Adviesraad voor Bioveiligheid
Conseil consultatif de Biosécurité

Advice of the Belgian Biosafety Advisory Council on the notification B/BE/18/BVW3 of the company Oragenics, Inc. for deliberate release in the environment of genetically modified organisms other than higher plants for research and development

11/09/2018
Ref. SC/1510/BAC/2018_0645

Context

The notification B/BE/18/BVW3 has been submitted by Oragenics, Inc. to the Belgian Competent Authority in June 2018 for a request of deliberate release in the environment of genetically modified organisms 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 phase 2, Multi-center, Randomized, Double-blind, Placebo-controlled Study to Assess the Safety and Efficacy of Topically-applied AG013 for the Attenuation of Oral Mucositis in Subjects With Cancers of the Head and Neck Receiving Concomitant Chemoradiation Therapy ".***

The primary objectives of this study is to evaluate the efficacy and to determine the safety and tolerability of the AG013 genetically modified *Lactococcus lactis* bacteria (dose-escalation) in patients undergoing chemoradiation for the treatment of head and neck cancer. AG013 is an investigational medicinal product (IMP) developed to reduce the symptoms of radiotherapy and/or chemotherapy induced oral mucositis, which results in a range of mucosal damage and which is a very frequently reported adverse effect associated with cancer treatment.

AG013 is the lyophilised powder of strain sAGX0085 with cryoprotectants formulated for oral administration. Strain sAGX0085 is a recombinant *L. lactis* genetically modified (GM) to secrete human Trefoil factor, which is encoded by the gene *htff1*. The latter has been stably inserted in the bacterial chromosome replacing the gene and the promoter encoding thymidylate synthase *thyA* gene, resulting in a strict tymine/thymidine dependency of sAGX0085. It is hypothesized that the GMO will secrete hTFF1 protein in the oral cavity after administration to the subject and reduce oral mucositis due to the healing and protective properties of the Trefoil factor. During the active treatment phase, which starts the first day of chemoradiation therapy and continues until two weeks following the last day or radiation therapy, subjects will rinse with a suspension containing AG013 (2×10^{11} CFU/15 mL) three times each day.

The parental strain of sAGX0085 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 in

the industrial manufacture of dairy products. This bacterium is a poor competitor and has a limited ecological niche. sAGX0085 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 13 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. Three experts 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. The SBB also took part in the evaluation of the dossier. The experts and the SBB 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 experts.

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 24 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 20 August 2018 and transmitted to the secretariat of the BAC on the same day. This complementary information was reviewed by the coordinator and the experts.

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 received two reactions from the public, the concern of which were not related to biosafety.

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 sAGX0085 including phenotypic and genetic stability of the transgenes were adequately described in the dossier.

3. The conditions of the release

In its list of questions addressed to the notifier, the BAC advised the notifier to further detail the instructions at the study site so as to inactivate by chemical means or incineration unused as well as used GMO. The BAC also advised to treat the "discarded mouth rinse" in a similar way as "an accidental spillage" by putting the mouth rinse in a standard detergent before discharge into the sink, to minimize the chances of a release of the GMO in the environment or a transfer to other persons (e.g. by close contact). The notifier agreed to adapt the patient documents accordingly by asking subjects to spit the discarded mouth rinse into a recipient containing a standard detergent soap or bleach before discarding it into a sink or toilet.

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 light of a statement of the notifier in regards replication ability of *L. lactis*, the BAC brought to the attention that scientific literature reports the presence or even infection of *L. lactis* in rare cases in humans. In its response the notifier clarified that the statement on the inability of sAGX0085 to multiply in mammals, except in gnotobiotic mice, should refer to observations following experimental inoculation. Furthermore, even though no additional *in vivo* auxotrophy studies were performed with sAGX0085, the BAC agrees with the notifier that *in vitro* studies with sAGX0085 and *in vivo* studies in preclinical setting with comparable engineered *thyA* deficient *L. lactis* strains indicated that any propagation of sAGX0085 following passage through the large intestine would be highly unlikely.
The BAC further noticed that transfer of the GMO to the blood stream through ulceration cannot be fully excluded but is also of the opinion that any associated adverse effect would be limited since studies indicate the incapacity of sAGX0085 to persist in blood stream. In the unlikely event of infection, the GMO can quickly and easily be inactivated with standard antibiotics.

In regards possible selective advantage of sAGX0085 associated to the expression of hTTF1 protein, it is noticed that hTFF1 is naturally abundantly expressed in epithelium of the stomach, hTFF1 peptide is a constituent of human saliva and serum and sAGX0085 has shown no direct growth advantage with the acquisition of htff1. Upon request of the BAC, the notifier also further informed on the relevance of using animal model to study the effect of hTFF1. hTFF1 is approximately 65% identical to mouse and rat TFF1. It is not known whether receptors for hTTF1 are similarly distributed in human and animal organs, but toxicological studies show that rat and dog cell lines are responsive to hTFF1.

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 and recommends the treatment of discarded mouth rinse in order to minimize the release of the GMO into the environment.

Conclusion

Based on the scientific assessment of the notification made by the Belgian experts, the Biosafety Advisory Council concludes that it is unlikely that AG013 developed for the attenuation of Oral Mucositis in cancer patients receiving concomitant chemoradiation therapy 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.

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 also taking into account the recommendations from the Biosafety Advisory Council for waste treatment.

- 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 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 AG013.

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/BVW3 (ref. SC/1510/BAC/18_0631)

Adviesraad voor Bioveiligheid
Conseil consultatif de Biosécurité

Compilation of comments of experts in charge of evaluating the dossier B/BE/18/BVW3
And comments submitted to the notifier

25 July 2018
Ref. SC/1510/BAC/2018_0631

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: Jozef Anné (KUL), Veronique Fontaine (ULB), Aline Baldo (Sciensano, SBB)
SBB: Katia Pauwels.

INTRODUCTION

Dossier **B/BE/18/BVW3** concerns a notification of the company Oragenics, Inc. 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 13/06/2018 and concerns a clinical trial with AG013, a recombinant *Lactococcus lactis* engineered to secrete human Trefoil factor, which is encoded by the gene htff1. 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 AG013.
The application concerns a phase II clinical trial involving topically-applied AG013 for the attenuation of oral mucositis in subjects with cancers of the head and neck receiving concomitant chemoradiation therapy. It is hypothetized that the GMO secretes hTFF1 protein in the oral cavity after administration to the subject and reduces oral mucositis due to the healing and protective properties of the Trefoil factor.

♦ 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 received from the experts

Remark: The comments below have served as basis for a list of questions that the Competent authority forwarded on 24/07/2018 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.

List of comments/questions received from the experts

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 1

Has evaluated this item and has no questions/comments.

Comment 2

Has evaluated this item and has no questions/comments.

Comment 3

Has evaluated this item and has no questions/comments.

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

2. INFORMATION RELATED TO THE VECTOR

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

Comment 1

Has evaluated this item and has no questions/comments.

Comment 2

Has not evaluated this item.

Comment 3

Has evaluated this item and has no questions/comments.

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

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 1

Has evaluated this item and has no questions/comments.

Comment 2

Has not evaluated this item.

Comment 3

Has evaluated this item and has no questions/comments.

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

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 1

Why is in Fig. 3 (technical dossier) the region pf pXXX shown as nnnn?

Comment 2

Has not evaluated this item.

Comment 3

Has evaluated this item and has no questions/comments.

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 1

In the Environmental Risk Assessment (AG013) file (page 8) it is mentioned "*L. lactis* lacks the ability to multiply *in vivo* in mammals, except in gnotobiotic mice", but it has been shown that:

(1) Immunocompromised persons can become infected by Lactococcus-associated peritonitis. Chonnam Med J. 2014 Aug; 50(2): 67–69.

(2) can be isolated from vaginal secretions Todorov SD, Botes M, Danova ST, Dicks LMT (2007) Probiotic properties of *Lactococcus lactis* subsp. *lactis* HV219, isolated from human vaginal secretions. J Applied Microbiol 103:629-639:

(3) microbiologic and molecular evidence for infection produced by *L. lactis* subsp. *lactis* in waterfowl. ((CDC EID journal Volume 7 Number 5—October 2001)

(4) Are patients with damaged hearth valves not at risk when bacteria come in the bloodstream due to wounds in the oral cavity? See Early *Lactococcus lactis* endocarditis after mitral valve repair: A case report and literature review. Infection. 2013 Aug;41(4):897-9 Rostagno C, Pecile P, Stefàno PL.

(5) A Case of Septic Shock Following Catheter-related Infection Caused by *Lactococcus lactis* subsp. *lactis* in an Adult Lab Med Online. 2016 Jul;6(3):187-190.

Can the applicants comment on this in relation to their sentence "*L. lactis* lacks the ability to multiply in vivo in mammals, except in gnotobiotic mice

Comment coordinator :

I agree with JA's comment (supported by scientific literature, as shown)

1. It is to be expected to have some transfer of the GMO to the blood stream through the ulceration
2. non pathogenic bacteria can develop in unusual niches or in immunocompromised people.

Concern somewhat limited by the fact that the GMO seems not to persist in blood stream.

Comment 2

Has evaluated this item and has no questions/comments.

Comment 3

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 1

The applicant mentions "In the event of an accidental spillage, the use of standard detergent (soap) or bleach immediately will completely eradicate the AG013 and decontaminate the affected area. AG013 is short-lived when dissolved in water at room temperature". Would it not be advisable to treat the "discarded mouth rinse" in a similar way as "an accidental spillage" by putting the mouth rinse in a standard detergent before discharge into the sink, as such the chance that the GMO is released in the environment will be minimalized.

Comment coordinator :

I agree with comment 1. Instructions should be given to patients

Comment 2

The applicant says that at the study site, any used and unused IMP containing GMO will be appropriated destroyed by set guidelines and according to the institutional standards. At the study site used and unused IMP containing GMO should be inactivated before disposal (by chemical means or by incineration).

Comment 3

Has evaluated this item and has no questions/comments.

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 1

See comments at 3.3.

What in case the GMO should colonize (as a consequence of lack of hand hygiene) the vaginal tract and express hTFF1 locally. Can this be investigated?

Comment coordinator :

This is related to the pertinence of the animal model: is the human trefoil1 factor active on rodents' receptor? Similarity between animal and human hTFF1l factor, similarity between the distribution of hTFF1 receptors in human and animal organs?

Comment 2

Has evaluated this item and has no questions/comments.

Comment 3

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 1

Has evaluated this item and has no questions/comments.

Comment 2

AG013 is non-replicative, non-infectious and non-pathogenic. AG013 is very much biologically contained. It can only survive in artificial laboratory cultures. Compared to the non-modified parental strain, it is totally dependent on the addition of thymine/thymidine to the culture medium (thymine-less death).

Comment 3

Has evaluated this item and has no questions/comments.

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

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

Comment 1

Has evaluated this item and has no questions/comments.

Comment 2

Has evaluated this item and has no questions/comments.

Comment 3

Has evaluated this item and has no questions/comments.

Comment coordinator :
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 1

The removal of *thyA* gene in the generation of AG013 confers an additional safety feature than the ones already present in MG1363. But can the amount of thymidine released from damaged host tissues and bacteria, nullify the additional safety feature (Richard A Proctor, Clinical Infectious Diseases 46(4):584-93, 2008)?

Comment coordinator :
Same concern as in comment 1, assuming that the GMO could enter the bloodstream through the ulcerations. It is not clear what selective advantage the expression of hTFF1 protein could confer to the GMO or any other microorganism, but this is not discussed in the technical dossier.

Comment 2

Compared to the non-modified parental strain, AG013 is totally dependent on the addition of thymine/thymidine to the culture medium (thymine-less death).

Comment 3

Has evaluated this item and has no questions/comments.

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 1

Has evaluated this item and has no questions/comments.

Comment 2

Has evaluated this item and has no questions/comments.

Comment 3

Has evaluated this item and has no questions/comments.

Comment coordinator :
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 1

Has evaluated this item and has no questions/comments.

Comment 2

AG013 contains no plasmids or conjugative transposons and is not able to transfer genetic material to other bacteria.

Comment 3

Has evaluated this item and has no questions/comments.

Comment coordinator :
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 1

Has evaluated this item and has no questions/comments.

Comment 2

Has evaluated this item and has no questions/comments.

Comment 3

Has evaluated this item and has no questions/comments.

Comment coordinator :
It is not clear what selective advantage the expression of hTFF1 protein could confer to the GMO or any other microorganism, but this is not discussed in the technical dossier.

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 1

Has evaluated this item and has no questions/comments.

Comment 2

Has evaluated this item and has no questions/comments.

Comment 3

Has evaluated this item and has no questions/comments.

Comment coordinator :
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 1

After discarding in the sink, it is not prescribed to decontaminate the sink to avoid transmission to other individuals: transfer to other persons eg. by close contact see comment above item 4

Comment coordinator :
Agrees with this comment.

Comment 2

Has evaluated this item and has no questions/comments.

Comment 3

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 1

Has evaluated this item and has no questions/comments.

Comment 2

The applicant says that at the study site, any used and unused IMP containing GMO will be appropriated destroyed by set guidelines and according to the institutional standards. At the study site used and unused IMP containing GMO should be inactivated before disposal (by chemical means or by incineration).

Comment 3

Has evaluated this item and has no questions/comments.

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

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

Comment 1

Has evaluated this item and has no questions/comments.

Comment 2

Has evaluated this item and has no questions/comments.

Comment 3

Has evaluated this item and has no questions/comments.

Comment coordinator :
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 1

Has evaluated this item and has no questions/comments.

Comment 2

Has not evaluated this item.

Comment 3

Has evaluated this item and has no questions/comments.

7. OTHER INFORMATION

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

Comment 1
None

Comment 2
None

Comment 3
None

References

(1) Chonnam Med J. 2014 Aug; 50(2): 67–69.

(2) Todorov SD, Botes M, Danova ST, Dicks LMT (2007) Probiotic properties of *Lactococcus lactis* subsp. *lactis* HV219, isolated from human vaginal secretions. J Applied Microbiol 103:629-639:

(3) CDC EID journal Volume 7 Number 5—October 2001)

(4) Early *Lactococcus lactis* endocarditis after mitral valve repair: A case report and literature review. Infection. 2013 Aug;41(4):897-9 Rostagno C, Pecile P, Stefàno PL.

(5) A Case of Septic Shock Following Catheter-related Infection Caused by *Lactococcus lactis* subsp. *lactis* in an Adult Lab Med Online. 2016 Jul;6(3):187-190.

(6) Richard A Proctor, Clinical Infectious Diseases 46(4):584-93, 2008