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/12/002
(c)	Date of acknowledgement of notification	06/02/2012
(d)	Title of the project	Oncolytic adenovirus therapy using a prostate-specific conditionally replication-competent adenovirus as an adjuvant treatment for localised prostate cancer
(e)	Proposed period of release	From 01/11/2012 until 01/11/2022

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)
Genus Mastadenovirus, species human Adenovirus subgenus C, serotype 5.

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

Genetic stability of GMO was confirmed by full genomic sequence analysis, a PCR specific for the GMO, a restriction digestion map analysis and immuno-blotting to confirm adenoviral E1A protein expression. The clinical GMO product contains a very small level of 1 ppm of E1B-containing variants originated from recombination of the GMO with the E1B region from the genome of the producer cells, as assessed by a semi-quantitative PCR.

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 environmental risks associated with the use of the GMO are negligible. The GMO is a strongly attenuated derivative from a commonly occurring cold virus with an unmodified tropism, and adverse effects are limited to the prostate only. There is a risk of transmission of this virus in case of an accident during administration, resulting in the potential exposure of pharmacy or trial personnel to high amounts of virus, and in case of exposure of third persons by transmission of virus that is shed via excreta, predominantly semen. Harmful effects of transmission of virus or recombinants to third persons are not to be expected due to the strongly attenuated pathogenicity of the GMO, which is restricted to prostate cells only, and the negligible level of potential recombinants. Therefore, from an environmental safety perspective risk management measures are not required. Nevertheless, because of ethical considerations the risk of overt transmission to third persons will be minimised by a number of procedures, including training of trial personnel, cleaning protocols, the use of protective clothing by trial personnel, routine precautions for handling of biological specimens and precautions to be taken by the patient.

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)	…
(ii)	genus	Mastadenovirus
(iii)	species	human Adenovirus
(iv)	subspecies	subgenus C
(v)	strain	human serotype 5 (Ad5)
(vi)	pathovar (biotype, ecotype, race, etc.)	…
(vii)	common name	human Adenovirus 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	Humans

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

5. (a) Detection techniques
Ad5 is detected by cell culture and/or (quantitative) PCR.

(b) Identification techniques
Ad5 is identified by sequencing, restriction digest analysis and 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	(.)
plants	(.)
other	(.)

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

Ad5 can cause a cold via infection of the mucosa and lymph nodes of the respiratory tract, with symptoms including fever, cough, nasal congestion and sore throat. Airway infections by Ad5 primarily occur in children. As a result, most adults will have experienced multiple Ad5 infections. In most cases, the infection is asymptomatic or mild and self-limiting, therefore not requiring treatment. In severely immuno-compromised individuals such as HIV infected patients, bone marrow transplanted patients and organ transplanted patients, systemic Ad5 infection can have serious and even fatal consequences primarily due to a low level of lymphocytes. In stem cell transplantation patients, intervention such as Cidofovir and immunotherapy is only recommended if lymphocyte counts are below 0.3x10E9/L, resulting in the inability of the patient's immune system to clear the adenovirus infection.

Adenoviruses are not associated with the development of cancer in humans. In a laboratory setting, Ad5 is able to transform cells but no tumorigenicity potential has been found in in vivo models.

Under normal circumstances the human serotype adenovirus does not infect other animal species. There are however some reports showing a certain degree of 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:
Ad5 replicates in about 24 hours.

(b) Generation time in the ecosystem where the release will take place:
In general, the generation time of an Ad5-derived of GMO is prolonged. The GMO will only replicate in prostate cells and not in non-prostate cells.

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

(c) Factors affecting reproduction:
The GMO is a human prostate-specific replicating adenovirus. Replication of the GMO and the resulting cytotoxic effect is restricted to human prostate cells only due to the incorporation of a human prostate-specific regulatory element. This is different compared to the parental virus Ad5, which does not replicate in a tissue-specific way (i.e. the parental virus will replicate in any infected human epithelial cell). The tropism of of the GMO has not been changed by the genetic modification and as such host specificity (humans only) and cell tropism (epithelial cells as highly preferential target cells) is comparable to that of the parental virus Ad5.

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	…	

(b) relevant factors affecting survivability:
Ad5 is quite stable and resistant to various chemical and physical agents. This allows for prolonged survival of Ad5 outside the host. In a laboratory setting, Ad5 can be stored frozen for years.

10. (a) Ways of dissemination
Ad5 spreads through direct contact, fecal-oral transmission, and occasionally waterborne transmission. Airway infections by Ad5 are usually transmitted by aerosols released by coughs and sneezes of an infected person. Studies in volunteers have shown that casual contacts between experimentally infected individuals and third persons do not result in horizontal transmission of adenovirus. Ad5 can persist as a latent infection for years in tonsils and adenoids, and can be shed in faeces for several months upon initial infection. There is no evidence for vertical transmission of Ad5. In a number of studies in mice, expression of CAR, the receptor that adenovirus uses to enter a target cell, on gonad cells was detected and infection of these cells was observed. However, this did not result in transmission of virus to the offspring.

(b) Factors affecting dissemination
Dissemination is affected by the level of GMO shed by treated patients, aerosol formation, and intimacy 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)
B/NL/08/008

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
Compared to the parental virus Ad5, the GMO differs in the following ways:
- The E1A gene is derived from human Adenovirus serotype 2
- The E1A promoter is replaced by a prostate-specific regulatory element
- The E1B and E3 genes are lacking

Replication of the GMO is restricted to prostate cells only because expression of E1A, the adenoviral protein required for replication, is controlled by a regulatory sequence comprising the human prostate specific antigen (PSA) enhancer, the human prostate specific membrane

antigen (PSMA) enhancer and the human T-cell receptor gamma-chain alternate reading frame protein (TARP) promoter. This regulatory sequence, composed of three prostate-specific elements (PSA, PSMA and TARP), is called PPT and is shielded from interfering adenoviral sequences by the mouse H19 insulator, called I. Since the regulatory element is only active in prostate cells, expression of E1A resulting in replication of the GMO and subsequently cytotoxicity will only occur in prostate cells. As such, the GMO can be used as a targeted treatment for prostate cancer. Since the GMO is not shielded from the immune system, rapid neutralization will occur in the circulation. Therefore, the primary target is localized prostate cancer.

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 GMO was constructed using the E1/E3-deleted AdEasy system. This system involves an adenoviral plasmid that contains all Ad5 sequences except nucleotides 1–3,533 (encompassing the E1 genes) and nucleotides 28,130–30,820 (encompassing the E3 gene). First, the human serotype 2 adenovirus E1A coding sequence was inserted in the so-called pShuttle vector encoding the prostate-specific I/PPT controlling sequence. The GMO was then generated from the pShuttle-I/PPT vector and the E1/E3-deleted AdEasy plasmid that delivers the adenoviral backbone.

(c) Host range of the vector

The plasmid vector replicates in E.coli bacteria.

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

Yes (X) No (.)

antibiotic resistance	(X)
other, specify	…

Indication of which antibiotic resistance gene is inserted
Kanamycin

(e) Constituent fragments of the vector
See above at C.4.b.

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

(i) transformation (.)
(ii) electroporation (.)
(iii) macroinjection (.)
(iv) microinjection (.)
(v) infection (.)
(vi) other, specify: DNA genome from the plasmid vector was obtained by restriction enzyme digestion, and transfected in Human Embryonic Kidney 293 producer cells supplemented with the adenovirus E1 gene. The GMO containing the full Ad5 genome with the modification listed above at C.2 is formed in these producer cells. Other components of the plasmid vector are not transferred into the GMO.

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 serotype 2 adenovirus E1A (Ad2 E1A) gene
- Human proximal T-cell receptor gamma-chain Alternate Reading frame Protein (TARP) promoter
- Human Prostate Specific Antigen (PSA) enhancer
- Human Prostate Specific Membrane Antigen (PSMA) enhancer
- Mouse H19 imprinting control region

(b) Source of each constituent part of the insert

- Ad2 E1A coding sequence was derived from a common lab strain of Ad2 (Genbank accession number J01917.1, pKGO-170 plasmid) that was isolated in 1953 from a spontaneously degenerating tissue culture of adenoid tissue removed from a 7 year old girl with hypertrophied tonsils and adenoids
- Proximal TARP promoter was amplified from human genomic DNA
- PSA enhancer was derived from the PSA73Luc plasmid
- PSMA enhancer was amplified from human genomic DNA
- Mouse H19 imprinting control region was derived from the pGEM-ICR plasmid

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

- Ad2 E1A interacts with various host intracellular proteins, such as key regulatory proteins, chromatin remodelling proteins and transcription factors, and in this way promotes transcription of the adenoviral genes and replication of the virus.
- TARP promoter + PSA enhancer + PSMA enhancer form a regulatory element that controls replication of the GMO, thereby allowing for replication in prostate cells only
- Mouse H19 imprinting control region acts as an insulator, thereby protecting the transgene expression cassette from interfering signals from the surrounding environment, such as the left inverted terminal repeat (LIRT), either by blocking the action of a distal enhancer on a promoter or by acting as a barrier to prevent silencing effects caused by nearby condensed chromatin.

(d) Location of the insert in the host organism

- on a free plasmid (.)
- integrated in the chromosome (.)
- other, specify: integrated in the genome of the GMO at the site 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 inserts were derived from existing plasmids or human genomic DNA.

2. Complete name

(i)	order and/or higher taxon (for animals)	Hominidae
(ii)	family name for plants	…
(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 (X) No (.) Unknown (.)
Specify: Reproduction of the GMO is controlled and will only occur in prostate cells.

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

Yes (X) No (.) Not known (.)
Specify: Horizontal transmission of the GMO may occur via infection through contact with body fluids containing shed GMO. Due to its prostate-specificity, the GMO will only replicate in prostate cells. Since the prostate is not a target organ for Ad5 upon normal infection, the GMO will not multiply and will be rapidly neutralized by the defense mechanisms, such as the immune system, of the infected individual. As such, no further spreading of the GMO to other persons will occur.

(d) is the GMO in any way different from the recipient as far as pathogenicity is concerned?
Yes (X) No (.) Not known (.)
Specify: The pathogenicity of the GMO is attenuated when compared to Ad5 due to controlled expression of E1A, limiting replication to prostate cells only, and deletion of the E3 genes that are involved in downregulating the human immune system.

2. Genetic stability of the genetically modified organism
Full genomic sequence analysis and identity testing by a PCR specific for the GMO was performed on the Master Virus Bank (MVB). Both testings confirmed the identity of the GMO based on comparison with a reference standard of the GMO. In addition, a restriction digestion map analysis as well as immunoblotting to assess expression of the adenoviral E1A protein confirmed the genetic stability of the Bulk Product. The presence of a wide range of viral contaminants has been excluded.
The GMO lacks the E1B region. A semi-quantitative PCR was used to test for the presence of E1B-containing variants originating from recombination of the GMO with the E1B region from the genome of the producer cells. The PCR had a sensitivity of 100 VP of E1B-containing virus in a background of 8x10E10 VP of the GMO, and showed amplification over a range of 5 logs (1x10E6 VP to 100 in a background of 8x10E10 VP of the GMO). The specificity and robustness of this assay were also confirmed. In the MVB, two Clinical Lots and the Pooled Bulk Clinical Product, E1B-containing variants were detected at a comparable level. The estimated level of E1B-containing variants in the final clinical product is 1 ppm, i.e. 1 VP per 1x10E6 VP GMO.

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)
The pathogenic effect of the GMO is identical to that of the parental virus. Through

replication and expression of adenoviral proteins, the infected cell eventually will be killed. The viral particles are released and can then infect neighbouring host cells. Due to controlled replication of the GMO, this cytotoxicity will only occur in infected human prostate cells, a desired effect since the GMO will be tested as an adjuvant to radical prostatectomy of prostate cancer. In contrast, for the parental virus Ad5 cytotoxicity will not be restricted to a specific tissue type since replication is not controlled.

Based on the preclinical results regarding the specificity of the GMO obtained from human primary cells and normal mice, it is highly unlikely that infection of non-prostate cells by the GMO will result in cytotoxicity due to the lack of replication. In the worst case scenario that the level of replication in non-prostate cells would be sufficient to induce a certain degree of cytotoxicity, the pathogenic effects will be comparable to those caused by the parental virus Ad5, for example cold symptoms in case of infection of airway epithelium. In immuno-competent individuals infections with Ad5 are asymptomatic and self limiting, requiring no treatment. Therefore, an important inclusion criterion for the proposed trial is that patients should be immuno-competent. As such, any GMO that will leak from the prostate into the circulation will be rapidly neutralised by natural intervention, namely through the patient's immune system. In this respect, it is important to note that the chance of infection of non-prostatic tissues by Ad[I/PPT-E1A] in the proposed trial is restricted due to the local route of administration, i.e. intraprostatic injection.

4. Description of identification and detection methods

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

The GMO can be detected using a GMO-specific quantitative PCR. If a sample tests positive, a specimen can be assessed further for the presence of infectious particles using a viral culture system can be performed. In this system 239 cells, which express the adenoviral E1 gene and therefore will allow for the replication of the GMO, and Hep2-cells are cultured in the presence of the specimen. A cytopathogenic effect (CPE) in the 293 cells after 48 h of culture and/or a positive immunofluoresence signal with an adenovirus-specific antibody in 293 cells in combination with no effect in the Hep2-cells will be considered as an indication for the presence of infectious GMO particles in the specimen.

(b) Techniques used to identify the GMO

GMO-specific quantitative PCR.

F. Information relating to the release

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

Prostate cancer is one of the most common malignancies in Western men. In the majority of these patients, localised prostate cancer is diagnosed. Localised prostate cancer is primarily treated by radical prostatectomy. However, for a substantial number of patients this treatment fails, resulting in growth and fatal metastasis of the tumor. Therefore, there is a strong need for improvement of the efficacy of radical prostatectomy, for instance through adjuvant therapy with an oncolytic adenovirus. An oncolytic adenovirus is a common human cold virus that has been modified in the laboratory in such a way that it can only replicate in specific target cells, such as tumor cells, and thereby kill these cells. In this project, the safety and tolerability of a novel prostate-specific oncolytic adenovirus will be studied in patients with localised prostate cancer prior to radical prostatectomy. Replication of this GMO is

controlled by a prostate-specific regulatory element. Because of this prostate-selective replication, the GMO will therefore only destroy prostate cells. This might facilitate the surgical removal of the prostate as well as induce anti-tumour immunity that may attack micrometastasis present at the time of surgery or recurrence after surgery. Besides the analysis of the safety of the novel GMO, the effects on the prostate and on the immune system of the patient will be extensively studied with a focus on the induction of anti-tumor immunity. In this way, this project will contribute to a better understanding of the working mechanism of oncolytic adenovirus therapy.

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):
Erasmus MC, Rotterdam, The Netherlands

(b) Size of the site (m^2): … m^2
(i) actual release site (m^2): 16 m^2
(ii) wider release site (m^2): unknown

The GMO will be administered to the patient via intraprostatic injection in a standard patient room by well-trained personnel in an outpatient unit. After the procedure, the patient is dismissed.

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

(d) 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:

The GMO will be administered at 1x10E11, 1x10E12 or 5x10E12 Virus Particles (VP) by intraprostatic injection under guidance of transrectal ultrasound in 4 deposits with a total volume of 1 ml. The first cohort of four patients will be treated with a dosage of 1x10E11 VP. If none of the patients experiences a dose limiting toxicity (DLT), the dose will be increased with one log to 1x10E12 VP for the next cohort of 4 patients. If a patient experiences a DLT, two additional patients will be enrolled at that dose level and the dose will be escalated if no more than 1 of 6 has a DLT. If DLT is seen in two patients at a dose level, that dose escalation will cease and the previous dose level will be expanded to include a total of six patients. If the dosages of 1x10E11 VP and 1x10E12 VP are tolerated well, the study will be extended with a third group of 4 patients treated with 5x10E12 VP.

(b) Duration of the operation:

The GMO injection procedure will take about 30-60 minutes in total. About three weeks after injection, the prostate will be surgically removed.

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

To avoid transmission of the virus in case of a spilling accident during administration, the following specific safety measures will be taken:

- Trial personnel will be instructed on the safe use of the GMO and the risks associated with working with an oncolytic adenovirus.
- The virus will be administered in a standard patient treatment room at the outpatient unit. The room will be equipped with appropriate materials for cleaning in case of a spilling accident.
- The procedure will be scheduled at the end of the day, allowing for extensive cleaning and disinfection of the room afterwards.
- During virus injection, trial personnel and the patient will wear appropriate clothing, including gloves, mouth mask and coat.
- The virus will be administered by a urologist experienced in the ultrasound-guided transrectal biopsy procedure that will be used for virus injection.
- A safety protocol for accidents will be available.

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

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 study will be the first to test this GMO in humans. Therefore, there are no data available on this GMO. Data from other trials testing oncolytic adenovirus therapy in patients with prostate cancer show that this type of GMOs is well tolerated and that shedding upon intraprostatic delivery very limited and shortlasting.

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

Not applicable

2. Anticipated mechanism and result of interaction between the released GMOs and the target organism (if applicable)

Exposure of human beings or the environment to the GMO is linked with far less harmful effects compared to the parental virus Ad5, since the GMO is a strongly attenuated, prostate-specific virus that does not express a transgene:

- Infection of third persons by this oncolytic virus after introduction into the environment can only result in a harmful effect, i.e. cytotoxicity, in the prostate of that person. The risk of a harmful effect in third persons is negligible, since infection with the GMO via the transmission routes known for Adenovirus will not result in infection of the prostate. For this event to occur, distribution of the virus via the circulation from the site of infection to the prostate is required. Systemically circulating Adenovirus is rapidly inactivated through the human immune system and binding to erythrocytes and thus infection of the prostate in a third person is highly unlikely.
- Due its prostate-specific replication, the GMO is not cytotoxic for airway epithelial cells and is not able to induce a common cold as the parental virus Ad5 does.
- The GMO only expresses common adenoviral proteins and does not express a transgene. Therefore, upon exposure there will be no harmful effect due to the expression of a transgene.
- The host specificity of the GMO is limited to humans only. Therefore, exposure of nature and animals to this virus will not result in harmful effects.

Exposure of human beings or the environment to the E1B-containing variants present in the clinical GMO product at a level of 1 ppm is linked with far less or comparable harmful effects compared to the parental virus Ad5:

- E1B-containing variants that have the I/PPT regulatory element resemble the GMO and harmful effects will be restricted to the prostate only.
- E1B-containing variants that have the wt E1A promoter instead of the I/PPT regulatory element resemble wt Ad5 but still lack the E3 gene. Infection of third persons with these variants can therefore result in a common cold, but due to the absence of the immunoregulatory E3 gene these variants will be far more rapidly neutralised by the immune system.
- E1B-containing variants only express common adenoviral proteins and do not express a transgene. Therefore, upon exposure there will be no harmful effect due to the expression of a transgene.
- The host specificity of E1B-containing recombinants is identical to the GMO, and as such is limited to humans only. Therefore, exposure of nature and animals to this virus will not result in harmful effects.

3. Any other potentially significant interactions with other organisms in the environment
Theoretically, but very unlikely, a human cell can be infected with both the GMO and wt Ad5. Recombination between the GMO and wt Ad5 might occur in this cell, which can result in various recombinants:

a. Replacement of the Ad2 E1A gene insert by the Ad5 E1A gene from the wt virus -> this will result in a recombinant with similar characteristics as the parental GMO.
b. Introduction of E1B gene in the GMO -> this will result in more efficient replication in prostate cells with a normal p53 pathway, resulting in the generation of more GMO- related particles in the prostate.
c. Introduction of the E3 gene in the GMO -> this will result in a GMO recombinant that is more resistant to the patient's immune system and may therefore persist longer.
d. Replacement of the I/PPT promoter by the wt Ad5 E1A promoter -> this will result in non-selective replication, which might result in more efficient generation of GMO-related particles in the prostate.

e. A combination of the events described at b-d, leading to the introduction of the E1B gene +/- the E3 gene in the GMO +/- the replacement of the I/PPT promoter by the wt Ad5 E1A promoter -> this will result in more efficient and non-selective replication, resulting in the generation of more GMO-related particles in the prostate.

These recombinants will be less or equally pathogenic compared to wt Ad5.

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

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

Give details

The GMO is less pathogenic compared to the parental virus Ad5 due to controlled replication. Only in case of recombination events as described at G3, the GMO can become more competitive. Since the tropism is unmodified compared to Ad5, invasiveness is unchanged.

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 can be shed into the environment via excreta of the treated patient. Taken the current knowledge on adenovirus-based therapy into consideration, together with the known human neutralisation mechanisms for systemic Ad5 (i.e. immune system and sequestration by erythrocytes), widespread distribution of the GMO to other organs upon intraprostatic delivery resulting in shedding of large amount of virus via various excreta, most likely will not occur in the proposed trial.

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

(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	…

Not applicable X

7. Likelihood of genetic exchange in vivo

(a) from the GMO to other organisms in the release ecosystem:
Exchange between the GMO and other organisms is highly unlikely (see G3).

(b) from other organisms to the GMO:
Exchange between the GMO and other organisms is highly unlikely (see G3).

(c) likely consequences of gene transfer:

Gene transfer between the GMO and the parental virus Ad5 will result in variants with unmodified tropism and attenuated or similar pathogenicity compared to Ad5.

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.):
This study will be the first to test this GMO in humans. Therefore, there are no data available on the behavior and characteristics of the GMO.

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
In order to gain scientific insight in the biodistribution and shedding of the GMO, blood and urine will be tested for the presence of virus DNA by PCR at day 1, 2, 4, 7, 14 and 21 after virus injection. For this purpose, a GMO-specific PCR using primers for the PPT promoter and the E1A sequence will be used. Furthermore, the presence of the GMO in the prostate and draining lymph nodes will be studied by immunochemistry about 3 weeks after virus injection.

2. Methods for monitoring ecosystem effects
There is a negligible risk of adverse effects due to the shedding of the GMO into the ecosystem. Therefore, 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 treated patient to other organisms. Therefore the transfer of donated genetic material will not be monitored.

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

5. Duration of the monitoring
See H1.

6. Frequency of the monitoring
See H1.

I. Information on post-release and waste treatment

1. Post-release treatment of the site
The treatment room and equipment used for administration of the GMO will be cleaned with an active chloride solution (250 ppm; 1 tablet Medacarine in 6 liter of water).

2. Post-release treatment of the GMOs
There is no need for post-release treatment of the GMO. Because of ethical considerations, patients are advised to use a condom during sexual intercourse in the period between GMO

injection and radical prostatectomy to minimise the risk of apparent exposure of third persons to a GMO.

3. (a) Type and amount of waste generated
Waste containing the GMO will be generated during virus preparation and injection (e.g. the syringe used for injection) and during clinical monitoring. Waste generated from the original vial and during administration will be disposed according to Appendix 8 of Dutch Regeling GGO (UN 3291 classification).

3. (b) Treatment of waste
See I3(a)

J. Information on emergency response plans

1. Methods and procedures for controlling the dissemination of the GMO(s) in case of unexpected spread
Hospital Hygiene Regulations are in place describing the measures to be taken in case of a spilling incident. A tissue soaked in an active chloride solution (1000 ppm; 1 tablet Medacarine in 1.5 liter of water) is place on the spilled GMO for 15 minutes. The person cleaning the spilled GMO should wear gloved to prevent manual contact with the spilled material. After 15 minutes, remaining liquid is absorbed using dry tissues. The tissues and the used gloves are disposed in a container for hospital waste (UN 3291). The person who has cleaned the spilled GMO should wash his/her hands extensively with water and soap.

2. Methods for removal of the GMO(s) of the areas potentially affected
See J1.

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
Patients will be monitored for the occurrence of serious adverse events (SAE) according to the clinical protocol: each SAE will be registered and evaluated, and health authorities will be notified when relevant.
Based on the environmental risk assessment, it is concluded that the environmental risks associated with the proposed clinical trial are negligible. Therefore, besides the precautions taken during the trial no specific plans for protecting human health and the environment are foreseen.