SUMMARY NOTIFICATION INFORMATION FORMAT FOR THE RELEASE OG GENETICALLY MODIFIED ORGANISMS OTHER THAN HIGHER PLANTS IN ACCORDANCE WITH ARTICLE 11 OF DIRECTIVE 2001/18/CE

ARI0002h

Clinical Trial CARTBCMA-HCB-01

(EudraCT No.: 2019-001472-11)

Version 1.0

18th June 2019

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: Spain
(b) Notification number: **B/ES/19/10**
(c) Date of acknowledgement of notification: 19/06/2019
(d) Title of the project: Pilot study of the infusion of differentiated autologous T-cells from peripheral blood, expanded and transduced with a lentivirus to express a chimeric antigen receptor with anti-BCMA (TNFRSF17) specificity humanized conjugated with the co-stimulatory region 4-1BB and signal-transduction CD3z (ARI0002h) in patients with relapsed/refractory multiple myeloma with previous treatment with proteasome inhibitor, immunomodulatory drug and anti-CD38 monoclonal antibody.
(e) Proposed period of release: September 2019-September 2023

2. Notifier

Name of institution or company: Fundació Clinic per a la Recerca Biomèdica

3. GMO characterisation

(a) Indicate whether the GMO is a:

viroid		(.)	
RNA virus		(.)	
DNA virus		(.)	
bacterium		(.)	
fungus		(.)	
animal			
-	mammals	(x): autologous T cells genetically modified	
-	insect	(.)	
-	fish	(.)	
-	other animal	(.)	specify phylum, class

other, specify (kingdom, phylum and class)

(b) Identity of the GMO (genus and species): T lymphocytes form multiple myeloma patients (*Homo sapiens sapiens*) transduced with the self-inactivating lentiviral vector pCCL to express the chimeric synthetic receptor against the BCMA antigen, which has been humanized, with 4-1BB as co-stimulatory domain and CD3ζ as signaling domain.

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

T lymphocyte transduction will be performed with lentiviral particles which contain the lentiviral vector pCCL-BCMA-4-1BB-CD3ζ without self-replicative capacity.
The lentiviral pCCL-BCMA-4-1BB-CD3ζ vector production will be performed through a third generation system, where the viral genes have been eliminated of the transfer vector and its 3' and 5' UTR regions have been modified to make it self-inactivating (viral LTRs inactive).

The DNA of the transfer vector, and the accessory plasmids for the lentiviral production will be produced at the Clean Rooms of the University of Barcelona, that will be responsible for their storage, amplification, and quality control for each new production. With this quality control plasmid sequencing will be performed to confirm their genetic stability during their amplification. Moreover, all quality controls related to the viral supernatant produced, such as absence of replication-competent lentiviruses (RCLs) in the productions, will be performed in the Clean Room.

Once the lentiviral vector transduction has been performed, the BCMA-4-1BB-CD3ζ sequence is stably integrated in the genome of T lymphocytes. The presence of this sequence and its stability will be confirmed by quantitative PCR (qPCR) in the T lymphocytes.

The chimeric receptor BCMA-4-1BB-CD3z expression is mediated through the internal promoter in T lymphocytes. T lymphocytes are totally differentiated and terminal cells with a half-life of years. However, in most clinical trials, their persistence is limited by an immunological reaction and by exhaustion of the T lymphocytes. In similar studies using an GMO to recognize BCMA, such as the one here, the GMO persistence has not been detected beyond 12 months. All the patients that will be included at this study will be monitored for 5 years, where the GMO persistence among other parameters will be evaluated.

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

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

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

The possible environmental impact is the GMO (T lymphocytes transduced with a chimeric receptor to express *BCMA-4-1BB-CD3z*) release. The GMO is not harmful for the environment, and is not able to survive without culture conditions ((37ºC, 5% CO_2, culture media enriched with human serum; or the patient's human body). Despite being a GMO, the modification does not confer any survival capacity outside culture conditions. It is impossible that either a cell or our GMO survives after having applied the confinement measurements, as they can't survive neither to the antiseptic products that are used in the facilities, nor in the environment outside culture conditions. There are no ecosystems where the GMO could disseminate, and in the patients there is no genetic modification of the germinal cells that could transmit it.
There are no interactions of the GMO with other external organisms because the patients who receive the GMO cannot have HIV. Only in the case that HIV infection occurs in the patients, recombinations could occur between the residual sequences of the lentiviral vector and the sequences of the wild virus. Therefore, HIV positive patients cannot benefit from this therapy.

The confinement measurements to avoid its dissemination to the environment consist on signaling the working area, proper clothes of the workers (disposable pajamas, gloves, hat and cover shoes), isolation of the working area in the case of leakage of the product (despite it is a hermetic system), and proper disinfection. These measurements are detailed in the technical report of facilities "Memoria técnica" of each production center (HCB-CDB and CUN).

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

1. Recipient or parental organism characterisation:

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

(select one only)

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

(specify phylum, class)

other, specify

2. Name
   (i) order and/or higher taxon (for animals): Phylum: Chordata; Class: Mammals; Order Primates: Family: Hominidae, Subfamily: Hominidae
   (ii) genus: *Homo*
   (iii) species: *Homo sapiens*
   (iv) subspecies: *Homo sapiens sapiens*
   (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 (.)

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

   Atlantic ..
   Mediteranean ..
   Boreal ..
   Alpine ..
   Continental ..
   Macaronesian ..

   (ii) No (X)
   (iii) Not known (.)

   (c) Is it frequently used in the country where the notification is made?
   Yes (.) No (X)

   (d) Is it frequently kept in the country where the notification is made?
   Yes (.) No (X)

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:

The natural habitat of the GMO (transduced T lymphocytes) is the hematopoietic environment of the patient that will receive.

5. (a) Detection techniques

The detection of the genetic modification is performed by molecular biology techniques: quantitative PCR (qPCR) and quantitative retro-transcription-PCR (qRT-PCR) and Western Blot. Moreover, flow cytometry will be used to detect transduced T lymphocytes with monoclonal antibodies against CD3, CD4, CD8 and the chimeric antigen receptor.

(b) Identification techniques

The same detection techniques are used here : qPCR, qRT-PCR, Western Blot and Flow cytometry.

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

Yes (X) No (.)

If yes, specify

According to RD 664/1997 of May 12th, about protection of workers against risks related to biological agents exposure during work time, the transduced T lymphocytes classified as Group II as they have the lentiviral vector integrated. However, there is not any possibility of producing competent replicative lentivirus. Both in the time of T lymphocyte transduction, during administration of the GMO to the patients and samples obtained from them a level II contention is required for its handling.

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

T lymphocytes that will undergo genetic modification will be from multiple myeloma patients and after the modification, they will be administrated back into patients as an autologous product. Patients and T lymphocytes will be tested to confirm the presence of any virus: HIV (*Human Immunodeficiency Virus*), HCV (*Hepatitis C Virus*) and HBV (*Hepatitis B Virus*). Positive patients for any virus will be excluded from the Clinical Trial.

8. Information concerning reproduction

(a) Generation time in natural ecosystems:

Transduced T lymphocytes cannot reproduce in any natural ecosystem, just in specific culture conditions or once they have been administrated into the patient. Transduced cells with the integrated vector will divide only when they recognize the tumor cell to eliminate them. In no case, germinal cells of the patient will be affected.

(b) Generation time in the ecosystem where the release will take place:
Not applicable

(c) Way of reproduction: Sexual .. Asexual ..
Not applicable

Factors affecting reproduction: Not applicable

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 capacity of the transduced T lymphocytes depends entirely on the hematopoietic system of the patient. Due to their differentiated and terminal state, they have a half-life of years. However, most clinical studies similar to the one mentioned here, have shown that the persistence of the GMO is limited due to an immunological rejection and to an exhaustion of the GMO, and this persistence has been detected no longer than 12 months.

10. (a) Ways of dissemination

Transduced T lymphocytes will engraft in the patient to attack the tumor multiple myeloma cells. There is no possibility that they can be transmitted between persons, and because they cannot survive in the environment, there is not any way of dissemination.

(b) Factors affecting dissemination

In the case that transduced T cells are administrated in a different individual than the patient, it is expected that the immune system of the individual will eliminate these cells in a few days.

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)

There are not any previous genetic modifications in the recipient organism that have been notified.

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 GMO are T lymphocytes modified to express a chimeric antigen receptor to recognize specifically BCMA in the tumor cells of multiple myeloma patients. It is expected that GMO after recognizing its target will proliferate creating an attack clone against the tumor, thus eliminating the myeloma disease of the patient. This strategy will increase the survival of the patient with a higher quality of life in comparison to conventional treatments.

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

The lentiviral vector pCCL-BCMA-4-1BB-CD3z is a vector based on the HIV-1 lentivirus. Lentiviral vectors used are of third generation (4 plasmids are required for its production, which increases the safety), improved (they contain sequences to improve their expression: cPPT, Central

Polypurin Tract and wPRE*, mutated posttranscriptional regulatory element of woodchuck hepatitis virus) and self-inactivating (deletions in the LTR regions make them inactive once they are integrated). These vectors are based on the HIV-I lentivirus where the accessory and some regulatory genes have been deleted. They present a different envelope to the wild virus, the VSV-G (Vesicular *stomatitis* virus G). These virus are defective in replication, it is not known the formation of neither wild virus nor Replication competent Lentivirus (RCLs).
These vectors are produced through co-transfection of 4 plasmids into 293T cells: Transfer vector (pCCL-cPPT-BCMA-4-1BB-CD3ζ–wPRE*), packaging vectors (rev and gag-pol) and envelope vector VSV (Figure 1).

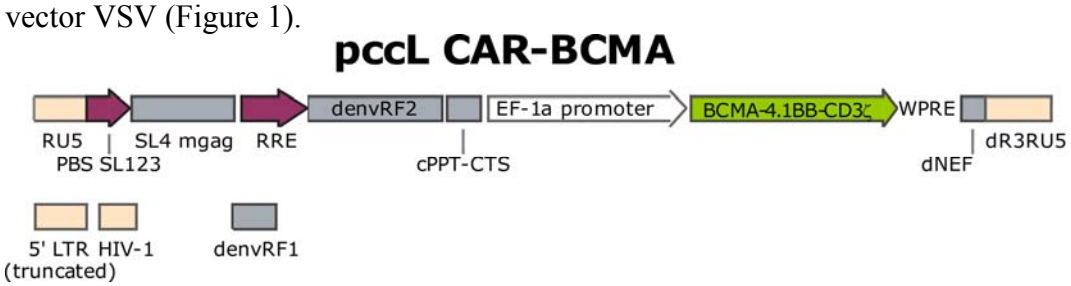

Figura 1: Scheme of the integrated gene transfer vector pCCL-cPPT-BCMA-4-1BB-CD3ζ.wPRE

(c) Host range of the vector

The lentiviral vector has been pseudo –typed with the VSV-G envelope, which enables to transduce a high number of cell types from different species. However, the vector handling will be performed in a laboratory with Level II confinement, and moreover, because the cells will be transduced *ex vivo*, will not occur any transduction of other cells from other organisms.

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

Yes (X) No (.)

antibiotic resistance (.)
other, specify

Tansduced T cells can be identified by flow cytometry with a Goat-Anti-IgGAM human antibody (ref: 504031, Inova diagnostics, Inc) conjugated to FITC. Moreover, it can be detected by Western Blot using an anti-CD3ζ antibody and by qPCR.

Indication of which antibiotic resistance gene is inserted: There is no integration of any gene that confer antibiotic resistance.

(e) Constituent fragments of the vector

The expression plasmid was kindly provided by Dr. Luigi Naldini ("A Third-Generation Lentivirus Vector with a Conditional Packaging System". J. Virol. 1998 Nov; 72(11): 8463–8471). It has a size of 9.402 bp in total including the gene of interest (BCMA-4-1BB-CD3z which has 1.466 bp). The important sequences are:

- *pUC19 replication origin:* a sequence from the pUC19 clininc plasmid where the plasmid replication is initiated.
- *F1 replication origin*: a particular sequence of the phage f1 where the plasmid replication is initiated.

- *Ampicillin resistance Gene (Amp^R)*: to select the positive clones that have acquired the plasmid. There ares no risks associated to Ampicillin in the final product because there are numerous steps and time between the use of Ampicillin and the administration.
- *Rev response element (RRE)*: a 350 nucleotides RNA fragment highly structured that binds to the accessory protein of the HIV-1 Rev protein to facilitate the mRNA export from the nucleus to the cytoplasm for transduction and envelope of virions.
- *EF-1α promoter*: for CARTBCMA transcription.
- *CARTBCMAh*: the gene of interest to introduce in the target cells.
- *Woodchuck Hepatitis Virus (WHP) Posttranscriptional Regulatory Element (WPRE)*: DNA sequence that when it is transcribed, it creates a ternary structure that improves the expression, and it is highly used in molecular biology to enhance the expression of genes through viral vectors (Zufferey, Donello et al. 1999).

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

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

The inserted fragment has the following regions that will be integrated, and which are between the two LTR (Figure 1 in section 4.B):

- 5'LTR truncated: Long Terminal Repeat (sequence derived from the lentivirus).
- HIV-1Ψ: packaging signal.
- RRE: *Rev Response Element*
- cPPT-CTS: C*entral Polypurine Tract:* it regulates the expression of the transgene.
- EF1α: internal promoter for the gene of interest.
- antiBCMA: cDNA coding for the chimeric protein anti-BCMA-4-1BB-CD3ζ
- Wpre*: mutated *Woodchuck pre-regulatory element*, or regulatory element of the *Woodchuck Hepatitis Virus*. It stabilizes and improved the expression of the transgene. It is mutated to improve the efficacy and safety of the sequence.
- 3'LTR (dR3RU5): Long Terminal Repeat (sequence derived from the lentivirus).

(b) Source of each constituent part of the insert

LTR: lentivirus
HIV-1Ψ: lentivirus.
RRE: lentivirus.
cPPT: lentivirus.
EF1α: human.
antiBCMA: human
Wpre, mutado: *Woodchuck Hepatitis Virus*.

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

3' LTR: *Long Terminal Repeat* (sequence derived from the lentivirus). The LTRs are made by the fusion of the regions U3-R-U5 which is produced after retro-transcription of the vector and before its integration. The wild LTR from the HIV-1 was mutated eliminating the U3, thus, the LTR is not able to stimulate gene expression neither in the plasmid nor in the integrated form after the retro-transcription. To synthetize the messenger RNA the promoter/enhancer CMV sequence (CMV IE-I prom) was added in 3' of the RU5 sequence. Thus, this promotor directs the expression of the infective RNA that will be packed in the infective capsids. This sequence will not be part of the virus, therefore, after its integration it will not be able to produce new infective particles. It is a self-inactivating vector (SIN).
HIV-1Ψ: packaging signal. Sequence with a third structure that forms 4 loops (SL1, SL2, SL3, SL4) which are required for the correct viral RNA incorporation in the capside.
RRE: *Rev Response Element.* Binds to Rev protein facilitating the mRNA export from the nucleus to the cytoplasm for transduction and envelope of virions.
cPPT: *Central Polypurine Tract*, tracto central de la polipurina. It regulates the expression of the transgene.
EF1α: internal promoter for the gene of interest. It is derived from the human gene *EEF1A1* which codes for the subunit alpha of the eucariotic elongation factor 1. This promoter has a high activity achieving a lasting expression of the transgene *in vivo*.
antiBCMA: gene that will be transduced to express a chimeric antigen receptor composed of an anti-BCMA that will recognize specifically BCMA expression in plasma cells of multiple myeloma, a co-stimulatory domain (4-1BB) that will make T lymphocytes proliferate after recognizing their target antigen (BCMA), and a signaling domain CD3ζ.
Wpre*: mutated *Woodchuck Hepatitis virus* (WHV) *post-transcriptional regulatory element*) is a sequence of the *Woodchuck Hepatitis virus* which has the capacity to stabilize mRNA increasing the amount of the generated protein. The wild version codes for the protein X related to hepatocarcinoma. The mutated version has eliminated all critical sites for expression of this protein.
5' LTR: The wild LTR of the HIV-1 was mutated deleting 18 bases of the promoter/enhancer region U3 (Δ18U3). Thus, the mutated LTR is not able to stimulate gene expression neither in the plasmid nor in the integrated form after retro-transcription. Therefore, after its integration it will not be able to form new viral particles. It is a *self -inactivating vector* (SIN).

(c) Location of the insert in the host organism

- on a free plasmid (.)
- integrated in the chromosome (X)
- other, specify

(d) 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)	…
(ii)	family name for plants	…
(iii)	genus	Hominidae/Retroviridae
(iv)	species	Homo/Lentivirus
(v)	subspecies	…
(vi)	strain	HIV-1
(vii)	cultivar/breeding line	…
(viii)	pathovar	…
(ix)	common name	Human and HIV-1 lentivirus

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 (X) No (.)

If yes, specify

According to RD 664/1997, May 12th, about workers protection against risks related to exposure to biological agents during work hours, HIV is classified as a biological agent type 3. However, part of its genome has been modified to eliminate the viral sequences required for its propagation, eliminating its infective capacity. Therefore its handling requires a confinement level 2.

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 genetic stability is very high. Once the vector is integrated in the genome of the T lymphocyte, thanks to the internal promoter, the chimeric receptor anti-BCMA-4-1BB-CD3z will be expressed and will allow the recognition of the myeloma cells that express BCMA.

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)

T lymphocyte transduction will be performed with the lentiviral vector antiBCMA-4-1BB-CD3ζ-wpre* which has no self-replicative activity.

Production of the lentiviral vector antiBCMA-4-1BB-CD3ζ-wpre* will be performed with a third generation system, where viral vectors have been eliminated of the transfer vector and its 3'UTR region has been modified to convert it into a self-inactivating vector (inactive viral LTRs)

The DNA of the transfer vector and the accessory plasmids required for the lentiviral production are produced in the Clean Rooms of the University of Barcelona, that will be responsible for its storage, amplification and quality control of each production. Sequence of the plasmids will be performed under these quality control measurements to confirm their stability during their amplification. Moreover, in this Clean Room, different quality controls in the supernatant of the lentivirus production will be performed, including, absence of replicative competent lentivirus (RCLs) in the productions.

After transduction with the lentiviral vector, the BCMA-4-1BB-CD3ζ sequence is stably integrated in the T lymphocyte genome. The presence of this sequence and its stability will be confirmed through quantitative PCR (qPCR) in the T lymphocytes.

The chimeric receptor BCMA-4-1BB-CD3ζ is expressed thanks to the internal promoter in the T lymphocytes. The T lymphocytes are differentiated and terminal cells with a half-life of a few years. However, majority of clinical trials similar to this one, have observed a limited persistence of the GMO due to an immunological reaction and to exhaustion of the GMO. Similar clinical studies to this one with a GMO to recognize BCMA, such as our GMO, the persistence of the GMO has not been beyond 12 months. All patients included in this study will be followed during 5 years, and among others, the persistence of the GMO will be evaluated.

4. Description of identification and detection methods

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

The detection of the genetic modification will be performed with molecular biology techniques: quantitative PCR (qPCR) and quantitative retrotranscription PCR (qRT-PCR) and Western Blot. Moreover, Flow Cytometry will be used to detect transduced T lymphocytes, using monoclonal antibodies against CD3, CD4 and CD8 and the chimeric antigen receptor.

(b) Techniques used to identify the GMO

The same techniques used for the detection will be used for identification: qPCR, qRT-PCR, Western Blot and Flow Cytometry.

F. Information relating to the release

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

The GMO is not released to the environment; it is administrated into multiple myeloma patients to recognize tumor myeloma cells expressing BCMA to eliminate them. The GMO is composed of T lymphocytes from multiple myeloma patients which have been transduced with the lentiviral vector antiBCMA-4-1BB-CD3ζ-wpre*. The therapeutic vector will integrate in the cell genome to express the chimeric receptor constitutively that will recognize specifically BCMA and leading to a proliferation of the T lymphocytes. These modified T lymphocytes will be administrated back into the patients, and the therapeutic protein will be expressed in the T lymphocytes and in the clones that arise after T cell proliferation due to recognition of the target antigen. Therefore, T cells and their clones will continue eliminating specifically tumor multiple myeloma cells expressing BCMA, and myeloma progression will be avoided and the quality of life of patients will be significantly improved.
This protocol is not expected to have any effect on the environment.

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 release wil be produced in the context of a clinical trial performed at different Hospitals (Hospital Clinic of Barcelona, Clinic Universitary of Navarra, Hospital Virgen del Rocío of Seville, Hospital Universitary of Salamanca, Hopital Virgen de la Arrixaca of Murcia).

(b) Size of the site (m^2): Not applicable

(i) actual release site (m^2):

(ii) wider release site (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:

It is expected that 30 patients will receive 0.3-6x10^6 OMG/kg.

(b) Duration of the operation:

We expect to recruit the patients in 24 months. Patients will be followed up 36 months in the study. The sample storage stops at 24 months follow up visit. The perrsistence of the OMG will be checked from the administration to month 24 of follow-up.

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

It is not possible that the GMO spreads as the modification of the T lymphocytes will be performed ex vivo and these will not be able to survive unless they are infused back into the patient. There is no possibility of dissemination; therefore special methods to prevent the spread are not contemplated.

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.

There are not any data regarding previous releases with the same GMO.

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)	Primates
(ii)	family name for plants	Hominidae
(iii)	genus	*Homo*
(iv)	species	*Homo sapiens*
(v)	subspecies	*Homo sapiens sapiens*
(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 transduced T lymphocytes will go through the peripheral blood to home into the bone marrow to recognize and kill the tumor cells. After the encountering with tumor cells there will be a proliferative response of the T lymphocytes to originate specific T cell clones that will eliminate the tumor cells. The interaction of the vector with patient cells is not expected. Likewise, biodistribution studies have been performed to ensure that other cells than the T lymphocytes could be transduced.

3. Any other potentially significant interactions with other organisms in the environment.

It is not expected any other interaction with other foreign organisms since the patients receiving the GMO have to be free of HIV to eliminate any possibility of recombination between our lentiviral vector and wild HIV.

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

As it is a clinical trial conducted in the Hospital setting, there aren't any possibilities that the GMO spreads to other ecosystems.
The clinical trial will be performed at Hospital Clinic of Barcelona (C/ Villarroel 170, 08036, Barcelona, España), Clinic Universitary of Navarra (Av. de Pío XII, 36, 31008 Pamplona, Navarra), Hospital Virgen del Rocío of Seville (Av. Manuel Siurot, S/n, 41013, Sevilla), Hospital universitary of Salamanca (Paseo de San Vicente, 182, 37007 Salamanca) and Hospital Virgen de la Arrixaca of Murcia (Ctra. Madrid-Cartagena, s/n, 30120 El Palmar, Murcia).

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:

Only in the case that there is HIV infection in the patient could exist recombinations between residual sequences of the lentiviral vector with wild viral sequences. To avoid it, positive HIV patients are excluded from this therapy.

(b) from other organisms to the GMO:
There is no possibility

(d) likely consequences of gene transfer:

The only consequence that could occur and that would be very unlikely, is that lentiviral sequences could incorporate into the wild virus, but it would not have relevant biological consequences.

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.):

They do not exist.

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

Not applicable.

H. Information relating to monitoring

1. Methods for monitoring the GMOs

Patients will be followed during 5 years after the administration of transduced T lymphocytes to evaluate the safety of the treatment. To do that, peripheral blood and bone marrow samples will be taken from patients starting on day 7 after the administration to evaluate hematological parameters and to quantify the presence of GMO by flow cytometry and by PCR, which is a highly sensitive technique to amplify and detect the sequences of interest.
Moreover, a long-term follow-up of patients will be performed for 5 years to confirm the absence of secondary effects by performing routine clinical analysis.

2. Methods for monitoring ecosystem effects

Not applicable as there will be no impact on the ecosystem.

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

The follow-up of patients will be performed for at least 5 years

6. Frequency of the monitoring

After the administration of the GMO, peripheral blood and bone marrow samples will be taken on day 7, 30, and 100. Then, at 6, 12, 18 and 24 months. Afterwards, samples will be taken annually.

I. Information on post-release and waste treatment

1. Post-release treatment of the site

The sponsor will provide instructions about the management, precautions and safety measures to train the participant sites in the post-release and waste treatment related to specific GMO of the current clinical trial.
The patients will be treated in rooms of the Hematology service of the different hospital centers, where they will remain at least 48 hours after the administration of the transduced T lymphocytes (GMO).
The release of the final product (GMO) will be performed by administration to the patient in a Hospital institution. Therefore, the place will adapt to the standards established in this center for this type of interventions. The place where GMO is prepared will be decontaminated, before and after handling, with a solution based on a conventional disinfectant.
All the personnel will be informed that T lymphocytes transduced with a lentiviral vector (GMO) are considered a level II biosecurity product and will be trained to handle and storage them following the appropriate standards for this level of biosafety (product handling, equipment and materials used, correct waste disposal, etc.). Any waste generated during the handling of the GMO or that may have been in contact with the product must be deposited in special biosecurity containers and incinerated afterwards.
The personnel must wear protective clothing, according to the following:

- Gowns must be worn
- Gloves must be used for any procedure that involved direct contact with the skin.
- All the equipment and work surfaces should be clean with bleach.
- The needles and syringes used should be discarded in biosecurity containers.
- After removing gloves, staff should wash their hands

GMO administration to the patient:

- Patients will be infused according to the usual protocols of the different hospitals participating in the clinical trial.

Management of the patient who is discharged after treatment

- No specific rules are contemplated once the patient is discharged. An informative letter will be provided to the participants.

Management of the patient who presents complications after treatment:

- Specific measures different from the usual ones in the Hospital are not contemplated.

Procedures to be followed by staff and visitors:

- Personnel that are punctured with needles that have had contact with the GMO must follow the standard procedures for this type of accident. The safety department as well as those responsible for the study must be informed.
- Any staff member involved in the trial who feels unwell should inform the safety department as well as those responsible for the study.

2. Post-release treatment of the GMOs

There are no special measures to prevent the spread of GMOs outside the place of release as transduced T lymphocytes cannot spread outside the patient's body. Therefore, the measures are the usual for patients who have been infused hematopoietic progenitors.

3. (a) Type and amount of waste generated

The waste generated are Type II, types of waste will be:

- Waste generated during the preparation and handling of the final product (T lymphocytes transduced with the lentiviral vector).
- Waste derived from the administration to the patient of the GMO.

- Waste derived from the cleaning of work areas.

The volume of waste generated will be the usual in a procedure of this type and large volumes are not expected. Most of the waste is taken by a specialized external company that takes the biological waste containers which have been sealed. The company will inactivate the waste by means of autoclaving and incineration. The liquid waste, which will not be more than 1L, will be treated with disinfectants.

3. (b) Treatment of waste

The proposed treatment for the different types of waste will be adapted to current regulations. In accordance with the provisions of Royal Decree 83/1999, which regulates the production and management of bio-sanitary and cytotoxic waste and in accordance with local legislation in each of the regions participating in the study (Catalonia, Navarre, Castile- Leon , Andalusia and Murcia).
Wastes are classified in:

- Type I and II: The materials are inactivated (liquids by disinfectants and solids by autoclaving) and disposed of as established.
- Type III: The waste is managed by an external company, registered and authorized for that purpose, according to the provisions of the aforementioned Royal Decree.

In general, the external company collects solid waste (such as gowns, masks, etc) previously sealed in black containers, which then are deactivated by autoclaving, and then conventionally incinerated. Liquid waste and surfaces will be treated with a suitable disinfectant. All other waste (bandages, swabs, etc.) will be incinerated by a external company hired by the different hospital centers of this study, in the same way as usual clinical waste.
In the event of any incident or accident, the CIOMG (Consejo Interministerial de Organismos Modificados Genéticamente, of the Ministry of Agriculture, Fisheries and Food) and the CNB (Comisión Nacional de Bioseguridad) of the "Ministry for the Ecologic Transition" must be informed immediately.

J. Information on emergency response plans

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

As a precautionary measure, containment measures of level 2 (class II) will be carried out at the place of release. The treated patients will be kept in the hospital for at least 48 hours.

As it has been established by the sponsor, in case of accidental release, the following measures will be performed:
Isolate the spill area; absorb the spilled solution with disposable paper towels or other type of absorbent material. It will be treated with 5% bleach, 0.5% sodium hydroxide solution or a disinfectant solution. Other wastes will also be collected with an adequate use of the picker, the spilled material will be collected and all the cleaning materials used in the contaminated place will be placed in a resistant disposable plastic bag. When all contaminated materials are outside the room, the area will be rinsed with clean water using additional disposable towels.

Upon completion of the cleaning, all contaminated materials will be placed in an appropriate manner, properly labeled, and disposed of as Type II waste in specific containers. Finally, remove the gloves, and wash your hands carefully with soap and clean water.

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

In case of spilling the product:

- In areas where the product is handled, stored and transported, there should always be a disinfectant available, such as bleach.
- In case of a spill of the product, the personnel that will clean must follow the rules specified in the previous point.
- All surfaces that have been contaminated have to be cleaned and disinfected.

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

See the previous section

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

In the clinical trial, patients will be monitored for 36 months. According to GCP in advanced therapies a long term follow-up is needed to detect long term adverse reaction that may occur; this long term follow-up period will last 15 years.. Due to the reasons set forth above, and in reference to the risk assessment, the drafting of specific plans to protect the environment will not be considered necessary. It will proceed according to the procedures of the center in these cases.