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	Czech Republic
(b)	Notification number	B/CZ/17/02
(c)	Date of acknowledgement of notification	16/11/2017
(d)	Title of the project	

Safety of CART19 cell manufactured by piggyBac mediated gene transfer for the therapy of lymphoid malignancies.

(e)	Proposed period of release	From 1/1/2018…. until 31/12/2020

2. Notifier

Name of institution or company:
Institute of Hematology and Blood Transfusion (IHBT)

3. GMO characterisation

(a) Indicate whether the GMO is a:

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

specify phylum, class
Human

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

Human in vitro expanded CD3+ T cells transduced with a piggyBac transposon vector carrying a Chimeric Antigenic Receptor (CAR) transgene. The CAR contains a scFv domain from an antibody recognizing human CD19 fused with hinge and transmembrane domains of human CD8 and with intracellular domains of the human genes 4-1BB and CD3-zeta.

(c) Genetic stability – according to Annex IIIa, II, A(10)
Yes. The CAR sequences will be stably integrated into the host genome.

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.
Modification of T cells is performed via electroporation of naked plasmid DNA in the GMP facility at UHKT. The administration of CART19 to patients is performed at a clinical unit at UHKT via intravenous infusion. Thus, an environmental impact is not expected since the release of the autologous CART19 is limited to patient administration in a hospital setting and CART19 can not survive in the environment.

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 …

2. Name

(i)	order and/or higher taxon (for animals)	homo sapiens
(ii)	genus	…
(iii)	species	…
(iv)	subspecies	…
(v)	strain	…
(vi)	pathovar (biotype, ecotype, race, etc.)	…
(vii)	common name	human

3. Geographical distribution of the organism

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

(b) Indigenous to, or otherwise established in, other EC countries:
(i) Yes (x), following points not applicable for human cells

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 (.) not applicable to human cells

(d) Is it frequently kept in the country where the notification is made?
Yes (.) No (.) not applicable to human cells

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	
not applicable to human cells	

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

5. (a) Detection techniques
Quantitative PCR and flow cytometry

(b) Identification techniques
Quantitative PCR and flow cytometry

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 (.) 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

The GMO is derived from autologous T cells isolated from the peripheral blood of lymphoma or leukemia patients. The CART19 cells cannot survive outside of the patient. The cells are not pathogenic and cannot persist or replicate in the environment or other organisms.

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:

Not applicable for human cells

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	not applicable for human cells

(b) relevant factors affecting survivability:
Human T cells require a precise combination of special media, growth factors, temperature and CO_2 for survival. Thus they will not survive outside the incubator or a host due to the substantially different conditions (temperature, pH, UV and a change in the biophysical and bio-chemical conditions).

10. (a) Ways of dissemination
CART19 cells can only be transmitted between individuals through injection. No dissemination in the environment is expected due to limited survival outside human body and lack of natural entry routes into the human body.

(b) Factors affecting dissemination
The immune system of people other than the donor will eliminate the blood cells.

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
The CART19 cells contain a transgene composed of a CD19-specific scFv, derived from the murine monoclonal antibody B-D3 fused with hinge and transmembrane domains of human CD8 and with intracellular domains of the human genes 4-1BB and CD3-zeta and a polyadenylation signal from bovine growth hormone. CAR is expressed form the ubiquitin C promoter (UbC). These CAR T cells recognize and lyse CD19-expressing target cells including both normal and malignant B 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 (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	(.)
cosmid	(.)
transposable element	(x)
other, specify …	

(b) Identity of the vector
piggyBac transposon

(c) Host range of the vector

The vector is a naked DNA plasmid and cannot spontaneously transduce cells.

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

Yes (x) No (.)

antibiotic resistance (x)

other, specify Modified cells can be identified by the detection of CAR using flow cytometry or by PCR for CAR sequences.

Indication of which antibiotic resistance gene is inserted

aph(3')-Ia conferring resistance to kanamycin. The resistance gene is encoded within the backbone of the plasmid and does not integrate into genomic DNA of lymphocytes.

(e) Constituent fragments of the vector

DNA sequences encoding CD19-specific scFv, derived from the murine monoclonal antibody B-D3 fused with hinge and transmembrane domains of human CD8 and with intracellular domains of the human genes 4-1BB and CD3-zeta. Regulatory sequences flanking the CAR gene : ubiquitin C promoter (UbC) and poly A signal form bovine growth hormone.

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

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

DNA sequence encoding a CD19-specific scFv, derived from the murine monoclonal antibody B-D3 fused with hinge and transmembrane domains of human CD8 and with intracellular domains of the human genes 4-1BB and CD3-zeta. The CAR is driven by the ubiquitin C promoter (UbC) and contains poly A signal form bovine growth hormone.. polyadenylation signal from bovine growth hormone . This sequence is flanked by 5'arm and 3'arm sequences specific to the piggyBac transposon and which enable integration into host DNA:

(b) Source of each constituent part of the insert

human ubiquitin C promoter (UbC)
3'arm and 5'arm piggyBac transposon vector sequences
scFv sequence from murine antibody
CD8, 41-BB, CD3zeta of human origin
polyadenylation signal from growth hormone of bovine origin

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

The transposon sequences flanking the CAR transgene mediate the insertion of the CAR into the host genome. CAR is d riven by the UbC promoter and is composed of the scFv binding domain which consists of the of the heavy and light chains of the murine B-D3 antibody, joined with a short linker to be expressed as a single chained protein. The scFv is linked to a hinge region of human CD8 and thereafter to the intracellular portions of CD3zeta and 4-1BB and polyadenylation signal from bovine growth hormone. The B-D3 antibody is specific for the target antigen CD19 present on the surface of normal and malignant B cells. The CD3-zeta mediates T cell activation following binding of scFv to the antigen CD19 on the surface of target cells, 4-1BB sequence delivers an additional, co-stimulatory signal that promotes T cell survival, persistence, and anti-tumor activity. Together, the signals lead to T cell activation, with proliferation, cytokine secretion and lysis of normal and malignant B cells expressing CD19.

(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 :

Non applicable, the piggyBac transposon is not an organism, but a mobile DNA element isolated from *trichoplusia ni.* It is delivered in the form of a DNA sequence encoded in a plasmid. The transposon can not be released form the cells because it is not a infectious agent (i.e. virus) but a fragment of DNA.

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 piggyBac transposon

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 (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 (.) 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
After integration, the CAR gene forms an integral part of host DNA

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 piggBac transposon encoding the CAR transgene is integrated in the T cell genome. Only the CAR sequence is expressed.

4. Description of identification and detection methods

(a) Techniques used to detect the GMO in the environment
PCR or flow cytomery

(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)
CD19 is a membrane-bound antigen expressed on all B cell differentiation stages, and on malignant B cells except some plasma cell populations. Therefore, T cells targeting CD19 antigen can be used to treat most CD19 positive B cell-derived malignancies such as B-ALL, CLL, non-Hodgkins lymphomas (NHL; such as diffuse large B cell lymphoma - DLBCL, follicular lymphoma – FL and mantle cell lymphoma – MCL). Several clinical trials have shown impressive results in otherwise refractory leukaemia and lymphoma using CD19-targeting CAR T cells.

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):
CART19 will be manufactured and administered in the Institute of Hematology and Blood transfusion, Prague, Czech Republic. The cells will be transported in sealed containers and unused material will be discarded.

(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
The patients will be treated in a hospital room in the Intensive care unit (ICU).

(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:
Maximal dose will be 1x10^8 of CART19 per patient administered in a single infusion, 12 patients will be treated.

(b) Duration of the operation:
Less than 1 hour.

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

Transfer of the GMO will be done in sealed containers. The hospital personnel will wear protective clothes, eye-wear, face mask and gloves. Detailed procedures for all steps in handling the GMO is described the biohazard instructions.

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.
Not applicable

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) Homo Sapiens
(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)
The autologous CART19 cells (GMO) will be infused into the patients from which the GMO is derived, following administration the CART19 will target malignant B cell lymphoma or leukemia cells.

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
Not applicable

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 …

Not applicable

7. Likelihood of genetic exchange in vivo

(a) from the GMO to other organisms in the release ecosystem:
…

(b) from other organisms to the GMO:
…

(c) likely consequences of gene transfer:

Activation of patients immune system and antitumor effect resulting in regression of tumors

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.):
None

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

H. Information relating to monitoring

1. Methods for monitoring the GMOs

The GMO will be monitored in the blood of patients by quantitative PCR and flow cytometry. Patients will be monitored for 2 years after the last CART19 cell infusion.

2. Methods for monitoring ecosystem effects
Not applicable.

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

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

5. Duration of the monitoring
Two years after last CART19 infusion.

6. Frequency of the monitoring
According to protocol, the patients are tested daily during the the first two weeks, than days 20, 25, 30, and then at months 3, 6, 9, 12, 15, 18, 21, 24 post treatment.

I. Information on post-release and waste treatment

1. Post-release treatment of the site
Disinfection of working area.

2. Post-release treatment of the GMOs
Remaining material will be destroyed as biohazard according to hospital guidelines.

3. (a) Type and amount of waste generated
Contaminated material used for infusion, including cryotubes, bags, syringes and tubing, catheters.

3. (b) Treatment of waste
Waste will be destroyed as biohazard according to hospital guidelines.

J. Information on emergency response plans

1. Methods and procedures for controlling the dissemination of the GMO(s) in case of unexpected spread
Standard policies and procedures have been implemented at IHBT for the treatment of medical waste which may contain bloodborne pathogens.

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

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 according to study protocol, adverse events will be evaluated and reported according to the procedures described in the protocol.