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GMO Deliberate Release Notification

INFORMATION FOR THE PUBLIC[1]

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).

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[1] *This document is in line with the "Guidelines To Compile The Public Dossier - Deliberate releases of genetically modified micro-organisms for experimental purposes (part B)" of the Biosafety Advisory Council (version of 26 februari 2003). Mandatory text is presented in italics.*

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1. REGULATORY FRAMEWORK AND AUTHORIZATION PROCEDURE

The release of genetically modified organisms (GMOs) in the environment is strictly regulated at European level by directive 2001/18/EC of 12 March 2001 repealing directive 90/220/EEC and at Belgian level by the Royal Decree of 21 February 2005 regulating the deliberate release and/or marketing of GMOs or products that contain GMOs into the environment repealing the Royal Decree of 18 December 1998.

To ensure the safe use of GMOs, the provisions of the Royal Decree above stipulate that the release of GMOs for experimental aims is prohibited without prior consent from the competent Minister. The decision is based on a thorough evaluation of the biosafety of the planned release, which is conducted by the Biosafety Advisory Council, composed of different Scientific Committees grouping independent experts from Belgian universities and governmental institutes.

To acquire the necessary authorization from the competent Minister, the company Intrexon T1D Partners, LLC. submitted an application dossier to the competent authority. On the basis of the advice of the Biosafety Council, the competent minister could grant permission to the company Intrexon T1D Partners, LLC. to conduct experiments with transgenic Lactococcus lactis AG019 as stipulated in the application ***B/BE/18/BVW5.***

The release will take place at locations in Flanders/ Brussels as a consequence of a clinical trial conducted at UZ Gasthuisberg (Leuven), UZ Antwerpen (Edegem) and UZ Brussel (Jette). It is expected to start on 01 August 2018 and to be completed by 31 August 2019.

2. DESCRIPTION OF THE GENETICALLY MODIFIED MICRO-ORGANISM (GMM):

The bacterium *Lactococcus lactis* strain MG1363 has been genetically modified to produce the therapeutic proteins human interleukin-10 (hIL-10) and human proinsulin (hPINS). These proteins are expected to stop the autoimmune destruction of pancreatic cells of patients recently diagnosed with type 1 diabetes mellitus (T1D). AG019 is the code for this GMM based drug product.

L. lactis is one of the most important microorganisms involved in the dairy industry. It is critical for manufacturing products like buttermilk, yogurt and cheese. *L. lactis* was originally isolated from raw milk and this is one of the few environments where it can survive. *L. lactis* can also be found in man and in animals, soil, effluent water and plants, but these environments do not sustain growth. *L. lactis* is non-disease causing (non- pathogenic) and does not build survival structures such as spores. The presence of the *il-10* and *pins* genes does not change that.

L. lactis strain MG1363 can no longer grow in milk or in any other natural environment. The genes coding for the enzymes required to use the necessary nutrients of milk are removed. As a consequence, MG1363 can only grow in artificially supplemented culture conditions.

The growth of the genetically modified MG1363 expressing hIL-10 and hPINS, is even more restricted: a gene needed for thymidine (a building block of DNA) production was removed from the bacteria genome. The in vitro culture of the recombinant strain is therefore dependent on addition of thymidine to the culture. Without thymidine, the bacterium cannot survive.

The gene construct introduced in AG019 genome also contains a signal to excrete the hIL-10 and hPINS proteins outside the bacterium. The bacteria will produce and excrete hIL-10 and hPINS in the intestines, to the benefit of the patient.

L. lactis bacteria are highly sensitive to acidic environments, such as the stomach and the duodenum (the part of the small intestine just after the stomach). To ensure that the bacteria can stay alive long enough to produce the hIL-10 and hPINS proteins, the bacteria were genetically modified to withstand this acidic environment.
Within a few days, the bacteria are released via the faeces. Only a limited number of bacteria survives the passage through the body and those that do will die soon afterwards.

3. TYPE AND PURPOSE OF THE ENVISAGED TRIAL:

A clinical trial, consisting of 2 phases, is planned:

- The first part of the trial will investigate the safety and tolerability of different doses of the AG019 genetically modified *L. lactis* bacteria in patients recently diagnosed with T1D
- The second part of the trial will investigate the safety and tolerability of different doses of the AG019 genetically modified *L. lactis* bacteria, combined with infusions of another drug under development called teplizumab, in patients recently diagnosed with T1D

AG019 is the code for the drug product containing the genetically modified bacteria. The AG019 bacteria are packaged in gastro-resistant capsules for oral intake. Two different doses of AG019 will be investigated in the first part of the study: 2 capsules per day and 6 capsules per day.

In the first part of the clinical trial, patients will take 2 or 6 capsules every day for 8 weeks.
In the second phase, patients will take 6 capsules every day for 8 weeks (if the safety profile of the 6 capsules per day regimen has been confirmed in the first part). In addition, during the first 12 days of this 8-week period, the patients will receive daily infusions of teplizumab. The patients in the second phase will either receive the drugs (AG019 + teplizumab), or will receive placebo. A placebo is a pharmaceutical formulation with no active ingredients that is not expected to have a therapeutic effect

Both trial phases are aimed at assessing safety, tolerability and potential activity of AG019, alone or in combination with teplizumab. In each phase, 24 patients will be recruited. This means that the clinical trial will enroll a total of 48 participants. Among these 48 patients, only 10 will be enrolled in Belgium.

The patients are selected according to very stringent criteria. This is why a multi-centre approach is required. The study is a so-called outpatient study i.e. the patients do not reside in the clinical trial centre (CTC) during the trial. In the hospital, the patients receive a treatment package with the drug and all necessary items, covering for a treatment interval set by the study protocol including user instructions. At regular times, the patient will return to the CTC for a study visit.

Experiments conducted in the laboratory and in animals have shown that this drug is safe and that the genetically modified bacteria composing this drug does not survive outside the human body.

4. RESEARCH AND DEVELOPMENT FRAMEWORK:

Type 1 diabetes mellitus (T1D) is an autoimmune disease in which beta cells in the pancreas – the cells in the body that create insulin – are broken down by the patient's own immune system. Although patients with type 1 diabetes are treated with insulin, it can still be difficult to maintain a good sugar balance. This

can lead to serious acute complications such as unconsciousness due to low blood sugar (insulin shock) or acidosis (diabetic coma). In the long term, an unsatisfactory blood sugar balance can lead to serious damage to the kidneys, eyes, nerves and heart. Therefore, it would be valuable if a treatment could be found that cure type 1 diabetes or is able to stop the degradation of the beta cells.

There is no known medical or surgical cure for T1D. Patients with T1D require lifelong insulin injections in order to maintain their sugar balance. Worldwide, many researchers and companies are trying to find a curative treatment for the disease.

Administration of hIL-10 and hPINS could be one such possible treatment. The main biological function of IL-10 is the limitation and termination of inflammatory responses and the regulation of the growth of several immune cells. It has been proposed that IL-10 plays a key role in modulating the immune response. Recombinant hIL-10 has been produced and tested in clinical trials for different indications, including rheumatoid arthritis, inflammatory bowel disease, psoriasis, organ transplantation, and chronic hepatitis C. By combining hIL-10 with hPINS, the treatment will only be directed towards the cells which are responsible for the destruction of the beta cells in the pancreas, the rest of the immune system will not be affected.

Injections of high doses of hIL-10 have been documented to cause side effects. These effects mainly include lowering the number of red blood cells (anemia), lowering the number of blood platelets (thrombocytopenia), headache and/or fever.

Intrexon T1D Partners, LLC. is developing the ActoBiotics® delivery system that is based on a living non-pathogenic *L. lactis* strain for local delivery of therapeutics in the gut. The ActoBiotics® delivery system reduces the need for high doses by localized delivery to the gastrointestinal tract. The microorganism passes through the gut within a few days after oral administration. The studies described here use a modified *L. lactis* strain secreting recombinant hIL-10 and hPINS.

Furthermore, the bacterium contains the gene for hIL-10, stably inserted in the chromosome where it replaces a gene needed for thymidine production (a building block of DNA). The in vitro culture of the recombinant strain is therefore dependent on addition of thymidine to the culture Without thymidine, the bacterium cannot survive.
The bacterium also contains the gene for hPINS, stably inserted in the chromosome.
Lastly, the bacteria have been modified to enable them to survive through the stomach and intestines, so they can stay alive long enough to produce and deliver the proteins.

Using mouse models of T1D, it was previously shown that the therapeutic dose of IL-10 and PINS can be reduced using localized delivery by a bacterium, genetically engineered to secrete the proteins. Administration of hIL-10 and hPINS in the gut by *L. lactis* caused a significant reduction in T1D in mice. The reduction was even greater when the L. lactis treatment was combined with an immune suppressing drug such as teplizumab.

The planned clinical study will be the first study in which this treatment is tested in human patients.

Based on its ActoBiotics® technology for local delivery of therapeutics in the gut, Intrexon T1D Partners, LLC. is developing a novel class of biopharmaceuticals with enhanced efficacy and a reduced side effect profile. ActoBiotics® can address a broad range of important diseases. During the past few years, the founding research team of Intrexon T1D Partners, LLC. has already obtained proof-of-concept with

ActoBiotics®-based products. Positive efficacy data were generated in animal models of inflammatory bowel disease, intestinal and oral mucositis, T1D and food allergy.

5. POTENTIAL BENEFITS OF THE PLANNED RELEASE:

The planned release is part of a clinical trial, which is the further step in the development of a new strategy to stop the progression of T1D. Currently, no curative treatments exist for T1D.

6. ASSESSMENT FOR POTENTIAL RISKS FOR THE HUMAN HEALTH AND ENVIRONMENT:

L. lactis is commonly found in and added to food products. *L. lactis* is one of the most important microorganisms involved in the dairy industry. The majority of the industrially produced bacteria do not survive outside the dairy environment. It is not classified as a hazardous organism. It does not produce survival structures such as spores.

L. lactis can be found in a whole range of environments, but these are not ecological niches. In spite of the wide spread use and massive discharge in the environment it has not been identified as invasive or disruptive. Growth can only be sustained in a selected number of favorable areas, containing the essential nutrients for its proliferation, such as milk. *L. lactis* does not multiply in or colonize humans or animals.

L. lactis strain MG1363 only grows in artificially supplemented culture conditions as the genes to use the necessary nutrients of milk are removed. MG1363 does not produce antibiotics, but is sensitive to a large range of them.

The hIL-10 and hPINS producing strain also lacks the ability to produce thymidine, without which it will die. It is highly unlikely that the genetically modified microorganism will reacquire the ability to produce thymidine. Also, it is unable to transfer the acquired genetic modifications to other microorganisms. No specific interactions with non-target organisms have been identified. AG019 will be released in compartments which are natural for *L. lactis,* essentially the human gastrointestinal tract and the sewage system.

It cannot be excluded that valuable biotopes, protected areas or drinking water supplies will be exposed to AG019. However, already today exposure to *L. lactis* is occurring. The modified strain has no additional features that make exposure more likely. On the contrary, as it is totally dependent on the presence of thymidine for not dying, any exposure will be even more limited in time. Whereas the release environment can be concluded to be similar to that normally encountered for *L. lactis*, the modifications characterizing the GMO make that the strain cannot survive in this habitat anymore.

Human IL-10 protein only triggers biological effects on human cells that have the appropriate receptors. These receptors are highly specific to the human species. Most other mammalian IL-10-specific receptors have little or no cross-reactivity with hIL-10 except for some simian and murine receptors. hPINS has very little biological activity and is not expected to induce any specific effects in humans, other than the effect on the immune system for which it is intended.

The hIL-10 and hPINS genes in the GMO are unique, synthetic genes which can be distinguished from the native hIL-10 and hPINS genes. They can be detected via a technique called polymerase chain reaction (PCR).

This notification concerns a deliberate release of GMO for experimental purposes. Therefore, as a general rule the use of this material for any other purpose is prohibited.

7. CONTAINMENT AND CONTROL

In the present clinical trial, the drug product (containing the bacteria) is manufactured as a powder, formulated as capsules packed in blisters. In the event that the packaging is disrupted, the powder quickly degrades after being in contact with moist and warmth. The microorganism is sensitive to temperatures above 40 °C, low pH, air drying, direct sunlight, UV, soap, bleaching agents, antibiotics and high salt concentration solutions. The quantity of a spillage will be limited. The affected area can be decontaminated with a standard detergent (soap) or bleach.

At the time of intake, there is no contact with the bacteria. The patient only receives the necessary material for a one treatment period (maximum 28 days). At the same time, instructions are provided and explained in order to ensure compliance to treatment.

In an outpatient clinical study, the administration of the investigational product occurs outside of the hospital (i.e. most probably at home). Once administered, the bacteria will follow the faecal flow. The administration and excretion (via faeces) is not necessarily limited to the home of the patient. In consequence, the national territory is considered as the wider potential release area. It can be expected that few days after the last treatment, the shedding of live bacteria stops. No specific treatment of the shedding environment is foreseen, as justified by the biological containment and the absence of any relevant impact on the environment. Also, the public at large usually has no access to the sewage system. If required, a standard antibiotic treatment would suffice to inactivate the bacteria.

At regular intervals, the patients will return to the hospital not only for examination but also to return drug packaging (whether empty or not used) and to receive a new treatment package. At the clinical trial centre, standard precautions are in place. Normal hygiene conditions for clinical staff handling patient's body fluids (in particular stools) 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.

Obviously the patient will be directly exposed to the bacteria. Other family members may be exposed directly or by contact with materials with shed bacteria. Standard hygienic practices should be sufficient to limit or prevent significant exposure.

8. RESPONSIBILITIES OF THE NOTIFIER:

The consent that could be given to the notifier by the competent Minister stipulates that the notifier takes complete civilian liability regarding the damage that could be caused by the deliberate release to the health of humans, animals, or environment.

9. INSPECTION BY THE PUBLIC AUTHORITIES:

Inspectors are in charge of inspecting the trials for compliance with the conditions specified in the consent and to investigate potential breaches of the consent. In case where mismanagement or fraud is identified specific sanctions will be imposed.

10. ACTIVITY REPORT:

At the end of the trial an activity report prepared by the notifier needs to be delivered to the competent authority. This activity report includes at least the following data:

- *the site and period of release,*
- *the precise nature of the actually released GMOs,*
- *the aim(s) of the trial,*
- *the measures that were taken to prevent unwanted release of transgenic material,*
- *if applicable, the measures that were taken to protect the subject (patient/animal) during administration of the GMO-containing study drug,*
- *if applicable, the measures that were taken to protect the relatives of the treated patients,*
- *the measures that were taken to protect the workers who had to manipulate the GMO-containing material,*
- *the method used for the destruction of the unused or contaminated material,*
- *the results obtained during the trial,*
- *an overview of the monitoring of patient/animal for GMO shedding,*
- *an overview of the monitoring of GMO or recombinant DNA in the environment.*

11. CONTACT:

If you have any comment on the public dossier or our activities or wish to obtain additional information on the deliberate release, please contact us at the following address.

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You can also have access to a summary of the notification (SNIF) on the web site of the Joint Research Centre of the European Commission (http://gmoinfo.jrc.it/). Comments can be addressed to the Commission via this web site.