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	**NL**
(b)	Notification number	B/NL/21/015
(c)	Date of acknowledgement of notification	17/06/2021
(d)	Title of the project	
(e)	**Clinical testing of TX200-TR101, an autologous antigen-specific chimeric antigen receptor (CAR) T regulatory cell therapy.**	
(f)	Proposed period of release	From 01/03/2020 until 31/12/2041

2. Notifier

Name of institution or company: **Sangamo Therapeutics France SAS**

3. GMO characterisation

(a) Indicate whether the GMO is a:

viroid	(.)
RNA virus	(.)
DNA virus	(.)
bacterium	(.)
fungus	(.)
animal	
- mammals	**(x) Human cells**
- insect	(.)
- fish	(.)
- other animal	(.)

specify phylum, class **Human**

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

Genus: Human
Species: Homo Sapiens

The GMO / IMP consists of human regulatory T cells transduced *ex vivo* with a replication-deficient self-inactivating lentiviral vector carrying a genetic element encoding the expression of a Chimeric Antigen Receptor (CAR).

(c) Genetic stability – according to Annex IIIa, II, A(10)
Yes – the vector is stably integrated into the host cell genome. As the transducing vector is self-inactivating, the integrated sequences cannot remobilize as viral vector.

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) **BE, FR, GB, DE**

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 **DE**
- Notification number B/../../… **(pending)**

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 B/../../…

7. Summary of the potential environmental impact of the release of the GMOs.
TX200-TR101 is concluded to have no potential environmental impact because:
(1) TX200-TR101 consists of genetically modified human cells which cannot survive in the environment outside of a human body or specialized conditions for containment or culture.
(2) The cells are for autologous use and are not likely to survive in any human recipient other than that from which they were derived.
(3) TX200-TR101 is only administered to patients within a hospital, and according to institutional control procedures, and will not be released into the wider environment.
(4) Residual transducing lentiviral vector has been demonstrated to be removed during the manufacturing process. The product to be used is also required to have been tested negative for the presence of any contaminating replication-competent viral vector.
(5) The transducing vector used is designed as self-inactivating, to block the possibility of remobilizing viral vector from transduced cells.

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

1. Recipient or parental organism characterisation: **the recipient organisms are autologous human regulatory T cells transduced *ex vivo* with a replication-deficient self-inactivating lentiviral vector carrying a genetic element encoding the expression of a Chimeric Antigen Receptor**.

(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)	**Primates**
(ii)	genus	Homo
(iii)	species	H. Sapiens
(iv)	subspecies	…
(v)	strain	…
(vi)	pathovar (biotype, ecotype, race, etc.)	…
(vii)	common name	Human

3. Geographical distribution of the organism

The following questions are not applicable to human cells.

(a) Indigenous to, or otherwise established in, the country where the notification is made:
Yes (.) 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 (.)
(iii) Not known (.)

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

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

4. Natural habitat of the organism

(a) If the organism is a microorganism

water (.)
soil, free-living (.)
soil in association with plant-root systems (.)
in association with plant leaf/stem systems (.)
other, specify …

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

5. (a) Detection techniques
Common techniques of blood cell analysis.

(b) Identification techniques
Common techniques of blood cell analysis.

6. Is the recipient organism classified under existing Community rules relating to the protection of human health and/or the environment?
Yes (.) No (**x**)
If yes, specify
…

7. Is the recipient organism significantly pathogenic or harmful in any other way (including its extracellular products), either living or dead?
Yes (.) No (**x**) Not known (.)

If yes:

(a) to which of the following organisms:

humans (.)
animals (.)
plants (.)
other (.)

(b) give the relevant information specified under Annex III A, point II. (A)(11)(d) of Directive 2001/18/EC

Human cells are not expected to be toxic to other humans or able to survive in any other organisms. The vector used to transduce the cells is self-inactivating, preventing remobilization. Patient's eligibility and safety screening for leukapheresis is performed according to the Commission Directives 2004/23/EC and 2006/17/EC. Patient screening samples and autologous blood leukapheresis material are tested for HIV, HBV, HCV and HTLV at a minimum. Only patients tested negative for adventitious agents will be included in the clinical trial. Overall, the infectious agent risks from the cells will therefore be no more than for any other human cells or tissues handled routinely within a hospital environment.

8. Information concerning reproduction:

Human cells are unable to survive or propagate in the environment.

(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:
Human cells are unable to survive outside of either a human body or specialized conditions for artificial culture or containment (proper-special media, temperature and CO2).

10. (a) Ways of dissemination
Human cells could only be disseminated to non-target individuals through accidental injection. No dissemination in the environment is possible due to fast inactivation.

(b) Factors affecting dissemination

Use according to the hospital's institutional control procedures should minimize the risk of any accidental injection, and if that should occur, the cells are not expected to survive within a non-genetically identical or non-immunosuppressed recipient. The immune system of people other than the donor will eliminate the patient-specific genetically modified regulatory T cells.

11. Previous genetic modifications of the recipient or parental organism already notified for release in the country where the notification is made (give notification numbers)

None.

C. **Information relating to the genetic modification**

1. Type of the genetic modification

(i)	insertion of genetic material	(**x**)
(ii)	deletion of genetic material	(.)
(iii)	base substitution	(.)
(iv)	cell fusion	(.)
(v)	others, specify …	

2. Intended outcome of the genetic modification

The transduction with the gene encoding the chimeric antigen receptor (anti-HLA-A2 CAR) allows T regulatory cell activation and immunomodulation through *in vivo* recognition of the HLA-A*02 antigen, which is introduced via a mismatch of HLA-A*02 positive donor organ that has been grafted into the HLA-A*02 negative recipient.
The immunosuppressive function of the genetically modified regulatory T cells is therefore expected to dampen effector and cytotoxic T cell activation responsible for the rejection of the graft whilst educating/modifying dendritic cells to present alloantigen they will sample from the graft to donor naive T cells in a non-immunogenic fashion, thus inducing tolerance of the renal allograft.

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 viral 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 (**x**) No (.)

antibiotic resistance (.)
other, specify
The vector and transduced cells can be identified based on the chimeric antigen receptor transgene. Transduced cells can also be identified from the expression of the chimeric antigen receptor.

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-HLA-A2 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 **Viral transduction**

5. If the answer to question B.3(a) and (b) is no, what was the method used in the process of modification?

(i) transformation (.)
(ii) microinjection (.)
(iii) microencapsulation (.)
(iv) macroinjection (.)
(v) other, specify …

6. Composition of the insert

(a) Composition of the insert

The insert corresponding to a humanized anti-HLA-A2 Chimeric Antigen Receptor contains a scFv domain specific to the target antigen HLA-A*02, a human CD8α hinge and transmembrane domain, and human CD28 and CD3ζ (T cell receptor ζ) intracellular signalling domains.

(b) Source of each constituent part of the insert
Mouse and human.

(c) Intended function of each constituent part of the insert in the GMO
Refer to 6(a).

(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**) **information provided below**
DNA virus (.)
bacterium (.)
fungus (.)
animal
- mammals (**x**) **human/murine, bovine – no relevant hazards**
- 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 **Lentivirus**
(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:

(c) 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):
Not applicable.

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 a group 3 organism. However, the sequences present in the GMO are not pathogenic and are incapable of producing infective virus.**

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 …

(d) is the GMO in any way different from the recipient as far as dissemination is concerned?
Yes (.) No (**x**) Not known (.)
Specify …

(e) 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 modified organism is genetically stable as the insert is stably integrated into the genome of the recipient cells.

3. Is the GMO significantly pathogenic or harmful in any way (including its extracellular products), either living or dead?
Yes (.) No (**x**) Unknown (.)

(a) to which of the following organisms?

humans (.)
animals (.)
plants (.)
other …

(b) give the relevant information specified under Annex III A, point II(A)(11)(d) and II(C)(2)(i)
The replication-deficient lentiviral vector genome is integrated as a provirus into the regulatory T cell genome. No new viral particles can be assembled in the final host cell due to the self-inactivation of the integrated insert and the absence of viral genes, such as gag/pol, that are necessary for viral replication. The replication-deficient lentiviral vector doesn't code for pathogenicity factors, cytokine-coding sequences, oncogenes, antibiotic resistance genes or otherwise hazardous inserts.

4. Description of identification and detection methods

(a) Techniques used to detect the GMO in the environment
Not applicable, as no environmental monitoring is considered necessary.

(b) Techniques used to identify the GMO
Identity is determined by PCR for the presence of the transgene.

F. Information relating to the release

1. Purpose of the release (including any significant potential environmental benefits that may be expected)
Clinical studies of the safety, mechanism of action and efficacy of TX200-TR101 in renal transplant recipients.

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**) **Not applicable.**
If yes, specify …

3. Information concerning the release and the surrounding area

(a) Geographical location (administrative region and where appropriate grid reference):
Administered to patients within hospitals in Europe.

(b) Size of the site (m^2): … m^2
Not applicable – administered within a hospital facility.
(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:
Not applicable.

(d) Flora and fauna including crops, livestock and migratory species which may potentially interact with the GMO
None.

4. Method and amount of release

(a) Quantities of GMOs to be released:
The maximum dose given to patients (single administration) will not exceed 10E8 CAR regulatory T cells.

(b) Duration of the operation:
Administration of TX200-TR101 will be completed within 90 minutes of thawing the product from frozen.

(c) Methods and procedures to avoid and/or minimise the spread of the GMOs beyond the site of the release
Sponsor instructions will detail procedures for handling and disposal activities, in addition to compliance with institutional standards and policies. The Sponsor instructions and institutional standards and policies are written with the aim of avoiding and/or minimizing spread of the GMO beyond the site of the release.

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

6. Relevant data regarding previous releases carried out with the same GMO, if any, specially related to the potential environmental and human health impacts from the release.
None.

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

None applicable.

1. Name of target organism (if applicable)
(i) order and/or higher taxon (for animals) **Not applicable**

(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)
Not applicable.

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

4. Is post-release selection such as increased competitiveness, increased invasiveness for the GMO likely to occur?
Yes (.) No (**x**) Not known (.)
Give details
The human cells will not survive outside, or disseminate from, the body of the treated patient.

5. Types of ecosystems to which the GMO could be disseminated from the site of release and in which it could become established
None – cells cannot survive or disseminate in the open 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 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 – there is no mechanism by which the transgene can transfer to other cells of the treated patient.

(b) from other organisms to the GMO:

None – there is no mechanism by which genetic material from the treated patient can transfer to the cells of the GMO.

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

8. Give references to relevant results (if available) from studies of the behaviour and characteristics of the GMO and its ecological impact carried out in stimulated natural environments (e.g. microcosms, etc.):
None – not considered applicable.

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

H. Information relating to monitoring

1. Methods for monitoring the GMOs
The presence of the GMO cells will be monitored in patient samples (specifically blood, tissue and urine samples) by PCR methods that detect the transgene and by flow cytometry methods that detect the expression of the anti-HLA.A2 directed chimeric antigen receptor.

2. Methods for monitoring ecosystem effects
None – not considered applicable.

3. Methods for detecting transfer of the donated genetic material from the GMO to other organisms
Not considered applicable, although theoretically the above PCR methods could be applied.

4. Size of the monitoring area (m^2)
… m^2
None – only the patient will be monitored for the GMO cells.

5. Duration of the monitoring
Patients will be monitored for the GMO cells after TX200-TR101 administration throughout the course of the studies, as indicated in the clinical protocol for each study .

6. Frequency of the monitoring
Patient monitoring for the GMO will be performed at several pre-defined timepoints after TX200-TR101 administration, as indicated in the clinical protocol(s).

I. Information on post-release and waste treatment

1. Post-release treatment of the site
Sponsor instructions will detail procedures for disposal and cleaning activities, in addition to compliance with institutional standards and policies. No special cleaning or disinfection procedures are required.

2. Post-release treatment of the GMOs
None.

3. (a) Type and amount of waste generated
Containers, bags, tubing and other contact materials.

3. (b) Treatment of waste
This will be disposed of as clinical waste in accordance with institutional procedures and additional instruction provided by the Sponsor.

J. Information on emergency response plans

1. Methods and procedures for controlling the dissemination of the GMO(s) in case of unexpected spread
Not applicable – the GMO cannot survive or disseminate in the open environment.

2. Methods for removal of the GMO(s) of the areas potentially affected
Cleaning according to institutional procedures and additional instruction provided by the Sponsor. However, no special decontamination procedures are required.

3. Methods for disposal or sanitation of plants, animals, soils, etc. that could be exposed during or after the spread
Not required – the GMO cannot survive or disseminate in the open environment.

4. Plans for protecting human health and the environment in the event of an undesirable effect
Only the treated patient is at risk of undesirable effects from exposure to the GMO, and any such instance will be handled according to the clinical study protocol and other documents and procedures associated with the conduct of the study.