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

A. General information

1. Details of notification
   (a) Member State of notification                        Sweden
   (b) Notification number                                B/../../…. (Duty of MPA)
   (c) Date of acknowledgement of notification            ../../… (Duty of MPA)
   (d) Title of the project
       Assessment of the Feasibility and Efficacy of Administering T-cells Expressing an anti-CD19 Chimeric Antigen Receptor to Children with Chemotherapy Resistant or Refractory CD19+ Leukemia.
   (e) Proposed period of release                         From May/2017/ until December/2021

2. Notifier

   Name of institution or company:                        Karolinska Institutet

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.
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 (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 KI CD19 CAR Pediatric transduced autologous T cells are limited to patient administration in hospital settings. According to the environmental risk assessment KI CD19 CAR Pediatric will not reach the environment at large. The overall risk of KI CD19 CAR 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), following questions not applicable to humans
      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)

   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 blood leukapheresis. 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.

   The source material is controlled for viral adventitious agents as per country specific guidances. Patients will at least be tested for HIV, HBV and HCV prior to blood donation and excluded from the clinical study if tested positive. 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:
       …
   (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 (fungi)
   (vi) eggs
   (vii) pupae
   (viii) larvae
   (ix) other, specify

   (b) **relevant factors affecting survivability:**
   The survival of human blood cells requires a complex combination of proper and special media, temperature and CO$_2$. The environmental conditions outside the host’s body are substantially different and are not appropriate for the cells’ survival (temperature, pH, UV, and a change in the biophysical and biochemical conditions).

10. (a) **Ways of dissemination**
   Human blood 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 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)**

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**
   KI CD19 CAR Pediatric is a novel, investigational, adoptive cancer immunotherapy whereby autologous T cells are genetically modified to express a transmembrane chimeric antigen receptor (CAR) to target CD19 on the cell surface of malignant B cells. The CD19 CAR T cells generated are activated following engagement with the CD19+ target which results in the elimination of malignant cells expressing CD19.

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 …

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 KI CD19 CAR Pediatric 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 signalling 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?**
**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 …
   - (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 (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 (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 ( )

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
   The CAR is introduced in the T cells via retroviral vector gene transfer and 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 …

(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**

   CD19 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)**

   B-cell malignancies consist of a heterogeneous group of leukemias and lymphomas and despite improvements in treatment strategies, many patients, both children and adults, still succumb to the diseases. Leukemia is the most common pediatric malignancy accounting for 30% of all pediatric cancers. Improvements in the treatment of pediatric acute lymphoblastic leukemia (ALL) have achieved cure rates of over 80%, however, leukemic relapse remains the most common cause of pediatric cancer death (Mateos et al., 2013). These children are considered for allogeneic hematopoietic stem cell transplantation (HSCT) based on the length of the first remission and the size of the minimal residual disease. Allogeneic HSCT is today curative for many adult and pediatric patients with therapy-resistant leukemia and lymphoma. The curative effect of HSCT results from both the radiation and/or chemotherapy in the conditioning regimen and a graft-versus leukemia effect of the new donor immune system (Rindgen et al., 2009). Over the last two decades, the number of HSCT performed worldwide has increased to over 20 thousand cases annually with a continuing upwards trend. Even if the overall survival has increased during the same period, there is still a significant risk of relapse. For pediatric ALL patients that relapse prior to or after HSCT, palliative care is the only remaining option in a substantial number of patients.

To address the problem of limited success with today’s available treatments for children with refractory ALL, novel cellular therapies using genetically modified, tumor-specific T-cells have gained significant attention.

The engineered autologous cell therapy 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).
The treatment with KI CD19 CAR Pediatric 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):**

   The apheresis will take place at the Karolinska University Hospital, in the Department of Transfusion Medicine, Huddinge, Sweden. Each patient’s respective leukapheresed product will be received by the Stem Cell Laboratory at the Karolinska University Hospital, where the CD19 CAR T cells will be prepared in the Stem Cell Laboratory GMP Facility. After production of CD19 CAR T cells, the cells will be transported to the patient care unit at the Karolinska University Hospital Astrid Lindgren Children’s Hospital (Dept. of Pediatric, Solna, Sweden) by a staff member from the Dept. of Transfusion Medicine or by a research nurse/physician.

   (b) **Size of the site (m²):** N/A (The administration site is a hospital room.)

   (i) actual release site (m²): … m²

   (ii) wider release site (m²): … m²

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

   No environmental sites outside the site of production and hospital room will be affected. Personal protective equipment will be used to avoid exposure to KI CD19 CAR of the personnel involved in the production and administration of the product. Containment measures during administration of KI CD19 CAR to the patients will exclude release of KI CD19 CAR into the environment.

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

   N/A

4. **Method and amount of release**

   (a) **Quantities of GMOs to be released:**

   KI CD19 CAR is a single infusion treatment. The KI CD19 CAR drug product is formulated to provide a target dose of 1-2 x 10⁶ CAR-positive T cells/kg 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**

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.

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) Human
   (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 purpose of administering KI CD19 CAR 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 KI CD19 CAR product. Exposure requires direct infusion of KI CD19 CAR. Immune-suppressed individuals other than the patients will not participate in the administration of KI CD19 CAR. Persons with a functional immune system would quickly eliminate KI CD19 CAR upon accidental injection.
Simple contact exposure to blood from treated patients will not result in transmission of KI CD19 CAR as KI CD19 CAR 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:  
       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.

II. **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 flow cytometry analysis, complemented by PCR analysis. 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 the KI CD19 CAR Pediatric Protocol. Since KI CD19 CAR 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 day 28 and 90, month 6, 12, and 24; then collected yearly for up to 10 years.

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 (m²)**
   Not applicable.

5. **Duration of the monitoring**
   Blood samples will be taken at several time points after infusion. See Section H1

6. **Frequency of the monitoring**
   See Section 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 GMOs**
   None.

3. **(a) Type and amount of waste generated**
   Empty tubes, plates, 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.

3. **(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 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 spread**
   There is no risk of environmental health hazard. KI CD19 CAR 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.

2. **Methods for removal of the GMO(s) of the areas potentially affected**
   Decontamination with disinfectants.
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 KI CD19 CAR.