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/11/001**
(c)	Date of acknowledgement of notification	**09/08/2011**
(d)	Title of the project	**Ex vivo retroviral transduction of human T lymphocytes with melanoma-specific T cell receptor genes**
(e)	Proposed period of release	**From 01/10/2011 until 01/10/2021**

2. Notifier

Name of institution or company: **Netherlands Cancer Institute (Nederlands Kanker Instituut)**

3. GMO characterisation

(a) Indicate whether the GMO is a:

viroid	(.)
RNA virus	(**X**) **Retrovirus**
DNA virus	(.)
bacterium	(.)
fungus	(.)
animal	
- mammals	(.)
- insect	(.)
- fish	(.)
- other animal	(.)

specify phylum, class …

(b) Identity of the GMO (genus and species)
The GMO released into the environment are ex vivo retroviral transduced, autologous T cells that are administered intravenously to patients.

Used retrovirus for transduction: Gammaretrovirus, Moloney murine leukemia virus

(c) Genetic stability – according to Annex IIIa, II, A(10)
MoMuLV infects only actively dividing cells. In mice, the virus is transmitted in the blood from infected mother to offspring. Transmission may also occur via germline infection. In vivo infection in humans appears to require direct injection with amphotropic or pseudotyped virus. Viral particles are extremely labile and do not survive on environmental surfaces.
In the transduced T cells there will be no viral particles present at the moment of administration to patients.

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 GMO released into the environment are ex vivo transduced, autologous T cells that are administered intravenously to patients. The viral vector is replication deficient and there will no longer be viral particles present at the moment of administration. In addition, the chance of recombination into replication competent retrovirus is very low.

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	(**X**)
DNA virus	(.)
bacterium	(.)
fungus	(.)
animal	
- mammals	(.)
- insect	(.)
- fish	(.)
- other animal	(.)
(specify phylum, class)	…

other, specify …

2. Name

(i)	order and/or higher taxon (for animals)	**Retroviridae**
(ii)	genus	**Gammaretrovirus**
(iii)	species	**Moloney murine leukemia virus**
(iv)	subspecies	**oncovirinae type C**
(v)	strain	**dl587rev mutant**
(vi)	pathovar (biotype, ecotype, race, etc.)	-
(vii)	common name	**MoMLV**

3. Geographical distribution of the organism

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

(b) Indigenous to, or otherwise established in, other EC countries:
(i) Yes (.) No (X)

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

Atlantic	..
Mediteranean	..
Boreal	..
Alpine	..
Continental	..
Macaronesian	..

(ii) No (.)
(iii) Not known (.)

(c) Is it frequently used in the country where the notification is made?
Yes (**X**) No (.)
Used in research laboratories.

(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	**murine virus, present in blood**

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

5. (a) Detection techniques
PCR, immunofluorescence

(b) Identification techniques
PCR, immunofluorescence

6. Is the recipient organism classified under existing Community rules relating to the protection of human health and/or the environment?
Yes (.) No (**X**)
If yes, specify
…

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:
Wild type virus is oncogenic in mice. In rhesus monkeys, lymphomas were observed several months after autologous transplantation of bone marrow stem cells transduced with a retroviral vector preparation containing replication competent virus. The data suggests a pathogenic mechanism in which chronic productive retroviral infection allowed insertional mutagenesis leading to cell transformation and tumor formation. To date, no documented clinical manifestations of disease have been noted in humans exposed to MoMuLV vectors.

The transduced T cells are not pathogenic.

(a) to which of the following organisms:

humans	(.)
animals	(**X**)
plants	(.)
other	(.)

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

8. Information concerning reproduction

(a) Generation time in natural ecosystems:
The formation of non modified viral particles can take place within 24 hrs

(b) Generation time in the ecosystem where the release will take place:
The used virus is replication deficient and can therefore not replicate

(c) Way of reproduction: **replication deficient**

(c) Factors affecting reproduction:
-

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:
-

10. (a) Ways of dissemination
The used virus is replication deficient. At the moment of infusion, no viral particles or RCR will be present. The transduced T cells can not survive outside the body.

(b) Factors affecting dissemination
The used virus is constructed in such a way that it is replication deficient and chance of recombination with other (retro)viruses is minimal.

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)
None

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 induce expression of the Mart-1 specific T cell receptor on transduced T cells, and thereby induce melanoma-specific T cell immunity.

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 …	

(b) Identity of the vector
The retroviral MP71-vector that will be used in this study is a Moloney Murine Leukemia Virus (Mo-MuLV) based vector. The MP71 leader sequence has been designed for increased safety in medical applications. All AUG codons which may aberrantly initiate translation and all viral coding sequences are removed, thereby reducing the probability of unwanted peptides and homologous recombination with retroviral genes.
The retroviral supernatant is produced in PG13 packaging cells and is pseudotyped with the Gibbon ape Leukemia Virus (GaLV) envelop.

(c) Host range of the vector
GaLV envelop: broad host range including human cells

(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; only present in original vector. No longer present in retroviral supernatant used for genetic modification of T cells.

(e) Constituent fragments of the vector
5' LTR, 5' untranslated region, splice donor site, packaging signal, splice acceptor site, T cell receptor beta chain, p2A linker, TCR alpha chain, 3' LTR

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

(i)	transformation	(.)
(ii)	electroporation	(.)
(iii)	macroinjection	(.)
(iv)	microinjection	(.)
(v)	infection	(.)
(vi)	other, specify	**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
Mart-1 specific T cell receptor (beta and alpha chain)

(b) Source of each constituent part of the insert
Derived from a T cell clone of a patient vaccinated with the Mart-1 peptide.

(c) Intended function of each constituent part of the insert in the GMO
Induction of Mart-1 specific T cell immunity

(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	…

2. Complete name

(i)	order and/or higher taxon (for animals)	**Human**
(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 (.) 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 (**X**) No (.) Not known (.)

Autologous T cells

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 **The patient will receive genetically modified, autologous T cells. These cells can not survive outside the body, similar to non modified autologous T cells.**

(b) is the GMO in any way different from the recipient as far as mode and/or rate of reproduction is concerned?

Yes (.) No (**X**) Unknown (.)

Specify **Genetic modification does not affect reproduction. Replication time of both modified and non modified T cells is approximately 24 hrs.**

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

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

Specify **The genetically modified autologous T cells do not contain any viral particles or RCRs and the GMO can therefore not disseminate.**

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

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

Specify **The genetically modified T cells are non-pathogenic. The only difference with non modified T cells is that they can now recognize Mart-1 positive tumor cells.**

2. Genetic stability of the genetically modified organism

The genetically modified T cells can survive up to several months after infusion.

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
T cell receptor transduced cells can be detected using PCR or flow cytometry

(b) Techniques used to identify the GMO
PCR or flow cytometry

F. Information relating to the release

1. Purpose of the release (including any significant potential environmental benefits that may be expected)
Inducing melanoma-specific T cell immunity and thereby treatment of melanoma.

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):
   Nederlands Kanker Instituut
   Plesmanlaan 121
   1066CX Amsterdam
   PO Box 90203
   1006 BE Amsterdam
   The Netherlands
   (GMP lab where ex vivo transduction is performed and hospital where modified cells are infused into patient).

   (b) Size of the site (m^2):
   (i) actual release site (m^2):
   (ii) wider release site (m^2):
   Not relevant

   (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
   Sections (b) – (d) above are not considered relevant. Environmental release of the transduced autologous T cells is not intended beyond the treatment of trial subjects. Subjects will be administered the product by injection at the Netherlands Cancer Institute / Antoni van Leeuwenhoek Hospital.

4. Method and amount of release

   (a) Quantities of GMOs to be released:
   Patients treated at the Netherlands Cancer Institute will receive between 2.5x10e8 and 2.5x10e10 transduced T cells. A maximum of 100 patients will be treated.

   (b) Duration of the operation:
   The duration of the clinical trial will not exceed ten years.

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

The TCR transduced T cells will be produced under Good Manufacturing Practice (GMP) conditions following Standard Operating Procedures (SOPs) of the BTU (BioTherapeutics Unit) at the Pharmacy Department of the Slotervaart Hospital. The manufacturing procedure is performed in a class B cleanroom (GMP EU Annex 1) of the BTU with strict air pressure hierarchy for combining aseptic conditions with containment. Handling of product with direct exposure to the environment is performed in a class A (GMP EU Annex 1) biohazard laminar down-flow cabinet within the BTU cleanroom. Performance of the cleanroom facility is monitored by measurement of the levels of microorganisms and particles both at operating state and at rest state.

Transport to the clinic, administration to the patient and handling of waste and patient samples will be in accordance with legal and institutional procedures of the Netherlands Cancer Institute.

5. Short description of average environmental conditions (weather, temperature, etc.)
see 4c

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 data available, because no previous releases have been carried out with the same gmo.

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 …
   (iv) species …
   (v) subspecies …
   (vi) strain …
   (vii) cultivar/breeding line …
   (viii) pathovar …
   (ix) common name …
   None, because the genetically modified T cells are not able to survive outside the patient.

2. Anticipated mechanism and result of interaction between the released GMOs and the target organism (if applicable)
   The genetically modified T cells are not able to survive outside the patient.

3. Any other potentially significant interactions with other organisms in the environment
   No

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
   The genetically modified T cells are not able to survive outside 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) …
   (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 homology between the used retroviral vector for transduction and endogenous (retro)viruses, so therefore no likelihood of genetic exchange.

   (b) from other organisms to the GMO:
   No homology between the used retroviral vector for transduction and endogenous (retro)viruses, so therefore no likelihood of genetic exchange.

   (c) likely consequences of gene transfer:
   -

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 MP71 vector, used to genetically modify T cells in this study, has been designed for increased safety in medical applications. In a clinical gene therapy study in Germany, HIV-infected patients were treated with autologous T cells transduced with MP91 PRE encoding an HIV entry inhibitory peptide, a vector closely related to MP71. Two years after starting treatment all patients were alive and healthy, the infusion of modified T cells was well tolerated without side effects. There is no evidence in this study for long term side effects of the infusion of genetically modified T cells. In addition, a study led by Prof. Hans Stauss (University College of London), human T cells will transduced with an MP71 vector encoding a WT-1 (Wilm's tumor antigen)-specific TCR. This trial was approved by the authorities in the UK, but has yet to be implemented.

9. Possible environmentally significant interactions with biogeochemical processes (if different from the recipient or parental organism)
None

H. Information relating to monitoring

1. Methods for monitoring the GMOs
   Transduced T cells can be detected using flow cytometry or PCR.

2. Methods for monitoring ecosystem effects
   Presence of GMOs could be monitored using PCR, but since environmental effects are not expected, this will not be performed.

3. Methods for detecting transfer of the donated genetic material from the GMO to other organisms
   Presence of GMOs could be monitored using PCR, but since transfer to other organisms is not expected, this will not be performed.

4. Size of the monitoring area (m^2)
   For detection of transduced cells within the patients, blood samples will be taken to at the hospital and analyzed at the clinical laboratory.

5. Duration of the monitoring
   Blood samples will be taken at several time points after infusion (1,2,3,6,12 months).

6. Frequency of the monitoring
   see above

I. Information on post-release and waste treatment

1. Post-release treatment of the site
After each production, the clean room facility will be thoroughly cleaned. All working surfaces will be disinfected using a 70% ethanol solution.
At the site of administration of the cell product to the patient, all used material will be disposed in accordance with legal and institutional procedures of the Netherlands Cancer Institute.

2. Post-release treatment of the GMOs
The genetically modified T cells will be infused into the patient.

3. (a) Type and amount of waste generated
During production, a maximum of 10 L of fluid waste is generated. In addition, solid waste (including plastic) is generated.
At the site of administration of the cell product tot the patient, solid waste such as syringes and gloves is generated.

3. (b) Treatment of waste
All waste is treated as biohazardous waste and is transported and disposed in accordance with legal and institutional procedures of the Netherlands Cancer Institute.

J. Information on emergency response plans

1. Methods and procedures for controlling the dissemination of the GMO(s) in case of unexpected spread
   The is no risk of environmental health hazard.
   All procedures are according to WIP procedures (Dutch working group for infection prevention) and standard hospital procedures. Site personnel responsible for spill cleanup will be instructed to wear personal protective equipment (protective gloves, safety glasses and clothing).

2. Methods for removal of the GMO(s) of the areas potentially affected
   All procedures are according to WIP procedures (Dutch working group for infection prevention) and standard hospital procedures. Spilled liquids will be absorbed with common absorbent materials and placed in appropriate containers for disposal.

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
   No risk of environmental health hazard.