PART 1 (COUNCIL DECISION 2002/813/EC)

SUMMARY NOTIFICATION INFORMATION FORMAT FOR THE RELEASE OF GENETICALLY MODIFIED ORGANISMS OTHER THAN HIGHER PLANTS IN ACCORDANCE WITH ARTICLE 11 OF DIRECTIVE 2001/18/EC

In order to tick one or several possibilities, please use crosses (meaning x or X) into the space provided as (.)

A. General information

1. Details of notification

(a)	Member State of notification	the Netherlands
(b)	Notification number	B/NL/11/003
(c)	Date of acknowledgement of notification	25/07/2011
(d)	Title of the project	Administration of donor specific T-cells transduced *ex vivo* using a retroviral vector coding for a leukemia-specific TCR, as a cellular treatment after allogeneic stem cell transplantation for patients with refractory or relapsed hematological malignancies.
(e)	Proposed period of release	From 03/2012 until 12/2017

2. Notifier

Name of institution or company: Leiden University Medical Center

3. GMO characterisation

(a) Indicate whether the GMO is a:

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

specify phylum, class Retrovirus

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

Retrovirus, Moloney murine leukemia virus (Mo-MuLV). …

(c) Genetic stability – according to Annex IIIa, II, A(10) (p24)

Stable. Risk group 1.(See E2, p9)
Not the wild type Moloney Leukemia virus is used, but a replication incompetent variant. Both the viral vector, as the inserted therapeutic gene (HA-1-TCR) were sequenced at the end of the production process, and no genetic mutations were observed.

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

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 Germany
- Notification number --

Studies published by Ott,M.G. et al Nat Med vol. 12; pp 401-9 (2006) and van Lunzen, J. Mol Ther vol. 15; pp 1024-33 (2007)

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

The environmental impact on release of the GMO into the environment is considered minor. The virus batch used for transduction of the donor cells does not contain replication competent retroviruses (RCR). Furthermore, no RCR will be present in the final cell product that will be transfused into the patient or will be produced after tranduction. This makes it very unlikely that the virus used in the study will spread through the environment.
The transfused patient is not allowed to donate blood. Therefore, the only feasible route of transmission of the transduced cells into the environment is via accidental blood contact of an individual other than the patient. If this occurs, the immune system of the person will recognize the foreign cells and will eliminate them from the body. Only in the case that the individual that has accidental blood contact is completely HLA-identical the immune cells will not recognize the transduced donor cells. However, the change that this will occur is considered unlikely. In addition, T-cells are not able to persist in the environment outside 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	(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)	n/a
(ii)	genus	Gamma retrovirus
(iii)	species	Murine leukemia virus
(iv)	subspecies	n/a
(v)	strain	dl58rev mutant
(vi)	pathovar (biotype, ecotype, race, etc.)	n/a
(vii)	common name	Mo-MuLV

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 (.)

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

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

(ii) No (X)
(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	in mice

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

5. (a) Detection techniques

Mo-MuLV can be detected using PCR

(b) Identification techniques

Mo-MuLV can be identified using sequencing, restriction analysis and/or 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

Classified as risk group 2 by the Dutch Ministry of Infrastructure and the Environment

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	(.)
animals	(X)
plants	(.)
other	(.)

(b) give the relevant information specified under Annex III A, point II. (A)(11)(d) of Directive 2001/18/EC (p24)

Mo-MuLV is an ecotropic virus and infects only dividing murine cells. In mice, the virus is transmitted in the blood from infected mother to offspring. Transmission may also occur via germline infection. Wild type (WT) virus is oncogenic in mice. In rhesus monkeys, lymphomas were observed in immunocompromised animals. The data suggests a pathogenic mechanism in which chronic productive retroviral infection allowed insertional mutagenesis leading to cell transformation and tumor formation. In vivo infection in humans appears to require direct injection with amphotropic or pseudotyped virus. However, in this study, we will use a replication deficient Mo-MuLV vector. To date, no documented clinical manifestations of disease have been noted in humans exposed to Mo-MuLV vectors.

8. Information concerning reproduction

(a) Generation time in natural ecosystems:
14-36 hrs (WT Mo-MuLV virus). Not relevant for the modified virus (MP71).

(b) Generation time in the ecosystem where the release will take place:
The WT Mo-MuLV virus is not released. The strain of Mo-MuLV (MP71) which is used is replication deficient. Since gag en pol have been deleted, the viral vector can infect cells only once.

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

(c) Factors affecting reproduction:
The WT Mo-MuLV virus is ecotropic and infects only murine cells. The modified Mo-MuLV that will be used in the clinical study (MP71) is pseudotyped with a GaLV envelope, and can infect only dividing cells of primate origin. The MP71 vector is replication deficient and since gag en pol have been deleted, the viral vector can infect cells only once.

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:

Temperature, UV-radiation, humidity, chemical disinfection

10. (a) Ways of dissemination

WT Mo-MuLV can be transferred in mouse from mother to child via blood or germ cells infection. The virus only infects dividing cells. Infections of humans can only occur if the virus is directly infused into the blood. Dividing, but not non-dividing, human cells can only be infected by pseudotyped Mo-MuLV, but not by wild type Mo-MuLV.

(b) Factors affecting dissemination
Patients that were treated with modified T-cells, will be excluded as donor for blood products.

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)
Previous release in Germany (Hildinger, J Virol 1999; Schambach, Mol Ther 2000; Ott, Nat Med 2006; van Lunzen, Mol Ther 2007)).

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 Mo-MuLV used for the study is replication deficient. From the original virus the gag, pol and env sequences are removed. The 3'and 5'LTR present in the construct are derived from myelosarcoma proliferative virus (MPSV) and the leader sequence from the murine embryonic stemm cell virus (MESV). The 5'leader sequence is designed for optimal safety in clinical studies. All AUG codons that could initiate unwanted translations and sequences coding for viral proteins are removed. Transgene expression is enhanced by introducing of a splice acceptor site in the 3'part of the leader sequence. The transgene is a T-cell receptor which will be expressed in the transduced cells.

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 (.) No (.) Partially present.

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

Retrovirus, Moloney Murine Leukemia Virus, MP71 vector

(c) Host range of the vector

Pseudotyped with Gibbon ape Leukemia Virus (GALV)-envelope; amphotropic (infects primate cells)

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

Yes (X) No (.)

antibiotic resistance (.)
other, specify T-cell receptor…

Indication of which antibiotic resistance gene is inserted

Not applicable

(e) Constituent fragments of the vector

The MP71 vector consists of the following constituent fragments (nt = nucleotide):

nt 1-625	MPSV 5' LTR. The LTR functions as a promotor and activates gene expression of the insert. The LTR is derived from myeloproliferative sarcoma virus (MPSV).
nt 441-1103	5' untranslated region derived from the vector SF71. This leader sequence originates from murine embryonic stem cell virus (MESV), and that virus is based on the Mo-MuLV dl587rev mutant. This leader sequence also contains the splice donor site, packaging signal and splice acceptor site, that ensure encapsulation of the RNA into virions, and enhance transgene expression.
nt 618-625	Splice donor site (derived from Mo-MuLV dl587rev mutant). Together with the splice acceptor site, this site forms an intron, that will be removed from the pre-mRNA. This splicing will result in enhanced mRNA processing and transgene expression.
nt 626- 1067	Packaging signal (derived from Mo-MuLV dl-587rev mutant). The packaging signal has an important role in regulating the RNA in virions in the packaging cellines. The packaging signal has no function in the modified T-cells.
nt 1068-1103	Splice acceptor site (derived from Mo-Mulv dl-587rev mutant). See splice donor site.
nt 1104-2941	Therapeutic gene*
nt 1104-1115	Start multiple cloning site (MCS).
nt 1116-2051	TCR beta chain
nt 2052-2099	T2A signal sequence.
nt 2100-2909	TCR alpha chain
nt 2910-2941	End of MCS. We have introduced a new MCS, consisting of BamHI, EcoRI, XhoI, ClaI en NotI restriction sites.
nt 2951-3588	MPSV 3' LTR. See MPSV 5'LTR.

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

(i)	transformation	(.)
(ii)	electroporation	(.)
(iii)	macroinjection	(.)
(iv)	microinjection	(.)
(v)	infection	(X)

(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

T-cell receptor (TCR) beta chain linked with a 2A signal peptide to TCR alpha chain.

(b) Source of each constituent part of the insert

TCR beta chain; Human-derived
T2A signal peptide; Thosea asigna virus-derived (insect virus, part of the Picornaviridae)
TCR alpha chain; Human-derived

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

The TCR is anti-leukemia specific. The T2A self-cleaving 2A sequence links the TCR alpha and beta chain, and results in stoichiometric production of both chains.

(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	(.)
-	insect	(.)
-	fish	(.)
-	other animal	(.)
	(specify phylum, class)	…
other, specify	human	

2. Complete name

(i)	order and/or higher taxon (for animals)	Primates
(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	Human

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 (.) No (X) Not known (.)
Specify

The introduced TCR can be present in healthy individuals, albeit in low frequencies.

(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

Modified T-cells behave similar as non-transduced T-cells, they remain dependent on antigen-specific stimulation of the TCR for proliferation. Antigen-specific stimulation via the introduced TCR results in similar signaling as the endogenous TCR that is already present in the modified T-cells.

(c) is the GMO in any way different from the recipient as far as dissemination is concerned?
Yes (.) No (X) Not known (.)
Specify

The retroviral supernatant that will be used to modify donor T-cells is RCR-free. Since human and murine retroviruses differ, it is highly unlikely that recombination with endogenous retroviruses will occur in T-cells. In addition, T-cells are largely unable to produce infectious virions. In addition, T-cells can not survive outside the human body.

(d) is the GMO in any way different from the recipient as far as pathogenicity is concerned?
Yes (.) No (X) Not known (.)
Specify

The introduced TCR has been chosen for its defined anti-leukemic specificity.

2. Genetic stability of the genetically modified organism

Stable. Risk group 1
Not the wild type Moloney Leukemia virus is used, but a replication incompetent variant. Both the viral vector, as well as the inserted therapeutic gene (TCR) were sequenced at the end of the production process, and no genetic mutations were observed.

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)

The MP71 Mo-MuLV vector used in this study is replication deficient. In addition, the retroviral supernatant that will be used in this study has been tested RCR-free. The chance of recombination with endogenous retroviruses present in the T-cells is very low, since human and murine retroviruses are very different. In addition, several recombinations at the same time at the right spots have to occur. This is highly unlikely to happen. Moreover, T-cells are largely unable to produce infectious virions. If a healthy individual accidentally receives TCR-modified T-cells meant for a patient with leukemia, most likely immune cells of the healthy individual will recognize the modified T-cells as foreign and will eliminate them. Only if the recipient, other than the patient for which the modified T-cells were generated, is completely HLA-matched, will the modified T-cells survive. However, theoretically there are more than 1 billion HLA-haplotypes, and every individual has 2 haplotypes. Thus, it is very unlikely that two individuals are completely HLA-matched. TCR-modified T-cells are not able to persist outside the human body.

4. Description of identification and detection methods

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

q-RT-PCR using primers specific for the introduced TCR will be used to detect the GMO in the environment.

(b) Techniques used to identify the GMO

Both tetramer staining and q-RT-PCR using primers specific for the introduced TCR will be used to identify the GMO in the environment.

F. Information relating to the release

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

   Introducing the TCR will result in reprogramming of donor T-cells with anti-leukemia specificity.

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 (X) No (.)

   If yes, specify

   The parental Moloney Murine Leukemia Virus is only infectious in *Mus musculus*. For this study, human T-cells will be modified with a pseudotyped retroviral vector (MP71).

3. Information concerning the release and the surrounding area

   (a) Geographical location (administrative region and where appropriate grid reference):

   Leiden University Medical Center, Albinusdreef 2, 2333 ZA Leiden, the Netherlands

   (b) Size of the site (m^2): … m^2
   (i) actual release site (m^2): 16 m^2 (Patient room)
   25 m^2 (laboratory of GMP facility)

   Production of the modified T-cells will take place in the Leiden University Medical Center (LUMC) in the Interdivisional GMP-facility of LUMC (IGFL), location J10-98. Administration of the modified T-cells will take place in the LUMC at the Department of Hematology, patient ward,location C-06-P

   (ii) wider release site (m^2): not applicable m^2

   (c) Proximity to internationally recognised biotopes or protected areas (including drinking water reservoirs), which could be affected:

   not applicable

   (d) Flora and fauna including crops, livestock and migratory species which may potentially interact with the GMO

   not applicable

4. Method and amount of release

   (a) Quantities of GMOs to be released:

   T-cells will be infected with $2x10^6$/ml infectious viral particles. The half-life of a retrovirus is around 10h. Modified T-cells will be cultured for another 10 days, and this will result in a reduction of viral particles of $4{,}8x10^9$ so that maximally 0.008

viral particles will be present in the final product. We aim to administer $3x10^6$ modified T-cells/kg body weight of the patient.

(b) Duration of the operation:

Six weeks before administration of the modified T-cells, patients will be transplanted with allogeneic stem cell transplantation. Modified T-cells will be administered in a timespan of 3h.

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

Transduction of the T-cells will take place at the IGFL in a laminar airflow (LAF) cabnet class II in a production room exclusively used for production of genetically modified therapeutics. During the production of the modified T-cells, personnel will wear gloves and surgical masks. TCR-modified T-cells will be transported to the patient ward following supplement 9 of the Dutch 'Ministerial Regulation on GMO'. During administration of the modified T-cells at the patient ward, personnel will wear gloves, surgical masks and water-repellent apron. The patient will also lie on a water-repellent matrass. After administration of the modified T-cells, the room will be thoroughly disinfected. Patients that were treated with modified T-cells are excluded for donation of blood products. All materials used in the procedure will be collected and send for destruction to an incinerator.

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 original MP71 vector is used in a clinical study for treatment of X-GCD in Germany. Because the SFFV LTR is a strong enhancer for gene expression in hematopoietic stem cells and myeloid progenitor cells this LTR is replaced by the MPSV LTR. This MSCV LTR vector has been used in a clinical study to treat HIV-patients with retroviral transduced T-cells. Two years after the start of this study no serious adverse events have been notified. There is no information on shedding.
From other studies using retroviral transduced T-cells (after stem cell therapy, kidney carcinoma patients, skin cancer patients) no serious adverse events are reported that are due to the transduced T-cells or the retroviral particles used to transduce the T-cells. Furthermore, no shedding of retroviral particles is reported in these studies.
It is reported that T-cells have only a low capacity of producing retroviral particles when infected with replication competent retrovirus. The produced virions were shown to be largely noninfectious.

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)	Primates
(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	Human…

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

   Not anticipated. T-cells will be modified with an anti-leukemic TCR. T-cells are unable to persist outside the human body. If the modified T-cells are accidentally transferred to another individual than the patient, most probably the modified T-cells will be recognized as non-self by the immune cells of that individual, and will eliminate the modified T-cells.…

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

   Not anticipated. The replication deficient MP71 Mo-MULV vector is highly instable on surfaces. Modified T-cells are unable to persist outside the human body. Cross-contamination with other species is highly unlikely.

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

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

   Give details
   The MP71 vector is a weakened, replication deficient Mo-MuLV based vector, that can only infect dividing cells once. The introduced TCR can be present in healthy individuals, albeit at a lower frequency. TCR modified T-cells still require antigen-specific triggering of their TCR to proliferate, similar to unmodified T-cells.

5. Types of ecosystems to which the GMO could be disseminated from the site of release and in which it could become established

   None. T-cells will be modified with retroviral supernatant that is RCR-free. Due to differences in homology between murine and human retroviruses, and the fact that several recombinations at the same time at the right spots have to occur, it is highly unlikely that RCRs will be produced in the patients. In addition, T-cells are uncapable of producing infectious virions. Morevoer, patients that have been treated with modified T-cells will be excluded from donating blood products. Modified T-cells will not survive outside the human body.

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:
Highly unlikely. The retroviral supernatant is RCR-free, and it is unlikely that RCRs will be produced in the patient (see G5). Accidental blood contact with non targeted organisms is highly unlikely.

(b) from other organisms to the GMO:
Highly unlikely. Since human and murine retroviruses differ, and for generation of new RCRs several recombinations at the same time at the right spots are needed, it is unlikely that this will occur in the patient cells.

(c) likely consequences of gene transfer:
Only if the introduced TCR is transferred to other T-cells can it be expressed at the cell surface, since expression of CD3 molecules is required. This will result in reprogramming of the T-cells with anti-leukemic specificity.

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.):
No off-target toxicity or toxicity related to the retroviral modification of T-cells has been observed in clinical trials treating in total 68 patients with either NY-ESO-TCR td (Robbins, J Clin Onc 2011), MART-1-TCR td (Morgan, Science 2006) or gp100-TCR td T-cells (Johnson, Blood 2009).

9. Possible environmentally significant interactions with biogeochemical processes (if different from the recipient or parental organism)
None. The MP71 Mo-MuLV particles haves a short half-life and are extremely labile on environmental surfaces (Mo-MuLV data sheet University of California, San Diego 1998). Modified T-cells are not meant for consumption, but for treatment of patients with refractory or relapsed hematological malignancies. Therefore, the modified T-cells will not be disseminated into the ecosystem, except through the previously described accidental blood contacts. Patients that were treated with modified T-cellsare excluded from donation of blood products. Modified T-cells can not survive outside the human body. …

H. Information relating to monitoring

1. Methods for monitoring the GMOs

   Both tetramer staining, as well as q-RT-PCR specific for the introduced TCR can be used to detect the modified T-cells in peripheral blood and bone marrow of the patient.

2. Methods for monitoring ecosystem effects

   After accidental blood contact, presence of modified T-cells can be verified using tetramer staining and q-RT-PCR.

3. See H2.

   After accidental blood contact, presence of modified T-cells can be verified using tetramer staining and q-RT-PCR.

4. Size of the monitoring area (m^2)

   Not applicable m^2

5. Duration of the monitoring

   Blood and bone marrow samples will be taken to monitor for presence of the modified T-cells. Blood samples will be withdrawn at day 1, day 3, day 7, week 3, week 6, week 9, week 12, week 15 and week 18 after administration. Bone marrow samples will be withdrawn at one week, 3 weeks, 6 weeks, 12 weeks and 18 weeks after administration of modified T-cells. Thereafter, patients will be monitored according to standard follow up procedures for allo-SCT patients.

6. Frequency of the monitoring

   See H5.

I. Information on post-release and waste treatment

1. Post-release treatment of the site

   The site of cell transduction and culture etc. will be disinfected using Clinisteril Plus. The patient room will be cleaned after the patient is discharged, When GMO is spilled, the spill site will be disinfected using 1000 ppm chlorine solution, preceding cleaning.

2. Post-release treatment of the GMOs

   No shedding is expected during the study.

3. (a) Type and amount of waste generated

Personal protective equipment (gloves, disposable coats), culture flasks, tubes, pipettes, pipet tips, gauzes, tissues, blood tubes, cover materials and dressings. Amount will be very small.

3. (b) Treatment of waste

Disposable waste will be collected in a bin suited for medical waste UN 3291 and will be sent to an incineration for direct incineration. Spills of the GMO will be treated with 1000 ppm chlorine solution or 1% SDS.

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 tissues. Subsequently the surface will be disinfected with tissues soaked in chlorine solution (1000 ppm). All material used during the procedure will be collected in a bin suited for medical waste UN 3291 and will be sent to an incineration for direct incineration.

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

   See J1

3. Methods for disposal or sanitation of plants, animals, soils, etc. that could be exposed during or after the spread

   Not applicable

4. Plans for protecting human health and the environment in the event of an undesirable effect

   Patients will be monitored for the occurrence of serious adverse events (SAE) according to the clinical protocol: each SAE will be registered and evaluated, and health authorities will be notified when relevant. Specific plans for protecting the environment are not considered necessary, for the reasons stated above.