Adviesraad voor Bioveiligheid
Conseil consultatif de Biosécurité

Advice of the Belgian Biosafety Advisory Council on the notification B/BE/18/BVW5 of the company Intrexon T1D Partners, LLC for deliberate release in the environment of genetically modified organisms other than higher plants for research and development

13/09/2018
Ref. SC/1510/BAC/2018_692

Context

The notification B/BE/18/BVW5 has been submitted by Intrexon T1D Partners, LLC to the Belgian Competent Authority in June 2018 for a request of deliberate release in the environment of genetically modified organisms (GMOs) other than higher plants for research and development according to Chapter II of the Royal Decree of 21 February 2005.

The planned activity concerns a clinical trial and the title of the notification is: ***"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)".***

The primary objectives of this study, consisting of two phases, is to investigate the safety, tolerability and potential activity of the AG019 genetically modified *Lactococcus lactis* bacteria, alone or combined with infusions of another drug under development called teplizumab, in patients recently diagnosed with type 1 diabetes mellitus (T1D). 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. 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. AG019 is an investigational medicinal product (IMP) developed to stop that autoimmune destruction.

AG019 is the lyophilised powder of bacterial strain sAGX0407 mixed with cryoprotectants, formulated for oral administration as gastro-resistant hard capsules. Strain sAGX0407 is a recombinant *L. lactis* genetically modified (GM) to secrete the therapeutic proteins human interleukin-10 (hIL-10) and human proinsulin (hPINS). The genes for hil-10 and hPINS have been stably inserted in the bacterial chromosome, the former replacing the gene and the promoter encoding thymidylate synthase *thyA* gene, resulting in a strict tymine/ thymidine dependency of sAGX0407. In addition, strain sAGX0407 was genetically modified to enable intracellular trehalose accumulation during the fermentation process (insertion of the gene for trehalose-6-phosphate phosphatase – otsB, deletion of the gene for trehalose-6-phosphate phosphorylase – trePP, and disruption of the gene for cellobiose-specific PTS system IIC

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component - ptcC). As a result, the GM bacteria are less sensitive to bile acid lysis during their passage through the gastrointestinal tract.
It is expected that through oral administration of AG019, the simultaneous delivery to the gastrointestinal (GI) tract of an antigen implicated in β-cell autoimmunity (hPINS) and a cytokine known to steer the immune system towards a state of tolerance (hIL-10) will be able to reverse T1D in humans by inducing and restoring long term tolerance to β-cell antigens. A combination of this treatment approach with a short treatment of generalized immunosuppression (using an immune suppressing drug such as teplizumab) is thought to further strengthen the therapeutic potential. In the first part of the clinical trial, patients will take 2 or 6 capsules containing AG019 (2 x 10^{11} CFU/dose or 6 x 10^{11} CFU/dose) every day for 8 weeks. In the second phase, patients will take 6 capsules every day for 8 weeks.

The parental strain of sAGX0407 is *Lactococcus lactis* subsp. *cremoris* MG1363, a non-pathogenic, non-invasive, non-colonizing Gram-positive strain that is incapable of survival outside of artificially supplemented laboratory conditions. *L. lactis* is primarily used to produce fermented foods such as the industrial manufacture of dairy products. This bacterium is a poor competitor and has a limited ecological niche. Strain sAGX0407 is devoid of the metabolic pathways that enable the use of milk carbohydrate and amino acid sources and is dependent on external supplementation of thymine or thymidine for growth and survival.

It is planned to conduct the trial in several clinical sites located in Brussels, the Flemish Region and Wallonia.

The dossier has been officially acknowledged by the Competent Authority on 20 June 2018 and forwarded to the Biosafety Advisory Council (BAC) for advice.
Within the framework of the evaluation procedure, the BAC, under the supervision of a coordinator and with the assistance of its Secretariat, contacted experts to evaluate the dossier. No expert from the common list of experts drawn up by the BAC and the Service Biosafety and Biotechnology (SBB) of Sciensano answered positively to this request. Only one expert from the SBB took part in the evaluation of the dossier.
The expert assessed whether the information provided in the notification was sufficient and accurate in order to state that the deliberate release of the genetically modified organism would not raise any problems for the environment, animal health or human health (people coming in contact with the treated patient and/or with the GMO) in the context of its intended use. See Annex I for an overview of all the comments from the expert.

The scientific evaluation has been performed considering following legislation:
- Annex II (principles for the risk assessment) and annex III (information required in notifications) of the Royal Decree of 21 February 2005.
- Commission Decision 2002/623/EC of 24 July 2002 establishing guidance notes supplementing Annex II to Directive 2001/18/EC.
The pure medical aspects concerning the efficacy of the medicinal product and its safety for the treated patient, as well as aspects related to social, economic or ethical considerations, are outside the scope of this evaluation.

On 25 July 2018, based on a list of questions prepared by the BAC, the Competent Authority requested the notifier to provide additional information about the notification. The answers from the notifier to these questions were received by the Competent Authority on 21 August 2018 and transmitted to the

secretariat of the BAC on the same day. This complementary information was reviewed by the coordinator and the expert, and was considered satisfactory.
In parallel to the scientific evaluation of the notification, the Competent Authority also made the dossier available on its website for the one-month public consultation foreseen in the abovementioned Royal Decree. The Competent Authority did not receive reactions from the public.

Summary of the scientific evaluation

1. The characteristics of the donor, the recipient or parental organism

The donor, recipient and parental organisms were found to be adequately described in the dossier.

2. Information related to the characteristics of the GMO and the medication

Information related to the molecular characteristics of strain sAGX0407 including phenotypic and genetic stability of the transgenes were adequately described in the dossier.

3. The conditions of the release

Information related to the conditions of the release were found to be adequately described in the dossier.

4. The risks for the environment or human health

There is no indication that the GMO itself is toxic, allergenic or pathogenic. The changes that were induced in the recipient strain 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.

In its list of questions addressed to the notifier, the BAC advised to further consider the risk for immune-compromised individuals or those who are immunosuppressed due to an underlying condition or therapy in case of swallowing entire capsules or (inadvertent) ingestion of accidentally opened capsules. The notifier clarified that, based on pre-clinical studies and previous experience, it is not expected that hIL-10 or hPINS will be absorbed into the systemic circulation. The delivery system in this study avoids the exposure to high systemic doses by localized expression in the GI tract. The doses are much lower and act only locally. The exposure is also limited in time as the bacteria are evacuated from the GI tract in a period of a few days following administration. Given that *L. lactis* does not colonize the GI tract, and that interleukin-10 has a short half-life, the exposure time to the secreted IL-10 will be limited in the unlikely event that it ends up in the systemic circulation. With regards to hPINS its sole purpose is to induce an antigen specific immune response and systemic injection of high doses of hPINS has been documented to induce little side effects. In addition, several exclusion criteria included in the protocol should prevent immune compromised patients to be enrolled in the study. Taken all these considerations into account the BAC agrees that the risk for immune-compromised individuals should be minimal.

The notifier was also requested by the BAC to address the potential risk and selective advantage posed by the acquisition of the hIL-10 gene by a pathogenic bacterium present in the GI tract as a result of horizontal gene transfer. Based on several considerations substantiated by scientific references the notifier concluded that mechanistically and physiologically it is highly unlikely that any cross-species transfer of genetic material from the GMO into other microbiota could occur. Available data also suggest the absence of a selective drive to acquire the hIL-10 gene i.e. the absence to gain a selective advantage upon its acquisition. The BAC agrees with these conclusions.

5. The monitoring, control, waste treatment and emergency plans proposed by the applicant

The Biosafety Advisory Council is of the opinion that the information provided is sufficient and does not raise safety concerns.
Upon request of the BAC the notifier confirmed that destruction of all used and unused IMP will be done at a dedicated drug destruction facility. In addition, the patient instruction leaflet already includes a request to always return used and unused medication to the site, including upon discontinuation of a patient.

Conclusion

Based on the scientific assessment of the notification made by the Belgian expert, the Biosafety Advisory Council concludes that it is unlikely that AG019 developed to stop autoimmune destruction of beta cells in the pancreas in patients recently diagnosed with type 1 diabetes mellitus (T1D), and administered alone or in association with teplizumab, will have any adverse effects on human health or on the environment in the context of the intended clinical trial provided that all the foreseen safety measures are followed. However, as a precautionary measure the Council emphasizes the importance of measures to avoid inadvertent intake of the IMP by people not enrolled in the clinical trial.

Therefore, the Biosafety Advisory Council issues a **positive advice with the following conditions**:

- The notifier and the investigators must strictly apply the clinical trial protocol, and all the safety instructions as described in the dossier. In addition, the patient instruction leaflet should clearly mention that the IMP should be stored in a lockable container at the patient's private home.

- Any protocol amendment has to be previously approved by the Competent Authority.

- The notifier is responsible to verify that each study centre has qualified personnel experienced in handling infectious material and that the investigator has the required authorizations to perform the clinical trial activities inside the hospital (laboratory, pharmacy, hospital room, consultation room...) according to the Regional Decrees transposing Directive 2009/41/EC on Contained use of genetically modified micro-organisms.

- The Biosafety Advisory Council should be informed within two weeks when the first patient starts the treatment and the last patient receives the last treatment.

- At the latest six months after the last visit of the last patient included in the trial, the notifier must send to the competent authority at the attention of the Biosafety Advisory Council a report with details concerning the biosafety aspects of the project. This report will at least contain:
    - The total number of patients included in the trial and the number of patients included in Belgium;
    - A summary of all adverse events marked by the investigators as probably or definitely related to the study medication;
    - A report on the accidental releases, if any, of AG019.

Dr. Corinne Vander Wauven
President of the Belgian Biosafety Advisory Council

Annex I: Compilation of comments of experts in charge of evaluating the dossier B/BE/18/BVW5 (ref. SC/1510/BAC/2018_0627)

Compilation of comments of experts in charge of evaluating the dossier B/BE/18/BVW5
And comments submitted to the notifier

26 July 2018
Ref. SC/1510/BAC/2018_0627

Mandate for the Group of Experts: Mandate of the Biosafety Advisory Council (BAC) of 13 June 2018.
Coordinator: Corinne Van der Wauven (Institut de Recherche Labiris)
Experts: Aline Baldo (Sciensano, SBB)
SBB: Didier Breyer, Katia Pauwels.

INTRODUCTION

Dossier **B/BE/18/BVW5** concerns a notification of the company Intrexon T1D Partners, LLC for deliberate release in the environment of genetically modified organisms other than higher plants according to Chapter II of the Royal Decree of 21 February 2005.
The notification has been officially acknowledged on 20/06/2018 and concerns a clinical trial with AG019, a recombinant *Lactococcus lactis* engineered to secrete human interleukin-10, which is encoded by the gene *hil-10*. The latter has been stably inserted in the bacterial chromosome replacing the gene and the promoter encoding thymidylate synthase thyA gene, resulting in a tymine/thymidine dependency of AG019. Other genes that have been inserted in the bacterial chromosome are the gene for human proinsulin (pins) and the gene for trehalose-6-phosphate phosphatase (*otsB*). The gene for trehalose-6-phosphate phosphorylase (*trePP*) is absent and the one for cellobiose-specific PTS system IIC component (*ptcC*) has been disrupted, thereby contributing to the accumulation of trehalose.
The application concerns a phase Ib/IIa clinical trial involving the oral administration of AG019 to patients with clinical recent-onset Type 1 Diabetes Mellitus (T1D). It is hypothetized that the proteins expressed by GMO will induce antigen specific immune tolerance and will increase the ability of the GMO to accumulate trehalose making it more resistant to bile acid lysis and prolonging the survival of the GMO in the GI tract.

♦ INSTRUCTIONS FOR EVALUATION

Depending on their expertise, the experts were invited to evaluate the genetically modified organism considered in the notification as regards its molecular characteristics and its potential impact on human health and the environment. The pure medical aspects concerning the efficacy of the medicinal product and its safety for the treated patient are outside the scope of this evaluation.
The comments of the experts are roughly structured as in
- Annex II (principles for the risk assessment) of the Royal Decree of 21 February 2005
- Annex III (information required in notifications) of the Royal Decree of 21 February 2005
- Commission Decision 2002/623/EC of 24 July 2002 establishing guidance notes supplementing Annex II to Directive 2001/18/EC.

List of comments/questions received from the expert

Remark: The comments below have served as basis for a list of questions that the Competent authority forwarded on 25/07/2018 to the notifier with a request to provide additional information. The comments or remarks highlighted in grey correspond to the questions addressed to the notifier.

1. INFORMATION RELATED TO THE CHARACTERISTICS OF THE DONOR, THE RECIPIENT OR PARENTAL ORGANISM

(e.g. possibility of natural transfer of genetic material to other organisms, pathological, ecological and physiological characteristics, indigenous vectors ...)

Comment

Has evaluated this item and has no questions/comments.

2. INFORMATION RELATED TO THE VECTOR

(e.g. description, sequence, mobilisation ...)

Comment

Has not evaluated this item.

3. INFORMATION RELATED TO THE CHARACTERISTICS OF THE GMO

3.1. Information related to the genetic modification

(e.g. methods used for the modification, description of the insert/vector construction ...)

Comment

Has not evaluated this item.

3.2. Information on the molecular characteristics of the final GMO

(e.g. number of copies of the transgenes, phenotypic and genetic stability of the transgenes, expression of the new genetic material, re-arrangements in the genome, inclusion or suppression of genetic material ...)

Comment

Has not evaluated this item.

3.3. Considerations for human, animal or plant health

(e.g. invasiveness and virulence, toxic or allergenic effects, possibility of survival outside of receiving host, other product hazards ...)

Comment

Has evaluated this item and has no questions/comments.

4. INFORMATION RELATING TO THE CONDITION OF RELEASE

(e.g. description of the activity, quantities of GMO to be released, workers protection measures, elimination of any contaminating material in the preparation of the GMO stock, elimination of the GMO at the end of the experiment ...)

Comment

The applicant says that given the biological containment which combines several inherent inactivation factors, no additional inactivation is foreseen at the clinical trial centers on top of normal practice. At the clinical trial center, capsules should be inactivated (by incineration) even if sAGX0407 is not able to survive in the environment due to the nutrient requirements (auxotrophy; biologically contained).
The subjects must return all unused capsules to the study site even in the case when a subject discontinues the study. The capsules should be inactivated by incineration.
At the patient's private home, the biological material should be stored in the refrigerator. This could lead to inadvertent use. However, the patient will be advised to store the doses in a lockable container.
The applicant should consider the risk related to an accidental intake of a capsule by children.

5. INFORMATION RELATED TO THE RISKS FOR THE ENVIRONMENT AND HUMAN HEALTH

5.1. Information on spread ("shedding") of the GMO from the treated patient/animal to other persons/animals or to the environment (including indirect/delayed effects due to vertical transmission to offspring).

(e.g. genetic transfer capability, routes of biological dispersal, target organisms ...)

Comment

Has evaluated this item and has no questions/comments.

5.2. Information on possible effects on human health resulting from interactions of the GMO and persons working with, coming into contact with or in the vicinity of the GMO release (carekeepers, patient relatives, immunocompromised people ...).

Comment

The applicant does not consider the risk for immune-compromised individuals and those who are immunosuppressed due to an underlying condition or therapy (e.g. HIV patients, transplant recipients, cancer patients undergoing certain therapy).
The applicant should consider the risk for immune-compromised persons in case of swallow entire capsules or ingest material from accidentally opened capsules.
It is also important to consider possible effects on the unborn child in the case of transmission to a pregnant woman.

5.3. Information on possible effects on animal health or on the environment.

Comment

Has evaluated this item and has no questions/comments.

5.4. Information on selective advantages or disadvantages conferred to the GMO compared to the parental organism.

Comment
Compared to the non-modified parental strain, sAGX0407 is totally dependent on the addition of thymine/thymidine to the culture medium (thymine-less death).

5.5. Information on the possibility of the GMO to reconvert to his wild type form and possible consequences for human health or the environment.

Comment
Has evaluated this item and has no questions/comments.

5.6. Information on the possibility of the GMO to exchange genetic material with other micro-organisms and possible consequences for human health or the environment.

Comment
Has evaluated this item and has no questions/comments.

5.7. Information on the possibility of gene transfer to other organisms and about the selective advantages or disadvantages conferred to those resulting organisms (possible consequences for human health or the environment).

Comment
Has evaluated this item and has no questions/comments.

6. INFORMATION RELATED TO THE MONITORING, SURVEILLANCE AND CONTROL, WASTE TREATMENT AND EMERGENCY PLANS PROPOSED BY THE APPLICANT

6.1. Monitoring plan proposed by the notifier and possibility to identify the occurrence of non-anticipated adverse effects.

(adequation between the monitoring plan and risks identified during the risk assessment, when appropriate measures to minimize the potential risks to offspring ...)

Comment
Has evaluated this item and has no questions/comments.

6.2. Surveillance and control of the release

(adequation between the procedures to avoid and/or minimise the spread of the GMO and risks identified during the risk assessment...)

Comment
Has evaluated this item and has no questions/comments.

6.3. Information on the waste generated by the activity and its treatment.

(e.g. type of waste, amount ...)

Comment

The applicant says that given the biological containment which combines several inherent inactivation factors, no additional inactivation is foreseen at the clinical trial centers on top of normal practice. At the clinical trial center, capsules should be inactivated (by incineration) even if the sAGX0407 is not able to survive in the environment due to the nutrient requirements (auxotrophy; biologically contained). The subjects must return all unused capsules to the study site even in the case when a subject discontinues the study. The capsules should be inactivated by incineration.

6.4. If applicable, information on the emergency plan(s) proposed by the notifier.

Comment

Has evaluated this item and has no questions/comments.

6.5 Information related to the identification of the GMO and the detection techniques

(e.g. identification methods and detection techniques, sensitivity, reliability and specificity of the proposed tests ..)

Comment

Has not evaluated this item.

7. OTHER INFORMATION

7.1 Do you have any other questions/comments concerning this notification that are not covered under the previous items?

Comment

None