard Operating Procedures (SOPs) will be in place in the event of spillage or accidental exposure.

2. Methods for removal of the GMO(s) of the areas potentially affected
Disinfection with alcohol or chlorine solutions

3. Methods for disposal or sanitation of plants, animals, soils, etc. that could be exposed during or after the spread
The study site will be an animal free area. Any affected materials will be autoclaved as per SOPs and disposed of with approved waste contractors.

4. Plans for protecting human health and the environment in the event of an undesirable effect
Standard medical management will be undertaken for any unwell patients based on their signs and symptoms and the results of investigations.