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 The Netherlands

(b) Notification number B/NL/16/006

(c) Date of acknowledgement of notification 15/04/2016

(d) Title of the project

Testing the safety and efficacy of KTE-C19 in patients with refractory or relapsed B-cell malignancies. KTE-C19 is a novel adoptive cellular immunotherapy for cancer whereby autologous T cells are genetically modified/transduced *ex vivo* by a replication-deficient retroviral vector to express anti-CD19 chimeric antigen receptors (CAR) on the surface of T cells to target malignant B cells expressing CD19 antigens.

(e) Proposed period of release From Q1-2016 until Q4-2041

2. Notifier

Name of institution or company: Princess Maxima Center for Pediatric Oncology

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 T cells**
-			

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

Human CD3+ T cells transduced with a replication-deficient gamma-retroviral vector (PG13-CD19-H3 Vector) to express a transmembrane CAR.

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

Yes

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

Yes (X) No (.)

If yes, insert the country code(s) The Netherlands, Germany and France.

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 N/A

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 (**X**) No ( )

If yes: USA IND No: 16278

- Member State of notification N/A
- Notification number N/A

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

An environmental impact is not expected as the release of the KTE-C19 transduced autologous T cells are limited to patient administration in hospital settings. According to the environmental risk assessment KTE-C19 will not reach the environment at large. The overall risk of KTE-C19 for people and the environment can be concluded to be negligible.

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) human
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)

If yes, indicate the type of ecosystem in which it is found:

Atlantic	(..)
Mediterranean	(..)
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: Human

5. **(a) Detection techniques**

Common techniques of blood cell analysis.

(b) Identification techniques

Common techniques of blood cell analysis.

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

Yes ( ) No (**X**)

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: N/A

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 patients. The production of both the replication-deficient retroviral vector and KTE-C19 (GMO), takes place in the USA, outside of the Netherlands. Only the final product (KTE-C19) that contains the engineered (genetically modified) anti-CD19 CAR T cells are entering the Netherlands. The genetically modified autologous T cells cannot survive outside of the patient from which the cells were derived. The cells are not pathogenic and do not persist or replicate in the environment or other organisms.

Patients will be tested for HIV, HBV and HCV prior to blood donation and excluded from the clinical trial if tested positive. In addition, the apheresis site is instructed to obtain additional viral serology assessments as per local guidelines. Nevertheless, patient autologous T cells should be handled as potentially containing infectious agents on the basis that pre-screening for blood borne pathogens is not exhaustive and cannot completely exclude the potential for such agents to be present.

8. **Information concerning reproduction**: Not applicable for human T cells

(a) Generation time in natural ecosystems:

N/A.

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

N/A

(c) Way of reproduction: Sexual … Asexual …

(d) 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:
The survival of human T cells, requires a complex combination of special media, temperature and CO_2. The environmental conditions outside the host (body) are substantially different and will not support the cells' survival (temperature, pH, UV and a change in the biophysical and bio-chemical conditions).

10. (a) Ways of dissemination
Human T cells can only be transmitted between individuals through injection. No dissemination in the environment is possible due to fast inactivation and lack of a natural entry route into the body.

(b) Factors affecting dissemination
The immune system of people other than the donor will eliminate the T cell product (the patient-specific genetically modified T 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
KTE-C19 is a novel, investigational, adoptive cancer immunotherapy whereby autologous T cells are genetically modified to express an anti-CD19 transmembrane CAR that targets

CD19 on the cell surface of malignant B cells. The CAR-modified T cell is activated following engagement with the CD19 target, resulting in elimination of the CD19 malignant cell.

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**

Replication-deficient gamma-retroviral vector: murine stem cell virus-based splice-gag vector (MSGV1) termed PG13-CD19-H3 Vector.

(c) **Host range of the vector**

The vector used is a hybrid retroviral vector consisting of the gag-pol accessory proteins from the Moloney murine leukemia virus (MoMLV) and the envelope from the gibbon ape leukemia virus (GALV), both contained and produced in the mouse cell line PG13. The backbone containing the transgene is MSGV1, that utilizes the long terminal repeats (LTR) from the murine stem cell virus (MSCV) and an extended gag region and splice site to improve retroviral titer and expression of the transgene across different cell types (Hughes et al. 2005). This backbone is compatible with the MoMLV retroviral accessory proteins. The PG13-CD19-H3 Vector produced in the PG13 cell line has a broad host range including rat, hamster, bovine, cat, dog, monkey and human cells (Miller et al. 1991).

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

Yes (X) No (.)

antibiotic resistance (.)

other, specify The vector encodes the anti-CD19 CAR which is expressed at the membrane surface of transduced T cells. Cell surface expression of the CAR can be detected by flow cytometric analysis of the transduced T cells, thereby providing an identifiable phenotype.

Indication of which antibiotic resistance gene is inserted N/A

(e) **Constituent fragments of the vector**

The backbone containing the CAR sequence is MSGV1, that utilizes the long terminal repeats (LTR) from the murine stem cell virus (MSCV) and an extended gag region and splice site to improve retroviral titer and expression of the transgene across different cell types (Hughes et al. 2005). Only the LTRs and the sequences contained in between are integrated in the genome of the transduced T cells as provirus. This provirus therefore, contains a 5'LTR serving as promoter, a partial gag sequence and packaging signal, a CAR sequence and a 3'LTR.

(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	N/A

6. Composition of the insert

(a) Composition of the insert

The PG13-CD19-H3 Vector encodes the anti-CD19 CAR. The process of retroviral-mediated transduction serves to integrate the CAR gene into the T cell genome.

The transfer plasmid MSGV1-FMC63-CD28z is an engineered construct that was used to generate an expression cell line that constitutively produces the PG13-CD19-H3 Vector. It comprises 5' and 3' long terminal repeats (LTRs) flanking a partial gag sequence, a retroviral packaging signal and the DNA sequence encoding the anti-CD19 CAR.

The anti-CD19 CAR constituent consists of the following domains linked as a single chimeric molecule:
A target-specific binding domain consisting of an antibody-derived single-chain variable fragment (scFv) specific for the target antigen CD19 expressed on the surface of normal and malignant B cells; the human T cell-derived activating domains CD3-zeta and CD28; and the transmembrane and hinge domains of human CD28.

(b) Source of each constituent part of the insert

The CAR construct utilised to produce KTE-C19 has been designed, optimised and initially tested at the Surgery Branch of the NCI (Kochenderfer et al. 2009, 2010). The scFv fragment was derived from the variable region of the anti-CD19 monoclonal antibody

FMC63 which is murine in origin. (Nicholson et al. 1997). The remainder of the CAR sequences, namely the hinge and transmembrane domains, CD3-zeta and CD28 signaling domains, are all of human origin, having been cloned from human T cells. The signalling domain of the CD3-zeta chain is of human origin and is essential for mediating T cell activation. The cytoplasmic domain of the CD28 costimulatory molecule is also included, since murine models and clinical studies have demonstrated the importance of CD28-mediated costimulation for optimal survival, persistence and anti-tumour activity of anti-CD19 CAR T cells (Kowolik et al. 2006). The CD3-zeta chain and CD28 fragments were cloned from human T cells into a contiguous chimeric single chain construct, and inserted in the MSGV1 plasmid.

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

Please refer to 6.a. (Composition of the insert) and 6.b. (Source of each constituent part of the insert).

- As per 4.e. (Constituent fragments of the vector) the retroviral integrase mediates the insertion of the retro-transcribed viral genome into the host genome via its interaction with the two LTRs, resulting in the integration of both LTRs along with all the nucleotide sequences found in between them, including the CAR. One of the LTRs serves as the promoter once the DNA is fully incorporated in the host genome, driving the expression of the CAR.
- Target Binding Domain: At one end of the CAR is a target binding domain of an antibody that is specific for the target antigen CD19 present on the surface of normal and malignant B cells. This domain extends out of the engineered T cell into the extracellular space, where it can recognise target antigens. The target binding domain consists of a single-chain variable fragment, or scFv, derived from an antibody comprising variable domains of heavy and light chains joined by a short linker. This allows the expression of the CAR as a single-chain protein.
- Transmembrane Domain and Hinge: This middle portion of the CAR links the scFv target binding domain to the activating elements inside the cell. This transmembrane domain “anchors” the CAR in the cell’s membrane. In addition, the transmembrane domain may also interact with other transmembrane proteins that enhance CAR function. In the extracellular region of the CAR, directly adjacent to the transmembrane domain, lies a “hinge” domain. This region of the CAR provides structural flexibility to facilitate optimal binding of the CAR’s scFv target binding domain with the target antigen on the cancer cell’s surface.
- Activating Domains: Located within the T cell’s interior are two regions of the CAR responsible for activating the T cell upon binding to the target cell. The CD3-zeta element delivers essential primary signal within the T cell, and the CD28 element delivers an additional, co-stimulatory signal that promotes T cell survival, persistence and anti-tumor activity (Kowolik et al. 2006). Together, these signals trigger T cell activation, resulting in CAR T cell proliferation and direct killing of CD19-expressing normal and malignant cells. In addition, T cell activation stimulates the local secretion of cytokines and other molecules that can recruit and activate additional anti-tumour immune cells.

(d) Location of the insert in the host organism

on a free plasmid	(.)
integrated in the chromosome	(X)

Integration of the insert takes place preferentially nearby transcriptional start sites (Aiuti et al. 2007).

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	(X)
	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)	Orthoretrovirinae; (subfamily Oncovirinae)
(ii)	family name for plants	…
(iii)	genus	Gammaretrovirus
(iv)	species	Murine stem cell virus
(v)	subspecies	Oncovirinae type C (subfamily)
(vi)	strain	…
(vii)	cultivar/breeding line	…
(viii)	pathovar	…
(ix)	common name	Gammaretrovirus

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 (.) No (X)

If yes, specify …

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

Yes (-) No (X) Not known (.)

Not applicable.

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 (.)

(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 (.)

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

Yes (.) No (X) Not known (.)

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

Yes (.) No (X) Not known (.)

2. Genetic stability of the genetically modified organism

The CAR is introduced in the T cells via retroviral vector gene transfer. After integration, the gene modified autologous T cells are genetically stable and form an integral part of the 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	N/A

(b) give the relevant information specified under Annex III A, point II(A)(11)(d) and II(C)(2)(i)

The replication-deficient retroviral vector genome is integrated as provirus in the T cell genome. No new viral particles can be assembled in the final host cell due to the absence in this proviral form of all the accessory proteins that confers infectivity and replicative potential to the retrovirus. In addition, the transgene inserted in the retroviral vector do not code for pathogenicity factors, cytokine-coding sequences, oncogenes, antibiotic resistance genes, or other hazardous inserts.

4. Description of identification and detection methods

(a) Techniques used to detect the GMO in the environment

CAR expression on transduced T cells can be detected using flow cytometry.

(b) Techniques used to identify the GMO

The GMO can be identified using flow cytometry. Integrated copies of the retroviral vector can be identified in T cells by qPCR.

F. Information relating to the release

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

As most advanced cancers eventually become refractory to conventional therapies, new treatment modalities are needed. Immunotherapy, which is based on the enhancement of an immune response against the tumour, is a promising approach to treating many cancer types. T cells play an important role in destroying diseased cells throughout the body. Studies with immune checkpoint inhibitors and tumour infiltrating lymphocytes have demonstrated the potential of T cells to treat cancer. T cells need to possess the appropriate specificity for the tumour, be present in sufficient numbers, and have the ability to overcome any local immunosuppressive factors to be effective. CAR-engineered T cells are a promising approach for cancer therapy (Kershaw et al. 2013).

Engineered Autologous Cell Therapy (eACT™) is a process by which a patient's own T cells are collected and subsequently genetically altered to recognise and target antigens expressed on the cell surface of specific malignancies (Kochenderfer et al. 2015). The ability to genetically engineer human T cells and use them to mediate cancer regression in patients has been demonstrated in a number of studies and has opened possibilities for the treatment of patients with a wide variety of cancer types including B cell malignancies expressing the CD19 antigen.

Early results from ongoing clinical trials in the USA have shown the potential for anti-tumour efficacy (Locke et al., 2015).

Treatment with KTE-C19 is not expected to have any effects on the environment, at large, neither negative nor positive.

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):

Apheresis will take place at Princess Maxima Center for Pediatric Oncology.

Each patient's respective leukapheresed product will be received by PharmaCell B.V., located at Maastricht, Netherlands, where it will be processed for lymphocytes enrichment, frozen and shipped to Kite Pharma, Inc. in the USA.

Modification of the patients' T cells using the PG13-CD19-H3 Vector encoding the anti-CD19 CAR gene (which constitutes a GMO) will take place at Kite Pharma, Inc. located at 1545 17th Street, Santa Monica, CA, 90404 USA.

The manufactured and purified autologous KTE-C19 T cell product, is shipped back to PharmaCell B.V., which upon release by a qualified person, acts as the site release across the countries in Europe. (In addition to the Netherlands, this may include Germany and France.)

The apheresis, infusion of KTE-C19 and subsequent follow-up will occur at the Princess Maxima Center for Pediatric Oncology for the pediatric ALL study. The lead investigator and the site address is as follows:

Pediatric ALL
Prof. Dr. R. Pieters
Princess Maxima Center for Pediatric Oncology
PO Box 113
3720 AC Bilthoven.

(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

Not applicable.

(c) Proximity to internationally recognised biotopes or protected areas (including drinking water reservoirs), which could be affected:

No environmental sites outside the hospital room will be affected. Containment measures during administration of KTE-C19 to the patients will exclude release of KTE-C19 into the environment. Personal protective equipment will be used to avoid exposure to KTE-C19 of the medical personnel involved in the administration of the product.

(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:

KTE-C19 is a single infusion treatment. The KTE-C19 drug product is formulated to provide a target dose of 2.0 x 10^6 CAR-positive T cells/kg (±20%) subject body weight

(b) Duration of the operation:

The complete administration procedure including preparation of the infusion system is expected to take less than 24 hours.

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

Kite Pharma is supplying an Investigational Product Manual which includes instructions for safe use, handling and disposal of KTE-C19 and materials.

All involved personnel on the site will be trained in best practices to be applied during administration and disposal of any biological product.

Disposal of waste will be according to the GMO guidelines and UN 3291 specific hospital waste.

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

Hospital treatment rooms have to fulfil hygiene conditions required for the treatment of immune-compromised patients. The investigational medicinal product, KTE-C19, is stored in vapour phase of liquid nitrogen at ≤-150°C until administration.

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.

None available.

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)	
(ii)	family name for plants	Human
(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 purpose of administering KTE-C19 final product is for the treatment of B-cell malignancies.

Targeting CD19 by anti-CD19 CAR expressing T cells has been shown to be effective in eliminating advanced B-cell malignancies and has the potential for a clinical benefit in patients otherwise beyond treatment.

3. Any other potentially significant interactions with other organisms in the environment
None expected.

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
None, except the dedicated patients who receive the autologous KTE-C19 product. Exposure requires direct infusion of KTE-C19. Immune-supressed individuals other than the patients will not participate in the administration of KTE-C19. Persons with a functional immune system would quickly eliminate KTE-C19 upon accidental injection. Simple contact exposure to blood from treated patients will not result in transmission of KTE-C19 as KTE-C19 is quickly inactivated under environmental conditions.

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: Highly unlikely.
None.

(b) from other organisms to the GMO: Highly unlikely.
None.

(c) likely consequences of gene transfer:
Not applicable.

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.):
No simulations other than early clinical trials as described above have been carried out.

9. **Possible environmentally significant interactions with biogeochemical processes (if different from the recipient or parental organism**)
None.

H. Information relating to monitoring

1. **Methods for monitoring the GMOs**
The presence, expansion, persistence, and immunophenotype of transduced anti-CD19 CAR T cells will be monitored in the blood of treated patients primarily by PCR analysis, complemented by flow cytometry. Expansion and persistence in peripheral blood will also be monitored by a CD19 CAR-specific quantitative polymerase chain reaction assay (qPCR). Blood will be collected according to Investigational product manual (IMP handling manual) and risk management plan.

Since KTE-C19 comprises retroviral vector transduced T cells, the presence of replication-competent-retrovirus (RCR) in the blood of treated subjects will be monitored. It is considered that the risk of RCR is low. It is included in the schedule of assessments in the clinical protocol that blood be drawn and evaluated at month 3, 6 and 12; then collected yearly for up to 15 years if measured positive at month 3, 6 or 12.

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)
Not applicable.

5. Duration of the monitoring
Blood samples will be taken at several time points after infusion (see H1).

6. **Frequency of the monitoring**
See H1.

I. Information on post-release and waste treatment

1. **Post-release treatment of the site**
All working surfaces that came into contact with the GMO will be disinfected using a 70% ethanol solution. The hospital room will be cleaned using the institutional standards for hospital room cleaning

2. **Post-release treatment of the GMO**s
None.

3. (a) **Type and amount of waste generated**

Empty bags and the used delivery system components (e.g., guide tube, cannula, injection needles and syringes), gauzes, personal protective equipment (e.g. gloves etc) and components used for collecting body fluids samples after administration.

(b) **Treatment of waste**

Sharps such as needles will be disposed of in adequate sharp containers and incinerated. Disposables such as syringes, tubing, catheters and surgery waste (gloves, compresses) will be treated as and disposed of as GMO waste. All the surgical materials (surgery tools, linens) will be collected and autoclaved before washing or will be treated as and disposed of as GMO waste. All non-disposable surgical equipment will be cleaned using a chemical disinfectant with proven virucidal activity (e.g. hypochlorite solution. 70% ethanol) and subsequently treated according to standard practice of the institution.

J. Information on emergency response plans

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

There is no risk of environmental health hazard. KTE-C19 for intravenous infusion will be prepared for administration. In case of spillage, the affected area, lineated with absorbing material, will be decontaminated using appropriate disinfectants. A spill kit will be available at all times during the administration procedure. Details are given in the Investigational Product Manual, describing the handling of the IMP, storage, and the administration procedures that will be handed over to the sites during the site initiation visit (prior to starting the study).

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

As per the GMO guidelines and the local hospital processes.

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

Not applicable since exposure of plants or animals is not expected.

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

Not applicable other than emergency response in case of accidental injection of medical personnel, which is disinfection of injection site and follow up in case of symptoms related to immune reaction against KTE-C19.