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/03/01
(c)	Date of acknowledgement of notification	22/07/2003
(d)	Title of the project	Gene therapy in aseptic prosthetic replacement loosening. A phase 1 study
(e)	Proposed period of release	From 01/11/2003 until 31/10/2004

2. Notifier

Name of institution or company: Academisch Ziekenhuis Leiden

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)

To assess genetic stability the vector DNA sequence of the transgene with its flanking regions is obtained by automated sequence analysis under contract. In a less laborious piece of work this is done by restriction analysis. By cutting viral DNA with restriction enzymes and analysing the fragments using agarose gel electrophoresis a pattern of bands for each enzyme can be observed when the gel is stained. By comparing the band patterns generated by several different enzymes chosen to cut throughout the viral genome with reference DNA cut with the same enzyme set, a qualitative similarity confirms the identity of the test article to be the same as the reference.

The presence of RCA in vector supernatant is tested by inoculation onto an adenovirus-susceptible cell line (human lung A549; ATTC CCL 185), and amplification by triple passage with examination for cytopathic effect (CPE) after each passage.

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.

None

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)	Family 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	Adenovirus 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:
…

5. (a) Detection techniques

To detect shedding of the viral vector or replication competent adenovirus Adenovirus subgroup C DNA is measured quantitavely in clinical materials using realtime PCR.

(b) Identification techniques

A reliable, reproducible and sensitive validated PCR was developed for detection of the NTR gene (CMV/NTR amplicon) using the recommended MV2F/NTR6R primer pair. This primer pair routinely allows for the detection of 0.1 copies positive control plasmid (NTR pDNA).

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 viruses (which includes serotype 5) usually cause mild upper respiratory infections in young children, although 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.

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. In laboratory situations animals have been infected with human serotype adenoviruses in experimental conditions. Outside these experimental conditions the risk for an animal te be infected by a human adenovirus is neglectable.

8. Information concerning reproduction

(a) Generation time in natural ecosystems:
The wildtype Ad5 replicates in approximately 20 hours. As the GMO, CTL 102, is replication incompetent there is no reproduction (outside helper cell lines).

(b) Generation time in the ecosystem where the release will take place:
The adenoviral vector CTL 102 lacks the E1 gene and is therefore replication incompetent. It can only replicate in permissive recombinant cells containing the adenovirus E1 gene.

(c) Way of reproduction: Sexual .. Asexual ..
Replication of CTL 102 is only possible in permissive recombinant cells containing the adenovirus E1 gene.

(c) Factors affecting reproduction:
CTL 102, lacks the E1 gene and thus is replication incompetent.

9. Survivability

(a) ability to form structures enhancing survival or dormancy: not applicable

(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:
CTL 102, as with the wild type adenovirus, may survive for weeks on a variety of surfaces. The survival time depends on the temperature and relative humidity (Mahl MC and Sadler C. Virus survival on inanimate surfaces Can.J.Microbiol. 21:819-823, 1974; Gordon Y.J. et al Prolonged recovery of dissicated adenoviral serotypes 5, 8, and 19 from plastic, and metal surfaces in vitro. Ophthalmology 100:1835-1839, 1993).

10. (a) Ways of dissemination
Dissemination through water and air may occur

(b) Factors affecting dissemination

Dissemination of the GMO can occur during processing of the vector and by shedding from the patients which have been treated.

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)

BGGO 00/02
BGGO 01/01
BGGO 01/04

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

Gene delivery is by a replication-defective strain of human adenovirus type 5 (Ad5) into which the *ntr* gene under the control of the CMV promoter has been inserted, together designated **CTL 102**. The vector has been engineered to remove the E1 (essential in replication) and E3 (transcription throughout infection) regions, which result in replication deficiency. Direct intraarticular injection of CTL 102 results in the infection of individual interfacecells by the replication-defective adenovirus, thereby delivering the *E. coli ntr* gene. Expression of *ntr* by the interfacecells renders the cells capable of activating the prodrug CB 1954 into 5-(aziridin-1-yl)-4-hydroxylamino-2-nitrobenzamide, a bifunctional alkylating agent which is capable of DNA interstrand crosslinking in interfacecells resulting in apoptosis or cell death. The toxicity of activated CB 1954 is not dependent upon cellular proliferation; CB 1954 can kill *ntr*-expressing cells regardless of their position in the cell cycle. This is particularly significant considering that most interfacecells are in the G_0 phase of the cell cycle, or cycle very slowly.

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 CTL 102 is derived is Adenovirus type 5. The coding capacity of Ad5 has been modified in three ways:

- Deletion of the E1 region, eliminating the orfs and control elements of E1A and E1B genes
- Deletion of the E3 region, eliminating the orf and control elements of the E3 gene.
- Insertion of an expression cassette bearing the *E.coli ntr* gene at the position of the E1 deletion
- The *ntr* expression cassette contains the cytomegalovirus (CMV) early promoter/enhancer to drive transcription of the *ntr* gene in transduced cells. It also carries the human beta-globin second intron and human complement 2B poly adenylation signal and transcription terminator sequences to ensure efficient expression of the gene product in CTL 102 infected cells.

(c) Host range of the vector

The adenovirus vector CTL 102 is replication incompetent. To survive and replicate it needs to infect a packaging cell line containing a functional E1 gene (i.e. HEK 293 or PER.C6 cells). These cell lines do not exist outside of the development or manufacturing laboratories.

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

Yes (x) No (.)

antibiotic resistance (.)
other, specify Expression of *ntr* by the interfacecells renders the cells capable of activating the prodrug CB 1954 into 5-(aziridin-1-yl)-4-hydroxylamino-2-nitrobenzamide, a bifunctional alkylating agent which is capable of DNA interstrand crosslinking in interfacecells resulting in apoptosis or cell death.

Indication of which antibiotic resistance gene is inserted
…

(e) Constituent fragments of the vector
see C4b

(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

E. coli ntr gene. The DNA sequence is confidential.

(b) Source of each constituent part of the insert

The *ntr* gene was amplified by PCR with genomic DNA of *E. coli* B/r as template and primers containing NotI and HindIII sites for subsequent cloning. The resultant plasmid, pTX0147, expresses *ntr* from the human CMV early promoter/enhancer and also carries the β-globin second intron and polyA sequences and a G418 selectable marker

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

The *ntr* gene expression cassette is composed of the following elements and functions:

CMV promoter and enhancer: required for the initiation of transcription upstream of the gene, it has no protein coding capacity and functions solely as a transcriptional control element. Cloned from human cytomegalovirus, this is a potent promoter with broad activity in most mammalian tissues and cell types, and as such is the ideal promoter for the expression of transgenes inthe with the target patient groups. The CMV promoter has been used extensively in clinical trials, including adenovirus-based vectors, and has a good safety record in man.

***ntr* gene**: contains the translation start site, open reading frame and translation stop site defining and encoding the *E.coli* nitroreductase protein.

Human β-globin intron 1: contains the 3' end of exon 1, the whole of intron 1 and the 5' end of exon 2 with the natural splice donor/acceptor signals at the intron/exon junctions. This sequence enhances expression of the *ntr* transgene by increasing the efficiency of polyadenylation at the 3' terminus of the mRNA. This sequence has no coding capacity expressed in the context of CTL 102 and is a common component of recombinant mammalian expression cassettes

Human complement factor C2 gene 3' region: contains the signals for termination of transcription and polyadenylation of the mRNA. This strong termination signal is required to match the strength of the CMV promoter. Polyadenylation of the 3' end of the mRNA is required for message stability in the cytosol. This sequence has no coding capacity.

(d) Location of the insert in the host organism

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

Integrated into the viral DNA at the site of the deleted E1 gene

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

2. Complete name

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

3. Is the organism significantly pathogenic or harmful in any other way (including its extracellular products), either living or dead?
Yes (x) No (.) Not known (.)
If yes, specify the following:
Although the E coli is normally present in the gastro-intestinal system, some strains of E coli can cause infections in other organsystems, e.g. cystitis, sepsis

(b) to which of the following organisms:

humans	(x)
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 (x) No (.)

If yes, specify class 2, with the exception of non-pathogenic strains

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

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

E. Information relating to the genetically modified organism

1. Genetic traits and phenotypic characteristics of the recipient or parental organism which have been changed as a result of the genetic modification

(a) is the GMO different from the recipient as far as survivability is concerned?

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

Specify …

(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

The GMO is replication incompetent. To replicate it needs the presence of a helper cell line. These cell lines are only present in laboratories of development and manufacturing.

(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

CTL 102 is not pathogenic, the E3 lytic determinants have been removed. Furthermore, the vector is replication incompentent decreasing pathogenicity.

2. Genetic stability of the genetically modified organism

The GMO is genetically stable. Production of replication competent adenovirus (RCA) is possible during amplification of the vector when there are overlapping parts in the vector and the helper cell line. In case of the manufacturing process of CTL 102 production of RCAs is impossible as there are no overlapping parts. The presence of RCAs is tested in the clinical batches. No batch has been tested positive for RCAs in samples of 10^9 particles.

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	(x)
plants	(.)
other	…

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

Goal of the study is to kill interface tissue using GDEPT. After administration of the prodrug CB 1954 cells expressing the gene nitroreductase will be killed by apoptosis. The intention is to kill only interfase cells, but as some of the virus will spread through the body, other cells will be trasnfected as well. When exposed to CB 1954 these cells will also be killed. 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

$TCID_{50}$ Assay: to give a measure of the infectivity of a virus sample. This method estimates its infectivity (Tissue Culture Infectious Dose) by calculating the dilution required to produce cytopathic effect (cpe) in 50% of replicate wells. A cell line that permits virus replication is exposed to serial dilutions of the virus and subsequently immunostained for transgene expression. Results are expressed as $TCID_{50}$ units per ml.

Plaque Assay: to determine the infectivity of the virus. A cell line that permits virus replication (e.g. 911 cells) is exposed to serial dilutions of the virus and subsequently cultured until viral plaques are visible. The plaques are counted and results expressed as plaque forming units (pfu) per ml of the virus stock.

Cell Kill Potency Assay: tests the ability of a batch of recombinant *ntr* -adenovirus to infect test cells (HNX14C) *in vitro* which then express nitroreductase. This test further demonstrates that the *ntr* produced is active by its ability to convert CB 1954 into the alkylating agent which kills the host cells.

(b) Techniques used to identify the GMO

Immunoassays: an ELISA has been used to determine levels of transgene expression following infection of cells with CTL 102. The ELISA specifically detects *ntr* protein, and

comparison of sample lysates with a standard curve allows the cellular concentration of *ntr* in the samples to be determined. In addition, cells that have been exposed to identical virus concentrations are immunostained. This procedure involves the visual detection of cellular *ntr* expression using anti-*ntr* antibodies. Immunostained sections are subjected to image analysis and successfully infecte cells are expressed as a percentage of total cells. This assay also provides further confirmation of identity of CTL 102 by demonstrating the presence of the *ntr* gene.

Polymerase Chain Reaction (PCR) for CTL 102 DNA: is the most sensitive measure of the presence of CTL 102 DNA prepared from tissue samples. Using primers optimised for the CMV/ntr expression cassette, single copy sensitivity is validated.

F. Information relating to the release

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

CTL 102/CB 1954 is a Gene-Directed Enzyme Prodrug Therapy (GDEPT) intended for the treatment of patients with aseptically loosened hip-endoprosthesis.

GDEPT is defined as:

- delivery to interface cells of a gene encoding an enzyme for activation of a prodrug
- expression of that gene within the interface cells leading to a local level of enzyme production
- exposure of the enzyme to the prodrug
- local activation of the prodrug to a highly cytotoxic agent
- killing of the cell containing the enzyme/ activated prodrug
- killing of adjacent cells (= bystander effect).

Direct intraarticular injection of CTL 102 results in the infection of individual interfacecells by the replication-defective adenovirus, thereby delivering the *E. coli ntr* gene. Expression of *ntr* by the interfacecells renders the cells capable of activating the prodrug CB 1954 into 5-(aziridin-1-yl)-4-hydroxylamino-2-nitrobenzamide, a bifunctional alkylating agent which is capable of DNA interstrand crosslinking in interfacecells resulting in apoptosis or cell death (unpublished data). The toxicity of activated CB 1954 is not dependent upon cellular proliferation; CB 1954 can kill *ntr*-expressing cells regardless of their position in the cell cycle. This is particularly significant considering that most interfacecells are in the G_0 phase of the cell cycle, or cycle very slowly.

CTL 102 will be investigated in patients with aseptically loosened hip-endoprosthesis. CTL 102 will be administered by direct injection in the joint to establish infection and expression of the delivered nitroreductase gene in the interfacecells. The study will monitor safety and tolerability of escalating doses of CTL 102 with administration of the chemically synthesised substrate CB 1954 (MW 252 Da) at a fixed dose

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):
Leids Universitair Medisch Centrum, Leiden

(b) Size of the site (m^2): … m^2
(i) actual release site (m^2):
Patients treated with GDEPT will be kept in an isolated room until urine, feces, nose and throat swabs are negative. The room is an isolated one bedded hospitalroom with private sanitary facilities. Reverse barrier nursing procedures will apply with staff in the side room waring gowns, gloves and face masks. ca 25 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:
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
n.a.

4. Method and amount of release

(a) Quantities of GMOs to be released:
The drug product, CTL 102 *injection*, containing up to 1ml nominal volume CTL 102 viral particles solution per dose (single use). The nominal mean potency of between 5×10^8 and 1×10^{12} particles/ml, and buffered at pH 7.4. Per patient one 1 ml aliquot will be used. In total 12 patients will be treated during the study.

(b) Duration of the operation:
November 2003 until October 2004

(c) Methods and procedures to avoid and/or minimise the spread of the GMOs beyond the site of the release
Side rooms will be cleaned daily by the nurses using an approved disinfectant (eg Virkon). Any accidental spillage of potentially infectious material will be dealt with in accordance with procedures in routine use in virus containment laboratories.

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.
The transfer of CTL 102 from injected patients to the external environment is not foreseen in the controlled and sanitary procedures and circumstances of their treatment. In other adenovirus trials, replication competent virus has never been detected in patient waste.

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 GDEPT approach to be used in this clinical trial involves the *in vivo* transfection and expression of *E. coli* nitroreductase enzyme (NTR) in the target interface tissue followed by local exposure to the prodrug CB 1954. Interface cells expressing nitroreductase convert CB1954 into a bifunctional alkylating agent which is capable of DNA interstrand cross linking resulting in apoptosis or cell death. It is anticipated that the local nitroreductase in the interface tissue will convert sufficient prodrug to active material(s) to cause interface tissue destruction. A recombinant, replication-deficient adenovirus is used as the vehicle to deliver the *ntr* gene, while tissue specificity is achieved via intraarticular injection. Gene expression is driven by the cytomegalovirus (CMV) promoter. The combined Ad-CMV-*ntr* system is termed CTL 102.

CTL 102 and CB 1954 together form the basis of a GDEPT programme for a clinical trial directed at the treatment of prosthetic loosening. The clinical trial will be a Phase I study in aseptic prosthetic replacement loosening. In this trial escalating intraarticular doses of CTL 102 will be administered at a fixed dose (concentration) of CB 1954. Safety will be the main objective, with some evidence of efficacy.

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

A theoretical risk is that the GMO infects a human cell that is already infected by a wild-type Ad5. The DNA of the GMO and wildtype virus can then possibly co-replicate resulting in a replication competent Ad5 containing the ntr gene. However, these co-replications have only been demonstrated in experimental conditions with very high MOIs.

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

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

Give details

As the GMO lacks the E1 sequence it is replication incompetent, which results in decreased competitiveness.

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:

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 ntr gene, leading to a local level of enzyme production.

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.):

25 patients with operable liver, head/neck or prostate tumours have been injected intra-tumourally with CTL 102 at doses ranging from 10^8 to $5x10^{11}$ particles. No treatment-related serious adverse events have been observed. ELISAs to detect viral proteins have been conducted on urine, stools and plasma samples 24hrs after injection, and the results showed no detectable shed virus at any dose.

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, urine, pharyngeal, nasal, and rectal samples. Samples will be assessed for Ad5-viruses using real-time PCR.

2. Methods for monitoring ecosystem effects

There is no 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 no 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)

patient's body

5. Duration of the monitoring

Until all outcomes are negative

6. Frequency of the monitoring

Until all outcomes are negative

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 Sodium dichloroisocyanurate solution (1 tablet Stafilex in 1 liter distilled water) and with ethanol 70%.

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

The GMO material is delivered in 1 ml aliquots. In the GMP-facility the GMO is prepared in a syringe. Waste material will be the aliquot, syringes, needles, and materials that come into contact with the GMO during preparation or administration, like gloves, towels.

3. (b) Treatment of waste

All waste material will be autoclaved.

J. Information on emergency response plans

1. Methods and procedures for controlling the dissemination of the GMO(s) in case of unexpected spread

In case of unexpected spread of the GMO the following measures will be taken:

- put on gloves;
- wipe up the spilled material using tissues soaked in a day-fresh solution of sodium dichloroisocyanurate (1 tablet of Stafilex dissolved in 1 liter distilled water);
- collect the tissues in the septobox;
- wash with ethanol 70%;
- collect all material that has been wasted and finally the gloves in the septobox;
- disinfect the hands with Baktosept and use sufficient contact-time. After this wash the hands with water and soap.

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

Same method as in J1.

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

In the clinical trial, the patients will be monitored for 5 years following the single-dose treatment for clinically significant adverse events. Specific plans for protecting the environment are not considered necessary, for the reasons stated above.