SNIF: GMOB-2023-19310

Domain:
GMO

Authorisation type:
Deliberate release of GMO or a combination of GMOs for any other purpose than for placing on the market

Application type:
Summary notification for the release of genetically modified higher organisms other than higher plants in line with Decision 2002/813/EC Annex, Part 1.

Recipient Member State:
Czech Republic

Competent Authority:
Ministry of the Environment of the Czech Republic

Notification number:
B/CZ/23/06

Acknowledgement date:
2023-10-23

A- General information

Details of notification

Details of notification
Safety of CAR123 cells manufactured by piggyBac mediated gene transfer for therapy of myeloid malignancies.
Member State of notification
Czechia
Title of the project
Safety of CAR123 cells manufactured by piggyBac mediated gene transfer for therapy of myeloid malignancies.
Proposed period of release
Starting date
2024-01-01
Finishing date
2027-12-31

Notifier

Name of institute or company
Institute of Hematology and Blood Transfusion
Email
info@uhkt.cz
Phone number
+420221977111
Website
www.uhkt.cz
Address
U Nemocnice 1, Prague 2
Post code
CZ-128 00
Country
Czechia

GMO characterisation

(a) Indicate whether the GMO is a:

Viroid
No
RNA virus
No
DNA virus
No
Bacterium
No
Fungus

No
Animal
Yes

Select from following options:

Mammal
Yes
Insect
No
Fish
No
Other animal
No
Other
No

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

Human T lymphocytes

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

Yes. The CAR sequences will be stably integrated into the host genome.

Is the same GMO release planned elsewhere in the Community (in conformity with Article 6(1)), by the same notifier?

No

Has the same GMO been notified for release elsewhere in the Community by the same notifier?

No

Has the same GMO been notified for release or placing on the market outside the Community by the same or other notifier?

No

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

The administration of GMO (CART123) to patients is performed at a clinical unit at IHBT via intravenous infusion. Thus, an environmental impact is not expected since the release of the autologous CART123 is limited to patient administration in a hospital setting and CART123 can not survive in the environment.

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

1. Recipient or parental organism characterisation

Indicate whether the recipient or parental organism is a:

Viroid
No
RNA virus
No
DNA virus
No
Bacterium
No
Fungus
No
Animal
Yes

Select from following options:

Mammal
Yes
Insect
No
Fish
No
Other animal
No
Other
No

2. Name

(i) Order and/or higher taxon (for animals)
Homo sapiens
(ii) Genus
Homo
(iii) Species
Sapiens
(iv) Subspecies
n/a
(v) Strain
n/a
(vi) Pathovar (biotype, ecotype, race, etc.)
n/a

3. Geographical distribution of the organism

(a) Indigenous to, or otherwise established in, the country where the notification is made:
yes
(b) Indigenous to, or otherwise established in, other EC countries:
yes
Indicate the type of ecosystem in which it is found:
continental
(c) Is it frequently used in the country where the notification is made?
No
(d) Is it frequently kept in the country where the notification is made?
No

4. Natural habitat of the organism

(a) Is the organism a microorganism ?
No
Specify
(b) Is the organism an animal?
Yes
Natural habitat or usual agroecosystem
Not applicable to human cells

5(a) Detection Techniques

Detection Techniques
Quantitative PCR and flow cytometry

5(b) Identification Techniques

Identification Techniques
Quantitative PCR and flow cytometry

6. Is the recipient organism classifies under existing Community rules relating to the protection of human health and/or the environment?

No

7. Is the recipient organism significantly pathogenic or harmful in any other way (including its extracellular products), either living or dead?

no

8. Information concerning reproduction

(a) Generation time in natural ecosystems:
Not applicable for human cells
(b) Generation time in the ecosystem where the release will take place:
Not applicable for human cells
(c) Way of reproduction
Asexual
(d) Factors affecting reproduction:
Not applicable for human cells

9. Survivability

(a) Ability to form structures enhancing survival or dormancy:

(i) endospores
No
(ii) cysts
No
(iii) sclerotia
No
(iv) asexual spores (fungi)
No
(v) sexual spores (fungi)
No
(vi) eggs
No
(vii) pupae
No
(viii) larvae
No
Other
Yes
Specify
Not applicable for human cells
(b) Relevant factors affecting survivability
CAR123 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). CAR123 can survive long-time only in the body of the recipient and can not survive in different hosts.

10(a) Ways of dissemination

CART123 cells can only be transmitted between individuals through injection. No dissemination in the environment is expected due to impossible survival outside human body and lack of natural entry routes into the human body.
10(b) Factors affecting dissemination
The immune system of people other than the donor will eliminate the CART123 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

Insertion of genetic material
Yes
Deletion of genetic material
No
Base substitution
No
Cell fusion
No
Other
No

2. Intended outcome of the genetic modification

To redirect modified T cells to recognize antigen CD123 on the surface of acute myeloid leukemia cells and kill them. CD20 surface antigen acts as a killswitch.

3(a) Has a vector been used in the process of modification?

Yes
3(b) If yes, is the vector wholly or partially present in the modified organism?
Yes

4. If the answer to 3(b) is yes, supply the following information

(a) Type of vector

Plasmid
No
Bacteriophage
No
Virus
No
Cosmid
No
Transposable element
Yes
Other
No
(b) Identity of the vector
piggyBac transposon
(c) Host range of the vector
The vector is a naked linear DNA and cannot spontaneously modify cells.
(d) Presence in the vector of sequences giving a selectable or identifiable phenotype
Yes
Antibiotic resistance
No

Other
Yes
Specify
Modified cells can be identified by the detection of CAR or CD20 antigen using flow cytometry or by PCR for CAR sequences.
(e) Constituent fragments of the vector
5arm and 3arm sequences specific to the piggyBac transposon and which enable integration into host DNA by the piggyBac transposase

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

(i) transformation
No
(ii) electroporation
Yes
(iii) macroinjection
No
(iv) microinjection
No
(v) infection
No
Other
No

6. Composition of the insert

(a) Composition of the insert

(1) CAR123 a Chimeric Antigenic Receptor composed of CD123 scFv fused with hinge and transmembrane domains of human CD8 and with intracellular domains of the human genes 4-1BB and CD3-zeta. (2) CD20 a surface antigen which serves as a safety precaution to enable a selective elimination of engineered CAR123 T cells with anti-CD20 therapeutic antibody rituximab. The expression of the inserts is driven by the ubiquitin C promoter (UbC) and ends with polyadenylation signal from bovine growth hormone. This sequence is flanked by 5arm and 3arm 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) 3arm and 5arm piggyBac transposon vector sequences CD20 antigen of human origin scFv sequence from murine antibody CD8, 41-BB, CD3zeta of human origin polyadenylation signal from growth hormone of bovine origin

(c) Intended function 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 The CAR123 vector expresses two ubiquitin promoter-regulated proteins: CD20 antigen and CAR123 construct, both separated by T2A sequence which induces ribosomal skipping during translation and thus two proteins CD20 and CAR123 are produced separately from a single mRNA. The CAR123 coding sequence is composed of the scFv binding domain which consists of the of the heavy and light chains of the murine monoclonal 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. Recognition of the target CD123 antigen by the

CAR leads to the activation and proliferation, cytokine secretion and killing of cells expressing the CD123 antigens such as acute myeloid leukemia cells by the CART123 cells. The CD20 protein is a surface antigen and serves as a safety precaution to enable a selective elimination of engineered CAR123 T cells with anti-CD20 therapeutic antibody rituximab.

(d) Location of the insert in the host organism

On a free plasmid
No
Integrated in the chromosome
Yes
Other
No

(e) Does the insert contain parts whose product or function are not known?

No

D. Information on the organism(s) from which the insert is derived

1. Indicate whether it is a:

Viroid
No
RNA virus
No
DNA virus
No
Bacterium
No
Fungus
No
Animal
No
Other
Yes
Specify kingdom, phylum and class
Non applicable, the piggyBac transposon is not an organism, but a mobile DNA element isolated from trichoplusia ni. It is delivered into the cells in the form of a linear DNA. The transposon can not be released form the cells because it is not a infectious agent (i.e. virus) but a fragment of DNA which can not self-replicate.

2. Complete name

(i) Order and/or higher taxon (for animals)
Multiple organisms - see C.6(a-c)
(ii) Family name (for plants)
Multiple organisms - see C.6(a-c)
(iii) Genus
Multiple organisms - see C.6(a-c)
(iv) Species
Multiple organisms - see C.6(a-c)
(v) Subspecies
Multiple organisms - see C.6(a-c)
(vi) Strain
Multiple organisms - see C.6(a-c)
(vii) Cultivar/Breeding line
Multiple organisms - see C.6(a-c)
(viii) Pathovar
Multiple organisms - see C.6(a-c)
(ix) Common name
n/a

3. Is the organism significantly pathogenic or harmful in any other way (including its extracellular products), either living or dead?

no
(b) are the donated sequences involved in any way to the pathogenic or harmful properties of the

organism?
no
Give the relevant information specified under Annex III A, point II, (A)(11)(d) of Directive 2001/18/EC
No pathological, ecological and physiological traits

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 related to exposure to biological agents at work?

No

5. Do the donor and recipient organism exchange genetic material naturally?

no

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?
no
Specify
(b) Is the GMO in any way different from the recipient as far as mode and/or rate of reproduction is concerned?
no
Specify
(c) Is the GMO in any way different from the recipient as far as dissemination is concerned?
no
Specify
(d) Is the GMO in any way different from the recipient as far as pathogenicity is concerned?
no
Specify

2. Genetic stability of the genetically modified organism

After integration, the CAR123 gene forms an integral part of host DNA

3. Is the GMO significantly pathogenic or harmfull in any way (including its extracellular products), either living or dead?

no
(b) Give the relevant information under Annex III A, point II (A)(11)(d) and II(C)(2)(i):
No pathological, ecological and physiological traits

4. Description of identification and detection methods

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

Clinical trial: CART123 a genetically- modified T cells targeting CD123 antigen can be used to treat malignancies such as B-ALL and AML.

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?

No

3. Information concerning the release and the surrounding area

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

CART123 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 (m2)

(i) actual release site (m2)
The patients will be treated in a hospital room in the Intensive care unit (ICU).
(ii) wider release area (m2)
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:

n/a

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

none

4. Method and amount of release

(a) Quantities of GMOs to be released:

Maximal dose will be no more then 5x10^8 of CART123 per patient administered in a single infusion of no more then 100ml volume, 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 are 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 organisms (if applicable)

Applicable?
No

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

Applicable?
Yes
Specify
The autologous CART123 cells (GMO) will be infused into the patients from which the GMO is derived, following administration the CART123 will target malignant 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?

no

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

n/a

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)
None
(ii) Family name (for plants)
None
(iii) Genus
None
(iv) Species
None
(v) Subspecies
None
(vi) Strain

None
(vii) Cultivar/Breeding line
None
(viii) Pathovar
None
(ix) Common name
None

7. Likelihood of genetic exchange in vivo

(a) from the GO to other organisms in the release ecosystem:
Not possible
(b) from other organisms to the GMO:
Not possible
(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 simulated natural environments (e.g. microcosms etc.):

n/a

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

n/a

H. Information relating to monitoring

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 CART123 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
PCR or flow cytometry
4. Size of the monitoring area (m2)
Human body
5. Duration of the monitoring
Two years after last CART123 infusion
6. Frequency of the monitoring
According to protocol, the patients are tested daily during the the first two weeks, than days 28, and then at months 3, 6, 9, 12, 15, 18, 21, and 24 post treatment.

I. Information on post release and waste 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

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 GMOs 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 enironment 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.