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/03/08 |
| (c) | Date of acknowledgement of notification | *07/Apr/2004*. |
| (d) | Title of the project | *A phase 1 dose escalation trial of MDX-010 in combination with CG1940 and CG8711 in patients with metastatic HRPC* |
| (e) | Proposed period of release | From *01/June/2004* until *01/Apr/2006*. |

2. Notifier

Name of institution or company:

*VU Medical Center*
*Department of medical oncology*
*De Boelelaan 1117, 1007 MB A'dam*
*The Netherlands*

3. GMO characterisation

(a) Indicate whether the GMO is a:

| | | |
|---|---|---|
| viroid | | (.) |
| RNA virus | | (.) |
| DNA virus | | (x) |
| bacterium | | (.) |
| fungus | | (.) |
| animal | | |
| - | mammals | (x) |
| - | insect | (.) |
| - | fish | (.) |
| - | other animal | (.) |

specify phylum, class *GM-CSF secreting allogeneic cellular vaccine cells*

(b) Identity of the GMO (genus and species)

*Allogeneic, irradiated prostate cancer cell lines PC3 and LnCAP transduced with*

*rAAV-GM-CSF. After transduction cells were cloned and selected for positive production of GM-CSF and negative testing voor AAV or helper viruses. Subsequently, cells were expanded and prepared for vaccination. Two prostate cancer cell lines are transduced for clinical use (CG1940 and CG8711).*
*A GM-CSF gene is ex vivo transferred into the prostate cancer cells using a replication deficient recombinant adeno-associated viral vector (Family Parvovirideae, genus Dependovirus, Species adeno-associated virus 2).*

(c) Genetic stability – according to Annex IIIa, II, A(10)
*Stable. The transduced cells are subsequently cloned by either single cell sorting (CG1940 cell line) or by picking an individual clone (CG8711 cell line). At this early stage of culturing in 96well plates, the clones are tested by PCR for the presence of wildype AAV and helper virus. No wildtype AAV or helper virus was detected. After expansion of the cells in culture, cells are tested for the production of GM-CSF (part of the release criteria).*

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 potential environment impact of the release of CG1940 and CG8711: the chance that the genetically modified cell lines CG1940 and CG8711 will contain replication-competent AAV can be disregarded due to the cloning procedure and the fact that the rAAV can not replicate in the presence of a helper virus since additional complementation of viral proteins is necessary. Therefore, excretion of the vector in the environment will not occur after administration of CG1940 and CG8711 to patients with prostate cancer. The cell lines are irradiated in order to prevent growth in patients. The risks of this clinical study for human and environment are scored as not harmful by the Dutch Commission on Genetic Modification (Advisenr. 040309-01).*

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

other, specify *two human prostate tumor cell lines, PC-3 (CG1940) and LNCaP (CG8711) were obtained from the American Type Culture Collection (ATCC). PC-3 was originally isolated from a bone metastasis and LNCaP from a prostate cancer lymp node metastasis.*

2. Name

| | | |
|---|---|---|
| (i) | order and/or higher taxon (for animals) | *Primates* |
| (ii) | genus | *Homo* |
| (iii) | species | *Sapiens* |
| (iv) | subspecies | *NA* |
| (v) | strain | *NA* |
| (vi) | pathovar (biotype, ecotype, race, etc.) | *Caucasian, site prostate* |
| (vii) | common name | *NA* |

3. Geographical distribution of the organism: *Not relevant (NR)*

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

(b) Indigenous to, or otherwise established in, other EC countries:
(i) Yes (.)

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

| | |
|---|---|
| Atlantic | .. |
| Mediteranean | .. |
| Boreal | .. |
| Alpine | .. |

Continental ..
Macaronesian ..

(ii) No (*NR*)
(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: *Not relevant (NR)*

(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 …

(b) If the organism is an animal: natural habitat or usual agroecosystem:
*Not relevant*

5. (a) Detection techniques
*Not relevant*

(b) Identification techniques
*Not relevant*

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 *Vaccine cells Biosafety level 1 (specified on www.atcc.org; ATCC numbers CRL-1435 (PC-3) and CRL-1740 (LNCaP)).*

7. Is the recipient organism significantly pathogenic or harmful in any other way (including its extracellular products), either living or dead?
Yes (.) No (*x*) Not known (.)

If yes:
(a) to which of the following organisms:

humans (.)
animals (.)
plants (.)
other (.)

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

*No pathological, ecological and physiological traits are present.*

8. Information concerning reproduction
*Not relevant; cells are lethally irradiated before administered to patients*

(a) Generation time in natural ecosystems:
*NA*
(b) Generation time in the ecosystem where the release will take place:
*NA*
(c) Way of reproduction: Sexual *NA*. Asexual *NA*

(c) Factors affecting reproduction:
*NA*

9. Survivability; *Not relevant for this product since no viral replication is possible in these cells.*

(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:
*NA*

10. (a) Ways of dissemination
*NA*

(b) Factors affecting dissemination
*NA*

11. Previous genetic modifications of the recipient or parental organism already notified for release in the country where the notification is made (give notification numbers)
…, B/../../…

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

1. Type of the genetic modification

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

2. Intended outcome of the genetic modification
*To insert sequences coding for the release of human genomic granulocyte-macrophage colony stimulating factor (hgGM-CS) leading to expression of GM-CSF from the transduced cells as a potentially therapeutic local immune adjuvant*

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 | (.) |
| bacteriophage | (.) |
| virus | (x) |
| cosmid | (.) |
| transposable element | (.) |
| other, specify | *r AAV-MD2-hgGM-CSF vector* |

(b) Identity of the vector
*rAAV-MD2-hgGM-CSF vector: Recombinant adeno associated virus (rAAV): family Parvovirideae, genus Dependovirus, Species adeno-associated virus 2*

(c) Host range of the vector
*human*

(d) Presence in the vector of sequences giving a selectable or identifiable phenotype
Yes (.) No (x)

| | |
|---|---|
| antibiotic resistance | (.) |
| other, specify | … |

Indication of which antibiotic resistance gene is inserted
…

(e) Constituent fragments of the vector
*Replication defective, recombinant AAV vectors are derived by replacement of the rep and cap genes with a heterologous transgene and the genetic control elements needed for its expression. The only viral DNA sequences retained by a rAAV vector are the viral ITRs, flanking the heterologous gene*.

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

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

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
*hg GM-CSF gene*

(b) Source of each constituent part of the insert
*The hgGM-CSF gene was amplified by polymerase chain reation (PCR) from a bacterial artificial chromosome (BAC). The amplified hgGM-CSF gene was non-directionally cloned into the EcoRI site of the vector, plox III-4 CMV. Resulting clones were screened by restrictions endonuclease digestion for the proper orientation of the insert, relative to the promoter. The resultant plasmid pSSV9-MD2-hgGGM-CSF contains the hgGM-CSF gene between the β-globin intron and the β-globin polyadenylation site.*

(c) Intended function of each constituent part of the insert in the GMO
*production of GM-CSF → stimulation immune response in Hormone Refractory Prostate Cancer patients*

(d) Location of the insert in the host organism

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

(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 (x)
- insect (.)
- fish (.)
- other animal (.)

(specify phylum, class) …

other, specify *the hgGM-CSF gene was derived from plox III-4 CMV/hgGM-CSF clone #5 by EcoRI restriction endonuclease digestion*

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 (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 (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 (x) No (.) Not known (.)
Specify *irradiated vaccine cells will die within 1 week and be eliminated by the immune system*

(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 *cells are not able to divide anymore due to lethal irradiation and the virus is replication incompetent.*

(c) is the GMO in any way different from the recipient as far as dissemination is concerned?
Yes (x) No (.) Not known ()
Specify *The prostate cancer cells are not able to disseminate anymore due to irradiation*

(d) is the GMO in any way different from the recipient as far as pathogenicity is concerned?
Yes (x) No (.) Not known (.)
Specify *Vaccine cells CG1940 / CG8711 are non pathogenic.*

2. Genetic stability of the genetically modified organism
*Vaccine cells are genetically stable and they will only survive maximally 1 week due to lethal irradiation of the cells and the cells are very immunogenic since they are allogeneic cells and produce GM-CSF (iimunostimulans). Hence, these cells will be destroyed by immune cells.*

3. Is the GMO significantly pathogenic or harmful in any way (including its extracellular products), either living or dead?
Yes (.) No (x) Unknown (.)

(a) to which of the following organisms?

humans (.)
animals (.)
plants (.)
other …

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

4. Description of identification and detection methods

(a) Techniques used to detect the GMO in the environment
*There is no foreseen nor expected effect on the environment. Irradiated vaccine cells are injected intradermally and locally degraded by the immune system. The cells cannot propagate nor be transmitted to persons other than the patient and thus pose no special public health risk . Therefore no techniques can be mentioned here.*

(b) Techniques used to identify the GMO
*Vector sequences in the vaccine cells can be detected by standard molecular techniques such as Southern hybridization or polymerase chain reaction. Once injected, the vaccine cells persist for approx. 96 hrs at the immunization site and are then eliminated by the immune system. During this time presence of the vaccine can be detected in some patients by a temporary modest increase of serum GM-CSF levels.*

## F. Information relating to the release

1. Purpose of the release (including any significant potential environmental benefits that may be expected)
*The vaccine cells (CG1940 / CG8711) are intended for the treatment of Hormone Refractory Prostate Cancer (HRPC) patients.*

*It is expected that the expression of hgGM-CSF by the vaccine cells CG1940 / CG8711 will provoke an immuneresponse against the tumorantigens, expressed by the vaccine cells. Administration of MDX-010 will possibly enhance the immune response.*

*Vaccine cells CG1940 / CG8711 will be given as an intradermal injection: injection sites will rotate between upper and lower extremities with each successive treatment. CG1940 should be administered on the right side and CG8711 on the left side whenever possible. MDX-010 will be administered over 90 minutes as an intravenous infusion. The study will monitor safety and toleratbility of escalating doses of MDX-010 in combination with CG1940 and CG8711 in patients with metastatic HRPC.*

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):
*Administrative region: Vrije Universiteit Medisch Centrum (VUMC), Amsterdam NL*

(b) Size of the site ($m^2$): … $m^2$

(i) actual release site ($m^2$): … $m^2$
*Patients administered with CG1940 / CG8711 and MDX-010 will be hospitalized only for the first treatment but at liberty to move around thereafter. Therefore it is difficult to define the actual release site.*
(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 relevant*

(d) Flora and fauna including crops, livestock and migratory species which may potentially interact with the GMO
*Not relevant*

4. Method and amount of release

(a) Quantities of GMOs to be released:
*Prime vaccination (visit 1) of $5*10^8$ cells per dose and boost vaccinations (visits 2-13) of $3*10^8$ cells per dose to HRPC patient.*

(b) Duration of the operation:
*26 weeks (13 vaccinations every 2 weeks)*

(c) Methods and procedures to avoid and/or minimise the spread of the GMOs beyond the site of the release
*None, the vaccine cells (GMO) are irradiated before administration so they can't duplicate in the patient; it is expected that the vaccine cells are not present any more 4 days after the administration.*

5. Short description of average environmental conditions (weather, temperature, etc.)
*In the NL there is an average sea-climate.*

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.
*No potential environmental and human health impacts are to be mentioned.*

## 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) | … |
| (ii) | family name for plants | … |
| (iii) | genus | Homo |
| (iv) | species | H.Sapiens |
| (v) | subspecies | … |
| (vi) | strain | … |
| (vii) | cultivar/breeding line | … |
| (viii) | pathovar | … |
| (ix) | common name | Man |

2. Anticipated mechanism and result of interaction between the released GMOs and the target organism (if applicable)
*Expression of GM-CSF from the vaccine cells at the immunization site is expected to attract cells of the immune system to that site and elicit an immune response directed towards tumor antigens expressed on the vaccine cells.*

3. Any other potentially significant interactions with other organisms in the environment
*None*

4. Is post-release selection such as increased competitiveness, increased invasiveness for the GMO likely to occur?
Yes (.) No (x) Not known (.)
Give details
…

5. Types of ecosystems to which the GMO could be disseminated from the site of release and in which it could become established
*Not relevant. Once injected, the vaccine cells persist for approx. 96 hrs at the immunization site of a patient and are then eliminated by the immune system of the patient.*

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) | *Not applicable* |
| (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:
*no exchange is expected*

(b) from other organisms to the GMO:
*no exchange is expected*

(c) likely consequences of gene transfer:
*no exchange is expected*

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.):
*Systemic antitumor immune responses have been observed in phase I/II studies (unpublished data).*

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

## H. Information relating to monitoring

1. Methods for monitoring the GMOs
*Immunomonitoring in patients (level of GM-CSF)*

2. Methods for monitoring ecosystem effects
*Not relevant*

3. Methods for detecting transfer of the donated genetic material from the GMO to other organisms
*Not relevant* …

4. Size of the monitoring area ($m^2$)
*Not relevant* … $m^2$

5. Duration of the monitoring
*Not relevant* …

6. Frequency of the monitoring
*Not relevant* …

## I. Information on post-release and waste treatment

1. Post-release treatment of the site
*No specific treatment is necessary, since risk is none existing and cells are injected into the dermis of the patient and once injected, the vaccine cells persist for approx. 96 hrs at the immunization site and are then eliminated by the immune system.*

2. Post-release treatment of the GMOs
*Not applicable*

3. (a) Type and amount of waste generated
*Not applicable* …

3. (b) Treatment of waste
*Not applicable*

## J. Information on emergency response plans

1. Methods and procedures for controlling the dissemination of the GMO(s) in case of unexpected spread
*Not applicable* …

2. Methods for removal of the GMO(s) of the areas potentially affected
*Not applicable* …

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
*Not applicable* …