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GMO Deliberate Release Notification

INFORMATION FOR THE PUBLIC[1]

PHASE 1b AND PHASE 2a CLINICAL TRIALS WITH AN hIL-10-EXPRESSING *LACTOCOCCUS LACTIS* (*L. LACTIS*)

European notification number
B/BE/07/BVW1

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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 ActoGeniX NV 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 ActoGeniX NV to conduct experiments with transgenic Lactococcus lactis *as stipulated in the application* ***B/BE/07/BVW1.***

The release will take place at locations in Flanders / Wallonia / Brussels as a consequence of clinical trials conducted at UZ Gasthuisberg, Leuven and Imelda vzw, Bonheiden. It is expected to start on 1 July 2008 and to be completed by 1 July 2011.

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

The bacterium *Lactococcus lactis* strain MG1363 has been genetically modified to produce the therapeutic protein human interleukin-10 (hIL-10). This protein will reduce the symptoms (such as pain and bloody diarrhea) of patients suffering from inflammatory bowel disease (IBD).

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 *hIL-10* gene does not change that.

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

The genetically modified MG1363 is even more restricted: the bacterium contains the gene for hIL-10, stably inserted in the chromosome where it replaces a gene needed for thymidine (a building block of DNA) production. This makes the recombinant strain dependent on addition of thymidine to the culture. Without thymidine, the bacterium cannot survive.

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

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 degrade soon afterwards.

3. TYPE AND PURPOSE OF THE ENVISAGED TRIAL:

Two types of trials are planned:

- The first trial is a Phase 1b "Randomized Placebo-Controlled Double-Blind Multi-Centre Study to Evaluate the Safety, Tolerability and Efficacy of AG011 in Subjects with Moderately Active Ulcerative Colitis".
- Secondly, a Phase 2a study is planned, which is a "Randomized Placebo-Controlled Double-Blind Multi-Centre Study to Evaluate the Safety, Tolerability, Pharmacodynamics and Efficacy of Multiple Dose Levels of AG011 in Subjects with Moderately Active Ulcerative Colitis".

AG011 is the code for the drug product. Two ways of administration will be used; capsules for oral intake and an enema formulation for rectal application.

In the Phase 1b study, a single dose level or a placebo will be administered rectally every day for four weeks. In the second trial, the administration to patients will be both oral and rectal, every day for eight weeks. The patients will be receiving either one out of three dose levels or a placebo.

Both trials are aimed at assessing safety, tolerability and efficacy of the drug. In all cases, 20 patients will be recruited per treatment group. This means that the two clinical trials will cover a total of 120 participants.

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

A previous trial has shown that this drug product is safe and does not survive outside the human body.

4. RESEARCH AND DEVELOPMENT FRAMEWORK:

Crohn's disease (CD) and ulcerative colitis (UC) are the two major forms of inflammatory bowel disease (IBD) of which the exact cause is unknown.
CD can affect any part of the gastrointestinal tract from mouth to anus. The main gastrointestinal symptoms for CD are abdominal pain, diarrhea (which may be bloody) or constipation, and weight loss.
UC is a disease of the large intestine or colon that includes characteristic ulcers in the colon. The main symptom of active UC is usually diarrhea mixed with blood, of gradual onset.

UC can be cured by surgical removal of the entire colon. There is no known medical or surgical cure for CD. A number of medical treatments are aimed at inducing and maintaining clinical remission (a state or period during which the symptoms of a disease are beaten down so as to leave a figure in relief).

Administration of hIL-10 is 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.

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.

ActoGeniX is developing the TopAct™ delivery system that is based on a living non-pathogenic *L. lactis* strain for local delivery of therapeutics in the gut. The TopAct™ 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.

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). This makes the recombinant strain dependent on addition of thymidine in culture. Without thymidine, the bacterium cannot survive.

Using different mouse models of colitis, it was previously shown that the therapeutic dose of IL-10 can be reduced using localized delivery by a bacterium, genetically engineered to secrete the protein. Administration of hIL-10 in the gut by IL-10-secreting *L. lactis* caused a significant reduction in colitis in mice. Furthermore, the *L. lactis*-mediated IL-10 delivery was compared to that of standard anti-inflammatory methods: five daily injections with, amongst others, recombinant IL-10, demonstrated that *L. lactis*-mediated IL-10 delivery required a much lower amount of IL-10.

An earlier Phase 1 clinical trial with TopAct™-delivered hIL-10 in CD patients in the Netherlands (Official GMO Ref. BGGO: 02/01; Ref. COGEM: CGM020823-02) showed good tolerability and safety. In addition, this study also provided an indication for clinical efficacy. This novel strategy avoids side effects and is biologically contained. Therefore; it is a suitable candidate as maintenance treatment for chronic intestinal disease.

The planned Phase 1b and Phase 2a clinical trials in UC are a continuation of this development. Based on the results of the earlier Phase 1 trial, this plan includes an *L. lactis* strain with increased expression of hIL-10 as compared to the first trial, in combination with two alternative modes of administration (capsules and enema).

Based on its TopAct™ technology for local delivery of therapeutics in the gut, ActoGeniX is developing a novel class of biopharmaceuticals with enhanced efficacy and a reduced side effect profile. TopAct™ can address a broad range of important diseases. During the past few years, the founding research team of ActoGeniX has already obtained proof-of-concept with TopAct™-based products. Positive efficacy data were generated in animal models of inflammatory bowel disease, intestinal and oral mucositis and food allergy.

5. POTENTIAL BENEFITS OF THE PLANNED RELEASE:

The planned release is a further step in the development of a new strategy to alleviate IBD. Current drugs to control the disease often show serious side effects when used for a long period.

This is also the case for hIL-10 when administered via injections. The proposed TopAct™ delivery system reduces these side effects. Moreover, the drug is released locally and only acts where it is needed. Therefore, the beneficial doses are much lower.

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 nutritionally favorable areas 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 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 genetic modification to other microorganisms.

In the clinical trials, the drug product (containing the bacteria) is available as a powder, formulated either as capsules packed in blisters or dissolved in a liquid in case of the enema. 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 (one dose). The affected area can be decontaminated with a standard detergent (soap) or bleach.

At the time of intake, there is no contact with the drug product in the case of capsules. In the case of the enema, brief contact with the powder and the solution is possible at time of reconstitution of the enema and when the applicator is opened for immediate application. Except for the reconstitution of the enema, there is no need for any additional manipulation, as the package that each patient will receive contains all doses ready for administration by the patient. The patient only receives the necessary material for a one week treatment period. At the same time, instructions are provided and explained in order to ensure compliance to treatment. Although patients with UC are used to the application of an enema, additional instructions will be provided recommending to uphold the enema for at least 2 hours.

In an outpatient 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. Patients will be recommended not to leave the country during the treatment due to the constraints imposed by the designs of the clinical trials. 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.

Once a week, the patients will return to the hospital not only for examination but also to return all material (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 when handling empty containers (although these will be collected and stored in prepared recipients) and possibly material with shed bacteria. Standard hygienic practices should be sufficient to limit or prevent significant exposure.

It cannot be excluded that valuable biotopes, protected areas or drinking water supplies will be exposed. 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.

hIL-10 expression only triggers an effect 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.

The hIL-10 gene in the GMO is a unique, synthetic gene which can be distinguished from the native hIL-10 gene. It can be detected via a technique called polymerase chain reaction (PCR).

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

7. 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.

8. 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.

9. 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 GMMs,*
- *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 GMM-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 GMM-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 GMM shedding,*
- *an overview of the monitoring of GMM or recombinant DNA in the environment.*

10. 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.