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: Germany

(b) Notification number: B/DE/19/PEI3506

(c) Date of acknowledgement of notification: 26/07/2018

(d) Title of the project

Clinical study CTX001-121 titled: "A Phase 1/2 Study to Evaluate the Safety and Efficacy of a Single Dose of Autologous CRISPR-Cas9 Modified $CD34^+$ Human Hematopoietic Stem and Progenitor Cells (CTX001) in Subjects with Severe Sickle Cell Disease"

(e) Proposed period of release

01.10.2018 – 31.07.2022

2. Notifier

Name of institution or company:

CRISPR Therapeutics AG
Baarerstrasse 14
CH 6300 Zug, Switzerland

3. GMO characterisation

(a) Indicate whether the GMO is a:

viroid	(.)
RNA virus	(.)
DNA virus	(.)
bacterium	(.)
fungus	(.)
animal	

- mammals (X) *(subject autologous CD34$^+$ cells)*
- insect (.)
- fish (.)
- other animal (.) specify phylum, class

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

The GMO/IMP (CTX001) consists of subject autologous peripheral blood-derived CD34$^+$ human hematopoietic stem and progenitor cells (hHSPCs) modified *ex vivo* using the CRISPR-Cas9 gene editing technology. The CRISPR-Cas9 editing components are transiently introduced into the target cell population by electroporation as a ribonucleoprotein complex consisting of Cas9 and a gRNA that targets the erythroid lineage-specific enhancer of the BCL11A gene. This process does not use plasmid or viral vectors. The CRISPR-Cas9 gene editing technology is intended to disrupt the erythroid lineage-specific enhancer of the BCL11A gene on chromosome 2 in a site-specific and permanent fashion in order to reactivate transcription and expression of γ-globin, resulting in increased levels of fetal hemoglobin (HbF) for the treatment of sickle cell disease.
The cells will be used only for therapeutic purposes in the same subject from whom the cells were obtained (autologous application).

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

The gene modification via CRISPR-Cas9 is not considered to have an influence on the genetic stability of the CD34$^+$ hHSPCs. Repair of the double strand break caused by CRISPR-Cas9 cleavage leads to the creation of Indels (Insertions and deletions) at the cleavage site. “On-target” Indels are confirmed by TIDE analysis (Tracking Indels by DEcomposition), which is part of CTX001 release specifications. With the selected gRNA, no “off-target” editing events have been detected during product development.

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

   Yes () No (X)

   If yes, insert the country code(s)
   Contained use notifications are planned in IT, GB, BE, and FR.

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 (decision pending)
- Notification number B/../../…

Contained use notifications were submitted in IT and UK.

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 US
- Notification number B/../../…; B/../../… (not applicable for US)

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

The IMP is not released into the environment. No environmental impact is expected from the highly controlled administration of CTX001 IMP to a limited number of subjects in clinical study CTX001-121.
The GMO/IMP (CTX001) consists of autologous genetically-modified $CD34^+$ cells modified *ex vivo*, which are intended for a single subject. CTX001 cells are used as a source of hHSPCs that will ensure a hematopoietic reconstitution after administration. Upon intravenous infusion, genetically-modified $CD34^+$ cells are expected to mainly migrate to lymphoid organs and to the bone marrow similar to their unmodified counterparts and will therefore not spread from the subject to another person or to the environment. The IMP is also not viable outside the body of the specific recipient. Additionally, no vectors are used in the manufacturing of the IMP, thus no vector shedding or transfer of any vector sequences can occur. In case the cells would be exposed to the environment, e.g. by being accidentally released from their container, they would not be viable, as they can only survive *ex vivo* under special cell culture conditions in a CO_2 incubator at 37°C in cell culture media.

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:

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	Homo sapiens
(iv)	subspecies	Homo sapiens sapiens
(v)	strain	$CD34^+$ Autologous Hematopoietic Stem Cells
(vi)	pathovar (biotype, ecotype, race, etc.)	N/A
(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)

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

Atlantic	X
Mediteranean	X
Boreal	X
Alpine	X
Continental	X
Macaronesian	X

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

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

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

4. Natural habitat of the organism

(a) If the organism is a microorganism

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

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

5. (a) Detection techniques

Common techniques of blood cell analysis (e.g. flow cytometry)

(b) Identification techniques

Common techniques of blood cell analysis (e.g. flow cytometry);
Labelled with donor/recipient code, donation identification number (DIN), collection center name and address, date and time of collection, collection and anti-coagulant volumes, ISBT128 product code, cell type ("HPC, APHERESIS"), sponsor name and address and the statement "for autologous use only." Some of this information will be barcoded.

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
…

8. Information concerning reproduction:

Not applicable for human cells. After administration to the subjects, CTX001 will proliferate mitotically, similar to non-transfected $CD34^+$ hHSPCs, to reconstitute the hematopoiesis of the subject.

(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: …

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 $CD34^+$ cells requires a complex combination of cell culture conditions in a CO_2 incubator at 37°C in cell culture media. The environmental conditions *ex vivo* are substantially different and will not support the cells' survival (temperature, pH, UV and a change in the biophysical and biochemical conditions).

10. (a) Ways of dissemination

Human $CD34^+$ cells can only be transmitted between individuals through infusion. No dissemination in the environment is expected as CTX001 will graft into the subject to reconstitute hematopoiesis. Apart from infusion there is no natural entry route into the body. Genetically modified cells can disseminate in the blood of treated patients. Risks from this are no greater than from unmodified human blood.

(b) Factors affecting dissemination

Not applicable. See above.

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**

This particular CRISPR-Cas9 approach leads to a site-specific double strand break (DSB) by Cas9. The permanent genetic editing results from the cell's endogenous non-homologous end-joining (NHEJ) repair pathway.

1. Type of the genetic modification

(i)	insertion of genetic material	(.)
(ii)	deletion of genetic material	(.)
(iii)	base substitution	(.)
(iv)	cell fusion	(.)
(v)	others, specify	Indel creation by endogenous NHEJ following Cas9 DSB

2. Intended outcome of the genetic modification

The goal of the genetic modification is to disrupt in a site-specific and permanent fashion the erythroid lineage-specific enhancer of B-Cell Lymphoma/Leukemia 11A (BCL11A). The permanent genetic editing results from the cell's endogenous NHEJ repair pathway at the targeted Cas9 cleavage site. After hematopoietic reconstitution, the reduced expression of BCL11A will reactivate transcription and expression of γ-globin, resulting in increased levels of HbF for the treatment of sickle cell disease.

3. (a) Has a vector been used in the process of modification?

Yes (.) No (X)

If no, go straight to question 5.

(b) If yes, is the vector wholly or partially present in the modified organism?

Yes (.) 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	(.)
cosmid	(.)
transposable element	(.)
other, specify	…

(b) Identity of the vector

…

(c) Host range of the vector

…

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

Yes (.) No (.)

antibiotic resistance	(.)
other, specify	…

Indication of which antibiotic resistance gene is inserted

…

(e) Constituent fragments of the vector

…

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

(i)	transformation	(.)
(ii)	electroporation	(.)
(iii)	macroinjection	(.)

(iv) microinjection (.)
(v) infection (.)
(vi) other, specify …

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

(i) transformation (.)
(ii) microinjection (.)
(iii) microencapsulation (.)
(iv) macroinjection (.)
(v) other, specify *Ex vivo* electroporation of subject autologous $CD34^+$ hHSPCs with CRISPR-Cas9 ribonucleoprotein (RNP) complex

6. Composition of the insert

This section is not applicable as no insert will be integrated by CRISPR-Cas9. No vector is used. The permanent genetic modification results from the specific editing process. Indels (insertions and deletions) are created by endogenous NHEJ following Cas9 DSB.

(a) Composition of the insert

There is no insert. The CRISPR-Cas9 editing components are transiently introduced into the target cell population by electroporation as a RNP complex consisting of a synthetic BCL11A-specific gRNA and Cas9 protein. This transient manipulation results in a site-specific DSB at the erythroid lineage-specific enhancer of the BCL11A gene on chromosome 2. The DSB will be repaired by the cell's endogenous NHEJ pathway to increase gamma-globin and thus HbF.

(b) Source of each constituent part of the insert

There is no insert. This section is not applicable.

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

There is no insert. This section is not applicable.

(e) Location of the insert in the host organism

There is no insert. This section is not applicable.

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

(f) Does the insert contain parts whose product or function are not known?
Yes (.) No (.) There is no insert. This section is not applicable.
If yes, specify …

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

This section is not applicable as no insert will be integrated by CRISPR-Cas9. No vector is used. The permanent genetic modification results from the specific editing process.

1. Indicate whether it is a:

viroid (.)
RNA virus (.)
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 …
(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 (.) Not known (.)
If yes, specify the following:

(b) to which of the following organisms:

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

(b) are the donated sequences involved in any way to the pathogenic or harmful properties of the organism
Yes (.) No (.) Not known (.)

If yes, give the relevant information under Annex III A, point II(A)(11)(d):
…

4. Is the donor organism classified under existing Community rules relating to the protection of human health and the environment, such as Directive 90/679/EEC on the protection of workers from risks to exposure to biological agents at work?

Yes (.) No (.)

If yes, specify …

5. Do the donor and recipient organism exchange genetic material naturally?

Yes (.) No (.) 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 gene modification via CRISPR-Cas9 is not considered to have an influence on the genetic stability of the $CD34^+$ hHSPCs. The site-specific cleavage by Cas9 forms a double strand break (DSB). The DSB is repaired by the cell's endogenous non-homologous end-joining (NHEJ) pathway and leads to the creation of Indels (Insertions and deletions) at the cleavage site. All "on-target" Indels are confirmed by TIDE analysis (Tracking Indels by DEcomposition), which is part of CTX001 release specifications. With selected gRNA no "off-target" editing events have been detected during product development.

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

The final GMO is not released into the environment. It is infused into the same subject from whom the autologous cells were originally obtained.

(b) Techniques used to identify the GMO

TIDE (Tracking Indels by DEcomposition) analysis

F. Information relating to the release

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

The IMP is not released into the environment. CTX001 IMP will be administered to a limited number of subjects in a highly controlled hospital environment in clinical study CTX001-121.
In case the genetically modified cells would be exposed to the environment, e.g. by being accidentally released from their container, they would not be viable, as they can only survive *ex vivo* under special cell culture conditions in a CO_2 incubator at 37 °C in cell culture media.

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): Not applicable

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

(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: Not applicable

4. Method and amount of release

(a) Quantities of GMOs to be released:

The GMO/IMP is not released into the environment. The GMO will be administered intravenously to the subjects at a concentration of at least 3 x 10^6 viable $CD34^+$ cells per kg (subject weight).

(b) Duration of the operation:

CTX001 will be thawed just prior to the scheduled infusion utilizing local site Standard Operating Procedures (SOPs) and infused within 20 minutes of thaw. On average each vial is thawed in 10-15 minutes in a water bath with a temperature between 37° to 40° C.

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

The GMO/IMP is not released into the environment. The GMO is administered intravenously into the subject under standard controlled conditions for hematopoietic stem cell transplant at the clinical site.
The GMO will be prepared at the GMP manufacturing facility and shipped cryopreserved to the clinical study sites in sealed dry nitrogen containers. The GMO will be stored at ≤ -135°C until shortly prior to the scheduled infusion. The administration will occur at the bedside of the subject within the hematopoietic transplantation unit of the site with restricted personnel access. Personnel responsible for the administration of the GMO have wide experience in stem cell transplantation, will receive training on the administration procedures and will follow Good Clinical Practice rules and institutional standard operating procedures. All waste will be destroyed according to hospital bio-hazard disposal procedures.

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

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

The $CD34^+$ hHSPCs will be obtained from the target organism (subject) and will be re-introduced into the same target organism after genetic modification. The interaction of the modified cells with the recipient are similar to interactions of unmodified cells from transplants. The GMO/IMP is not released into the environment.

1. Name of target organism (if applicable)

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

2. Anticipated mechanism and result of interaction between the released GMOs and the target organism (if applicable)

   The GMO/IMP (CTX001) consists of genetically-modified $CD34^+$ cells modified *ex vivo*, which are intended for a single, subject autologous application. CTX001 cells are used as a source of hHSPCs that will ensure a hematopoietic reconstitution after administration. Upon intravenous infusion, genetically-modified $CD34^+$ cells will mainly migrate to lymphoid organs and to the bone marrow and reconstitute the hematopoiesis of the subject with increased levels of HbF for the treatment of sickle cell disease.

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 GMO/IMP is not released into the environment. CTX001 cells are not viable in the environment outside of the intended recipient and *ex vivo* they can only survive under special cell culture conditions.

5. Types of ecosystems to which the GMO could be disseminated from the site of release and in which it could become established

   The GMO/IMP is not released into the environment. CTX001 cells are not viable in the environment outside of the intended recipient and *ex vivo* they can only survive under special cell culture conditions.

6. Complete name of non-target organisms which (taking into account the nature of the receiving environment) may be unintentionally significantly harmed by the release of the GMO

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:

None

(b) from other organisms to the GMO:

None

(c) likely consequences of gene transfer:

None

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.):
Not applicable

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 manufacturing, the gene modification frequency of the GMO/IMP is monitored by TIDE analysis.

Upon infusion, subjects will be tested as per the assessment schedule for $CD34^+$ cell count, percentage of edited cells, α- and γ-globin assessment, and quantification of HbF-containing cells.

2. Methods for monitoring ecosystem effects

Not applicable. The GMO/IMP is not released into the environment.

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

Not applicable. The GMO/IMP is not released into the environment.

4. Size of the monitoring area (m^2)

Not applicable. The GMO/IMP is not released into the environment.

5. Duration of the monitoring

The GMO/IMP is not released into the environment. The subjects will be followed-up for a period of up to 15 years after the treatment.

6. Frequency of the monitoring

The GMO/IMP is not released into the environment. Subjects are monitored continuously during the conditioning and transplant portion of the protocol, as they are hospitalized during that time. Upon discharge, subjects will be monitored during monthly study visits during the first 6 months following administration of the GMO, then quarterly for the remainder of the first year and finally every 6 months during the second year following GMO treatment. Subjects will then be enrolled in a long-term follow-up protocol with twice yearly visits for 5 years and yearly visits thereafter up to 15 years after the treatment.

I. Information on post-release and waste treatment

1. Post-release treatment of the site

The GMO/IMP is not released into the environment. It is administered intravenously into the subject under standard controlled conditions for hematopoietic stem cell transplantation at the clinical site.

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

All manipulations of the $CD34^+$ cells and the IMP are carried out under controlled conditions in a licensed GMP manufacturing facility located in the EU.

2. Post-release treatment of the GMOs

The GMO/IMP is not released into the environment. The IMP is infused into the subject as a one-time therapeutic treatment.

3. (a) Type and amount of waste generated

Waste is generated such as vials containing the genetically modified $CD34^+$ hHSPCs, tubing, gloves, paper towels, needles, syringes, cotton balls, dry adhesives, and disposable garments. Sharps (needles etc.) will be stored in different specific containers appropriately labelled. Waste and residues generated during handling of the IMP are minimal and expected for this type of procedure.

3. (b) Treatment of waste

All waste is destroyed according to hospital 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

   It is not possible for the IMP to spread into the environment as CTX001 cells are not viable outside of the intended recipient and *ex vivo* they can only survive under special cell culture conditions.

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

   In case of accidental spill of the CTX001 cells, hospital decontamination and cleaning procedures are applied. All waste is destroyed according to hospital bio-hazard disposal procedures.

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

   Subjects are monitored for 24 months after CTX001 infusion according to the clinical protocols. Subjects will be enrolled in a long-term follow-up protocol for up to 15 years after the treatment. Additionally, CTX001 cells are not viable outside of the intended recipient and *ex vivo* they can only survive under special cell culture conditions. Therefore, no undesirable effects are expected for the environment.