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	Belgium
(b)	Notification number	B/BE/18/BVW5
(c)	Date of acknowledgement of notification	20/06/2018.
(d)	Title of the project	A prospective, multi-center, Phase 1b/2a study to assess the safety and tolerability of different doses of AG019 administered alone or in association with teplizumab in subjects with clinical recent-onset Type 1 Diabetes Mellitus (T1D).
(e)	Proposed period of release	From 01/08/2018 until 31/08/2019

2. Notifier

Name of institution or company: Intrexon T1D Partners, LLC.

3. GMO characterisation

(a) Indicate whether the GMO is a:

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

specify phylum, class: …

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

Lactococcus lactis

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

L. lactis strains have been used in food production. No particular factors have been identified. The growth of *L. lactis*, in particular of MG1363, is largely determined by the specific ecological niche.

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

Yes () No (X)

If yes, insert the country code(s):

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

Yes (.) No (X)

If yes:
- Member State of notification …
- Notification number 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 …
- Notification number B/../../…

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

The GMO is an environmentally contained strain of *Lactococcus lactis*. It is only able to grow in artificial laboratory cultures and is totally dependent on supplementation of thymine/ thymidine to the medium.
The GMO will be administered orally to patients with recent-onset type 1 diabetes mellitus (T1D). The organism does not colonize the gastrointestinal tract. Live organisms are likely shed in stools at low levels for about 3 days. Shedding will constitute the release of the organism and potentially, it could be released into the sewage system. Normal hygiene (hand washing) is considered sufficient to prevent transmission from person to person.

The GMO has no selective advantage in the environment. It is not invasive and does not persist in the environment. The potential for exchange of genetic material is extremely low, as the organism does not harbor plasmids or conjugative transposons and phage replication is severely hindered as it is not able to produce thymidine.

In vitro studies performed with this GMO, as well as clinical data obtained with similar GMOs, have consistently demonstrated that these types of GMOs are safe and well tolerated, and demonstrated not only that the biological containment strategy was safe but also provided indications for clinical efficacy.

In summary, the risk assessment for this study shows a very low risk associated with administering the GMO to patients. The risk to other humans is negligible and the risk to the environment is estimated to be practically zero.

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 (X)
fungus (.)
animal:
- mammals (.)
- insect (.)
- fish (.)
- other animal (.)

specify phylum, class: …

other, specify …

2. Name

(i)	order and/or higher taxon (for animals)	Lactobacillales
(ii)	genus	*Lactococcus*
(iii)	species	*L. lactis*
(iv)	subspecies	subsp. *cremoris*
(v)	strain	MG1363
(vi)	pathovar (biotype, ecotype, race, etc.)	
(vii)	common name	

3. Geographical distribution of the organism:

(a) Indigenous to, or otherwise established in, the country where the notification is made:

Yes (.) No (X) Not known (.)

While the wild type *L. lactis* is indigenous and globally present, *L. lactis* subsp. *cremoris* MG1363 (hereafter referred to as '*L. lactis* MG1363' or MG1363') is a strain incapable of survival outside of artificially supplemented laboratory conditions.

(b) Indigenous to, or otherwise established in, other EC countries:

(i) Yes (.)

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

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

(ii) No (X)

(iii) Not known (.)

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

Yes (X.) No (.)

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

Yes (X) 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	

L. lactis MG1363 can only grow in artificially supplemented media and is restricted to laboratory cultures.

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

5. (a) Detection techniques

Standard microbial techniques.
Molecular techniques based on 16S rRNA PCR and sequencing.

Specific culture media requirements.

(b) Identification techniques

Same as above:

- Standard microbial techniques.
- Molecular techniques based on 16S rRNA PCR and sequencing.

L. lactis MG1363 can only grow in artificially supplemented media and is restricted to laboratory cultures.

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

Yes () No ()

If yes, specify:

EFSA introduced the concept of "Qualified Presumption of Safety" (QPS) in relation to a generic approach for safety assessment of micro-organisms used in food/feed and the production of food/feed additives. In 2013, a first list of microorganisms with QPS recommendation was published. Since 2013, this list is reviewed by EFSA's Panel on Biological Hazards (BIOHAZ) annually. *Lactococcus lactis* received QPS recommendation in 2013 as a gram-positive non-sporulating bacteria. Last scientific opinion on the update of the list of QPS-recommended biological agents intentionally added to food or feed as notified to EFSA, published in March 2017, concluded that there is no need to change the QPS recommendation of *L. lactis* (EFSA, 2017).

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

(a) Generation time in natural ecosystems:

L. lactis MG1363 is restricted to artificial laboratory growing conditions. In optimal culture circumstances, the generation time is 30 minutes.

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

The GMO will be released in the sewage system after administration to patients. *L. lactis* MG1363 is not able to grow outside the laboratory.

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

(d) Factors affecting reproduction:

L. lactis MG1363 can only grow in artificially supplemented culture conditions.

9. Survivability

(a) ability to form structures enhancing survival or dormancy:

(i)	endospores	(.)
(ii)	cysts	(.)
(iii)	sclerotia	(.)
(iv)	asexual spores (fungi)	(.)
(v)	sexual spores (fungi)	(.)
(vi)	eggs	(.)
(vii)	pupae	(.)
(viii)	larvae	(.)
(ix)	other, specify	none

(b) relevant factors affecting survivability:

L. lactis MG1363 can only grow in artificial laboratory conditions.

10. (a) Ways of dissemination

Dispersal of the bacteria is essentially passive.

(b) Factors affecting dissemination

No specific factors.
Passive dissemination with medium. The survival time outside of the laboratory is very short.

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)

B/BE/07/BVW1

C. Information relating to the genetic modification

1. Type of the genetic modification

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

2. Intended outcome of the genetic modification

The gene for human interleukin-10 (*hil-10*) has been inserted in the bacterial chromosome, replacing the *thyA* gene and promoter encoding thymidylate synthase. The accompanying regulatory sequences are aimed at secreting hIL-10.
The gene for human proinsulin (*pins*) has been inserted in the bacterial chromosome. The accompanying regulatory sequences are aimed at secreting hPINS.
The gene for trehalose-6-phosphate phosphatase (*otsB*) has been inserted in the bacterial chromosome; aimed at removing the phosphate from trehalose-6-phosphate to produce free trehalose.
The gene for trehalose-6-phosphate phosphorylase (*trePP*) is absent, aiming at accumulate exogenously added trehalose.
The gene for cellobiose-specific PTS system IIC component (*ptcC*) is disrupted, aiming at retention of trehalose after accumulation.
A constitutive promoter preceeds the putative phosphotransferase genes in the trehalose operon (*trePTS*) to potentiate trehalose uptake.

Upon administration to patients, the proteins are targeted to induce antigen specific immune tolerance in recent-onset T1D patients.
Deleting the *thyA* gene resulted in strict thymine/thymidine dependency, not only for growth but also for survival of the GMO (*thymine-less death*).
The ability of the GMO to accumulate trehalose during the productin process makes the GMO more resistant to bile acid lysis and therefore prolongs the survival of the GMO in the GI tract.

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 (.) No (X)

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

6. Composition of the insert

(a) Composition of the insert

Insertion of a gene encoding a secretion leader fused to the *hil-10* gene, encoding hIL-10 (UniProt P22301, aa 19-179, variant P2A).
Insertion of a gene encoding a secretion leader fused to the *pins* gene, encoding human proinsulin (UniProt P01308, aa 25-110).
Insertion of trehalose-6-phosphate phosphatase gene (*otsB*, Gene ID 1036914)

(b) Source of each constituent part of the insert

The promoters and the secretion leader are from *L. lactis* MG1363.
hil-10 is a synthetic gene derived from the *hil-10* gene with codon optimization for expression in *L. lactis*.
pins is a synthetic gene derived from the human preproinsulin gene.
Trehalose-6-phosphate phosphatase gene is a synthetic gene derived from *E. coli*.

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

The promoters are used to drive expression of the *hil-10*, *pins* and *otsB* genes. The secretion leader encodes an extracellular secretory protein that enables the GMO to secrete hIL-10 and hPINS in the gastrointestinal tract after administration to the patient. The hIL-10 protein is a tolerance promoting cytokine. Human proinsulin is an autoantigen involved in type 1 diabetes.

Trehalose-6-phosphate phosphatase removes the phosphate from trehalose-6-phosphate to produce free trehalose. Intracellular accumulation of free trehalose protects *L. lactis* from bile toxicity.

(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 (.)
DNA virus (.)
bacterium (X)
fungus (.)
animal:

- mammals (.)
- insect (.)
- fish (.)
- other animal (.)

specify phylum, class: …

other, specify: synthetic (copying human IL-10 and pins) and *E. coli* (otsB)

2. Complete name

hIL-10 and hPINS:

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

otsB:

(j)	order and/or higher taxon (for animals)	Bacteria
(ii)	family name for plants	…
(iii)	genus	Escherichia
(iv)	species	…
(v)	subspecies	…
(vi)	strain	Escherichia coli DH5-alpha
(vii)	cultivar/breeding line	…
(viii)	pathovar	…
(ix)	common name	E. coli

This strain of E. coli was developed in the laboratory, for laboratory cloning procedures, and therefor has no 'natural' environment or habitat as it does not exist in nature. (https://microbewiki.kenyon.edu/index.php/DH5-Alpha_E.coli)

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 () No (X)

If yes, specify: Class 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 (X) No (.) Not known (.)

If yes, specify:

The GMO is dependent on addition of thymine/thymidine to the growth medium.

(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) Not known (.)

If yes, specify: …

(c) is the GMO in any way different from the recipient as far as dissemination is concerned?

Yes (.) No (X) Not known (.)

If yes, specify: …

(d) is the GMO in any way different from the recipient as far as pathogenicity is concerned?

Yes (.) No (X) Not known (.)

If yes, specify: …

2. Genetic stability of the genetically modified organism

The strain sAGX0407 is stored in Intrexon Actobiotics culture collection, position number 1757, date of deposit June 5th, 2012. Genetic stability of *L. lactis* strain sAGX0407 was documented on 90 sAGX0407 siblings obtained following a simulation of growth to obtain 10^{16} CFU biomass, containing trehalose (Van Huynegem, 2017). This process, starting from a single colony until the end of the simulation of the bulk fermentation corresponded with 61.76 generations of growth. Subsequent to this simulation:

- As sAGX0407, all of the 90 tested sAGX0407 siblings were unable to grow in thymidine-deficient medium, confirming the preservation of the *thyA*-negative containment system.
- As sAGX0407, all of the 90 tested sAGX0407 siblings showed adequate hPINS and hIL-10 secretion potency.
- As sAGX0407, all of the 90 tested sAGX0407 siblings showed adequate bile resistance capacity after growth to saturation in medium that is supplemented with 500 mM trehalose.
- All of the 90 tested sAGX0407 siblings showed identical growth curves to sAGX0407 in GMAPFLT + 500 mM trehalose medium.
- PCR analysis revealed that for all of the 90 tested sAGX0407 siblings, the modified loci (*pins*, *hil-10* (Δ*thyA*), P*xxx*>*trePTS* (Δ*trePP*), *ptcC* and *otsB*) had the excepted sizes. Furthermore, DNA sequencing of these PCR fragments revealed that for all of the 90 tested sAGX0407 siblings, the DNA sequence of the modified loci *pins*, *hil-10* (Δ*thyA*), P*xxx*>*trePTS* (Δ*trePP*), *ptcC* and *otsB* was identical to that of sAGX0407.

Quality of the material is monitored during storage and production. As the insertion occurred on the bacterial chromosome, the stability of the genetic trait is expected to be similar to any other chromosomal trait. No instability created by transposons is anticipated.

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

Yes (.) No (X) Not known (.)

(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)

L. lactis are non-pathogenic bacteria, critical in manufacturing dairy products such as buttermilk, yogurt and cheese. In spite of the widespread use and massive discharge in the environment, *Lactococci* have not been identified as invasive or disruptive. Although they can be found in very diverse sources (soil, manure, waste water), the bacteria depend on particular nutritional components for growth. MG1363 is restricted even more and as such confined to artificially supplemented culture conditions. On top of that, the GMO is dependent on thymine/thymidine/ supplementation.

There is no indication that the GMO itself is toxic, allergenic or pathogenic. The changes that were induced in the recipient strain MG1363 as well as in the GMO, do not affect the basic toxic or allergenic features. In the unlikely event of infection, the GMO can quickly and easily be inactivated with standard antibiotics.

Systemic injection of high doses of hIL-10 has been documented to cause important side effects. The delivery system in this study avoids the exposure to high systemic doses by localized expression in the gastrointestinal tract. The doses are much lower and act only locally. The exposure is also limited in time as the bacteria are evacuated from the gastrointestinal tract in a period of a few days following administration.

hPINS has little biological or pharmacological activity. Its sole purpose is to induce an antigen specific immune response. Systemic injection of high doses of hPINS has been documented to induce little side effects. In addition, the delivery system in this study avoids the exposure to high systemic doses by localized expression in the gastrointestinal tract. The doses are very low and act only locally. The exposure is also limited in time as the bacteria are evacuated from the gastrointestinal tract in a period of a few days following administration.

L. lactis does not colonize the gastrointestinal tract.

4. Description of identification and detection methods

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

- Through PCR amplification of 16S rRNA and subsequent sequencing of the PCR fragment, the species identity of sAGX0407 was established as *Lactococcus lactis* subspecies *cremoris* MG1363 during the manufacturing of the master cell bank. In addition, the presence of the *hIL-10* gene, the *pins* gene and the absence of the *thyA* gene was also demonstrated by sequencing.
- Enzyme-Linked ImmunoSorbent Assay (ELISA) was used to quantify the levels of hIL-10 and hPINS secreted by *L. lactis* sAGX0407.

(b) Techniques used to identify the GMO

- PCR and sequencing methods result in clear-cut identifications.
- Detection limit of the hIL-10 ELISA is 5 pg/ml; the assay is specific with respect to various other cytokines, growth factors, etc. The detection limit of the hPINS ELISA is 30 pg/ml.

F. Information relating to the release

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

   The proposed clinical study consists of 2 phases:

   - Phase 1b: this open label part of the study will investigate the safety and tolerability of different doses of AG019, in 2 age groups (≥18 years of age and 12-17 years of age, respectively)
   - Phase 2a: this randomized, double blind part of the study will investigate the safety and tolerability of different doses of AG019, in association with teplizumab, in 2 age groups (≥18 years of age and 12-17 years of age respectively).

   AG019 is the lyophilized powder of the GMO, formulated for oral administration as a gastro-resistant capsule, hard.

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 (X) No (.)

   If yes, specify:

   The parental strain MG1363 can only grow in laboratory conditions. The GMO will be administered to patients orally and will follow the normal flow of faeces. As these are outpatient studies, the shedding will occur at the patient's home or elsewhere.

3. Information concerning the release and the surrounding area

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

   Following sites will be initially included in the proposed Phase 1b/2a study:
   - UZ Gasthuisberg, Leuven, Belgium
   - UZ Brussel, Brussel, Belgium
   - UZ Antwerpen, Edegem, Belgium

   However, the identity and coordinates of the patients will not be known to the notifier. In addition, shedding will occur mainly during the evacuation of stool. This is not necessarily limited to the home of the patient. In consequence, the national territory is considered as the wider potential release area. Patients will be recommended not to leave the country during the treatment due to the constraints imposed by the design of the clinical trials.

(b) Size of the site (m^2): … m^2

(i) actual release site (m^2): … m^2
(ii) wider release site (m^2): … m^2

Not applicable

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

The proximity of significant biotopes, protected areas or drinking water supplies can not be excluded as possible sites of release. However, the only route for exposure would be via the disposal of stool, which would in any event not be expected to reach such areas. In addition, if this would be the case, one can expect that already today exposure to *L. lactis* is occurring as it is a natural component of dairy products. The GMO has no additional features that make exposure more likely, on the contrary, the strict dependence on specific components and the self-eliminating thymine/thymidine dependency makes any exposure even more limited in time.

(d) Flora and fauna including crops, livestock and migratory species which may potentially interact with the GMO

L. lactis does not interact with fauna and flora. No involvement in particular environmental processes is known.

4. Method and amount of release

(a) Quantities of GMOs to be released:

A total of 48 patients are involved in the study. In the Phase 1b portion, in each of the 4 cohorts, 2 single dose patients and up to 6 repeat dose patients will be treated. Single dose patients will receive a dose of 2 or 6 capsules for one day. Repeat dose patients will receive 2 or 6 daily capsules for 8 weeks.
In the Phase 2b portion, in each of the 2 cohorts, 10 repeat dose patients will receive 6 daily capsules for 8 weeks.
On the total of maximum 48 patients to enroll in the whole study, 10 will be enrolled in Belgium; the other 38 in the US.

(b) Duration of the operation:

Recruitment of the first participants is expected to start in August 2018. Completion of recruitment will depend on availability of participants fulfilling the selection criteria and is estimated to take until end of June 2019. For each individual participant, a treatment (release) period of 8 weeks is envisaged. Therefore, the expected release period of the GMO will be from 01/08/2018 to 31/08/2019.

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

Each patient will receive a treatment package containing ready-to-use doses for a limited period, corresponding to the interval between follow-up visits (a maximum interval of 4 weeks is foreseen).
In the event that the packaging would be disrupted, the powder quickly degrades when in contact with moist or warmth. The organism is sensitive to temperatures above 40°C, low pH, air drying, direct sunlight, UV, soap, bleaching agents, antibiotics and high salt. The quantity of a spillage will be limited (one capsule) and the affected area can be decontaminated with a standard detergent (soap) or bleach.

Patients are examined regularly. Normal hygiene conditions for clinical staff handling patient's body fluids (in particular stool) should be sufficient. Disposable gloves and disposable wipes should be used when handling devices for analysis and biopsies. All waste material should be handled as hazardous medical waste.
While shedding of live bacteria during the treatment period and up to a few days after the last application is realistic, the biological containment and the absence of relevant impact is deemed sufficient not to warrant any specific treatment of the shedding environment.

If required, a standard antibiotic treatment would suffice to inactivate the bacteria.

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

Environmental conditions will be those of the sewage system.

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.

sAGX0407 has never been released in the environment.
However, another ActoBiotics® developed by Intrexon Actobiotics, AG011, has been studied in a Phase1b/2a in Crohn's disease patients under deliberate release in Belgium and in other European countries.
Presence and kinetics of the strain release in the stool of patients were assessed by conventional culturing and quantitative PCR. Compared to the amount of intake, a significant decrease in amount of culture forming units (CFU) was detected in faeces.
The results obtained demonstrated the efficacy of the chosen biologic containment strategy.
Moreover, two other ActoBiotics® have previously been evaluated by the Belgian Agency and approved for a clinical trial under the "contained use-only" framework:

- AG013: L. lactis bacteria secreting human trefoil factor 1, EudraCT number 2012-000621-50
- AG014: L. lactis bacteria secreting certolizumab (anti-TNFα antibody), EudraCT number 2014-000190-39

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):

The target organisms are a specific group of patients (with Crohn's disease and ulcerative colitis).

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

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

Human patients will ingest the GMO orally. The organism will reach the gastrointestinal tract and will produce hIL-10 and hPINS. The proteins are expected to induce antigen specific immune tolerance and thus revert or delay the autoimmune beta cell destruction which causes T1D.

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

hIL-10 expression only triggers an effect on human cells that have the appropriate receptors. These receptors are highly specific and other organisms that might react to IL-10 have specific receptors with little or no cross-reactivity towards hIL-10.
hPINS has no significant bioactivity, it is merely used in order to induce an antigen specific immune response.

4. Is post-release selection such as increased competitiveness, increased invasiveness for the GMO likely to occur?

Yes (.) No (X) Not known (.)

Give details:

Compared to the wild type *L. lactis* and the parental strain MG1363, the GMO is reduced in its capacities.

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

Once administered, the GMO passes the intestines and will be evacuated via stool and eventually via the sewage system. The GMO is not able to survive, let alone establish, in this 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

(i)	order and/or higher taxon (for animals)	No specific interactions with non-target organisms have been identified.
(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:

MG1363 does not contain plasmids or conjugative transposons. The GMO is thymine/thymidine dependent, severely hindering phage replication. Therefore, transduction of modified genetic material via phages is very unlikely.
Genetic elements could be released in the environment upon lysis and might be taken up by other bacteria. In the case of the GMO, the likelihood of release of intact naked DNA is reduced as *thymine-less death* triggers the degradation of DNA before the actual cell lysis.

(b) from other organisms to the GMO:

MG1363 can only act as a recipient of conjugative transposition.
The only relevant risk is transfer of an intact *thyA* inwards. In the *Bacteriae* and *Archaeae*, *thyA* genes do not reside on plasmids, so plasmid borne mobility of *thyA* inwards is impossible. Theoretically, the gene for thymidine production might be regained via homologous recombination with a natural strain. This has not been demonstrated to be possible. Also, once released in the environment, the bacteria no longer grow or replicate. Hence, no selection for *thyA* is possible.

(c) likely consequences of gene transfer:

In the highly unlikely event that *hil-10* or *pins* is transferred to other organisms, it would give no selective advantage to those organisms.

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.):

Information not available.

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

No potential interactions with biogeochemical processes have been identified.

H. Information relating to monitoring

1. Methods for monitoring the GMOs

The *hIL-10* gene in the GMO is a unique, synthetic gene that can be distinguished from the native *hIL-10* gene and detected via PCR. A method has also been developed to distinguish between live and dead bacteria. ELISA methods are available to detect hIL-10 and hPINS expressed by sAGX0407.
The gene encoding a fusion of the secretion leader with the pins gene is a unique, synthetic gene that can be distinguished from the native preproinsulin gene and detected via PCR.

2. Methods for monitoring ecosystem effects

Not planned.

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

To detect the hypothetical transfer of donated genetic material to other organisms, PCR aimed at the *hIL-10* gene and/or *hPINS* gene can be used.

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

Not relevant.

5. Duration of the monitoring

The last time point for, monitoring of the GMO in stool samples is 8 days after the end of the treatment.
No other monitoring at the exception of treated patient faeces and blood sampling has been planned.

6. Frequency of the monitoring

Monitoring is planned at baseline (before the start of the treatment), at the last day of treatment and at 2, 4, 6 and 8 days after treatment cessation.

I. Information on post-release and waste treatment

1. Post-release treatment of the site

The clinical trial centers will disinfect equipment and surfaces according to standard medical procedures.
Patients will leave the clinical setting during treatment. Although shedding of live bacteria will occur, the biological containment and the absence of relevant impact are deemed sufficient not to warrant any specific treatment of the shedding environment.

2. Post-release treatment of the GMOs

Given the biological containment which combines several inherent inactivation factors, no additional inactivation is foreseen. If required, a standard antibiotic treatment would suffice to inactivate the bacteria.

3. (a) Type and amount of waste generated

Two types of waste possibly carrying living GMOs are identified:

- Disposable materials that have been exposed to bacterial material (e.g. empty containers, patients' disposable gloves, wipes, etc.).
- Faeces and faecal traces, hygienic wipes, disposed of in sewage system.

(b) Treatment of waste

Disposable items will be inactivated as hazardous medical waste by incineration or other validated method.
Faeces end up in the sewage system. The biological containment does not require additional treatment. Moreover, the sewage treatment system is designed to eliminate bacteria.

J. Information on emergency response plans

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

Unexpected spread would mainly be limited to accidental opening of the packaged materials, releasing the lyophilized powder. Application of standard detergent (soap) or bleach would be sufficient to eradicate the GMOs and decontaminate the affected area.

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

Idem.

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

Idem. No specific sanitation measures are foreseen.

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

The bacteria can be inactivated with several treatments. Furthermore, the biological containment system is expected to eliminate the bacteria in a short period after the release. In addition, there are no indications of possible undesirable effects on the environment.
L. lactis bacteria, and thus the GMO, are sensitive to all groups of commonly used antibiotics.

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