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	France
(b)	Notification number	B/FR/13/GT07
(c)	Date of acknowledgement of notification	21/10/2013
(d)	Title of the project	

Utilisation de lymphocytes T (LT) génétiquement modifiés, exprimant le gène inductible de la caspase 9 humaine (iCASP9) et le gène de sélection delta CD19 dans le traitement de la réaction du greffon contre l'hôte (GvHD) lors de la greffe hématopoïétique allogénique. Etude Side by Cide

Use of genetically modified T-lymphocytes expressing the inducible human caspase 9 gene (iCASP9) and the selection gene ΔCD19 for the treatment of Graft versus Host Disease (GvHD) in allogeneic haematopoietic transplantation: Study Side by Cide

(e)	Proposed period of release	From 01 /03/2014 until 30/03/2017

2. Notifier

Name of institution or company: Centre Hospitalier Régional Universitaire de Besançon

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 …

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

Human T lymphocytes expressing the iCasp9 (suicide gene) and ΔCD19 cell surface marker (selection gene).

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

We have previously shown in our first study, based in use of modified T lymphocytes expressing a suicide gene (HSV-TK), that the modified T lymphocytes circulated more than 10 years post-transplant.

For iCasp9 system, knowledge is not yet sufficient to establish the stability of long-term transgene. Indeed, the scientific community has only two years of feedback in the study of Di Stasi, published in NEJM in 2011

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.

No environmental impact because the cells die in the external environment.

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

1. Recipient or parental organism characterisation:

T lymphocytes are human origin.

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

(select one only)

viroid (.)
RNA virus (.)
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) …
(ii) genus …
(iii) species …
(iv) subspecies …
(v) strain …
(vi) pathovar (biotype, ecotype, race, etc.) …
(vii) common name …

3. Geographical distribution of the organism

(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

(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?
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
The lymphocytes are modified with iCasp9 suicide gene and membrane cell surface marker ΔCD19.

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.iCasp9.2A.ACD19 consists of iCasp9 linked, via a cleavable 2A-like sequence, to truncated human CD19 (ΔCD19). iCasp9 consists of a human FK506-binding protein (FKBP12; GenBank AH002 818) with an F36V mutation, connected via a Ser-Gly-Gly-Gly-Ser linker to human caspase 9 (CASP9; GenBankNM 001229). The F36Vmutation increases the binding affinity of FKBP12 to the synthetic homodimerizer, AP20187 or AP1903.

(c) Host range of the vector

Retroviral vector.

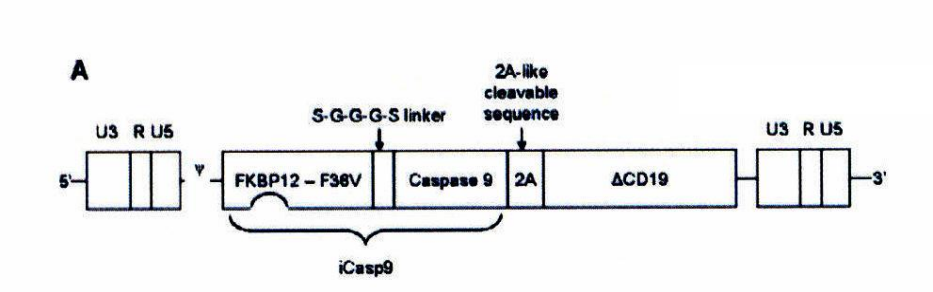
(d) Presence in the vector of sequences giving a selectable or identifiable phenotype
Yes X No (.)

antibiotic resistance (.)
other, specify membrane marker trunked human CD19

Indication of which antibiotic resistance gene is inserted
…

(e) Constituent fragments of the vector

Figure1: Structure of SFG.iCasp9.2A.Δ CD19.



The transgene consists of a suicide gene, inducible caspase 9 (iCasp9), and a selectable marker, truncated CD19 (ΔCD19), linked by a 2A-like sequence, which encodes a cleavable peptide. iCasp9 consists of a drug-binding domain (FKBP12-F36V) connected via a short linker (SGGGS) to human caspase 9.

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

(i)	transformation	(.)
(ii)	electroporation	(.)
(iii)	macroinjection	(.)
(iv)	microinjection	(.)
(v)	infection	X (transduction)
(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
iCasp9: consists of human FK506-binding protein with an FV36 mutation, connected via a Ser-Gly-Gly-Gly-Ser linker to human caspase 9.
The 2A-like sequence encodes a 20 amino acid peptide from Thosea Asigna insect virus.
ΔCD19: consists of full-length CD19 truncated at amino acid 333, which shortens the intracytoplasmic domain from 242 to 19 amino acids, and removes all conserved tyrosine residues that are potential sites for phosphorylation.

(b) Source of each constituent part of the insert
iCasp9 and ΔCD19 are human origin.

2A-like sequence is synthetic.

(c) Intended function of each constituent part of the insert in the GMO
iCasp9 : is used as suicide gene
ΔCD19: is used as cell surface marker of gene modified cells for cell selection

(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

The ΔCD19 and iCasp9 genes are of human origin.
The 2A-like sequence is synthetically

1. Indicate whether it is a:

viroid (.)
RNA virus (.)
DNA virus (.)
bacterium (.)
fungus (.)
animal
- mammals (.)
- 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 (.) 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

We have previously shown in our first study, based in use of modified T lymphocytes expressing a suicide gene (HSV-TK), that the modified T lymphocytes circulated more than 10 years post-transplant.

For iCasp9 system, knowledge is not yet sufficient to establish the stability of long-term transgene. Indeed, the scientific community has only two years of feedback in the study of Di Stasi, published in NEJM in 2011

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 …

(c) 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

Not applicable (the cells die in the external environment).

(b) Techniques used to identify the GMO

PCR and cytometry

F. Information relating to the release

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

The GMO will be injected to patients for whom a bone marrow transplant is required. The bone marrow will be T & B lymphocytes depleted.

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):
The administration of the cells will be held in a controlled atmosphere chamber at Hematology Division (Bone Marrow unit graft) of the University Hospital of Besançon (France).

(b) Size of the site (m^2): about 250 m^2
(i) actual release site (m^2): 20 m^2
(ii) wider release site (m^2): … m^2

(d) Proximity to internationally recognised biotopes or protected areas (including drinking water reservoirs), which could be affected:
Not applicable

(e) 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:
Maximum 20.10^e6 cells / kg of patient body weight.

(b) Duration of the operation:
20 minutes

(c) Methods and procedures to avoid and/or minimise the spread of the GMOs beyond the site of the release
The injection of the GMO will be taken in confined chamber.

5. Short description of average environmental conditions (weather, temperature, etc.)
Not applicable

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.
Irrelevant

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

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)
Not applicable

3. Any other potentially significant interactions with other organisms in the environment
Not applicable

4. Is post-release selection such as increased competitiveness, increased invasiveness for the GMO likely to occur?
Yes X No (.) 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
Not 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 …

7. Likelihood of genetic exchange in vivo

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

(b) from other organisms to the GMO:
Irrelevant

(c) likely consequences of gene transfer:

The retroviral vector is completely devoid of genes gag, pol and env which are necessary for its replication.

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.):
Irrelevant

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

H. Information relating to monitoring

1. Methods for monitoring the GMOs
Blood samples will be taken according to a defined schedule.
Cytometry and Molecular analysis (PCR for RCR, iCasp9 expression…)

2. Methods for monitoring ecosystem effects
A detection of replication competent retrovirus RCR will be performed on blood samples.
PCR for iCasp9 expression gene

3. Methods for detecting transfer of the donated genetic material from the GMO to other organisms
A detection of replication competent retrovirus RCR will be performed on blood samples.

4. Size of the monitoring area (m^2)
… m^2
Not applicable

5. Duration of the monitoring
3 years.

6. Frequency of the monitoring
Detection of RCR will be taken each 6 months.

I. Information on post-release and waste treatment

1. Post-release treatment of the site
All the material used for the injection will be autoclaved.

2. Post-release treatment of the GMOs
Not applicable

3. (a) Type and amount of waste generated
Solid and liquid waste.

3. (b) Treatment of waste
The waste will be autoclaved in the site of the production before incineration.

J. Information on emergency response plans

1. Methods and procedures for controlling the dissemination of the GMO(s) in case of unexpected spread
   The cells die in the external environment.

2. Methods for removal of the GMO(s) of the areas potentially affected
   In case of accidental spillage, the infected premises will be washed by standard disinfectant bleach.

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

   Not applicable (the cells die in the external environment).

4. Plans for protecting human health and the environment in the event of an undesirable effect
   Not applicable (the cells die in the external environment).