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	**NL**
(b)	Notification number	**B/NL/05/006**
(c)	Date of acknowledgement of notification	**24/11/2005**
(d)	Title of the project	**Adjuvant IL-12 immuno-gene therapy prior to radical prostatectomy in patients with prostate cancer**
(e)	Proposed period of release	**From 01/07/2006 until 01/07/2008**

2. Notifier

Name of institution or company: **Erasmus MC Rotterdam, 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 …

(b) Identity of the GMO (genus and species)
Mastadenovirus (genus), human adenovirus (species). The GMO is a replication incompetent recombinant adenovirus (human serotype 5) containing the human gene for interleukin-12 (referred to as Adv/IL-12)

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

The complete vector containing both p35 and p40 subunits of IL-12 connected with an IRES was completely sequenced using specific primers. All sequencing reactions were performed with Sequenase using ^{33}P dATP. Reactions were separated by PAGE electrophoresis and the sequence read using a digitizing pallet with MacDNAsis software. The resulting fragments were arranged with contig manager and the complete sequence of the IL-12 p35 and p40 genes as well as the IRES sequence and the junctions with the cloning vector pCA3 arranged.

The clinical grade virus preparation tested negative for other potential virus contaminants including EBV, CMV, HIV-1, HCV, HTLV-1, HTLV-2, HBV, Adventitious virus, and parvovirus. Bacterial, fungal and mycoplasma screens were also negative. The replication competent assay for recombinant adenovirus was also negative when tested with up to 10^{10} VP.

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.

The adenovirus is localized in the nucleus of the infected cell, as an episomal form, without known risk of integration in the genome of the infected cell. Considering that the recombinant adenovirus has lost all replicative capability, the viral DNA will be naturally eliminated when the infected cell dies. Based on its physiology, the injected tumor can be considered as a contained environment. From the site of intra tumoral injection, viral DNA has however been detected in some organs like liver and lungs or spleen in preclinical studies. As well, we cannot exclude a very limited presence of viral DNA in biological fluids like urine, faeces or saliva. But considering the nature of this viral DNA, infectious or not, and the non-replicative character of Adv/IL12, the probability of a biological risk for public health or environment is very low.

It is theoretically possible that recombination with a wild type adenovirus harbored by the patient could reintroduce the E1a region back into the virus. Such a recombinant virus that contained the RSV and/or IL-12 genes and the regained E1 sequence would be too large for packaging in the viral protein coat and would not be recoverable.

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)	**Adenoviridae**
(ii)	genus	**mastadenovirus**
(iii)	species	**human adenovirus**
(iv)	subspecies	**subgroup C**
(v)	strain	**human serotype 5 (ad5)**
(vi)	pathovar (biotype, ecotype, race, etc.)	…
(vii)	common name	**human serotype 5 (ad5)**

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	**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 **man**	

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

5. (a) Detection techniques
Detection of adenovirus type 5 is done by cell culture and (quantitative) PCR.

(b) Identification techniques
(quantitative) PCR

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
Group/class 2 under directive 90/679/EEC (Protection of workers from risks related to exposure to biological agents at work)

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	(**x**)
plants	(.)
other	(.)

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

Infection by wild-type Ad5 results in acute mucous-membrane infection of the upper respiratory tract, eyes lymphoid glands and nodes, with mild symptoms similar to those of the common cold. Exposure to C type adenoviruses is widespread in the population; the majority of adults are seropositive for this type of adenovirus.
Under normal circumstances the human serotype adenovirus does not infect other animal species. There are however some reports showing adenovirus replication in experimental animals. There are no reports showing the presence of adenoviral DNA in invertebrate species or plants.

8. Information concerning reproduction

(a) Generation time in natural ecosystems:
20-24 hrs for wild-type adenovirus type 5. (In contrast, Adv/IL-12 is a replication deficient vector, only capable of reproduction in permissive helper cell lines containing the adenovirus E1 gene).

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

(c) Way of reproduction: Sexual .. Asexual ..
Adv/Il-12 can only be produced in permissive helper cell lines.

(c) Factors affecting reproduction:
Adv/Il-12 lacks the E1 gene and is therefore a replication incompetent vector.

9. Survivability

(a) ability to form structures enhancing survival or dormancy:**n/a**

(i)	endospores	(.)
(ii)	cysts	(.)
(iii)	sclerotia	(.)
(iv)	asexual spores (fungi)	(.)
(v)	sexual spores (funghi)	(.)
(vi)	eggs	(.)
(vii)	pupae	(.)
(viii)	larvae	(.)
(ix)	other, specify …	

(b) relevant factors affecting survivability:
Survival of adenoviruses (including Adv/IL-12) is mainly affected by temperature followed by relative humidity. Adenoviruses may survive for several weeks under optimal conditions (Abad FX et al; Appl Environ

Microbiol. 1994 Oct;60(10):3704-10, Mahl MC et al; Can J Microbiol. 1975 Jun;21(6):819-23.).

10. (a) Ways of dissemination
Through air and water.

(b) Factors affecting dissemination
Dissemination may occur through aerosol formation during processing of the vector and shedding by treated patients.

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/../../…

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
Adv/Il-12 differs from wildtype Ad5 by a complete deletion of the E1 gene and a deletion and insertion in the E3 domain. Adv/IL-12 is constructed from the adenoviral shuttle vector pCA3 containing the human interleukin-12 gene and the pJM17 plasmid containing the adenoviral backbone.
Intratumoral injection of Adv/Il-12 will thus lead to the expression of IL-12.

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

The parent virus from which Adv/IL-12 is derived is human adenovirus type 5. The coding capacity of Ad5 has been modified in three ways:

• Deletion of the E1 region (base 342 – 3523), including the start- and stop codons.

• Deletion and insertion in the E3 region, eliminating the expression of the E3b gene.

• Insertion of the pCA3 shuttle vector containing the human interleukin-12 gene at the position of the E1 deletion

• The pCA3 shuttle vector contains the human cytomegalovirus immediate early (HMV IE) promoter to drive transcription of the IL-12 gene in transduced cells. It also carries the polyadenylation signal (SV40) and the internal ribosomal entry site (IRES) which connects the IL-12 subunits, the p35 subunit (α-chain) and the p40 subunit (β-chain).

(c) Host range of the vector

Adv/IL-12 is replication incompetent and only replicates in permissive helper cell lines which do not exist outside the laboratory.

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

Yes (.) No (.)

antibiotic resistance (.)

other, specify **Expression of IL-12 by prostate tumour cells may lead to an immune response resulting in subsequent tumour cell death.**

Indication of which antibiotic resistance gene is inserted

n/a

(e) Constituent fragments of the vector

The adenoviral shuttle vector pCA3, containing the IL-12 transgene, and the pJM17 plasmid, containing the adenoviral backbone.

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

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

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
Human gene for interleukin-12 (IL-12). IL-12 is a heterodimeric molecule composed of two subunits, the p35 subunit (α-chain) and the p40 subunit (β-chain).

(b) Source of each constituent part of the insert
The IL-12 subunits are derived from a human B-lymphoblast cell line (NC-37) purchased from the American Type Culture Collection (ATCC CCL 214).

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

- **Human cytomegalovirus immediate early (HMV IE) promoter**: **required for the initiation of transcription of the gene, it has no protein coding capacity and functions solely as a transcriptional control element regulating the expression of the IL-12 gene.**
- **IL-12gene**: **IL-12 is a heterodimeric molecule composed of two subunits, the p35 subunit (α-chain) and the p40 subunit (β-chain), linked by the internal ribosomal entry site (IRES). Il-12 expression in cells leads to an immune response.**
- **SV 40 pA: polyadenylation signal.**

(d) Location of the insert in the host organism

- on a free plasmid (.)
- integrated in the chromosome (.)
- other, specify: **Integrated in the vector genome, at the position of the deleted 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 (.)
DNA virus (.)
bacterium (.)
fungus (.)
animal
- mammals (.)
- insect (.)

- fish (.)
- other animal (.)

(specify phylum, class) …

other, specify: **The IL-12 gene in the Adv/IL-12 vector was originally isolated from a cDNA library derived from mRNA from a human B lymphoblast cell line (NC-37).**

2. Complete name

(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	…

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 (.) No (.) Not known (**x**)

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 (.) No (.) Unknown (.)
Specify **Adv/Il-12 is replication incompetent and only replicates in permissive helper cell lines which do not exist outside the laboratory.**

(c) is the GMO in any way different from the recipient as far as dissemination is concerned?
Yes (.) No (**x**) Not known (.)
Specify …

(d) is the GMO in any way different from the recipient as far as pathogenicity is concerned?
Yes (**x**) No (.) Not known (.)
Specify **The Adv/IL-12 vector is replication incompetent decreasing pathogenicity.**

2. Genetic stability of the genetically modified organism
The GMO is genetically stable. The replication competent assay for recombinant adenovirus was negative when tested with up to 10^{10} VP in the clinical batches.

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 …

(b) give the relevant information specified under Annex III A, point II(A)(11)(d) and II(C)(2)(i)
There is the potential that Adv/IL-12 virus may escape the tumor bed and is disseminated via the blood supply to transduce other sites in the patient's body.

In animal studies with escalating doses up to 2.5 x 10^8 PFU injected into the mouse dorsolateral prostate a low level of viral spread (detectable only by PCR) was detected in testis, blood and liver in less than 10% of the animals. Spread to the gut and lung or sperm was not detected. No obvious pathologic abnormalities were observed on microscopic examination. The liver is in principal at greatest risk of damage as this organ takes up the majority of blood borne virus.

4. Description of identification and detection methods

(a) Techniques used to detect the GMO in the environment
Specific PCR, culture on complementation cell line.

(b) Techniques used to identify the GMO
Specific PCR.

F. Information relating to the release

1. Purpose of the release (including any significant potential environmental benefits that may be expected)
Non specific immunotherapy by interleukin 12 expressed from a recombinant adenovirus (Adv/IL-12) intended to treat patients with prostate cancer. The phase 1 study will monitor safety and tolerability of escalating doses of Adv/IL-12.

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
N/a; Adv/IL-12 is administered by intratumoral injection.
(a) Geographical location (administrative region and where appropriate grid reference):
…

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

(c) Proximity to internationally recognised biotopes or protected areas (including drinking water reservoirs), which could be affected:
…

(d) Flora and fauna including crops, livestock and migratory species which may potentially interact with the GMO
…

4. Method and amount of release

(a) Quantities of GMOs to be released:

Injection of $1x10^{10}$, $1x10^{11}$ or $1x10^{12}$ Virus Particles/patient

(b) Duration of the operation:
Several minutes are required for intraprostatic vector injection.

(c) Methods and procedures to avoid and/or minimise the spread of the GMOs beyond the site of the release
The viral vector Adv/IL-12 is suspended in a small volume (<2.0 ml) of buffer by trained and dedicated personnel of the hospital pharmacy staff in a dedicated biohazard room, and is contained in a septum vial. The syringe is loaded from the vial with no virus vector loss. Pharmacy to patient transport is performed in a double sealed biohazard box. Intratumoral injection is made and the amount of leakage along the needle track is likely to be very minute. In the event of accidental spills of the virus from the vial common disinfectants can be used to inactivate the agent according to a Standard Operation Procedure file that is available in the outpatient room. All personnel are instructed on the safe use and potential biological hazards of the virus vector before the procedures are performed. The Adv/IL-12 vector is unlikely to produce viral infection in others, as it is replication defective.

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

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.
Based upon animal and human studies using recombinant human IL-12 intravenously or in genetically engineered fibroblasts, potential complications may include: fever / chills, headache, nausea and vomiting, anemia, neutropenia, lymphopenia, thrombocytopenia, hyperglycemia, and hypoalbuminemia.

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)
Infection of tumour cells by the recombinant adenovirus followed by the expression of human interleukin 12 and a subsequent anti-tumoral immune response.

3. Any other potentially significant interactions with other organisms in the environment
It is theoretically possible that recombination with a wild type adenovirus harbored by the patient could reintroduce the E1a region back into the virus. Such a recombinant virus that contained the RSV and/or IL-12 genes and the regained E1 sequence would be too large for packaging in the viral protein coat and would not be recoverable.

4. Is post-release selection such as increased competitiveness, increased invasiveness for the GMO likely to occur?
Yes (.) No (**x**) Not known (.)
Give details
The propagation and the replication of the vector needs the use of complementation helper cells and specific culture media.

5. Types of ecosystems to which the GMO could be disseminated from the site of release and in which it could become established
After intra prostatic injection of Adv/IL-12 patients will be asked to collect their urine followed by addition of chloric acid for inactivation of any potentially shedded virus. Also patients will be asked to use a condom during sexual intercourse. By these measures, the risk of dissemination of viral particles is neglectible.

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 **n/a**

(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:
Apart from the treated patient, no exchange would be expected.

(b) from other organisms to the GMO:
No exchange between other organisms and the GMO will be expected.

(c) likely consequences of gene transfer:
Cells transfected by the GMO are intended to express the IL-12 gene.

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.):
Adv/IL-12 is currently being studied in a Phase I trial entitled "Phase I study of adenoviral vector delivery of the IL-12 gene in men with local recurrence of prostate cancer after irradiation therapy" at the Department of Urology of Baylor College of Medicine, Houston, USA (principal investigator Prof. Brian J. Miles). In this trial, patients in whom curative radiotherapy has failed and who therefore have no other treatment option will receive a single intra prostatic injection of Adv/IL-12 as a single therapeutic treatment. The first group of 3-5 patients will be treated with $1x10^{10}$ VP and in the absence of serious adverse effects the dose will be escalated in subsequent groups of 3-5 patients by increasing the dosage in a half log scale up to $5x10^{12}$ VP, or until unacceptable toxicity is reached (grade 3 or greater).

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

H. Information relating to monitoring

1. Methods for monitoring the GMOs
To assess whether any of the vector is shed from the target organ systemically or in the environment patients will be monitored on blood and urine samples. Samples will be assessed for Ad5-viruses using quantitative PCR and cell cultures.

2. Methods for monitoring ecosystem effects
There is a negligible risk for shedding of virus into the ecosystem. Therefore the ecosystem effects will not be monitored.

3. Methods for detecting transfer of the donated genetic material from the GMO to other organisms
There is a negligible risk for transfer of donated genetic material from the patient to other organisms. Therefore the transfer of donated genetic material will not be investigated.

4. Size of the monitoring area (m^2)
n/a m^2

5. Duration of the monitoring
Urine samples will be tested for the presence of adenoviral particles until virus testing of urine is negative for two consecutive time-points.

6. Frequency of the monitoring
Urine samples will be tested for the presence of adenoviral particles until virus testing of urine is negative for two consecutive time-points.

I. Information on post-release and waste treatment

1. Post-release treatment of the site
Post-release treatment of the site is not necessary. In case of spillage of the GMO this should be cleaned thoroughly with a chloric acid solution.

2. Post-release treatment of the GMOs
Not required.

3. (a) Type and amount of waste generated
Waste material will be the aliquot containing the GMO, syringes, needles, and materials that come into contact with the GMO during preparation or administration, like gloves, towels ed.

3. (b) Treatment of waste
Waste will be destroyed according to local regulations of infected hospital waste.

J. Information on emergency response plans

1. Methods and procedures for controlling the dissemination of the GMO(s) in case of unexpected spread
Spilled GMO will be wiped up using tissues and inactivated with a chloric acid solution.

2. Methods for removal of the GMO(s) of the areas potentially affected
Spilled GMO will be wiped up using tissues and inactivated with a chloric acid solution.

3. Methods for disposal or sanitation of plants, animals, soils, etc. that could be exposed during or after the spread
n/a

4. Plans for protecting human health and the environment in the event of an undesirable effect
Patients will be monitored for the occurrence of serious adverse events (SAE) according to the clinical protocol: each SAE will be registered and evaluated by the hospital staff and the sponsor of the study, and health authorities will be notified when relevant.