Summary of the file, bound to European committee

Origin : mentioned in article 2, in accordance with the decision of the Council on 3rd October 2002.

A General Information
B Nature of the GMOs contained in the product
C Predicted behaviour of the product
D Information relating to previous releases
E Information relating to the monitoring plan

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	*Germany*
(b) Notification number	*B/DE/10/PEI1279/*
(c) Date of acknowledgement of notification	*02.02.2011*
(d) Title of the project	

TK008: Randomized phase III trial of haploidentical HCTwith or without an add back strategy of HSV-Tk donor lymphocytes in patients with high risk acute leukemia

(e) Proposed period of release	*from Nov 2012 to Nov 2015*

2. Notifier

Name of institution or company:	*MolMed SpA* *Via Olgettina 558 20132* *Milano (Italy)*

3. GMO characterisation

(a) Indicate whether the GMO is a:

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

specify phylum, class	*gp+Am12 cells producing retroviral vector encoding for HSV-tk Mut 2 and ΔLNGFR genes*

(b) Identity of the GMO (genus and species):
Murine cell with MCH=H-2q haplotype. The cells are obtained from ATCC (ATCC cat # CRL 9641)
(c) Genetic stability – according to Annex IIIa, II, A(10):
The GMO is genetically stable as demonstrated by serial passages in culture for up to 15 months and corresponding to 50 passages

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

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

7. Summary of the potential environmental impact of the release of the GMOs.
The GMO is manipulated under biohazard laminar flow hood only, in case of accidental dissemination on the lab bench, the operator wearing gloves and prescribed lab suites inactivate the GMO with sodium ipochloride. In normal working conditions there no risks, since the GMO is not to be manipulated with cutting tools (syringes, glass pipettes) that can provide a means of inoculation of viral particles.
In case of environmental dissemination of the GMO or of the produced viral particles, the survival of both will be extremely limited and the chance of infection of any other species in the absence of proper experimental conditions approaches zero.
If, for any reason, an operator working in adequate and prescribed conditions wounds himself, this should be a very superficial one. In this case, the chance that a productive infection could take place would be very limited as dependent on the following limiting factors: i) the low number of free viral particles present in the final cell suspension (estimated lower than 1/105 cells) ii) the need of actively replicating cells for cell infection and in case of replicating cells, infection may take place only in the presence of specific agents (e.g. protamine) which facilitate viral adhesion to the cell membrane and entry iii) the inactivation of the free viral particles by the complement system of immune competent, healthy individuals Further, even if this very unlikely event occurs, the expression of both the therapeutic and of the marker transgenes will not be associated with any specific risk since i) the ΔLNGFR gene is truncated into its intracytoplasmic portion and therefore incapable to signal transduction and downstream cell activation ii) the WT, parental HSV is able to infect humans and HSV-Tk expression is not related to any pathological effect in infected cells iii) HSV-Tk ability to trigger a suicide pathway in replicating cells constitutes an added safety mechanism which can be activated by GCV administration in case of accidental infection iv) HSV-Tk is an heterologous protein regarded as non-self so that Tk+ cells are rapidly cleared by the immune system of an healthy individual by the activation of a specific $CD8^+$ cells response

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 : *gp+env Am12 avaliable at ATCC (CRL-9641) (Markowitz D et al. Virology 167:400, 1998)*

2. Name

(i) order and/or higher taxon (for animals)	*Murinae*
(ii) genus	*Mus*
(iii) species	*Mus Musculus*
(iv) subspecies	
(v) strain	*NIH Swiss*
(vi) pathovar (biotype, ecotype, race, etc.)	*NA*
(vii) common name	*Mouse*

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 (*x*) No ()
(d) Is it frequently kept in the country where the notification is made?
Yes (*x*) No ()

4. Natural habitat of the organism: *Not Relevant. It is used in laboratory only and requires specific culture medium*

(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 Use in vitro only with specific culture medium

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

5. (a) Detection techniques *Not relevant*
(b) Identification techniques
The microorganism has an aplotye MHC=H-2q and two plasmids (pgagpolgpt and penvAm) encoding for gag/pol and env proteins.

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: *Biosafety Level 1as specified on attcc.org for the ATCC cat # 9641*

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

8. Information concerning reproduction*: Not relevant. The GMO is unable to reproduce in environments other than culture conditions*

(a) Generation time in natural ecosystems:

(b) Generation time in the ecosystem where the release will take place:

(c) Way of reproduction:
Sexual.
Asexual
(c) Factors affecting reproduction:

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: *Not Applicable. The GMO is unable to survive in environments other than culture conditions*

10. (a) Ways of dissemination: *Not Applicable. The GMO is not for deliberate release in the environment.*

(b) Factors affecting dissemination
Not Applicable.

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):
The backbone LXSN is derived from MoLV (Miller AS Biotechniques 7:980, 1989) has been firstly modified to generate the SFCMM-3 vector (Notification # MM/IC/Op.2-20) with a length in the plasmid form of 7306 bp. The SFCMM-3 Mut2 vector has been generated by site directed mutagenesis introducing in the SFCMM-3 vector encoding for the WT HSV-TK a silent T→C transition at bp 1996. The length of the SFCMM-3 Mut 2 vector in its plasmid form is of 7306 bp. The table below reports the notification history and dates of authorization for MolMed:

Notification Id	Date of authorization
MI/IC/OP.IIA/98-03	14/07/98
MI/IC/OP.IIA/98-03 a and b	14/09/98
MI/IC/OP.IIA/98-03 c and d	26/02/99
MI/IC/OP.IIA/98-03 f	02/03/00
MI/IC/OP.IIA/98-03 g	25/07/00
MI/IC/OP.IIA/00-07	16/05/00
MI/IC/OP.IIA/01-01 and 02	16/06/01
MI/IC/OP.2/02-017 – 024	11/09/02
MI/IC/OP.3/02/004	29/07/94

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
Production of retroviral vectors defective for reproductive ability and encoding for the surface marker gene Low Nerve Growth Factor Receptor truncated of its cytoplasmic tail (ΔLNGFR) and for the Herpes Simplex Virus Thymidine Kinase (HSV-Tk) gene.

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: *SFCMM-3 Mut2. Recombinant retroviral vector: family Retroviridae, genus: Gammaretrovirus Species: Murine leukemia-related retrovirus*

(c) Host range of the vector: *Amphotropic spectrum able to transduce human cells*

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

Yes (*x*) No ()

antibiotic resistance (.)

other, specify: *endocytoplasmic truncated form of the Low Nerve Growth Factor Receptor (ΔLNGFR)*

Indication of which antibiotic resistance gene is inserted

…

(e) Constituent fragments of the vector *(please refer to attachement 1 and 2 for restriction map and sequence of the vector)*

a. *Structural genes: 700bp fragment of gag gene,*
b. *ΔLNGFR gene:*
c. *HSV-Tk gene*
d. *Long Terminal Repeats*
e. *SV40 Promoter*

(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

(b) Composition of the insert
   a. *Structural genes: 700bp fragment of gag gene,*
   b. *ΔLNGFR gene: The fragment has been inserted in the Hind III(5')/NaeI restriction site of the vector*
   c. *HSV-Tk gene: The fragment has been inserted in the Upa unique restriction site of the vector*
   d. *Long Terminal Repeats*
   e. *SV40 Promoter*

(c) Source of each constituent part of the insert
   a. *700bp fragment of gag gene: MoMSV*
   b. *ΔLNGFR gene: Homo Sapiens*
   c. *HSV-Tk Mut2: Herpes Simplex virus type I strain CL101*
   d. *LTR: Moloney Murine Leukemia Virus*
   e. *SV40 Promoter: Simian Virus 40*

(d) Intended function of each constituent part of the insert in the GMO
   a. *700bp fragment of gag gene: the starting codon ATG modified in a stop codon TAG so that no functional transcript can be generated*
   b. *ΔLNGFR gene: the gene is expressed as surface molecule on the cell membrane and thus functions as marker gene for positively transuded cells recognized by FACS analysis. The truncation of the endocytoplamic tail impairs downstream signalling upon antibody binding*
   c. *HSV-Tk gene: The protein is used as negative selection marker to eliminate via suicide mechanism positively transduced cells in presence of ganciclovir. A silent T → C mutation was introduced in position 1996 of the wild type gene to avoid the splicing phenomenon by inactivation the splice donor site and thus minimizing resistance to GCV (Garin M et al, Blood 97:122, 2001).*
   d. *LTR: regulatory element for HSV-Tk gene*
   e. *SV40 promoter: regulatory element for ΔLNGFR gene*

(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) …

(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:

(a) 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 (*x*) No ()

If yes, specify Level 2

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 () No (*x*) Unknown (.)
Specify

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

Yes (*x*) No (.) Not known ()
Specify the GMO is able to produced infective viral particles

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

Yes () No (*x*) Not known (.)
Specify:

2. Genetic stability of the genetically modified organism
The packaging cell line Am12 producing viral vectors made starting from a LXSN backbone has been demonstrated stable up to 15 month in culture corresponding to 50 passages without any modification have been 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)
…
4. Description of identification and detection methods
(a) Techniques used to detect the GMO in the environment: *Expression analysis of the inserted genes*
(b) Techniques used to identify the GMO: *Constitutive expression of transgenes*

F. Information relating to the release
1. Purpose of the release (including any significant potential environmental benefits that may be expected): *Production of supernatants containing the retroviral vector encoding for the HSV-Tk and ΔLNGFR genes*
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: MolMed Via Olgettina 58 20312 Milano. Urban localization in part of the entire building.

(b) Size of the site (m^2): … m^2
(i) actual release site (m^2): … 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 relevant. No proximity to protected area or biotopes.*

(d) Flora and fauna including crops, livestock and migratory species which may potentially interact with the GMO:
Not Relevant. The GMO is not for deliberate release in the environment and manipulated in restricted areas only with specific measures to avoid dissemination, so no interaction with flora or fauna is possible

4. Method and amount of release
(a) Quantities of GMOs to be released: *Maximal GMO concentration in culture: $0.2x10^6$ cell/cm^2*
(b) Duration of the operation: *Processes entailing manipulation of GMO lasts from 10 to 28 days*
(c) Methods and procedures to avoid and/or minimise the spread of the GMOs beyond the site of the release:
Cells producing the retroviral vector are cultured in flasks, roller bottles, cell factories or bioreactor in adequate cell culture medium at 37°C 5% CO2 for 1-3 weeks. The supernatant is then harvested filtered, filled and stocked at -80°C. All manipulations are performed under biohazards laminar flow hood or, whenever possible in a closed system.
All manipulation for cell seeding, harvest, filtration and filling are performed under biohazard laminar flow hood. All wastes are inactivated with sodium ipochcloride. Whenever possible, all wastes are gathered in closed containers specific for potentially infective biologic materials and treated by a specialized company.

5. Short description of average environmental conditions (weather, temperature, etc.):
Mediterean 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.
The GMO has been manipulated at the site since 1998 (date of first notification MI/IC/OP.IIA798-03). 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): Haploidentical donor T lymphocytes

(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): *the GMO integrate in host cell chromosome as proviral sequence following transduction. Transduced Tk+ positevley cells are aimed at helping immureconstitution*

of immunosuppressed patients with additional GvL activity. In case of GvHD, cycling Tk+ can be eliminated by GCV administration

3. Any other potentially significant interactions with other organisms in the environment
None. Retroviral particles cannot interact with other organism in the environment.

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 GMO is not for deliberate release and it has no fitness to survive and colonize in any ecosystem*

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.
In case of accidental release of the GMO the likelihood of dissemination in the environment and infection of any other species approaches zero since, in the absence of specific experimental conditions, the GMO infectivity potential is negligible.
(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: *no gene transfer may occur either from the GMO to other organisms in the ecosystem and viceversa since infectivity and survival of the GMO in the environment is negligible.*

(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.):
A phase I/II studies has been performed and results reported in Ciceri et al. Lancet Oncology 2009, 10(5): 489

9. Possible environmentally significant interactions with biogeochemical processes (if different from the recipient or parental organism)

H. Information relating to monitoring

1. Methods for monitoring the GMOs: *Constitutive expression of the transgenes*
2. Methods for monitoring ecosystem effects: *Analysis of the inserted genes*
3. Methods for detecting transfer of the donated genetic material from the GMO to other organisms: *Constitutive expression of the transgenes*
4. Size of the monitoring area (m^2)

… m^2

5. Duration of the monitoring: *Not relevant*
6. Frequency of the monitoring: *laminar flow hoods and HVAC plant are checked twice a year.*

I. Information on post-release and waste treatment

1. Post-release treatment of the site: *Areas and equipment where manipulation occurs are cleaned after manipulation with with disinfecting and decontaminating agents.*
2. Post-release treatment of the GMOs:
3. (a) Type and amount of waste generated: *solid and liquid wastes*
3. (b) Treatment of waste: *Wastes are inactivated with sodium ipochloride. Whenever possible they are gathered in specific closed containers and treated as potentially infective biologic material.*

J. Information on emergency response plans

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

The GMO is to be manipulated in restricted area only and under specific procedures. In this light, dissemination of the GMO in the environment is a very unlikely event and emergency plans are not in place.

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

In case of accidental spillage these will be treated as a level 2 biohazardous spill, they will be absorbed onto sand, earth or any suitable adsorbent material. Transfer to a container for disposal and finally the spillage area is cleaned using soap and water and then treat with a bleach solution

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.