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 : ES

(b) Notification number : B/ES/17/18

(c) Date of acknowledgement of notification : 22 September 2017

(d) Title of the project

Study bb2121-MM-001 titled "A Phase 2, Multicenter Study of the Efficacy and Safety of bb2121 in Subjects with Relapsed / Refractory Multiple Myeloma "

(e) Proposed period of release

01/12/2017 to 01/02/2020

2. Notifier

Name of institution or company:

The Sponsor of Study bb2121-MM-001 is Celgene Corporation. Bluebird bio Inc. with its wholly owned subsidiary bluebird bio France is collaborating with Celgene Corporation for the development of the product bb2121 and supplies the lentiviral vector for bb2121.

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	
other, specify (kingdom, phylum and class)				

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

Homo Sapiens

The GMO, referred to as bb2121, is defined as anti-BCMA CAR+ T cells, where human autologous T Cells are transduced with a recombinant lentivirus (Anti-BCMA02 CAR lentiviral vector).

Anti-BCMA02 CAR lentiviral vector: a recombinant HIV-1 RNA lentivirus manufactured with multiple plasmids designed to express all the packaging components to generate a modified recombinant lentiviral vector. Anti-BCMA02 CAR lentiviral vector packaged RNA transcript encodes for a chimeric antigen receptor (CAR) that recognizes the cell-surface marker B cell maturation antigen (BCMA).

Autologous T cells: obtained by apheresis from patients diagnosed with BCMA-expressing multiple myeloma.

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

The inserted genetic material is stably integrated into the genome of the patient's T cells, and is not capable of replication. The T cells are differentiated. Even if the insert were to be transferred, it encodes the chimeric antigen receptor and lacks HIV viral genes or any other pathogenic genes, thereby posing negligible risk to unintended organisms.

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): FR, DE, BE, IT

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

Yes (X) No ()

If yes:

- Member State of notification : FR
- Notification number: DUO#4004

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:

- Member State of notification : United Stated
- Notification number: NIH protocol # 1507-1443

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

No environmental impact is expected from the administration of bb2121 drug product to subjects in clinical trial bb2121-MM-001

The bb2121 drug product consists of autologous T cells transduced with the Anti-BCMA02 CAR lentiviral vector encoding the chimeric antigen receptor specific for B cell maturation antigen (BCMA), also known as human tumor necrosis factor (TNF) receptor superfamily member 17. bb2121 drug product supplied to the clinical site for infusion into the patient via intravenous route. Thus, an environmental impact is not expected as the release of the transduced autologous T cells is limited to patient administration in a hospital setting and will not reach the environment at large. Transduced cells are not viable in the environments outside of the patient. The Anti-BCMA02 CAR lentiviral vector is attenuated, replication incompetent and it degrades rapidly in the environment.

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

1. Recipient or parental organism characterisation:

The following information is provided for the patient as the parental organism.

(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

The following information is provided for the patient as the parental organism

(i) order and/or higher taxon (for animals)

Homo sapiens

(ii) genus

Not applicable

(iii) species

Not applicable

(iv) subspecies

Not applicable

(v) strain

Not applicable

(vi) pathovar (biotype, ecotype, race, etc.)

Not applicable

(vii) common name

Human

3. Geographical distribution of the organism

The following information is provided for the patient as the parental 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 points not applicable for human cells

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

Atlantic

Mediteranean

Boreal

Alpine

Continental

Macaronesian

(ii) No (.)

(iii) Not known (.)

(c) Is it frequently used in the country where the notification is made?

Yes (.) No () not applicable to human cells

(d) Is it frequently kept in the country where the notification is made?

Yes (.) No () not applicable to human cells

4. Natural habitat of the organism

The following information is provided for the patient as the parental 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 (.)

in association with animals ()

other, specify:

not applicable to human cells

(b) If the organism is an animal: natural habitat or usual agroecosystem:

Human

5. (a) Detection techniques

The following information is provided for the parental organism.

Quantitative PCR and common techniques of blood cell analysis (e.g. flow cytometry)

(b) Identification techniques

The following information is provided for the parental organism.

Quantitative PCR and common techniques of blood cell analysis (e.g. flow cytometry)

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

Yes (.) No (X)

The recipient organism is *Homo sapiens*

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 (.)

The recipient organism is *Homo sapiens.*

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 T cells isolated from the peripheral blood of multiple myeloma patients. The T cells cannot survive outside of the patient. The cells are not pathogenic and cannot persist or replicate in the environment or other organisms. Patients are tested for HIV during screening and excluded from the clinical trial if tested positive.

8. Information concerning reproduction

The following information is provided for the parental organism.

(a) Generation time in natural ecosystems:

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

(c) Way of reproduction: Sexual .. Asexual ()

(d) Factors affecting reproduction: not applicable for human cells

9. Survivability

The following information is provided for the parental organism.

(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: Human T cells require a combination of special media, temperature and CO2 for survival. Thus they will not survive outside the host with the substantially different (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 expected due to fast inactivation and lack of a natural entry route into the body. (b) Factors affecting dissemination

11. The immune system of people other than the donor will eliminate the blood cells. 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

The information provided in this section relates to the autologous T cells that are genetically modified by transduction with the Anti-BCMA02 CAR lentiviral vector

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 goal of the genetic modification is to add one or more copies of the gene for a chimeric antigen receptor specific to BCMA (CAR; the "therapeutic gene") to the autologous T cells by transduction. In vivo the transduced cells will express the therapeutic gene and become capable of recognising and responding to BCMA on the surface of B cells. BCMA is consistently expressed on plasma cells and myeloma cells from multiple myeloma patients. The presence of T cells capable of recognizing BCMA-expressing B cells is expected to result in reduction in the tumor load and improvements in the overall survival of treated patients as a result of T cell activation.

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

Anti-BCMA02 CAR lentiviral vector is used to transduce autologous T cells. This vector uses the murine leukemia virus-derived MND promoter to drive expression of the chimeric receptor, a multi-domain protein consisting of the extracellular antigen recognition domain (VL and VH), the CD8α hinge domain, a transmembrane domain (CD8 TM), and the intracellular CD137 co-stimulatory (4-1BB) and CD3zeta chain signaling domains.

The Anti-BCMA02 CAR lentiviral vector does not encode for any HIV proteins; the only HIV derived sequences in the transcript are the repeat regions that have been made self-inactivating by deleting promoter/enhancer sequences and attenuated regions of the proteins and element that aid in the production, packaging, or transfer of the transcript containing the therapeutic gene. Additional sequences are derived from human genes encoding components of the T cell receptor.

(c) Host range of the vector

Lentiviral vectors of this type are capable of transducing animal and insect cells. However, it is important to emphasize that the Anti-BCMA02 CAR lentiviral vector is not replication competent and does not encode any pathogenic genes

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

Yes (X) No (.)

antibiotic resistance (.)

other, specify : The therapeutic gene product (anti-BCMA T cell receptor) is identified by flow cytometry, and lentiviral vector back-bone sequences are identified and quantified by qPCR.

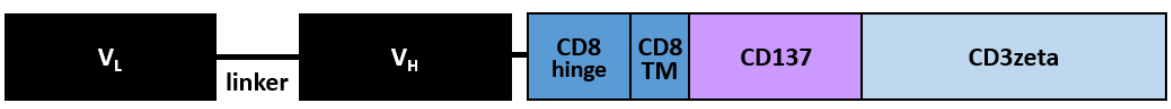
Indication of which antibiotic resistance gene is inserted : None. No antibiotic resistance genes are present in the Anti-BCMA02 CAR lentiviral vector.

(e) Constituent fragments of the vector

The therapeutic anti-BCMA02 CAR gene product is a chimeric receptor, a multi-domain protein consisting of the extracellular antigen recognition domain (V_L and V_H), the CD8α hinge domain, a transmembrane domain (CD8 TM), and the intracellular CD137 co-stimulatory (4-1BB) and CD3zeta chain signaling domains. It is composed of an anti-BCMA02 single chain variable fragment (scFv) operably linked to T cell signaling domains by a CD8α transmembrane and truncated hinge region. The scFv was constructed by connecting the heavy and light variable fragments from an anti-BCMA antibody clone fused with a short flexible linker sequence. The T cell signaling domains include endodomains from CD3ζ, a component of the T cell receptor complex, and CD137 (4-1BB). The anti-BCMA02 CAR gene is under the transcriptional control of the myeloproliferative sarcoma virus enhancer, negative control region deleted, dl587rev primer-binding site substituted (MND) promoter. An amino terminus CD8α signal peptide shuttles the anti-BCMA02 CAR to the surface of an engineered T cell. T cells engineered with the anti-BCMA02 CAR molecule gain recognition and cytolytic function to cells expressing BCMA including multiple myeloma and some lymphoma tumors.

A schematic of the anti-BCMA CAR is shown below.

Anti-BCMA Chimeric Antigen Receptor



Abbrev.: V_L, light chain variable domain; V_H, heavy chain variable domain; TM, transmembrane

(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

This is summarized under 6(c), below.

(b) Source of each constituent part of the insert

This is summarized under 6(c), below.

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

The following table provides the composition of the insert, the source of each key constituent part and its function. The insert also includes synthetic non-functional linker sequences to ensure functionality of the insert.

No functional HIV genes are encoded in the anti-BCMA02 CAR (bb2121) drug product insert. The insert encodes sequences necessary for the expression and production of the therapeutic CAR gene. The HIV sequences that are retained are necessary for the packaging and delivery of the insert. The insert has no replication function and does not encode any pathogenic genes. Anti- BCMA02 CAR lentiviral vector and bb2121 drug product are both tested to ensure the absence of replication competent lentiviral vector.

Insert Component	Source	Function
HIV-1 Repeat, unique 5'site PBS and Ψ packaging sequences	pNL4-3; GenBank Reference Accession #M19921.2 (Maldarelli et al., 1991)	Required for insertion of provirus DNA into the chromosome
HIV-1 gag region	pNL4-3 GenBank Reference Accession #M19921.2 (Maldarelli et al., 1991)	Secondary structures required for vector packaging.
HIV-1 Central Polypurine Tract (cPPT)	pNL4-3 GenBank Reference Accession #M19921.2 (Maldarelli et al., 1991)	Required for reverse transcription
HIV-1 env region Rev Response Element (RRE)	PgTAT-CMV GenBank Reference Accession #M14100.1 (Malim et al, 1988)	Binding site for Rev, for efficient packaging of the vector RNA
MND promoter (see 4(e), above)	pccl-c-MNDU3c-x2 (Challita et al., 1995)	Promoter drives T cell-specific expression
Anti-BCMA02 scFv (VL-linker-VH)	Synthetic	Therapeutic gene

CD8a hinge and Transmembrane region	GenBank Reference Accession # NM_001768 (Milone et al., 2009)	Ensures correct T cell receptor conformation
CD137 (4-1BB) signaling domain	GenBank Reference Accession # NM_001561 (Milone et al., 2009)	Ensure correct T cell receptor function
CD3-ζ signaling domain	GenBank Reference Accession # NM_000734 (Milone et al., 2009)	Ensure correct T cell receptor function
HIV-1 unique 3' region and repeat region.	pNL4-3; GenBank Reference Accession #M19921.2 (Maldarelli et al., 1991)	Required for insertion of provirus DNA into the chromosome

References are as follows:

Challita, P.M., Skelton, D., el-Khoueiry, A., Yu, X.J., Weinberg, K., and Kohn, D.B. (1995). Multiple modifications in cis elements of the long terminal repeat of retroviral vectors lead to increased expression and decreased DNA methylation in embryonic carcinoma cells. J Virol 69, 748-755.

Maldarelli, F., Martin, M.A., and Strebel, K. (1991). Identification of posttranscriptionally active inhibitory sequences in human immunodeficiency virus type 1 RNA: novel level of gene regulation. J Virol 65, 5732-5743.

Malim, M.H., Hauber, J., Fenrick, R., and Cullen, B.R. (1988). Immunodeficiency virus rev trans-activator modulates the expression of the viral regulatory genes. Nature 335, 181-183.

Milone, M.C., Fish, J.D., Carpenito, C., Carroll, R.G., Binder, G.K., Teachey, D., Samanta, M., Lakhal, M., Gloss, B., Danet-Desnoyers, G., et al. (2009). Chimeric receptors containing CD137 signal transduction domains mediate enhanced survival of T cells and increased antileukemic efficacy in vivo. Mol Ther 17, 1453-1464.

(d) Location of the insert in the host organism

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

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

Yes (.) No (X)

If yes, specify

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

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

This section is not applicable.
The donor, Anti-BCMA02 CAR lentiviral vector, is an artificial organism. This vector uses the murine leukemia virus-derived MND promoter to drive expression of the chimeric receptor, a multi-domain protein consisting of the extracellular antigen recognition domain (VL and VH), the CD8α hinge domain, a transmembrane domain (CD8 TM), and the intracellular CD137 co-stimulatory (4-1BB) and CD3zeta chain signaling domains. The Anti-BCMA02 CAR lentiviral vector does not encode for any HIV proteins; the only HIV derived sequences in the transcript are the repeat regions that have been made self-inactivating by deleting promoter/enhancer sequences, and attenuated regions of the proteins and element that aid in the production, packaging, or transfer of the transcript containing the therapeutic gene. Additional sequences are derived from human genes encoding components of the T cell receptor.

(i) order and/or higher taxon (for animals) …

(ii) family name for plants …

(iii) genus …

(iv) species …

(v) subspecies …

(vi) strain …

(vii) cultivar/breeding line …

(viii) pathovar …

(ix) common name …

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

Yes (.) No (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 (X) No (.)

If yes, specify

Group 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

After integration, the CAR gene form an integral part of autologous T cells' 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)

4. Description of identification and detection methods

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

Cells transduced with Anti-BCMA02 CAR lentiviral vector (i.e. bb2121 drug product) are not released into the environment, and are not stable under uncontrolled environmental conditions. They are infused in to the subject from whom the autologous cells were originally obtained, and are detected using flow cytometry with a labelled antibody specific to the anti-BCMA CAR. Detection of the Anti-BCMA02 CAR lentiviral vector is conducted by immunochemistry, using an enzyme linked immune-sorbant assay (ELISA).

(b) Techniques used to identify the GMO

Quantitative PCR measures the amount of integrated vector in recipient cells. Flow cytometry is used to confirm expression and identify cells expressing the therapeutic gene product (the CAR). ELISA is used to identify the lentiviral vector.

F. Information relating to the release

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

It will be administered intravenously into subjects enrolled in bb2121 studies and will be administered for the treatment of multiple myeloma under highly controlled conditions for cell transplant at the clinical study site. The transduced cells may migrate to the bone marrow or may remain in the peripheral circulatory system post-infusion.

The bb2121 drug product is manufactured at a cGMP manufacturing site in the US. Autologous T cells are collected from the subject at the clinical study site and transported to the manufacturing facility where they are transduced with Anti-BCMA02 CAR lentiviral vector to produce the final bb2121 drug product. Each lot of Drug Product is tested to ensure identity and purity prior to release. In addition, each Drug Product lot is tested to confirm the absence of active, replication competent lentivirus (RCL). The released drug product is then transported from the drug product manufacturing site back to the clinical site under controlled conditions, where it is stored prior to infusion.

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

The bb2121 drug product (transduced T cells) will be administered intravenously into subjects enrolled in bb2121 studies and will be administered for the treatment of multiple myeloma under highly controlled conditions for cell transplant at the clinical study site. The transduced cells may migrate to the bone marrow or may remain in the peripheral circulatory system post-infusion.

The Anti-BCMA02 CAR lentiviral vector is released under highly controlled and isolated conditions (in vitro) at the US GMP manufacturing site to transduce autologous T cells ex vivo. The site of release of the Anti-BCMA02 CAR lentiviral vector is not its natural habitat. The cell culture conditions, containing autologous T cells, have been designed specifically to facilitate the expansion of the T cell population. The vector is attenuated, replication incompetent and it also degrades rapidly in the environment.

3. Information concerning the release and the surrounding area

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

-Clínica Universidad De Navarra

-Institut Catala d'Oncologia (ICO)

- Hospital Universitari Germans Trias i Pujol (HUGTP)

(b) Size of the site (m^2):

The patients will be treated in a hospital room

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

(ii) wider release site (m^2):

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

Not applicable

(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:

It is planned that up to 450 million T cells containing the gene for the anti-BCMA chimeric antigen receptor will be administered intravenously to patients.

The quantity of lentiviral vector used in the controlled conditions of the drug product manufacturing process varies depending on the number of cells available for transduction.

(b) Duration of the operation:

Only during infusion of the patient during the clinical trial

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

The bb2121 drug product containing T cells transduced with Anti-BCMA02 CAR lentiviral vector is administered intravenously into the subject under standard controlled conditions for cell transplant at the clinical site.

All clinical waste is destroyed according to hospital's procedures for the disposal of bio-hazardous waste.

All residues from processing are destroyed in accordance with the procedures of the production plant for the disposal of waste at biological risk. The bb2121 drug and the lentiviral vector of the CAR anti-BCMA02 are produced outside the EU in accredited and controlled plants, which meet the established requirements of the EU's correct manufacturing standards.
Detailed procedures for all steps in handling the GMO is described the biohazard instructions (Annex V.A).

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.

Not applicable.
bb2121 manufactured with Anti-BCMA02 CAR lentiviral vector is being studied in ongoing study CRB-401 in the USA. For additional information, please refer to Section 4 of the Investigator's Brochure version 3 (dated 24AUGUST2017).

Reference:
Berdeja, J.G., Lin, Y., Raje, N., Siegel, D., Munshi, N., Turka, A., Lam, L.P., Quigley, M.T., and Kochenderfer, J.N. (2016). Clinical remissions and limited toxicity in a first-in-human multicenter study of bb2121, a novel anti-BCMA CAR T cell therapy for relapsed/refractory multiple myeloma, Eur J Cancer, 68 Suppl 1:S5 [abstract]

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

This section is not applicable. The target organism is the recipient. The transduced autologous T cells that comprise bb2121 and the Anti-BCMA02 CAR lentiviral vector are not released into the environment.
Not applicable. In all the document

1. Name of target organism (if applicable)

(i) order and/or higher taxon (for animals) …Homo sapiens

(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)

bb2121 drug product contains autologous T cells transduced with the Anti-BCMA02 CAR lentiviral vector encoding a chimeric anti-BCMA T cell receptor gene. Upon infusion to the patient, the cells may migrate to the bone marrow or remain in circulation. It is expected that bb2121 will have a therapeutic effect in patients with multiple myeloma expressing B cell maturation antigen (BCMA). Transduced cells are not viable in the environments outside of the subject

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

Possible interaction with other foreign organisms as HIV present in the patients is extremely low as no HIV+ patients are exposed to bb2121. Subjects are screened prior to acceptance into the current bb2121 clinical study. No bb2121 product is made from HIV positive subjects, therefore eliminating the possibility of recombination of the LVV with HIV. The transduced cells are not viable outside of the body of the treated subjects. The Anti-BCMA02 CAR lentiviral vector degrades rapidly in the environment. Therefore no undesirable effects are expected. The administration of the GMO product to immunocompetent people leads to rejection of the GMO cells.

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

Since this is a clinical trial there is no possibility to disseminate the GMO to any other ecosystem. All residues from processing are destroyed in accordance with the procedures of the production plant for the disposal of waste at biological risk. All clinical waste is destroyed according to hospital's procedures for the disposal of bio-hazardous waste.

bb2121 drug product consists of autologous T cells transduced with the Anti-BCMA02 CAR lentiviral vector encoding the chimeric anti-BCMA T cell receptor gene. Transduced cells are not viable in the environments outside of the subject. The Anti-BCMA02 CAR lentiviral vector is attenuated, replication incompetent and it also degrades rapidly in the environment.

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

None. This section is not applicable.

(i)	order and/or higher taxon (for animals)	…
(ii)	family name for plants	…
(iii)	genus	…
(iv)	species	…
(v)	subspecies	…
(vi)	strain	…
(vii)	cultivar/breeding line	…
(viii)	pathovar	…
(ix)	common name	…

7. Likelihood of genetic exchange in vivo

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

The bb2121 drug product is made with a replication defective vector that inserts the proviral DNA encoding the chimeric antigen receptor stably into the genome of the autologous T cells. Neither the insert nor the vector is capable of replication. Therefore, gene transfer to unintended organisms is not anticipated and is extremely low for the following reasons:

1) Potential risks to the treated subject include the theoretical risk of generation of a replication competent lentivirus (RCL). However, it is important to note that all HIV viral genes have been removed from the proviral sequence, and replaced with a human therapeutic gene, thereby making the risk of RCL negligible. No new viral particles can be assembled and shredded from 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 lentivirus.

2) No HIV+ patients exposed to bb2121

   Subjects are screened prior to acceptance into the current bb2121 clinical study. No bb2121 product is made from HIV positive subjects, therefore eliminating the possibility of recombination of the LVV with HIV.

3) No intact, infectious anti-BCMA02 LVV particles are present in the bb2121 drug product

   The anti-BCMA02 LVV is not directly administered to human subjects. Instead, the anti-BCMA02 LVV material is used to transduce the autologous T cells during the bb2121 manufacturing process. The amount of residual infectious LVV particles present in the final bb2121 drug product has been estimated. Based on the calculations, no intact, infectious anti-BCMA02 LVV particles are expected to be present in the bb2121 drug product. Details of the calculations are outlined below.

   - The bb2121 manufacturing process consists of 3 primary steps (PBMC preparation, cell culture, and drug product formulation and cryopreservation) which are carried out sequentially. T cell transduction occurs at Day 1 of the 10-day cell culture step. The target multiplicity of infection (MOI) ratio used for transduction is specific to the lot of anti-BCMA02 LVV, and is selected to achieve comparable drug product results. At

a typical target MOI of 20, the infectious titer introduced to cell culture is calculated to be: (Target MOI) x (Total Cell Number) = (20) x (100 x 10^6 cells at Day 1 of cell culture) = 2 x 10^9 active particles.

- Following transduction, the cell culture is maintained for 9 additional days. Starting at Day 3, fresh growth medium is added to maintain cell concentration and to ensure proper nutrient level for T cell growth. Free anti-BMCA02 LVV particles that are not incorporated into the autologous T cells degrade rapidly in cell culture. Under cell culture conditions (i.e. 37 ^oC), the half-life of lentiviral particles is expected to be less than 10 hours (Zhang, 2004). Based on this half-life estimation, the reduction fold of active anti-BCMA02 LVV particles during the 9-day cell culture is calculated to be 2 ^ ((9 days) x (24/10 half-lives per day)) = 3.18 x 10^6 fold.
- On Day 10, the cells are harvested from cell culture, washed, and formulated and cryopreserved at vapor phase of liquid nitrogen (≤ -140 ^oC). During the drug product processing steps at Day 10, any residual active LVV particles present in cell culture are diluted extensively during the post-harvest washing steps, resulting in further dilutions of > 2,000 fold.
- Taken together, the cumulative reduction fold of free, active LVV particles throughout the bb2121 manufacturing process is calculated to be (3.18 x 10^6) x 2,000 = 6.36 x 10^9 fold. The theoretical amount of infectious particles present in the bb2121 drug product is calculated to be (2 x 10^9 active particles) / (6.36 x 10^9 fold reduction) = 0.31 particles per entire drug product lot. Therefore, no infectious anti-BCMA02 LVV particles are expected to be present in the bb2121 drug product. As part of QC release testing, all bb2121 lots are tested to confirm the absence of active, replication competent lentivirus (RCL).

4) The anti-BCMA02 LVV is replication incompetent by design, and is incapable of mobilizing and replicating pathogenic HIV-1 genes

The anti-BCMA02 CAR LVV is designed to efficiently deliver the target anti-BCMA02 CAR transgene to human T cells without the capacity to replicate HIV-1 genes. Active infection and transfer of HIV-1 genes require the LVV to become replication competent. As described in literature (Schambach, 2013), lentiviral vectors offer a number of biosafety features that minimize the formation of replication competent species. Anti-BCMA02 CAR LVV is a replication defective, self-inactivating (SIN), third generation HIV-1 based LVV. As mentioned in C.4(b) and C.6(c) above, this LVV is engineered ex vivo such that the HIV genes necessary for transcription and expression are separated into multiple plasmids during transient transfection of producer cells, therefore greatly minimizing the probability for recombination. In addition, the anti-BCMA02 CAR LVV does not contain the natural promotor necessary to induce HIV. The LVV genome is completely devoid of intact viral long-terminal repeat (LTR) sequences through the use of SIN LTRs, making it impossible to regenerate a functional lentiviral genome due to the critical role of these LTR sequences in the virus life cycle. The replication-deficient lentiviral vector genome is integrated as provirus in the host T cell genome. No new viral particles can be assembled and shredded from the final host cell due to the absence in this proviral transgene form of all the accessory proteins that confers infectivity and replicative potential to the lentivirus. In addition, the transgene inserted in the lentiviral vector does not code for pathogenicity factors, cytokine-coding sequences, oncogenes, antibiotic resistance genes, or other known hazardous inserts. Therefore, the hypothetical recombination with wild-type HIV should not yield variants containing novel putatively dangerous structures, since the only exogenous gene present in the Anti-BCMA02 CAR lentiviral vector is a chimeric T cell antigen receptor gene.

(b) from other organisms to the GMO:

The bb2121 drug product will exist as differentiated T cells in the subject. While it is always possible that human subjects are infected with other organisms, there is no added risk to the subject as the Anti-BCMA02 CAR lentiviral vector does not encode any viral or pathogenic genes. Anti-BCMA02 CAR lentiviral vector is a self-inactivated lentiviral vector.

(c) likely consequences of gene transfer:

Once bb2121 drug product is created, no further gene transfer is anticipated.

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.):

The Anti-BCMA02 CAR lentiviral vector is used to transduce ex vivo autologous T stem cells in the controlled and insulated manufacturing laboratory setting. The transduced T cells that comprise bb2121 drug product are infused into the corresponding subject. Neither is viable in the environment. Neither will be released into the environment.

No clinical evidence of aberrant HIV-1 gene transfer and replication from LVV to date. The lentiviral vector gene delivery system has been studied extensively for the shredding of transduced viral particles and the appearance of atypical replication-competent virus. The shredding of intact, replication-incompetent viral particles from lentiviral vector transduced cells has been reported in preclinical studies as summarized in literature (Schambach, 2013), and the functional consequences other than the transmission of reporter cassettes, or true adverse events caused by such transmission events, have not yet been described (Scaramuzza, 2012). Nevertheless, aberrant gene transfer has not been observed with clinical-grade lentiviral vectors in a gene therapy setting (Cesani, 2015), and to date no adverse events reported in the ongoing Phase 1 bb2121 study can be attributed to the off-target transmission of the anti-BMCA02 transgene. Also, no detectable RCL has been reported for subjects treated with LVV (Sastry, 2009), or to date in the ongoing Phase 1 bb2121 study.

bb2121 is being studied in ongoing study CRB-401 in the USA, as described in Berdeja, et al. (2016) [Eur J Cancer, 68 Suppl 1:S5]

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

Upon infusion into the subject, CAR-positive T cells will be detected using cytometric methods for identification and quantification of the therapeutic cell type.

Upon manufacturing, the Anti-BCMA02 CAR lentiviral vector is measured cell culture techniques and flow cytometry.

2. Methods for monitoring ecosystem effects

Not applicable. The Anti-BCMA02 CAR lentiviral vector and bb2121 drug product are not released into the environment.

3. Methods for detecting transfer of the donated genetic material from the GMO to other organisms

Not applicable. The Anti-BCMA02 CAR lentiviral vector and bb2121 drug product are not released into the environment. Moreover, the administration of the GMO product to immunocompetent people leads to rejection of the GMO cells.

4. Size of the monitoring area (m2)

Not applicable. The Anti-BCMA02 CAR lentiviral vector and bb2121 drug product are not released into the environment.

5. Duration of the monitoring

Subjects will be followed for disease status, AEs, clinical status, and laboratory parameters for up to 24 months in study bb2121-MM-001.

Long-term bb2121-related toxicity, and viral vector safety as well as disease status (in patients who have not progressed), survival status and subsequent anti-MM therapies will continue to be monitored under a separate Long-term Follow-up (LTFU) protocol for up to 15 years after the last bb2121 infusion as per competent authority guidelines.

6. Frequency of the monitoring

The frequency of monitoring is at baseline, Days -5, -3 and -2, Days 0, 1, 2, 3, 4, 7, 9, 11, 14, 21 and monthly thereafter until Month 24. Please see Table 2 of the protocol concept sheet bb2121-MM-001 for more details.

I. Information on post-release and waste treatment

1. Post-release treatment of the site

The bb2121 drug product (transduced T cells) is not released in the environment. It is administered intravenously into the subject under standard controlled conditions for cell transplant at the clinical site.

All clinical waste is destroyed according to hospital bio-hazard disposal procedures.

All manufacturing waste is destroyed according to the manufacturing facility bio-hazard disposal procedures. bb2121 drug product and Anti-BCMA02 CAR lentiviral vector are manufactured outside the EU.

2. Post-release treatment of the GMOs

The bb2121 drug product and Anti-BCMA02 CAR lentiviral vector are not released into the environment. bb2121 is infused into the patient as a one-time therapeutic treatment.

3. (a) Type and amount of waste generated

The waste generated following treatment of patients with bb2121 drug product is minimal and consists mainly of residual cells remaining in the infusion bag.

The waste generated following manufacturing of bb2121, i.e. following transduction of the autologous T cells with the Anti-BCMA02 CAR lentiviral vector is minimal and consists of residual cells or residual process solution. The waste is minimized as the efficacy of the product is highly dependent on the number of autologous cells that are transduced.

The waste generated following manufacturing of the Anti-BCMA02 CAR lentiviral vector is minimal and consists of residual process solutions that may have contacted the lentiviral vector and residual inactivated viral particles.

All waste is destroyed according to hospital or manufacturing facility bio-hazard disposal procedures after appropriate disinfection.

(b) Treatment of waste

All waste is destroyed according to hospital or manufacturing facility bio-hazard disposal procedures.

J. Information on emergency response plans

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

bb2121 (drug product) is not viable in the environment outside of the body of the treated patient. It is not possible for the drug product to spread into the environment. The Anti-BCMA02 CAR lentiviral vector is used to transduce ex vivo the autologous T cells in the controlled and insulated manufacturing laboratory setting. It degrades rapidly in the environment.

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

In case of accidental spill of the transduced cells or the Anti-BCMA02 CAR lentiviral vector, hospital or manufacturing facility decontamination and cleaning procedures are applied.

Waste is disinfected by appropriate products (e.g., paraformaldehyde, aqueous bleach, detergent based disinfectant, or hydrogen peroxide).

The study team at site, which will be involved in the study drug product administration will be fully trained to the study requirements and to the hospital's procedures

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

No plant, animal or soil will be in the manufacturing facility or the transplant unit where bb2121 is administered to the subject.

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

The bb2121 drug product (transduced cells) and the Anti-BCMA02 CAR lentiviral vector do not encode any pathogenic gene. The transduced cells are not viable outside of the body of the treated subjects. The Anti-BCMA02 CAR lentiviral vector degrades rapidly in the environment. The

administration of the GMO product to immunocompetent people leads to rejection of the GMO cells. Therefore no undesirable effects are expected