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/17/PEI3148
(c)	Date of acknowledgement of notification	25/07/2017
(d)	Title of the project	Treatment of patients with relapsed or refractory CD19+ lymphoid disease with T lymphocytes transduced by RV-SDF.CD19.CD28.4-1BBzeta retroviral vector – a unicenter Phase I/II clinical trial (HD-CAR-1)
(e)	Proposed period of release	From 01/JUL/2018 until 03/JUN/2020

2. Notifier

Name of institution or company: Universitätsklinikum Heidelberg

3. GMO characterisation

(a) Indicate whether the GMO is a: CD19 Chimeric antigen receptor T cells (CD19.CAR T cells): human T lymphocytes from patient were transduced with a retroviral vector to generate CD19.CAR T cells.

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

specify phylum, class …

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

Mammals: human T lymphocytes

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

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.
CD19.CAR T cells can only survive in cell culture or in the patient, who is donor and recipient of the T cells (autologous CAR T cells).

B. Information relating to the recipient or parental organism from which the GMO is derived

1. Recipient or parental organism characterisation: The patient is the donor of the human T cells and recipient of the CD19.CAR T cells (autologous CAR T cells)

(a) Indicate whether the recipient or parental organism is a:

(select one only)

viroid (.)
RNA virus (.)
DNA virus (.)
bacterium (.)
fungus (.)
animal
- mammals (X) → Human
- insect (.)

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

other, specify …

2. Name: Human T cells from peripheral blood mononuclear cells
   (i) order and/or higher taxon (for animals) …
   (ii) genus …
   (iii) species …
   (iv) subspecies …
   (v) strain …
   (vi) pathovar (biotype, ecotype, race, etc.) …
   (vii) common name …

3. Geographical distribution of the organism Human T cells from peripheral blood mononuclear cells of patients at the University Hospital Heidelberg.

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

   (c) Is it frequently used in the country where the notification is made?
   Yes (.) No (.)

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

4. Natural habitat of the organism Human T cells from peripheral blood mononuclear cells of the recipient patient (autologous T cells)

   (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:
…

5. (a) Detection techniques

(b) Identification techniques

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

7. Is the recipient organism significantly pathogenic or harmful in any other way (including its extracellular products), either living or dead? Human T cells from peripheral blood mononuclear cells of patient. Patient is donor of the T cells and recipient of the autologous CD19.CAR T cells.
Yes (.) No (.) 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

(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

(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

(b) Factors affecting dissemination
…

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
…

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
SFG retroviral vector: replication deficient retrovirus, could only be integrated once. The retroviral vector is not able to survive outside of cells because it is not stable and cannot reproduce. Aerogen transfer is not possible.

(c) Host range of the vector
Most mammalian cells.

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

Yes (X) No (.)

antibiotic resistance	(.)
other, specify	With PCR and flow cytometry

Indication of which antibiotic resistance gene is inserted

(e) Constituent fragments of the vector
…

(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
scFvCD19, CD28, CD137 (4-1BB) and CD3zeta chain

(b) Source of each constituent part of the insert
scFv CD19 comes from a murine antibody (FMC63).
CD28, CD137 (4-1BB) and CD3zeta Chain are of human origin.

(c) Intended function of each constituent part of the insert in the GMO
CD28 and CD137 (4-1BB) are for the costimulation of T cells.
CD3zeta is for the recognition of the TCR
scFv CD19 is providing specificity in recognition of the CD19 antigen on the surface of leukemia and lymphoma cells.

(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 …

Mouse: Mammalia → Theria → Euteria → Rodentia → Mudidae → Murinae

Human: Hominoidea → Hominidae → Homo

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 (.) 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 (.) Not known (X)
Specify: CD19.CAR T cells underwent robust in vivo T cell expansion, persisted at high levels for at least 6 months in blood and bone marrow, continued to express functional receptors on cells with a memory phenotype, and maintained anti-CD19 effector function in vivo.

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

(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 DNA is stable integrated in the transduced T cells. These transduced CAR T cells will be injected in the respective patient.

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
Detection of the transduced T cells (CAR T cells) via flow cytometry with specific antibodies: environment = blood of the patient

(b) Techniques used to identify the GMO
Identification of the transduced T cells (CAR T cells) via flow cytometry with specific antibodies: environment = blood of the patient

F. Information relating to the release

1. Purpose of the release (including any significant potential environmental benefits that may be expected)
The transduced cells will be injected in the respective patient from whom the cells were isolated and cultured for transduction (= release).

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):
…

(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:
…

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

4. Method and amount of release

(a) Quantities of GMOs to be released:
…

(b) Duration of the operation:
…

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

5. Short description of average environmental conditions (weather, temperature, etc.)
…

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.
…

G. Interactions of the GMO with the environment and potential impact on the environment, if significantly different from the recipient or parent organism

CD19.CAR T cells can only survive in cell culture or in the patient, who is donor and recipient of the T cells (autologous CAR T cells).

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	…

2. Anticipated mechanism and result of interaction between the released GMOs and the target organism (if applicable)
CD19.CAR T cells, which were injected in the patients, recognize and bind the CD19 transmembran molecule of CD19-positive cancer cells.

3. Any other potentially significant interactions with other organisms 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
No

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

(b) from other organisms to the GMO:
No

(c) likely consequences of gene transfer:
No

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.):
Clinical trials, which used the same CAR T cell vector to generate CD19.CAR T cells: 1. Prof. Malcolm Brenner and Dr. Carlos Ramos from the Baylor´s College of Medicine in USA; 2. Dr. Angelica Loskog and Dr. Gunilla Endblad, Uppsala, Sweden.
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
Flow cytometry analysis

2. Methods for monitoring ecosystem effects
…

3. Methods for detecting transfer of the donated genetic material from the GMO to other organisms
…

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

5. Duration of the monitoring
…

6. Frequency of the monitoring
…

I. Information on post-release and waste treatment

1. Post-release treatment of the site
…

2. Post-release treatment of the GMOs
…

3. (a) Type and amount of waste generated
Cell culture medium, buffer, plastic ware like pipettes, pipette tips, plates, cell culture flasks → liquid waste (not more than 100 ml per day) and solid material

3. (b) Treatment of waste
All material which is used will be autoclaved and discarded subsequently.

J. Information on emergency response plans

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

Work will take place in a laminar air flow safety cabinet class 2 in a clean room. Contamination will be cleaned with appropriate disinfection materials which will be autoclaved. All waste will be also autoclaved.

2. Methods for removal of the GMO(s) of the areas potentially affected
Contamination will be cleaned with appropriate disinfection materials and all waste will be autoclaved.

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

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