ANNEX, PART 1 of COUNCIL DECISION 2002/813/EC

SUMMARY NOTIFICATION INFORMATION FORMAT FOR PRODUCTS CONTAINING GENETICALLY MODIFIED ORGANISMS OTHER THAN HIGHER PLANTS IN ACCORDANCE WITH ARTICLE 11 OF DIRECTIVE 2001/18/EC

MB-CART20.1 Melanoma
Multicenter Phase I trial of MB-CART20.1 for the Treatment of Patients with Metastatic Melanoma

A. General information

1. Details of notification

(a)	Member State of notification	Germany
(b)	Notification number	B/DE/17/PEI3183
(c)	Date of acknowledgement of notification	01.09.2017
(d)	Title of the project	Multicenter Phase I trial of MB-CART20.1 for the Treatment of Patients with Metastatic Melanoma
(e)	Proposed period of release	From Q1 2018 until Q4 2020

2. Notifier
Miltenyi Biotec GmbH, Friedrich-Ebert-Straße 68, Bergisch Gladbach, Germany

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

(b) Identity of the GMO (genus and species)
T cells transduced with a replication-deficient lentiviral vector harbouring the chimeric antigen receptor for targeting CD20.

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

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

Yes (x) No (.)

If yes, insert the country code(s) DE

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 ( ) No (x)

If yes:

- Member State of notification not applicable
- Notification number not applicable

7. Summary of the potential environmental impact of the release of the GMOs.
An environmental impact is not expected as the clinical trial with the MB-CART20.1 is limited to patients treated in hospital settings under safe application conditions. Treated patients do not shed MB-CART20.1 into the environment. According to the environmental risk assessment MB-CART20.1 does not compromise either human health or environment safety.

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	..
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 (.)

(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 cell analysis (EP 2.7.24) and presence of provirus and transgene (real-time qPCR)

(b) Identification techniques
Common techniques of cell analysis (EP 2.7.24) and presence of provirus and transgene (real-time qPCR)

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
Patients will be tested for HIV, HBV and HCV prior to leukapheresis and would be excluded from the clinical study if tested positive. In addition, patients have to be free of overt signs of viral, bacterial, fungal or parasitic infection.

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 (funghi)	(.)
(vi)	eggs	(.)
(vii)	pupae	(.)
(viii)	larvae	(.)
(ix)	other, specify …	

(b) relevant factors affecting survivability:
The survival of human blood cells outside the respective autologous human host is not possible unless special laboratory conditions and growth media are applied.

10. (a) Ways of dissemination
No dissemination of blood cells in the environment is possible due to fast inactivation.

(b) Factors affecting dissemination
If injected into people other than the donor, these blood cells would be eliminated through the respective immune system.

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)
Not applicable

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 desired mode of action for CAR T cells consists in the physiological response of T cells to their cognate antigen, meaning the target mediated killing of CD20 positive cells as well as the release of pro inflammatory cytokines by the MB-CART20.1 cells. Killing and cytokine release are mediated by activation of the signalling cascade of the chimeric antigen receptor upon recognition and binding to CD20 expressing cells.

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 HIV-1-derived lentiviral vector of the 3rd generation.

(c) Host range of the vector
VSV-G pseudotyped and thus able to transduce many different non-dividing human and animal cells.

(d) Presence in the vector of sequences giving a selectable or identifiable phenotype
Yes ( ) No (x)

antibiotic resistance	(.)
other, specify	

Indication of which antibiotic resistance gene is inserted
…

(e) Constituent fragments of the vector
Self-inactivating replication deficient lentiviral vector including an expression cassette for the expression of an anti-CD20 directed chimeric antigen receptor.

(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
In order to generate the target construct (anti-) CD20 CAR, the scFv sequence derived from mouse monoclonal antibody connected by an intra-chain linker. The resulting targeting domain was then linked in frame to human hinge and transmembrane (TM) domains, human co-stimulatory domain and human signaling domain sequence. A human leader sequence was included to facilitate secretory pathway-mediated CAR expression on the cell surface. The CAR encoding DNA sequence was codon optimized.

(b) Source of each constituent part of the insert
HIV (lentiviral vector LTRs, major 5' splice donor (SD), packaging sequence, rev response element, central polyurine tract and SIN, hCMV promoter/enhancer, murine and human transgene, as indicated above.

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

(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 (.)
- 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	Retrovirus
(iv)	species	Human Immunodeficiency Virus
(v)	subspecies	…
(vi)	strain	HIV-1
(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 (x) No (.) Not known (.)
If yes, specify the following: Causing AIDS

(b) to which of the following organisms:

humans	(x)
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 Wild type HIV is classified as group 3 organism. However, the replication-defective lentiviral vector used for transduction of T cells is not pathogenic anymore as no infectious viral particles can be produced after transduction.

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 (anti-) CD20 CAR is introduced into the T cell via the Lentiviral Vector (anti-) CD20 CAR. Therefore, the (anti-) CD20 CAR DNA sequence is 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 gene-modified T cells express a chimeric antigen receptor (CAR) directed against CD20. The gene-modified T cells possess conventional properties of antigen-reactive T cells meaning that upon encounter with a $CD20^+$ target, the CAR^+ T cells will receive activation signals that will trigger normal T cell response. CD20 CAR signaling will lead to the expansion of CAR^+ T cells and the release of pro-inflammatory cytokines (such as IFNγ and IL-2). Target cells expressing the CD20 antigen will undergo cell death mainly mediated by cytolytic degranulation and granzyme/perforin release and Fas/Fas-L interaction by CAR^+ T cells. The replication-deficient lentiviral vector genome is integrated as provirus in the T cell genome. No new viral particles can be assembled in the final host cell since most HIV sequences have been deleted.

4. Description of identification and detection methods

(a) Techniques used to detect the GMO in the environment
Patient monitoring for persistence of MB-CART20.1 will be performed using common techniques of flow cytometric cell analysis.

(b) Techniques used to identify the GMO
Identity of MB-CART20.1 will be determined using common techniques of flow cytometric cell analysis with a specific detection reagent.

F. Information relating to the release

1. Purpose of the release (including any significant potential environmental benefits that may be expected)
The Phase I clinical trial is a single arm, prospective, multicenter, open label, dose escalation study at two sites with approximately 15 patients with unresectable stage III or stage IV melanoma using autologous MB-CART20.1 to assess:
- The feasibility, safety, and toxicity of adoptive cell therapy using autologous CD20 CAR transduced T cells, MB-CART20.1
- The safety, and toxicity of adoptive cell therapy as per adverse events (AE) reporting classified according to the Common Terminology Criteria for Adverse Events, CTCAEv.4.0 and Lee et.al. 2014.
- Preliminary evidence of response to treatment (Number of patients with Complete Response; Partial Response; Stable Disease; Progressive Disease).
- B cell aplasia
- Immunophenotyping /Persistence of transduced MB-CART20.1

The clinical trial with MB-CART20.1 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 clinical trials will take place at hospital sites in Germany.

(b) Size of the site (m^2): The administration site is a hospital room
(i) actual release site (m^2): … m^2
(ii) wider release site (m^2): … m^2

(c) Proximity to internationally recognised biotopes or protected areas (including drinking water reservoirs), which could be affected:
No environmental sites outside the hospital room will be affected. Safe handling of MB-CART20.1, including personal protection of health care professionals, decontamination measures and safe disposal, prevent exposure of people with the IMP other than the patient and release into the environment of the IMP.

(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:
MB-CART20.1 is delivered freshly after manufacturing and administered intravenously (i.v.) as single dose with a final volume adapted to the patients' weight as slow infusion depending on the final volume over a time period of approximately 15 minutes. The minimal dose is $1x10^5$ MB-CART20.1 cells per kg BW, the maximum dose is $1x10^7$ MB-CART20.1 cells per kg BW.

(b) Duration of the operation:
Administration through an i.v. infusion line will take approx. 15 minutes.

(c) Methods and procedures to avoid and/or minimize the spread of the GMOs beyond the site of the release
Safe handling, decontamination and disposal procedures comparable to contained use applications are in place.

5. Short description of average environmental conditions (weather, temperature, etc.)
The administration sites will be strictly controlled rooms as all treatment sites for immune compromised individuals, with restricted access to health care professionals involved in the treatment of the 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.
CAR T cells have been used successfully in clinical trials for several years. The majority of current and closed clinical trials using CAR T cells were performed with CAR T cells targeting the B cell surface marker CD19. For all CD19 CAR T cell trials similar toxicities have been reported in the patients: cytokine release syndrome, neurological toxicities and B cell aplasia. Of most clinical interest is the persistence and maintenance of functionality of the CAR T cells within the body. CAR T cells expressing second generation CAR constructs have been found to persist in the body for at least 11 months to years for patients with complete response. Results of only a few clinical trials with CD20 CAR T cells are published up to date. There are no cases described in the literature which showed the malignant transformation of a mature CAR genetically modified T cell. Additionally, Carl June and co-workers at UPenn analyzed the malignant potential of CAR modified mature CD4 T cells and followed up more than 500 patient-years after introducing gamma-retroviral vector-engineered T cells and did not find any evidence of vector-induced immortalization of T cells.

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 MB-CART20.1 behaves like conventional T cell with no tissue selectivity or defined tropism. The IMP will be capable of circulating through the body and tissues comparable to non-modified T cells. Presence of the CD20 target on B cells and melanoma stem cells will lead to an accumulation of the CD20 CAR transduced T cells in areas where the target cells are present (e.g. in secondary lymphoid organs where B cells are present). CAR T cells expressing second generation CAR constructs have been found to persist in the body for at least 11 months to several years for patients with complete response. T cells have the advantage to spread in the entire body to seek and destroy targets expressing their cognate antigen.

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
Treated patients do neither shed MB-CART20.1, nor Lentiviral Vector (anti-) CD20 CAR or RCL. They are advised to use HIV-infection control measures and not to donate blood, tissues or organs. In case of an accidental bleeding, MB-CART20.1 cells are inactivated through drying. Taking blood form treated individuals does not require safety measures in addition to the ones applied for the safe handling of human blood.
Outside of the host, MB-CART20.1 is sensitive to and rapidly killed both by physical inactivation (dehydration and heat) and disinfectants (lipid solvents and mild detergents). The genetic modification does not affect survivability in a different environment outside the host organism.

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
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:
   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 behavior and characteristics of the GMO and its ecological impact carried out in stimulated natural environments (e.g. microcosms, etc.):
Clinical trials as described above have been carried out and no ecological impact has been detected.

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

H. Information relating to monitoring

1. Methods for monitoring the GMOs
No procedures for controlling MB-CART20.1 are planned. The MB-CART20.1 parental organism is T cell, not a human pathogen and not zoonotic under natural conditions; MB-CART20.1 is only modified to selectively recognize CD20+ melanoma stem cells. Because the MB-CART20.1 cells remain as normal as the parental T cells, if exposure occurs this will not lead to replication and shedding to those not intended to receive the treatment. Patients will be instructed not to donate blood, organs, tissues and cells for transplantation.
As MB-CART20.1 has been demonstrated to be free of transduction-competent Lentiviral Vector (anti-) CD20 CAR and Lentiviral Vector (anti-) CD20 CAR to be negative for RCL, no shedding of such viral vectors from treated patients is expected. Monitoring for lentiviral vectors is, therefore, not required.

2. Methods for monitoring ecosystem effects
See Section H1

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
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
Work surfaces will be decontaminated using a chemical disinfectant. Any regular, ethanol-based hospital disinfectant can be used. No other treatment of the administration site after administration of MB-CART20.1 will be necessary. In case of an incidental spill, the same decontamination measures will be applied. As health care professionals administering MB-CART20.1 are protected to prevent exposure, and sharps are not involved in the context of MB-CART20.1 application, no measures other than decontamination of the affected area and disposal as indicated above are required.

2. Post-release treatment of the GMOs
None

3. (a) Type and amount of waste generated
Infusion bags and infusion lines.

3. (b) Treatment of waste
Unused or damaged MB-CART20.1 as well as materials having been in contact with the IMP will be disposed of safely as other blood products according to hospital waste disposal practices for potentially infectious material.

J. Information on emergency response plans

1. Methods and procedures for controlling the dissemination of the GMO(s) in case of unexpected spread
Safe handling measures as indicated above will prevent a spill of MB-CART20.1. However, if a spill occurs, decontamination and disposal procedures will be followed to prevent release of MB-CART20.1 into the environment.

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.

4. Plans for protecting human health and the environment in the event of an undesirable effect
Not applicable.