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	Germany
(b)	Notification number	B/DE/18/PEI3432
(c)	Date of acknowledgement of notification	22/05/2018
(d)	Title of the project	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
(e)	Proposed period of release	Approximately from July 2018 until June 2019

2. Notifier

Name of institution or company: Oragenics, Inc.
4902 Eisenhower Blvd., suite 125
Tampa, FL 33634
United States

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)

The species used to construct the GM strain is *Lactococcus lactis*, formerly named *Streptococcus lactis*, subspecies *cremoris*. The particular recipient strain, *L. lactis* MG1363, is a derivative of the natural isolate *L. lactis* National Collection of Dairy Organisms (NCDO) 712, a strain widely used as cheese starter culture (Johansen *et al.*, 2003). Gasson et al. described the removal of all of the 5 different plasmids that were present in *L. lactis* strain NCDO 712 by protoplast-induced curing, resulting in the plasmid-free *L. lactis* strain MG1363 (Gasson et al. 1983).
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 (X) No ( )

If yes, insert the country code(s): BE, DE

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 (X) No (.)

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

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

The GMO will be administered as part of a clinical study, orally as a mouth rinse to patients with head and neck cancer (HNC) receiving chemoradiation therapy (CRT), to treat oral mucositis (OM). The organism does not colonize the gastrointestinal tract. Live organisms are likely to be released in the sewage system when the mouth rinse is discarded into the sink or toilet. It is unlikely they will be shed in stools as the mouth wash is not expected to be swallowed in the current study. Normal hygiene (hand washing) is considered sufficient to prevent transmission from person to person however study subjects will be provided with instructions on how to safely prepare, administer and dispose of the GMO.

AG013 has been found to be generally safe and well tolerated when applied topically in both single and multiple-dose clinical investigations. No deaths or treatment-related SAEs have been observed. There have also been no adverse events leading to discontinuation from any study. Overall, there were no safety findings that would preclude continued clinical development of AG013 for the treatment of oral mucositis.

The GMO is a biologically 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 is non pathogenic, non infectious. 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 harbour plasmids or conjugative transposons and phage replication is severely hindered as it is not able to produce thymidine.

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 also estimated to be negligible.

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 ()

(ii) No (X) *idem as 3.A*

(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: NA

5. (a) Detection techniques

L. lactis is detected by standard microbial techniques and molecular techniques based on PCR and sequencing. The bacteria can also be grown using specific culture media.

(b) Identification techniques
In contrast to wild type *L. lactis*, MG1363 (GMO parental strain and the GMO) cannot grow on media containing lactose as the sole carbon source and casein as the sole source of amino acids. Consequently, MG1363 is not capable of coagulating milk. Full genome sequencing of MG1363 and the GMO have demonstrated that no extra-chromosomal elements nor lactose/casein metabolic pathways are present, which identifies these strains from wild type *L. lactis.*

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

Yes ( ) No (X)

If yes, specify:

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

L. lactis bacteria are critical for manufacturing dairy products like buttermilk and cheese. The widespread use of these products indicates that they are non-pathogenic. Despite their widespread use and massive discharge in the environment, they have not been identified as invasive or disruptive. Although *L. lactis* can be found in very diverse sources (soil, manure, waste water), the bacteria depend on particular nutritional components for growth. *L. lactis* strain MG1363 is restricted even further to artificially supplemented culture conditions.

As stated above, *L. lactis* is a food-grade micro-organism and has a long history of safe use in the food industry. Therefore, infection is highly unlikely. Nevertheless, an extensive literature search was completed and is repeated on a yearly basis.

According to the literature review, bacteria can have some potential for pathogenic interactions in patients with co-morbidities, with consumption of unpasteurized dairy products reported in some cases. Starting from the taxonomic identification of *L. lactis* in the 19th Century, up until 2006, approximately 18 cases of *L. lactis* infection have been reported. The authors of the review indicate that this does not imply specific pathogenic traits attributable to *L. lactis*. All reported cases were proficiently cured by standard antibiotics therapy. Likewise, not a single fatality has ever been attributed to consumption of *L. lactis*.

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 is being used in a clinical trial. It will be released in the sewage system after administration to study subjects. *L. lactis* MG1363 (GMO parental strain) is not able to grow outside the laboratory due to dependence on an external source of thymine or thymidine.

(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

For wild type *L. lactis*, horizontal human-to-human dispersion is not part of reproductive biology, which makes that dispersal of the bacteria is essentially passive. For the recipient strain, MG1363, an organism with growth limitations when compared to wild type *L. lactis*, this is assumed not to be different. No dissemination studies have been done.

(b) Factors affecting dissemination

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

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 Trefoil Factor 1 (*htff1*) has been stably inserted in the bacterial chromosome, replacing the *thyA* gene and promoter encoding thymidylate synthase. The accompanying regulatory sequences are aimed at secreting human Trefoil Factor 1 (hTFF1). Upon administration to participants, the protein is targeted to reduce oral mucositis.
Deleting the *thyA* gene resulted in strict thymine/thymidine dependency, not only for growth but also for survival of the GMO (*thymine-less death*).

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

The insert consisted of a bacterial promoter, a secretion sequence leader, the *htff1* gene and a non-coding sequence downstream of the *thyA* coding sequence.

(b) Source of each constituent part of the insert

The promoter was isolated from a highly expressed *L. lactis* MG1363 gene, identified by proteomic analysis of most prominent protein bands.
The *htff1* gene is synthetic and has been derived from the human gene. It has been codon optimized for efficient expression in the GMO, *L. lactis*
The secretion sequence leader and the non-coding sequence downstream of *thyA* are from *L. lactis* MG1363.

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

The promoter is used to drive expression of the *htff1* gene. The secretion leader sequence encodes an extracellular secretory protein that enables the GMO to secrete hTFF1 protein in the oral cavity after administration to the study subject.
The hTFF1 protein is aimed at reducing oral mucositis and has been found to have healing and protective properties.

(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) (promoter)
fungus (.)
animal:
- mammals (.)
- insect (.)
- fish (.)
- other animal (.)
  specify phylum, class: …

other, specify: man *(htff1, synthetic gene)*

2. Complete name

htff1 gene

(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

promoter

(j)	order and/or higher taxon (for animals)	
(ii)	family name for plants	…
(iii)	genus	*Lactococcus*
(iv)	species	*lactis*
(v)	subspecies	*cremoris*
(vi)	strain	MG1363
(vii)	cultivar/breeding line	…
(viii)	pathovar	…
(ix)	common name	

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: …

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 insertion has occurred on the bacterial chromosome, which was confirmed by PCR amplification.

Analysis of the genetic stability of the GMO (*L. lactis* strain sAGX0085), obtained by repeated sequential dilution and growth to saturation, was performed after a minimum of 100 generations of growth. The genetic stability was analysed by four parameters:

- Inability of sAGX0085 to grow in thymidine-deficient medium (showing the efficiency of the biological containment system).
- Unchanged hTFF1 secretion by sAGX0085.
- Stability of the modified locus using PCR analysis with specific oligonucleotides.
- DNA sequence verification of the expression cassette of *L. lactis* strain sAGX0085.

The experiment concluded that genetic stability of *L. lactis* strain sAGX0085 was absolute for all of these parameters.

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 bacteria are critical for manufacturing dairy products like buttermilk and cheese. The widespread use of these products indicates that they are non-pathogenic. Despite their widespread use and massive discharge in the environment, they have not been identified as invasive or disruptive. Although *L. lactis* can be found in very diverse sources (soil, manure, waste water), the bacteria depend on particular nutritional components for growth. *L. lactis* strain MG1363 is restricted even further to artificially supplemented culture conditions.

As stated above, *L. lactis* is a food-grade micro-organism and has a long history of safe use in the food industry. Therefore, infection is highly unlikely. Nevertheless, an extensive literature search was completed and is repeated on a yearly basis. According to the literature review, bacteria can have some potential for pathogenic interactions in patients with co-morbidities, with consumption of unpasteurized dairy products reported in some cases. Starting from the taxonomic identification of *L. lactis* in the 19th Century, up until 2006, approximately 18 cases of *L. lactis* infection have been reported. The authors of the review indicate that this does not imply specific pathogenic traits attributable to *L. lactis*. All reported cases were proficiently cured by standard antibiotics therapy. Likewise, not a single fatality has ever been attributed to consumption of *L. lactis*.

All available reports on infection with *L. lactis*, including the review article, stipulate that the limited potential for pathogenic interactions should not question the role of *L. lactis* bacteria in the food industry, nor in medical applications. In nonclinical development of the GMO (AG013), it has been demonstrated that it is unable to survive in complement preserved human serum. In addition, during development of AG013, IV inoculation into neutropenic rats or colitic mice did not result in any signs or symptoms suggesting active infection or sepsis. Nevertheless, for AG013, an antibiotics susceptibility/resistance profile was determined, so in

the unlikely event of infection, the bacteria can easily and quickly be inactivated by standard antibiotics treatment.

4. Description of identification and detection methods

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

- The auxotroph sAGX0085 strain (AG013) only grows on thymidine or thymine containing culture media,
- PCR amplification of the insert using specific probes, and
- Enzyme-Linked ImmunoSorbent Assay (ELISA) of the secreted protein.

(b) Techniques used to identify the GMO

- Through PCR amplification of 16sRNA and subsequent sequencing of the PCR fragment, the species identity of sAGX0085 can be established as *Lactococcus lactis* subspecies *cremoris* MG1363 and was completed during the manufacturing of the master cell bank. In addition, the presence of the *htff1* gene and the absence of the *thyA* gene was also demonstrated by sequencing.
- ELISA (LLOQ: 5 pg/ml) was used to quantify the levels of hTFF1 secreted by the sAGX0085.

F. Information relating to the release

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

Oragenics' overall objective of the development program is to establish AG013 as a therapeutic option, and gain marketing approval, for reduction of the signs and the symptoms of radiation therapy and/or chemotherapy induced oral mucositis.

The proposed Phase 2 clinical trial is a 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.

AG013 is the lyophilized powder of the GMO, formulated for oral administration as a mouth rinse.

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 participants as a mouth rinse and will be expectorated into a sink or toilet. As these are outpatient studies, the GMO is expected to be shed will occur at the participant's home or elsewhere.

3. Information concerning the release and the surrounding area

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

While the location of the clinical trial centres is known, the identity and coordinates of the participants will not be known by the notifier. In addition, the bacteria will be released via the sewage system via the sink or toilet after using the mouth rinse. The mouth rinse is not expected to be swallowed and the shedding in faeces is not expected.
Administration of the GMO by study subjects is not necessarily limited to their home. In consequence, the national territory is considered as the wider potential release area.

(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 cannot be excluded as possible sites of release. However, the only route for exposure would be via the sewage system, 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. In comparison, the GMO would be considerably diluted out. 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 MG1363 is not known to interact with fauna and flora. No involvement in particular environmental processes are known.

4. Method and amount of release

(a) Quantities of GMOs to be released:

Study subjects that receive chemoradiation therapy, will rinse three times per day with AG013 mouth rinse beginning from the start of radiotherapy until 2 weeks following

its completion. The active treatment phase lasts for 7 to 9 weeks, depending on the duration of radiotherapy.

The efficacy and safety of AG013 will be compared to a placebo. After screening, patients will be randomized to either an AG013 treatment arm or a placebo arm according to a 1:1 randomization ratio.

A total of 200 participants will be involved in the study worldwide: 140 in the US and 60 in Europe divided equally between Belgium and Germany. Therefore, in Belgium and in Germany, 15 subjects per country will be recruited in the AG013 treatment group.

Based on the treatment design and the number of subjects planned to be enrolled, it can be estimated that a maximum quantity of $6.4x10^{14}$ colony forming units (CFU) will be released in Belgium as well as Germany (supposing that every AG013 bacteria in the mouth rinse is released viable, which is impossible and a worst-case scenario).

(b) Duration of the operation:

Recruitment of the first participants in Belgium is expected to start in July 2018. Completion will depend on availability of subjects fulfilling the selection criteria and could take until June 2019 (Last Patient Completes Treatment) and July 2020 (Last Patient Completes Long-term Follow-up, equals last patient last visit). In the Phase 2 study, each participant will be treated for 7-9 weeks (depending on the participant's prescribed chemoradiation therapy).

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

The AG013 lyophilised powder is packed in child-proof bottles. Participants will be instructed how to prepare and administer the AG013 MR. The first administration of AG013 will be witnessed at the clinical trial centre. Detailed instructions for dose preparation and dispensing as well as a questions and answers booklet with detailed instructions on the actions to be taken in case of spillage or accident will be provided to subjects. The participants will receive a supply of doses for only one week (+ 1 spare day), which reduces the amount of bacteria outside of a control clinical setting.

In the event that the packaging would be disrupted, the powder quickly degrades when in contact with moisture 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 bottle contains one mouth rinse) 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 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 as per local institutional requirements and guidelines.

The GMO is expected to remain viable for up to 24 hours in the oral cavity after administration and may be shed in saliva. The risk of shedding via the fecal route is expected to be insignificant as patients are not required to swallow AG013. It is expected to be held for 30 seconds in the mouth and then discarded into the sink or toilet. During the Phase 1 healthy volunteer studies, live bacteria were not detected in fecal matter and so the risk of shedding via this route is very low. The biological containment and the absence of relevant impact by AG013 is deemed sufficient not to warrant any specific treatment of the shedding environment.

All used and unused study drug bottles that may still contain GM material will be returned by the participant to the clinical trial centre.

If required, a standard antibiotic treatment would suffice to inactivate the bacteria. In the event of spillage, standard disinfectants can be used.

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.

To date, AG013 has been studied in humans in a Phase 1b study in the US and a Phase 1 pharmacokinetic (PK) study in healthy volunteers in Belgium.

The Phase 1b study was a multicenter, single-blinded, placebo-controlled, sequential dose-escalation study that evaluated the safety, tolerability, and PK profile of AG013 in subjects experiencing OM during induction chemotherapy (CT) for the treatment of Head and Neck Cancer (HNC) [ClinicalTrials.gov Identifier: NCT00938080].

A total of at least 21 subjects were planned to be enrolled in 3 successive groups of at least 7 subjects each (at least 5 subjects were assigned to AG013 and at least 2 subjects were assigned to placebo). An independent data and safety monitoring board (DSMB) reviewed the safety results from each group prior to dose escalation. The study achieved its primary objective by demonstrating that AG013 was generally safe and well tolerated. The incidence of sepsis due to AG013 was followed as an event of special interest in this clinical study and no subjects experienced an adverse event of this type.

The Phase 1 PK study in healthy volunteers was a single-center, open-label Phase 1 study to assess the effect of food/beverage and to characterize the pharmacokinetics of single and multiple oral doses of AG013 in healthy subjects. Ten subjects were enrolled in the study.

AG013 was generally safe when applied by mouth rinse once or three times on one day. There was no evidence for systemic exposure neither to live AG013 bacteria (blood) nor to hTFF1 secreted (serum) and there was no recovery of live AG013 bacteria in feces.

The 2 completed phase I studies support safe administration of AG013 for attenuation of OM in patients with cancer of head and neck receiving concomitant chemoradiation therapy.

Moreover, another similar developed compound has been studied in a Phase 1b/2a in Crohn's disease patients under deliberate release in Belgium and in Sweden. 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 CFU was detected in faeces.

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 head and neck cancers receiving chemotherapy and/or radiotherapy).

(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 participants will use the GMO as a mouth rinse. Participants will rinse with the suspension for 30 seconds, three times each day using the assigned IMP. The suspension will then be expectorated into a sink or toilet. The organism will produce hTFF1 in the mouth. The protein is expected to alleviate oral mucositis.

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

No specific interactions with non-target organisms have been identified. AG013 bacterial strain sAGX0085 will be present in compartments which are natural for *L. lactis*, essentially the human gastrointestinal tract and sewage system. With the exception of *htff1*, no other change has occurred, therefore it is expected that the impact will be similar to that of *L. lactis*. Again, the possible interactions will be more limited given the additional attenuated features and reduced life expectancy of AG013 strain sAGX0085 compared with wildtype *L. Lactis.*

The therapeutic protein hTFF1 generated by AG013 is non-toxic. It binds to salivary mucins and forms a mucus layer over the epithelia of the mouth, acting as a physical barrier against bacteria and noxious environmental agents. Moreover, TFF peptides have wound-healing properties and are important in protecting and healing mucosal tissues. In the pre-clinical

trials healthy and diseased hamsters, rats and dogs have been administered AG013 without adverse effects. No local or systemic pathogenic effects were documented after chronic administration of AG013.

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 will be directly released into the sewage system, with a possible minimal amount, passing the intestines and evacuating via stool, eventually also being released into 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:

L. lactis 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 this 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:

L. lactis 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 *htff1* 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. Taking into consideration the manner, scale and environment of release, the mode of transmission and survivability of the parent organism (L. lactis and MG1363 strain), the available data relating to shedding of AG013 in clinical trials and the risk management measures in place, it is considered that the risk of exposure of an unintended recipient and the natural environment to AG013, or a genetic variant of AG013 is very low.

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 *httf1* gene in the GMO is a unique, synthetic gene that can be distinguished from the native *httf1* gene and detected via PCR. A method has also been developed to distinguish between live and dead bacteria. An ELISA method is available to detect hTFF1 expressed by sAGX0085.

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 *httf1* gene can be used.

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

Not relevant.

5. Duration of the monitoring

Standard monitoring of the patients will occur as per the clinical study protocol.
The GMO will only be monitored in the USA. The last time point for monitoring of the GMO in buccal smears and blood samples is 2 weeks after the last day of IMP dosing. No other monitoring with the exception of treated patient buccal smears and blood sampling has been planned.

6. Frequency of the monitoring

Patients will be monitored as per the monitoring plan in the clinical study protocol.

In the USA, blood and buccal smears will be collected at specific time points in a subset of subjects (15 subjects on active and 15 subjects on placebo) who consent separately. Monitoring is planned at baseline (before the start of the treatment). During the clinical trial samples will be collected at week 3 and 5, on the last day of radiotherapy, on the last day of IMP dosing and 2 weeks after the last IMP dosing.

I. Information on post-release and waste treatment

1. Post-release treatment of the site

The clinical trial centres will disinfect equipment and surfaces according to standard medical procedures.
Participants will leave the clinical setting during treatment and will be trained and given instructions on storage, preparation, administration and destruction of bottles/packaging that

have come in to contact with AG013. As well as instruction in the event of spillage of AG013 as a result of breakage of the glass bottles.
Although shedding of live bacteria will occur via sewer system, 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.
In case of spillage, the affected area can be decontaminated with a standard detergent (soap) or bleach.

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, wipes, etc.).
- Spat out bacterial suspension, hygienic wipes, disposed of in sewage system.

(b) Treatment of waste

Disposable items will be appropriately destroyed by set guidelines and according to institutional standards. Due to the non-pathogenic, non-replicating nature and limited survival capacity of the GMO, no additional treatment is required. 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 or the suspended liquid. Application of standard detergent (soap) or bleach would be sufficient to eradicate the GMOs and decontaminate the affected area. Special instructions will be provided to the study subject.

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

In case of spillage, the affected area can be decontaminated with a standard detergent (soap) or bleach.

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

This is not applicable. AG013 is being used as part of a clinical study and plants, animals and soils are not expected to be exposed to the GMO. The GMO has limited survival capacity.

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.

K. References

Johansen, E., Challenges when transferring technology from Lactococcus laboratory strains to industrial strains. Genet Mol Res, 2003. 2(1): p. 112-6.

Gasson, M.J., Plasmid complements of *Streptococcus lactis* NCDO 712 and other lactic streptococci after protoplast-induced curing. J Bacteriol, 1983. 154(1): p. 1-9.