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	Netherlands
(b)	Notification number	B/NL/08/008
(c)	Date of acknowledgement of notification	03/10/2008
(d)	Title of the project	A Phase I/II Trial of a Conditionally Replication-Competent Adenovirus (Delta-24-RGD) Administered by Convection Enhanced Delivery in Patients with Recurrent Glioblastoma Multiforme.
(e)	Proposed period of release	From 01/01/2010. until 01/01/2013

2. Notifier

Name of institution or company: Erasmus Medical Center

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

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

Full genomic sequence analysis and genetic stability analysis of the 24 base pair deletion in the adenoviral E1a region by TaqMan®-based qPCR were done on the Master Viral Bank and on the Purified Filtered Bulk Product. Restriction digestion and molecular weight analysis by loading on an agarose gel for electrophoresis, followed by band size comparison to known molecular weights of marker bands was done on the Master Viral Bank and the Final Vialed Product.

The limit of detection of the qPCR technique was determined to be <15 copies of wild-type Ad5E1a in 3x 10^4 vp/reaction, which is equivalent to less than 1 wild type Ad5E1a copy per 2,000 Ad5-Delta24-RGD copies. The test was valid with an R^2=0.998 and Ct values for all three negative controls were > 40. No wild type E1a DNA was detected in the filtered final bulk virus test sample analyzed in triplicate (no amplification beyond the threshold fluorescence Ct > 40). A review of the amplification plot indicated that no nascent amplification below the threshold was noted for the three test replicates. Thus, absence of wild type E1a copies, and thus of wild type adenovirus, was confirmed in three independent test samples from the final filtered bulk GMO product, with a 1:2,000 sensitivity.

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	(X)	No	(.)

If yes:

- Member State of notification USA
- Notification number NCT00562003 and NCT00805376 (ClinicalTrials.gov Identifiers)

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

The potential environmental impact of the GMO is minor. It cannot be excluded that the GMO is shed from the primary recipient. Although the GMO has an expanded infection tropism compared to wild type adenovirus, transmission to a secondary recipient will be as efficient as transmission of wild type adenovirus, because the cells most likely to come in contact with the transmitted GMO and susceptible to infection are upper respiratory tract cells already very efficiently infected via natural virus uptake. The infection tropism modification of the GMO is not needed to pass the dose threshold for infection and can therefore be considered redundant in this respect. Importantly, because the GMO has cancer cell specific replication properties it will not

replicate in healthy cells of a secondary recipient and further dissemination is thus prevented. Possible toxicity to the infected cells in the secondary recipient will be similar or less than that of wild type adenovirus, which is itself already a mild pathogen.
Theoretically, but very unlikely and never observed in practice, the GMO could recombine with wild type adenovirus in a recipient infected with both viruses. In this case, a GMO derivative could be formed with wild type replication properties and expanded infection tropism. Further horizontal transmission of such a theoretical GMO derivative via respiratory tract infections is not expected to be any different from that of wild type adenovirus, causing the same mild symptoms and being self-limiting. However, such a newly formed GMO derivative may be less harmless than the wild type virus if this virus would in any way (other than the natural transmission route) reach other tissues in other individuals. Although animal experiments do not suggest that the tropism modification changes its biodistribution, it cannot be excluded that a newly formed GMO derivative with wild type replication properties and expanded tropism could be more pathogenic than wild type adenovirus on certain cells in the human body.

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	subgroup 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
Human adenovirus is detected by cell culture and/or (quantitative) PCR.

(b) Identification techniques
Human adenovirus 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

Over 50 distinct human adenovirus (Ad) serotypes have been isolated, which vary in their pathogenicity. The subgroup C adenoviruses (which includes serotype 5) usually cause mild upper respiratory, gastrointestinal and ocular infections in young children. Shedding via the gut following acute infection can occur, and latent infection of the adenoids and tonsils is also recognised. However, life-long immunity to the specific serotype is thought to be the usual outcome of primary infection. Exposure to subgroup C adenoviruses is widespread in the population; the majority of adults are seropositive for this type of adenovirus. In immune-competent adults, infections with Ad5 are mostly asymptomatic and usually self-limiting. Under normal circumstances other animals than humans are not susceptible to human serotype adenoviruses and no diseases in other animals can be demonstrated caused by the human serotype.

8. Information concerning reproduction

(a) Generation time in natural ecosystems:
Adenovirus serotype 5 replicates in approximately 24 hours.

(b) Generation time in the ecosystem where the release will take place:
In cancer cells, the GMO replicates with a generation time similar to that of wild type Ad5. In healthy cells, its replication capacity is strongly inhibited.

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

(c) Factors affecting reproduction:
The reproduction of the GMO is restricted compared to wild type Ad5 due to the Delta24 modification in the E1A region. This modification abrogates the capacity of adenovirus encoded E1A protein to bind pRB pocket protein family members. This prevents release of E2F from E2F-pRB complexes, which in turn prevents activation of adenoviral genes and genes involved in DNA synthesis and progression through cell cycle. Consequently, the GMO is incapable of inducing host cells to pass the G2/M checkpoint and can therefore replicate only in cells with a defective pRb pathway. A defective pRb pathway is a hallmark of cancer. There are indications that a deregulated pRb pathway might also be implicated in other diseases, including Parkinson's disease and Alzheimer disease. Furthermore, it has been suggested that pRb activity may also be inhibited in hyperproliferative smooth muscle cells during restenosis after balloon angioplasty. Thus, pRb pathway defects are very common in

cancer cells and could also exist in other types of diseased cells. In contrast, pRb defects have not been described to occur in healthy cells. Consequently, the GMO exhibits strongly reduced replication property compared to wild type Ad5 in quiescent normal human cells. However, it cannot be excluded that the GMO could perhaps also reproduce in actively proliferating normal cells progressing through cell cycle in the human body.

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:
At ambient temperature and high relative humidity adenoviruses may survive for several weeks on various surfaces. In frozen condition, the virus can be kept for years.

10. (a) Ways of dissemination
The common route of Ad5 transmission is via inhalation or uptake in the eye of aerosols produced through couching/sneezing by infected individuals. Under these moist conditions, transmission is effective. Adenovirus transmission can also occur via the fecal-oral route, but this transmission route requires intimate contact and is unusual.

(b) Factors affecting dissemination
Dissemination is affected by the dose shed, the production of aerosols and the 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/03/01, B/NL/05/006

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

The GMO contains a full-length Ad5 genome with the following two modifications. First, a 24 base pair deletion (Delta24) was introduced in the pRb-binding CR2 domain of the E1A gene. This modification renders adenovirus replication defective in normal cells with intact Rb protein. As a result, the GMO exhibits selective replication in cells with a defective pRb pathway, most notably cancer cells. Second, a short sequence encoding an integrin-binding RGD-4C peptide was inserted in the fiber gene. This modification allows cell infection via high-affinity binding via several types of integrins. As a result, also cells that do not express high-affinity receptors for the virus and are therefore difficult to infect with Ad5, but that do express these integrins can be infected with the GMO. Glioblastoma multiforme cells in brain tumors exhibit the characteristics (pRb pathway defects; low adenovirus receptor expression, high integrin expression) for which the GMO was designed. The GMO is intended to replicate efficiently and selectively in glioblastoma multiforme cells in the brain of patients injected with the GMO, thereby killing these cancer cells effectively.

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 is made from the plasmid vector pVK526 (Suzuki et al., Clin. Cancer Res. 7, 120-126, 2001). This plasmid contains a bacterial origin of replication, an ampicillin resistance gene and a full-length Ad5 genome with the following two modifications:

- a 24 base pair deletion (Delta24) in the pRb-binding CR2 domain of the E1A gene
- a short synthetic sequence insert encoding an integrin-binding RGD-4C peptide in the fiber gene

(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
Ampicillin

(e) Constituent fragments of the vector
see 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 : Upon release of the Ad5-derived DNA genome from the plasmid vector by restriction enzyme digestion, it is transfected into A549 human epithelial lung carcinoma cells. In these susceptible cells, the GMO is formed. The GMO contains only the full-length Ad5 genome with the two modifications. The 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
Synthetic DNA sequence encoding a cyclic RGD-4C motif.

(b) Source of each constituent part of the insert
Synthetic

(c) Intended function of each constituent part of the insert in the GMO
Extension of infection range to include human cells with low expression of high-affinity adenovirus receptor, but high integrin expression.

(d) Location of the insert in the host organism

- on a free plasmid (.)
- integrated in the chromosome (.)

- other, specify : integrated in the fiber gene ORF on the virus DNA

(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 synthetic DNA (D 2 – D 5 are not applicable)

2. Complete name

(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 …

3. Is the organism significantly pathogenic or harmful in any other way (including its extracellular products), either living or dead?
Yes (.) No (.) 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 (.) 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 (.)
If yes, specify …

5. Do the donor and recipient organism exchange genetic material naturally?
Yes (.) No (.) 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 …

(b) is the GMO in any way different from the recipient as far as mode and/or rate of reproduction is concerned?
Yes (X) No (.) Unknown (.)
Specify Due to the Delta24-modification, the GMO cannot replicate in quiescent normal cells with intact Rb pathway. Thus, in healthy cells the reproduction of the GMO is reduced compared to the parental virus Ad5. The RGD-4C modification broadens the infection-tropism of the virus. Also cells that do not express high-affinity adenovirus receptors and are therefore difficult to infect with Ad5, but do express certain types of integrins can be infected with the GMO. This modification could thus potentially increase the reproduction of the GMO compared to wild type Ad5. Notably, the broadening of infection by the RGD-4C modification does not affect the attenuation of replication by the Delta24-modification. Normal cells lacking high-affinity adenovirus receptors but expressing integrins, which would not be infected efficiently by wild type Ad5, can be infected by the GMO, but the GMO will not replicate in these cells if they are non-dividing. Therefore, the RGD-4C modification is considered not to increase the reproduction rate. Overall, the reproduction of the GMO is reduced compared to the parental virus Ad5.

(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 the natural Ad5 transmission route, i.e., uptake of aerosols created by coughing or sneezing via inhalation or the eye. Since this uptake is already very efficient via the natural Ad5 receptors, the RGD-4C modification in the GMO is irrelevant in this respect. In contrast, the Delta24 modification attenuates virus replication in normal cells, thus reducing further spread of the GMO from

secondary recipients. Thus, if the GMO is released into the environment, it is expected to transmit horizontally to secondary recipients like wild type Ad5, but due to its attenuated replication property it will extinguish, thus reducing further dissemination.

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

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

Specify Due to the Delta24-modification, the GMO cannot replicate in quiescent normal cells with intact Rb pathway. Thus, the replication-tropism of the GMO is narrowed compared to the parental virus Ad5. The already low pathogenicity of wild type Ad5 is therefore further reduced. The RGD-4C modification broadens the infection-tropism of the virus. Also cells that do not express high-affinity adenovirus receptors and are therefore difficult to infect with Ad5, but do express certain types of integrins can be infected with the GMO. This modification could thus potentially increase the pathogenicity of the GMO compared to wild type Ad5. Following high-dose systemic administration to cotton rats, the GMO did not show altered biodistribution compared to a control virus lacking the RGD-4C infection-tropism modification. Thus, apparently cells that do not express natural Ad5 receptors in sufficient amounts to allow effective infection, but that do express integrins to which RGD-4C can bind to mediate virus uptake are not detectably reached in steady-state healthy tissues. The GMO is therefore not considered more pathogenic than wild type Ad5 to steady-state healthy cells and tissues that can be reached via the circulation. Notably, the broadening of infection by the RGD-4C modification does not affect the attenuation of replication by the Delta24-modification. Normal cells lacking high-affinity adenovirus receptors but expressing integrins, which would not be infected efficiently by wild type Ad5, can be infected by the GMO, but the GMO will not replicate in these cells if they are non-dividing. Thus, the infection-tropism modification could in theory increase pathogenicity of the GMO compared to wild type Ad5 only on actively dividing cells that do not express the natural Ad5 receptors in sufficient amounts to allow effective infection, but that do express integrins to which RGD-4C can bind to mediate virus uptake. Glioblastoma multiforme cells fulfill these requirements and are thus expected to experience increased pathogenicity by the GMO compared to Ad5. This is the intended effect of the RGD-4C inclusion in the GMO. Apart from gliobastoma multiforme cells or other cancer cells with low adenovirus receptor expression, we envision two types of cells that could potentially experience increased pathogenicity of the GMO compared to Ad5. The first type of cell in the human body that could be subject to increased pathogenicity compared to Ad5 (i.e., because it is difficult to infect with Ad5, expresses integrins, undergoes active cell cycle and can be reached via the blood circulation) is an activated endothelial cell in an angiogenic blood vessel wall. Angiogenesis occurs in tumors, where this possible increased pathogenicity is therefore considered a positive attribute of the GMO. Angiogenesis however also takes place during wound healing and the menstrual cycle. Productive infection of these cells with the GMO could in theory inhibit new blood vessel formation by damaging newly formed vessel wall cells. Therefore, the GMO could perhaps inhibit wound healing and regeneration of the endometrium. Experimental evidence for this is lacking. The second type of cell in the human body that could be subject to increased pathogenicity compared to Ad5 is a stimulated peripheral blood mononuclear cell (PBMC). It has been described that the GMO at high dose could decrease proliferation of human PBMC with interleukin-2 and PHA, thus indicating possible toxicity of the GMO. However, analysis of virus copy numbers indicated that the GMO had not replicated in these cells.

2. Genetic stability of the genetically modified organism

The GMO is stable. The GMO is produced in the A549 cell line. The MCB was tested and found negative for a range of viral contaminants, including Ad5 by quantitative PCR for E1A and hexon genes. Complementation by or recombination with wild type E1A during propagation of the GMO on A549 cells can therefore be excluded. Full genomic sequence analysis and genetic stability analysis of the 24 base pair deletion in the adenoviral E1A region by TaqMan®-based qPCR were done on the Master Viral Bank and on the Purified Filtered Bulk Product. Absence of wild type E1A copies, and thus of wild type adenovirus, was confirmed in three independent test samples from the final filtered bulk GMO product, with a 1:2,000 sensitivity (see A 3 c).

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 GMO will be administered to the brain. The main physiological process expected to take place there is lytic replication in and destruction of cancer cells. Normal brain cells are post-mitotic. Due to its replication attenuation in non-cancerous cells, the GMO is expected to be less pathogenic in this locale than the mild pathogen Ad5. However, leakage of the GMO from the brain is possible to the cerebrospinal fluid and/or the blood. The likelihood for this to occur is unknown. Therefore, we discuss the worst case scenario, assuming that this will happen. It is expected that – similar to what is known for Ad5 - most of the GMO that may have leaked into the circulation will be sequestered and destructed by Kupffer cells in the liver. A biodistribution study following intravenous injection in cotton rats where the GMO was compared to a control virus lacking the RGD-4C modification revealed almost identical distributions for the two viruses over blood and tissues. This suggested that the impact of the RGD-4C modification on the spreading of the virus through the body is minimal. However, measuring virus copy numbers in total tissues does not allow detection of differences in infection of individual cell types within the tissue. Therefore, we cannot exclude that the GMO may exhibit a changed pathogenicity at the individual cell level. If the GMO leaks from the brain into the circulation it might infect integrin-expressing normal cells that are difficult to infect with Ad5, possibly endothelial cells lining the blood vessels. Because these cells are usually non-cycling, replication of the GMO in these cells will be strongly reduced compared to Ad5. Thus, although more cell types could perhaps become infected by the GMO than by Ad5, its overall pathogenicity is considered lower. Under certain conditions, healthy endothelial cells will be activated to proliferate. This takes place in tumor blood vessels, during wound healing and the menstrual cycle. Productive infection of these cells with the GMO could in theory inhibit new blood vessel formation by damaging newly formed vessel wall cells. This could thus inhibit wound healing and regeneration of the endometrium. Experimental data on infection of endothelial cells during normal angiogenesis in healthy tissues with wild type Ad5 and the GMO are lacking. The impact of the GMO modifications in this respect are therefore unknown. Furthermore, also infection without replication may evoke an immune response against infected cells expressing early viral proteins. Similar to infections with Ad5, infection with the GMO will be self-limiting due to an evoked immune response.

4. Description of identification and detection methods

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

Presence of the GMO DNA is detected by specific, quantitative PCR, using an amplification primer pair specific for the adenovirus fiber gene and a TaqMan® fluorescent probe specific for the RGD-4C encoding insert in the fiber gene. Functional assays for viable virus, which do not discriminate between the GMO and wild type adenovirus, include an infection assay and a plaque assay. In these assays, a cell line susceptible to adenovirus infection and replication is exposed to serial dilutions of the virus. In the infection assay, the cells are stained for adenovirus hexon protein expression and results are expressed as infectious units per ml. In the plaque assay, the cells are cultured until viral plaques are visible. The plaques are counted and the results are expressed as plaque forming units per ml. To confirm the identity of the GMO in the functional assay, the quantitative PCR can be done on plaque-containing cultures.

(b) Techniques used to identify the GMO

Specific quantitative PCR described under a.

F. Information relating to the release

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

Malignant brain tumors, especially glioblastoma multiforme (GBM) are among the most aggressive human tumors that exist. The median survival after diagnosis is approximately one year, despite maximal aggressive treatment. Novel therapies for this devastating disease are urgently warranted, due to an unmet need for an effective therapy. One of the key problems in GBM treatment is its invasive character, which causes the tumor to consist of two components: a solid component that can be safely resected with high precision neurosurgical techniques; and a diffuse component, consisting of infiltrated tumor cells in the surrounding healthy brain, inaccessible for surgery. Due to the existence of the Blood-Brain Barrier, drug based therapies such as chemotherapy remain insufficiently effective. The GMO is a genetically modified adenovirus that owes strong anti-tumor efficacy due to its capability of selective replication in cancer cells, resulting in direct and very efficient cell kill (tumor cell lysis). Because the expression level of high-affinity adenovirus receptors is usually low on glioma cells, the GMO incorporates an integrin binding peptide (RGD-4C peptide) in its fiber capsid protein. This peptide binds with high affinity to several types of integrins present on the surface of mammalian cells, including glioma cells. Infiltrated brain tumor cells around the solid brain tumor mass show high integrin expression, whereas in surrounding normal brain integrin expression is absent. The GMO will be delivered to infiltrating tumor cells in the diffuse part of the tumor in patients with GBM using a new method called Convection Enhanced Delivery. This method consists of very slow continuous infusion through thin catheters that are temporarily inserted in the brain. This should enhance convection or flow of interstitial fluid in between the brain cells, carrying the GMO over long distances up to several centimetres throughout the brain tissue. The clinical trial aims to acquire preliminary clinical safety and efficacy data with the GMO in this setting. The ultimate aim is to develop an effective treatment against recurrent GBM.

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 Medical Center, Rotterdam

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

Administration of the GMO to the patient will be done in a dedicated room with isolated care, according to the WIP (Werkgroep Infectie Preventie) regulations. Virus administration will continue for a period of 2 to 3 days. Staff inside the room will adhere to the gowning and operating guidelines for barrier nursing to prevent infections, which are similar to routine universal precautions taken during care of virus-infected patients. After ending the infusion and removing the catheters the patient will remain in isolated care until urine, pharyngeal and anal swabs are negative for the GMO for at least three consecutive days and catheter entry wounds on the head are confirmed to be dry.

(ii) wider release site (m^2): … m^2
Not applicable.

(c) Proximity to internationally recognised biotopes or protected areas (including drinking water reservoirs), which could be affected:
Not applicable. Patients will be kept in an isolated room until viral shedding to the environment is excluded.

(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:
In the phase I dose finding part of the study, patients will receive a single infusion at one of six dose levels ranging from 1*10e7 to 1*10e11 viral particles. Three to six patients are treated at each dose level. In the phase II part of the study, 12 patients will receive the maximal tolerated dose (MTD), or the highest dose if the MTD is not reached.

(b) Duration of the operation:
Virus infusion will continue over a period of 50-68 hours, depending on the number of intracranial catheters used for infusion.

(c) Methods and procedures to avoid and/or minimise the spread of the GMOs beyond the site of the release
The viral solution will be prepared in the hospital pharmacy in a class 2 flow cabinet and brought into 2 to 4 different syringes. Each syringe will be sealed and transported to the isolated room of the patient where it will be placed in a special microinfusion syringe-pump and will be connected to the patients externally placed catheter by a tubing, via a luer-lock. Each catheter will be connected to a different syringe. After

infusion, the catheters will be removed from the head of the patient by withdrawing them from the insertion wound without interrupting the connection to tubing and syringe. The complete sets of catheter, tubing and syringe will be packed and sealed and destroyed according to SOP of Erasmus MC for contaminated material. The insertion openings in the skin of the head of the patient, from which the catheters have been removed, will be stitched as to reach complete closure and covered with sterile gaze and a fluid-resistant sterile dressing (tegaderm). The patient will remain in isolated care until excreta and secreta are confirmed negative for the GMO for at least three consecutive days and catheter entry wounds on the head are confirmed to be dry. Excreta and secreta of patients obtained during this period will be handled as (GMO) contaminated material and will be either used or stored for research purposes or destroyed according to SOP for (GMO) contaminated material.

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.
In a phase I study conducted in the USA (University of Alabama in Birmingham), the GMO was administered to patients with ovary cancer once a day for three days by intraperitoneal infusion. No severe toxicity related to the virus administration was observed so far. No relevant data related to the potential environmental impact of the release are available.
A phase I trial with the GMO administered to GBM tumors is currently ongoing in the USA (MD Anderson Cancer Center study number ID01-310). Data on possible release into the environment are not yet available. In this trial, patients are allowed to go home within a few days after virus infusion and return regularly for analysis. Patients with an operable tumor keep the catheter in place and are readmitted to the hospital after two weeks to have the tumor and catheter surgically removed.

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	…

2. Anticipated mechanism and result of interaction between the released GMOs and the target organism (if applicable)
The GMO is anticipated to infect brain tumor cells in the brain of GBM patients, to replicate selectively in these cells and to kill these cells by lysis. Lysed cancer cells will release GMO progeny virus that can also infect and kill brain tumor cells. The main objective of the trial is to investigate the safety of administering the GMO to the brain of patients with GBM and to obtain a first indication of the anti-tumor efficacy of the procedure.

3. Any other potentially significant interactions with other organisms in the environment
There is a theoretical, but highly unlikely, risk that the GMO and a wild type adenovirus infect the same human cell and recombine to form a new GMO with wild type replication properties and expanded (RGD-4C – integrin interaction mediated) tropism. This newly formed GMO might be less harmless than wild type adenovirus.

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 has strongly decreased competitiveness compared to wild type adenovirus due to the Delta24-modification in the E1a gene that attenuates replication in non-cancerous cells. Notably, the broadening of infection by the RGD-4C modification does not affect the attenuation of replication by the Delta24-modification. Thus, increased infection does not alleviate the decreased competitiveness of the GMO in non-cancerous cells. However, in the unlikely recombination event described under G 3, the newly formed GMO with natural replication properties and expanded tropism could exhibit increased competitiveness compared to wild type adenovirus.

5. Types of ecosystems to which the GMO could be disseminated from the site of release and in which it could become established
Patients will be kept in an isolated room until virus shedding is excluded. Therefore there will be no risk for the environment regarding exposure to the GMO.

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

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

(c) likely consequences of gene transfer:

Transfer of wild type E1A to the GMO or transfer of the RGD-4C modification to wild type adenovirus would create a new GMO with wild type replication properties and expanded infection tropism. Infection with such a theoretical GMO will probably be self-limiting due to existing immunity against adenovirus. It can however not be excluded that this newly formed GMO might be less harmless than wild type adenovirus.

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.):
The GMO has been and is currently being administered to patients with cancer of the ovary or GBM in two different phase I clinical trials in the USA (see F 6). Results of these studies have not yet been reported.

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 the GMO is shed from the brain systemically or in the environment, patients will be monitored on whole blood, cerebrospinal fluid, urine, pharyngeal, and rectal samples. Viral DNA is isolated and detected by quantitative PCR specific for the RGD-4C modification present in the adenovirus fiber gene. In case samples are positive by PCR, they are tested for functional virus by infection and plaque assay. If plaques occur, they will be tested for presence of the GMO using the quantitative PCR method.

2. Methods for monitoring ecosystem effects
There is no risk for shedding of virus 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
In case the PCR specific for the RGD-4C modification present in the adenovirus fiber gene is positive on DNA isolated from plaques cultured from patient samples, this DNA will also be subjected to a PCR specific for the E1 region followed by PCR-product restriction analysis specific for the Delta24-modification (the 24 nucleotide deletion removes a BstXI restriction digestion site). Presence of the RGD-4C modification and absence of the Delta24-modification demonstrates the highly unlikely exchange between the GMO and wild type adenovirus.

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

5. Duration of the monitoring
Once before virus administration; then daily starting the day after virus administration until samples are negative for three consecutive days; thereafter at least after 1 and 2 weeks. Blood will also be analyzed after 1 and 3 months.

6. Frequency of the monitoring
See H 5.

I. Information on post-release and waste treatment

1. Post-release treatment of the site
The isolation room will be cleaned with an active chlorine solution (1000 ppm; one tablet Medicarine or Suma Tab D4 in 1,5 liter water).

2. Post-release treatment of the GMOs
Shedding of the GMO from the patient will be monitored. Until no shedding is seen the patients are isolated. Treatment of the GMO is not required.

3. (a) Type and amount of waste generated
Waste includes the complete sets of catheters, tubing and syringes used for virus administration, containing the remaining volume virus aliquot that is not infused; wound-dressings, bed linen, disposable cleaning cloths, disposable clothing worn by the patient, disposable crockery, personnel and visitors, etc; and excreta and secreta of patients.

3. (b) Treatment of waste
Disposable waste and feces will be collected in a special container and destroyed according to procedures for (GMO) contaminated hospital waste (UN 3373). Non-disposable waste will be autoclaved or disinfected with active chlorine solution (1000 ppm; one tablet Medicarine or Suma Tab D4 in 1,5 liter water). To urine, one tablet Medicarine or Suma Tab D4 is added and after at least 5 minutes incubation it is disposed via the toilet.

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 with dry tissues. Subsequently, the surface will be disinfected with tissues soaked in active chlorine solution (1000 ppm; one tablet Medicarine or Suma Tab D4 in 1,5 liter water). All collected materials and gloves worn during execution of the cleaning procedure are disposed according to procedures for (GMO) contaminated hospital waste (UN 3373).

2. Methods for removal of the GMO(s) of the areas potentially affected
See J 1.

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. Specific plans for protecting the environment are not considered necessary, for the reasons stated above.