THE UNIVERSITY OF WESTERN ONTARIO
BIOHAZARDOUS AGENTS REGISTRY FORM
Approved Biohazards Subcommittee: November 21, 2008
Biosafety Website: www.uwo.ca/humanresources/biosafety/

This form must be completed by each Principal Investigator holding a grant administered by the University of Western Ontario or in charge of a laboratory/facility where the use of Level 1, 2 or 3 biohazardous agents is described in the laboratory or animal work proposed. The form must also be completed if any work is proposed involving animals carrying zoonotic agents infectious to humans or involving plants, fungi, or insects that require Health Canada (HC) or Canadian Food Inspection Agency (CFIA) permits.

This form must also be updated at least every 3 years or when there are changes to the biohazards being used.

Containment Levels will be established in accordance with Laboratory Biosafety Guidelines, 3rd edition, 2004, Health Canada (HC) or Containment Standards for Veterinary Facilities, 1st edition 1996, Canadian Food Inspection Agency (CFIA).

Completed forms are to be returned to Occupational Health and Safety, (OHS), (Support Services Building, Room 4190) for distribution to the Biohazard Subcommittee. For questions regarding this form, please contact the Biosafety Officer at extension 81135. If there are changes to the information on this form (excluding grant title and funding agencies), modifications must be submitted to Occupational Health and Safety. See website: www.uwo.ca/humanresources/biosafety/

PRINCIPAL INVESTIGATOR Dr William Barnett
SIGNATURE
DEPARTMENT Department of Medicine - Gastroenterology
ADDRESS 339 Windermere Rd. London ON, N6H 5A5
PHONE NUMBER 519-685-8500 x 33757
EMAIL william.barnett@lhsc.on.ca

Location of experimental work to be carried out: Building(s) LHSC-UH Room(s)_____

*For work being performed at Institutions affiliated with the University of Western Ontario, the Safety Officer for the Institution where experiments will take place must sign the form prior to its being sent to Occupational Health and Safety (See Section 12.0, Approvals). For research being done at Lawson Health Research Institute, London Regional Cancer Program, Child and Parent Research Institute, or Robarts Research Institute, a University Biosafety Committee member can also sign as the Safety Officer for the Institution.

FUNDING AGENCY/AGENCIES: Sponsored Clinical Trial - Actogenix NV
GRANT TITLE(S): Protocol AG011-mpcu-201-A Phase 2a Randomized Placebo Controlled Double-Blind, Multicentre Dose Escalation Study to Evaluate the Safety, Tolerability Pharmacodynamics & Efficacy of AG011 in Subjects with Moderately Active Ulcerative Colitis

PLEASE ATTACH A BRIEF DESCRIPTION OF YOUR WORK THAT EXPLAINS THE BIOHAZARDS USED AND HOW THEY WILL BE USED. PROJECTS SUBMITTED WITHOUT A SUMMARY WILL NOT BE REVIEWED.

Names of all personnel working under Principal Investigators supervision in this location:

Dr Melanie Beaton

1.0 Microorganisms

1.1 Does your work involve the use of microorganisms or biological agents of plant or animal origin (including but not limited to viruses, prions, parasites, bacteria)? O YES ☒ NO Bactena, not plant or animal origen
If no, please proceed to Section 2.0

Do you use microorganisms that require a permit from the CFIA? O YES ☒ NO
If YES, please give the name of the species. _____
What is the origin of the microorganism(s)? Recipient strain used in genetic engineering is derivative of L. Lactis, originally isolated from raw milk,
Please describe the risk (if any) of escape and how this will be mitigated:
Genetic Engineering has incorporated a biological containment Strategy, described in attached documents.

Please attach the CFIA permit.
Please describe any CFIA permit conditions:

1.2 Please complete the table below:

Name of Biological agent(s)*	Is it known to be a human pathogen? YES/NO	Is it known to be an animal pathogen? YES/NO	Is it known to be a zoonotic agent? YES/NO	Maximum quantity to be cultured at one time? (in Litres)	Source/ Supplier	Health Canada or CFIA Containment Level
Lactococcus Lactis ~~AGX0037~~	O Yes ☒ No	O Yes ☒ No	O Yes ☒ No	NA	Actogenix NV	O 1 O 2 O 3 Biosafety Class 1
	O Yes O No	O Yes O No	O Yes O No			O 1 O 2 O 3
	O Yes O No	O Yes O No	O Yes O No			O 1 O 2 O 3
	O Yes O No	O Yes O No	O Yes O No			O 1 O 2 O 3

*Please attach a Material Safety Data Sheet or equivalent from the supplier.

2.0 Cell Culture

2.1 Does your work involve the use of cell cultures? O YES ☒ NO
If no, please proceed to Section 3.0

2.2 Please indicate the type of primary cells (i.e. derived from fresh tissue) that will be grown in culture in the table below

Cell Type	Is this cell type used in your work?	Source of Primary Cell Culture Tissue	AUS Protocol Number
Human	O Yes O No		Not applicable
Rodent	O Yes O No		
Non-human primate	O Yes O No		
Other (specify)	O Yes O No		

*** DESCRIPTION MUST BE ATTACHED TO THIS FORM OR PROJECT WILL NOT BE REVIEWED***

2.3 Please indicate the type of established cells that will be grown in culture in the table below.

Cell Type	Is this cell type used in your work?	Specific cell line(s)*	Supplier / Source
Human	O Yes O No		
Rodent	O Yes O No		
Non-human primate	O Yes O No		
Other (specify)	O Yes O No		

*Please attach a Material Safety Data Sheet or equivalent from the supplier. (For more information, see www.atcc.org)

2.4 For above named cell types(s) indicate HC or CFIA containment level required O 1 O 2 O 3

3.0 Use of Human Source Materials

3.1 Does your work involve the use of human source materials? O YES O NO
If no, please proceed to Section 4.0

3.2 Indicate in the table below the Human Source Material to be used.

Human Source Material	**Source/Supplier /Company Name**	**Is Human Source Material Known to Be Infected With An Infectious Agent? YES/NO**	**Name of Infectious Agent (If applicable)**	**HC or CFIA Containment Level (Select one)**
Human Blood (whole) or other Body Fluid		O Yes O No		O 1 O 2 O 3
Human Blood (fraction) or other Body Fluid		O Yes O No		O 1 O 2 O 3
Human Organs or Tissues (unpreserved)		O Yes O No		O 1 O 2 O 3
Human Organs or Tissues (preserved)		O Yes O No		O 1 O 2 O 3

4.0 Genetically Modified Organisms and Cell lines

4.1 Will genetic modifications be made to the microorganisms, biological agents, or cells described in Sections 1.0 and 2.0? O YES O NO If no, please proceed to Section 5.0

4.2 Will genetic modification(s) involving plasmids be done? O YES, complete table below O NO

Bacteria Used for Cloning *	Plasmid(s) *	Source of Plasmid	Gene Transfected	Describe the change that results

* Please attach a Material Data Sheet or equivalent if available.

4.3 Will genetic modification(s) involving viral vectors be done? O YES, complete table below ⊗NO

Virus Used for Transduction *	Vector(s) *	Source of Vector	Gene Transfected	Describe the change that results

* Please attach a Material Safety Data Sheet or equivalent.

4.4 Will genetic sequences from the following be involved?

- HIV O YES, please specify _____ O NO
- HTLV 1 or 2 or genes from any Level 1 or Level 2 pathogens O YES, specify _____ O NO
- SV 40 Large T antigen O YES O NO
- E1A oncogene O YES O NO
- Known oncogenes O YES, please specify _____ O NO
- Other human or animal pathogen and or their toxins O YES, please specify _____ O NO

4.5 Will virus be replication defective? O YES ⊗NO

4.6 Will virus be infectious to humans or animals? O YES ⊗NO

4.7 Will this be expected to increase the containment level required? O YES ⊗NO

5.0 Human Gene Therapy Trials

Not a Viral Vector

5.1 Will human clinical trials be conducted using the viral vector in 4.0? O YES O NO
If no, please proceed to Section 6.0 If YES attach a full description of the make-up of the virus.

5.2 Will virus be able to replicate in the host? O YES O NO

5.3 How will the virus be administered? Not a virus

5.4 Please give the Health Care Facility where the clinical trial will be conducted: LHSC-UH

5.5 Has human ethics approval been obtained? ⊗YES, number: 15342 O NO O PENDING

6.0 Animal Experiments

6.1 Will live animals be used? O YES ⊗NO If no, please proceed to section 7.0

6.2 Name of animal species to be used _____

6.3 AUS protocol # _____

6.4 Will any of the agents listed be used in live animals O YES, specify: _____ O NO

*** DESCRIPTION MUST BE ATTACHED TO THIS FORM OR PROJECT WILL NOT BE REVIEWED***

7.0 Use of Animal species with Zoonotic Hazards

7.1 Will any of the following animals or their organs, tissues, lavages or other body fluids including blood be used?

- Pound source dogs O YES O NO
- Pound source cats O YES O NO
- Cattle, sheep or goats O YES O NO
- Non- Human Primates O YES, please specify species _____ O NO
- Wild caught animals O YES, please specify species & colony # _____ O NO
- Birds O YES O NO
- Others (wild or domestic) O YES, please specify _____ O NO

8.0 Biological Toxins

8.1 Will toxins of biological origin be used? O YES X NO If no, please proceed to Section 9.0

8.2 If YES, please name the toxin(s)_____
Please attach information, such as a Material Safety Data Sheet, for the toxin(s) used.

8.3 What is the LD_{50} (specify species) of the toxin_____

9.0 Insects Requiring CFIA Permits

9.1 Do you use insects that require a permit from the CFIA? O YES X NO
If no, please proceed to Section 10.0

9.2 If YES, please give the name of the species. _____

9.3 What is the origin of the insect? _____

9.4 What is the lifestage of the insect? _____

9.5 What is your intention? O Initiate and maintain colony, give location: _____
O "One-off" use, give location: _____

9.6 Please describe the risk (if any) of escape and how this will be mitigated:
_____
_____
_____

9.7 Please attach the CFIA permit.

9.8 Please describe any CFIA permit conditions:
_____
_____
_____

*** DESCRIPTION MUST BE ATTACHED TO THIS FORM OR PROJECT WILL NOT BE REVIEWED***

10.0 Plants Requiring CFIA Permits

10.1 Do you use plants that require a permit from the CFIA? O YES ⊘ NO
If no, please proceed to Section 11.0

10.2 If YES, please give the name of the species. _____

10.3 What is the origin of the plant? _____

10.4 What is the form of the plant (seed, seedling, plant, tree…)? _____

10.5 What is your intention? O Grow and maintain a crop O "One-time" use

10.6 Do you do any modifications to the plant? O YES O NO
If yes, please describe: _____
_____

10.7 Please describe the risk (if any) of loss of the material from the lab and how this will be mitigated:
_____
_____
_____

10.8 Is the CFIA permit attached? O YES O NO

10.9 Please describe any CFIA permit conditions:
_____
_____
_____

11.0 Import Requirements

11.1 Will any of the above agents be imported? ⊘ YES, please give country of origin Belgium
If no, please proceed to Section 10.0 O NO

11.2 Has an Import Permit been obtained from HC for human pathogens? Not a pathogen O YES O NO

11.3 Has an import permit been obtained from CFIA for animal or plan pathogens? O YES O NO

11.4 Has the import permit been sent to OHS? O YES, please provide permit # _____ O NO
other - New Substance Notification # EAU 439

12.0 Training Requirements for Personnel Named on Form

All personnel named on the above form who will be using any of the above named agents are required to attend the following training courses given by OHS:

Hospital employees have received WHMIS + employee Health & Safety training; They follow hospital policies + SOPs for biosafety & waste management.

- ~~Biosafety~~
- ~~Laboratory and Environmental/Waste Management Safety~~
- WHMIS (Western or equivalent)
- Employee Health and Safety Orientation

As the Principal Investigator, ~~I~~ have ensured ~~that~~ all of the personnel named on the form who will be using any of the biohazardous agents in Sections 1.0 to 9.0 have been trained.

SIGNATURE _____

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13.0 Containment Levels

11.1 For the work described in sections 1.0 to 9.0, please indicate the highest HC or CFIA Containment Level required. O 1 O 2 O 3

13.2 Has the facility been certified by OHS for this level of containment?

O YES, permit # if on-campus_____
O NO
O NOT REQUIRED

14.0 Procedures to be Followed – Employees will follow LHSC procedures for safety & disposal of materials

14.1 As the Principal Investigator, I will ensure that this project will follow the Western Biosafety Guidelines and Procedures Manual for Containment Level 1 & 2 Laboratories (and the Level 3 Facilities Manual for Level 3 projects). I will ensure that UWO faculty, staff and students working in my laboratory have an up-to-date Hazard Communication Form, found at http://www.wph.uwo.ca/

SIGNATURE _____ Date: 2009-04-07

15.0 Approvals

UWO Biohazard Subcommittee: SIGNATURE: _____
Date: _____

Safety Officer for Institution where experiments will take place: SIGNATURE: _____
Date: _____

Safety Officer for University of Western Ontario (if different from above): SIGNATURE: _____
Date: _____

Approval Number: _____ Expiry Date (3 years from Approval): _____

Special Conditions of Approval:

*** DESCRIPTION MUST BE ATTACHED TO THIS FORM OR PROJECT WILL NOT BE REVIEWED***

Joan Morris

From: Decebal Bora [decebal.bora@actogenix.com]
Sent: Monday, October 06, 2008 11:36 AM
To: Mark Vaeck; Bernard Coulie; Annegret Van der Aa; Luc Bereau; Lothar Steidler; Patrick Rudelsheim (Perseus); Tim De Smedt; Klaas Vandenbroucke; Sabine Neirynck; Patrick Dhaese; Sam Corveleyn; Pieter Rottiers; Peter Vanhoenacker; Michelle DeLaCroix
Subject: FORWARD AS RELEVANT - EAU-439 - NSN - Approval

Attachments: pic15862.jpg

pic15862.jpg (3 KB)

Dear all;
Please find enclosed the following email from the New Substance Assessment and Control Bureau.
"Health Canada concluded that there is no suspicion of toxicity. You may begin importing the substance after today. Joëlle will be sending you an official "No Suspicion of Toxic" letter with instructions for DSL listing shortly."
Herein, congratulations to all those who contributed to the meeting with this important milestone.
With best regards

Decebal Bora
Pharm. D. - M.P.H.
Director Regulatory Affairs

ActoGeniX N.V.
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Tel: +32 (0)9 261 06 00
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decebal.bora@actogenix.com
www.actogenix.com

-----Original Message-----
From: Kathrina Yambao [mailto:kathrina_yambao@hc-sc.gc.ca]
Sent: maandag 6 oktober 2008 17:25
To: Decebal Bora
Cc: Dr. Brian Feagan
Subject: Re: EAU-439 - NSN - status

Hello Dr. Bora,

My apologies, I just came back to the office from a week vacation. HC concluded that there is no suspicion of toxicity. You may begin importing the substance after today. Joëlle will be sending you an official "No Suspicion of Toxic" letter with instructions for DSL listing shortly (the NDSL do not apply to new biotech substances).

Regards,

Kathrina Yambao
Senior Biologist
Biotechnology Section
New Substances Assessment and Control Bureau Health Canada 8th Floor, Room A822, MacDonald Building
123 Slater Street
Ottawa, Ontario, K1A 0K9
P.L. 3508D

Tel.: (613) 948-4881

"Decebal Bora"
<decebal.bora@actogenix.com>

2008-10-01 08:59 AM

To
"Kathrina Yambao"
<kathrina_yambao@hc-sc.gc.ca>

cc
"Dr. Brian Feagan"
<bfeagan@robarts.ca>

Subject
EAU-439 - NSN - status

Dear Dr Yambao;
In view of the forthcoming end of assessment (October 6, 2008) of the referred to file (see attachment), we would be grateful to you to let us know whether we can expect a positive outcome of the said evaluation before or by October 6, 2008. It is our understanding that past October 6, 2008, we may begin importing the related substance should there be no action taken by the Canadian government.
For your information, we have received a No Objection Letter from Health Canada - BGTD for our CTA on July 31, 2008 (and we have addresses appropriately within 30 days the binding comments and most of the non binding ones)(see attachment)..

As you can imagine, we would thank any positive feed back from you that would enable us initiate the clinical trial in Canada as swifly as possible by reducing the time-dependenc of all the related administrative paper work (such but not limited to Faxback forms with BGTD, notification to relevant EU authorities of the shipment schedule to Canada, etc.).

In addition, in the event of a positive outcome of the procedure, we'd like to hereby confirm that we wish to be enlisted on the appropriate lists (i.e. per our understanding the Non Domestic Substance List). Hence, we would be grateful to you keeping us informed on the process to follow.

Should you need any additional information, please do not hesitate to contact us,
Looking forward to hearing from you soon, Yours sincerely,

Decebal Bora

Pharm. D. - M.P.H.

Director Regulatory Affairs

(Embedded image moved to file: pic15862.jpg)

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decebal.bora@actogenix.com

www.actogenix.com

From:	Annegret Van der Aa
To:	tina.cyr@cbsa.gc.ca;
CC:	Luc Bereau; Sam Corveleyn; Decebal Bora; Joan Morris; Jennifer Royce;
Subject:	ActoGeniX" AG011 GMO import into Canada
Date:	Friday, July 25, 2008 11:38:35 AM
Attachments:	MSDS AG011 v1.0 20080611.doc image001.jpg

Dear Tina,

We want to follow-up on the telephone discussion we had Wednesday regarding importation of ActoGeniX material (genetically modified organisms (GMO)) for a phase 2a Clinical Trial) into Canada.

ActoGeniX (AGX) material (product code AG011) is a freeze-dried living genetically modified *Lactococcus lactis* bacterium expressing human interleukin-10 (hIL-10), for oral and rectal administration and comprising a biological containment system (avoiding multiplication and survival of the bacterium).

Therefore, our products fall under the following classification:

1. Tariff classification 3002.90.99 (**Chapter 30 - PHARMACEUTICAL PRODUCTS - 30.02 Human blood; animal blood prepared for therapeutic, prophylactic or diagnostic uses; antisera and other blood fractions and modified immunological products, whether or not obtained by means of biotechnological processes; vaccines, toxins, cultures of microorganisms (excluding yeasts) and similar products. 3002.90.00 –Other** 99 - - - - - *-Other*)
2. Dangerous good classification: class 9 (because it is a GMO)
3. Biosafety classification: class 1

The current situation for Canada is depicted below:

- Ongoing assessments:

Agency	Document under review	Submission Date	Outcome expected by
Health Canada	Clinical Trial Application (CTA)	June 30, 2008	August 1, 2008
Environment Canada	New Substance Notification (NSN)	June 9, 2008	October 6, 2008

- Import information

Name of the importer	Robarts Research Institute PO Box 5015, 100 Perth Drive N6A 5K8 London – ON Tel.: (519) 663-3400 Fax: (519) 663-3807
Proposed port of entry into Canada	Pearson International Airport 3111 Convair Drive Toronto - ON
Central Depot	DiLan Clinical Packaging Ltd (Contact Jennifer Royce) 1 Rimini Mews LSN 4K1 Mississauga –ON Tel: (905) 363-2100 Fax: (905) 363-2101

Once the approval is granted, we plan to import AGX material from UK into Canada with the following documents attached to the shipment:

- No Objection Letter from Health Canada
- CTA fax-back form - clinical trial material(s) – needed for biotechnological products
- NSN approval authorizing the import into Canada
- QP release certificate
- Certificate of Analysis
- Airway bill
- Commercial Invoice
- Directions for use of the investigational medicinal products

Would you mind confirming whether the above quoted documents are sufficient for the import into Canada? In attachment, we will also send you the MSDS. If the document does not get through your firewall, please let us know so we can fax it to you. If you would like to receive other documentation, please let us know!

In addition, we would appreciate if you could inform us about the CBSA department we should contact in case of additional questions.

Finally, we want to thank you for your help.

Should you need any further information, do not hesitate to come back to us.

With best regards,

Annegret

Annegret Van der Aa, PhD
Clinical Project Manager
ActoGeniX

ActoGeniX NV
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: Annegret.Vanderaa@actogenix.com

robarts
CLINICAL TRIALS

November 10, 2008

Ms. Joelle Pinsonnault Cooper, Notification Specialist,
Environmental Assessment Unit (EAU)
c/o New Substances Division
Science and Technology Branch
Environment Canada
Fontaine Building, 8th Floor
Gatineau QC
K1A 0H3

Courier: Director, New Substances Division
Department of the Environment
Fontaine Building, 8th Floor
200 Sacré-Coeur Boulevard
Gatineau QC
J8X 4C6

Dear Ms. Pinsonnault Cooper:

Commencement of Import
Reference to: NSN Submission Number - EAU 439

Please accept this letter in relation to the New Substance Notification (EAU 439) for genetically modified *Lactococcus lactis* (*L. lactis*).

In response to the email received from Dr. Kathrina Yambao dated October 6, 2008 confirming Health Canada's conclusion of no suspicion of toxicity and allowing the import of product containing the substance, we wish to inform the EAU that product for use in our approved Clinical Trial was first shipped into Canada on October 16, 2008.

Please note that this submission contains Confidential Third Party information. Disclosure may result in material of financial loss or prejudice of competitive position. See Section 20, *Access to Information Act*.
Should you have questions, please do not hesitate to contact Decebal Bora (ActoGeniX N.V.) at +32 (0)9 261 06 00 or +32 (0)474 807 870 (cell).

Sincerely,

Dr. Brian Feagan
Robarts Research Institute

cc: Decebal Bora – Pharm.D. - MPH
Director Regulatory Affairs
ActoGeniX N.V.

Michelle DeLaCroix, RAC
Associate Director, CTA Group
CanReg Inc.

1.3 PHYSICOCHEMICAL PROPERTIES AND CLINICAL FORMULATION

1.3.1 Physicochemical properties

L. lactis is a non-pathogenic, non-invasive, non-colonizing Gram-positive bacterium and is primarily used to produce fermented foods. Prior to the industrial use in the manufacture of dairy products, *L. lactis* may have been a commensal to specific plants. No colonization of any other ecological niche has ever been reported.

The recipient strain used in the genetic engineering of sAGX0037 was *L. lactis* subsp. *cremoris* MG1363. *L. lactis* MG1363 is a derivative of the natural isolate *L. lactis* National Collection of Dairy Organisms (NCDO) 712, which was originally isolated from raw milk and is ecologically contained to foods, primarily dairy products. Importantly, MG1363 is devoid of the metabolic pathways that enable the use of milk carbohydrate and amino acid sources and can therefore no longer grow in its ancestral ecologic niche.

Furthermore, inherent to the genetic engineering of sAGX0037, the thymidylate synthase gene (*thyA*) was replaced by an hIL-10 expression cassette. As such, sAGX0037 became dependent on external supplementation of thymine or thymidine for growth and survival. In contrast to other auxotrophies, lack of thymine/thymidine will induce suicide in a *thyA*-deficient (thyA$^-$) strain. Furthermore, the desired transgene is inserted within the *thyA* locus in such a way that -in the hypothetical event that an intact *thyA* gene was reacquired by homologous recombination with wild-type *L. lactis*-, the transgene would be deleted. Finally, antibiotic resistance genes and plasmid vectors, both environmental concerns with some genetically modified organisms (GMO's), are not present in sAGX0037.

The combination of restrictions as described above, ensures a fail-safe environmental containment strategy: sAGX0037 can no longer propagate outside of artificially supplemented laboratory cultures, and will die quickly once released.

1.3.2 Clinical formulation

The DS is an amorphous, slightly yellow, freeze-dried powder of *L. lactis* sAGX0037 and cryoprotectants, at a concentration of No Less Than (NLT) 6.4 x 10^{11} Colony Forming Units (CFU)/g. For the upcoming Phase 2a study, the clinical formulations of the AG011 DP consist of an oral, solid dosage form and a rectal, liquid dosage form.

The oral, solid dosage form comprises delayed-release, enteric-coated capsules formulated at 1.2 x 10^{10} CFU or 1.2 x 10^{11} CFU respectively per capsule.
The rectal, liquid dosage form consists of a powder formulation and an enema solution (0.9% NaCl in purified water) for resuspension, which -when reconstituted- contains 2.4 x 10^{10} CFU/100 ml, 2.4 x 10^{11} CFU/100 ml or 1.2 x 10^{12} CFU/100 ml respectively, dependent on the dose level.

ActoGeniX	DRC DENYS RESEARCH CONSULTANTS	robarts CLINICAL TRIALS

PROTOCOL SYNOPSIS

Protocol Number: AG011-MDUC-201

A Phase 2a Randomized, Placebo-Controlled, Double-Blind, Multi-Center Dose Escalation Study, to Evaluate the Safety, Tolerability, Pharmacodynamics and Efficacy of AG011, in Subjects with Moderately Active Ulcerative Colitis

Version:	**Version 3.0, incorporates Amendment 02**
Version date:	**26/05/2008**
Version status:	**Final**
Previous version date:	**14/05/2008**
Sponsor:	ActoGeniX NV. Technologiepark 4 9052 Zwijnaarde Belgium
Contract Research Organizations:	Denys Research Consultants bvba Proeftuinstraat 84 9000 Gent Belgium
	Robarts Clinical Trials Robarts Research Institute P.O. Box 5015 100 Perth Drive London, Ontario, Canada N6A 5K8

1. TITLE

A Phase 2a, Randomized, Placebo-Controlled, Double-Blind, Multi-Center Dose Escalation Study, to Evaluate the Safety, Tolerability, Pharmacodynamics and Efficacy of AG011, in Subjects with Moderately Active Ulcerative Colitis.

2. OBJECTIVES

2.1. THE PRIMARY OBJECTIVES ARE:

- to assess the safety and tolerability of AG011 in subjects with moderately active ulcerative colitis (UC),
- to assay for fecal excretion of AG011 and assess the environmental containment strategy, and
- to obtain pharmacodynamic (PD) data (biomarkers) of AG011 in a subset of subjects (Belgian subjects only).

2.2. THE SECONDARY OBJECTIVE IS:

- to determine the efficacy of AG011 in reducing inflammation in the colon, as measured by clinical observations and endoscopy.

3. STUDY DESIGN

This is a Phase 2a, randomized, double-blind, placebo-controlled, multi-centre dose escalation study. A total of 60 subjects with moderately active UC will be evaluated.

Subjects will be entered sequentially into one of three dose groups, starting from the lowest dose group. Within each of the first two dose groups, 15 subjects will be entered. Within the highest dose group, 30 subjects will be entered.

Within each dose group, subjects will be randomly assigned in a 2:1 ratio to receive either AG011 or placebo for 28 days.

A follow-up clinic visit will occur 4 weeks after the subject completes study treatment. All subjects will be contacted by phone for long-term follow-up 40 weeks after the last dose.

4. STUDY CONTINUATION AND DOSE ESCALATION

Timely monitoring of safety data is planned for the study, such that subject enrolment can continue without interruption for the purpose of data collection between dose groups. Safety and tolerability will be closely monitored by the Clinical Safety Specialist (CSS) assigned to the study. The CSS will review adverse events and laboratory safety data and report any safety concerns to the Sponsor and a Data Safety Monitoring Committee (DSMC).

If at anytime during the study a subject becomes severely anemic (hemoglobin < 8.5 g/dL) or the platelet count falls below 100 x 10^9/L (100,000/mm^3) or any serious adverse event (SAE) is reported, the DSMC will convene to assess the abnormal findings and/or SAE and a decision will subsequently be made regarding study continuation.

At least 8 subjects must have safely completed study treatment for 28 days at a specific dose level, prior to escalation to the next dose group. The DSMC will convene to assess safety data when 8 subjects have completed study treatment for 28 days at the specific dose level. The role of the DSMC for the study will be complete when all subjects in the study have completed study treatment.

5. STUDY POPULATION

60 subjects will be enrolled from approximately 14 centers in North America and Europe.

5.1. MAIN CRITERIA FOR INCLUSION INCLUDE:

(1) Male or non-pregnant, non-lactating females, 18 years of age or older. Females of child bearing potential must have negative serum or urine pregnancy tests at the screening visit and throughout the study, and must use a hormonal (oral, implantable or injectable) or barrier method of birth control throughout the study. Females unable to bear children must have documentation of such in the case report form (i.e. tubal ligation, hysterectomy, or post-menopausal [defined as a minimum of one year since the last menstrual period]).

(2) Documented diagnosis of UC, with a minimum disease extent of 15 cm from the anal verge.

(3) Presence of friability on endoscopy, with minimum of Grade 2 (modified Baron score) changes at approximately 15 cm or more from the anal verge.

(4) Minimum Mayo Clinic Disease Activity Score of 5, with a score of at least 1 on both the stool frequency and rectal bleeding components.

(5) Receiving 5-aminosalicylic acid (5-ASA) treatment for at least two months and a stable dose of oral 5-ASA for at least two weeks prior to randomization. Concurrent treatment with prednisone, or equivalent glucocorticoid ≤ 20 mg/day is acceptable as follows:

a. minimum dosing of 4 weeks prior to screening AND
b. stable dose for 2 weeks prior to screening AND
c. expected to remain on a constant dose during the trial.

Use of 5-ASA compounds is not required for those subjects who have failed treatment with 5-ASA compounds, or are allergic or intolerant.

(6) Hepatic function (aspartate transaminase (AST), alanine transaminase (ALT), total bilirubin, alkaline phosphatase, lactatedehydrogenase (LDH)) ≤ 2 times the upper limit of the normal range.

(7) Adequate renal function, as evidenced by serum creatinine ≤ 1.5 times the upper limit of the normal range.

(8) Hemoglobin ≥ 10 g/dL.

(9) Absolute neutrophil count (ANC) ≥ 1.5 x 10^9/L (1,500 mm^3).

(10) Lymphocyte count ≥ 0.1 x 10^3/µL.

(11) Platelet count ≥ 100 x 10^9/L (100,000/mm^3).

(12) Ability of subject to participate fully in all aspects of this clinical trial.

(13) Written informed consent must be obtained and documented.

5.2. MAIN CRITERIA FOR EXCLUSION INCLUDE:

(1) Exhibiting severe ulcerative colitis as defined by the following criteria:

≥6 bloody stools daily with one or more of the following:

a. oral temperature > 37.8 °C or > 100.0 °F,
b. pulse > 90/min,
c. hemoglobin < 10 g/dL.

(2) Crohn's disease.

(3) History of colectomy or partial colectomy.

(4) *C. difficile* positive at screening visit or treated for *C. difficile* within the 4 weeks prior to randomization.

(5) Treatment with antibiotics or probiotics at screening.

(6) Treatment with cyclosporine, methotrexate, azathioprine, 6-MP, infliximab, adalimumab or other immunosuppressants/biologics within 4 weeks prior to randomization.

(7) Use of rectal steroids or 5-ASA enemas within 2 weeks prior to randomization.

(8) Clinically significant active infection.

(9) Known chronic liver disease.

(10) Serious underlying disease other than UC in the opinion of the investigator.

(11) Alcohol or illicit drug consumption, which in the opinion of the investigator, may interfere with the subject's ability to comply with the study procedures.

(12) Active psychiatric problems, which in the opinion of the investigator, may interfere with the subject's ability to comply with the study procedures.

(13) History of malignancy other than basal or squamous cell cancer of the skin that has been removed, or carcinoma in situ of the cervix that has been adequately treated.

(14) History of dysplasia in colonic biopsies.

(15) Receiving any investigational therapy or any approved therapy for investigational use within 30 days or 5 half-lives prior to randomization (whichever is longer).

(16) Pregnant or lactating women.

(17) Prior enrolment in the current study and had received study treatment.

6. TREATMENTS, DOSAGE AND ADMINISTRATION

Three dose levels of AG011 will be evaluated.

Subjects assigned to the AG011 group, at the first dose level, will receive orally administered AG011 capsules at a total daily dose of 2.4×10^{10} CFU, plus co-administration of AG011 given rectally in the form of an enema at a fixed daily dose of 2.4×10^{10} colony forming units (CFU).

At the second dose level, subjects will receive orally administered AG011 capsules at a total daily dose of 2.4×10^{11} CFU, plus co-administration of AG011 given rectally in the form of an enema at a fixed daily dose of 2.4×10^{11} CFU.

At the highest dose level, subjects will receive orally administered AG011 capsules at a total daily dose of 7.2×10^{11} CFU, plus co-administration of AG011 given rectally in the form of an enema at a fixed daily dose of 1.2×10^{12} CFU.

Subjects assigned to the respective placebo groups will receive orally administered appearance- and taste-matched placebo capsules plus rectally administered, identical appearing placebo enema.

Oral treatment will be administered twice daily, either one capsule (for the first two dose levels) or three capsules (for the highest dose level) given in the morning and in the evening before the meal. The enema treatment will be given once daily at bedtime. All subjects will receive study treatment for 28 days.

7. STUDY EVALUATIONS

See the **Time and Event Schedule**, included at the end of this document, for the performance and timing of study evaluations.

7.1. SAFETY EVALUATIONS

Safety evaluations include documentation of adverse events, physical examination, vital signs, clinical laboratory evaluations (hematology, chemistry, urinalysis), human Interleukin-10 (hIL-10) and anti-hIL-10 antibodies, and stool diary (stool frequency, consistency and presence of gross blood).

7.2. FECAL EXCRETION OF AG011 AND ENVIRONMENTAL CONTAINMENT EVALUATIONS

For measuring fecal excretion of AG011 and validating the environmental containment strategy, the presence of live and dead *Lactococci* will be followed by monitoring 16S rDNA. Measurement of 16S rRNA will allow for the calculation of the ratio of viable versus dead *Lactococci*. Since the synthetic hIL-10, as present in AG011, is unique to the genetically modified (GM) *Lactococcus lactis* strain, this will be monitored (using synthetic hIL-10 mRNA and synthetic hIL-10 DNA) to identify the ratio of viable versus dead GM bacteria.

7.3. PHARMACODYNAMIC (PD) EVALUATIONS

PD evaluations include an assessment of the levels of a wide array of relevant cytokines, chemokines and other biomarkers for IL-10 receptor activation, in blood and colonic biopsy samples. PD evaluations will be performed in a subset of subjects (Belgian subjects only).

7.4. EFFICACY EVALUATIONS

Efficacy evaluations include flexible sigmoidoscopy (modified Baron score), colonic biopsies (modified Riley score and histological assessment of inflammation), disease activity assessments (Mayo Clinic Disease Activity Score, Ulcerative Colitis Clinical Score, investigator and subject global ratings), and evaluation of C-reactive protein (CRP) and fecal calprotectin.

8. STATISTICAL METHODS

All subjects who receive at least one dose of study drug, will be evaluated for safety and environmental containment analyses. Among the subset of subjects with PD evaluations performed, those who provided sufficient biomarkers data to reliably estimate PD parameters will be evaluated for PD analyses. Descriptive statistics will be presented.

All subjects who receive at least one dose of study drug and have undergone one post-randomization efficacy assessment, will be included in the efficacy analyses. Data obtained from the three dose groups will be pooled. The primary efficacy evaluation will be by the modified Baron score, an ordered categorical variable (scores range from 0 to 4). Scores obtained at the Day 29 visit from subjects assigned to AG011 will be compared with those assigned to placebo using the Wilcoxon rank-sum statistic. Statistical tests will be two-sided and performed at the 0.05 level of significance.

This is a Phase 2a study in which 60 subjects will be evaluated. This sample size is deemed to be reasonable to address the objectives of this early phase study. Forty subjects (10 from each of the first 2 dose groups and 20 from the highest dose group) will have received active treatment and 20 subjects (5 from each of the first 2 dose groups and 10 from the highest dose group) will have received placebo. The evaluation of 60 subjects (40 active and 20 placebo) would provide 84% power at the 0.05 level of significance, to detect a difference in the distributions of the modified Baron scores (Grades 0/1/2/3/4) in the two groups obtained at the Day 29 visit, where the distribution of the grades for the AG011 group is assumed to be 0.40/0.30/0.20/0.08/0.02, compared respectively to the distribution for the placebo group 0.13/0.21/0.33/0.26/0.07, based on data obtained from a previous trial of a biologic therapy for UC.

9. TIME AND EVENTS SCHEDULE

Study Period	Screen	Treatment					Post-Treatment		
Visit	1	2	3	4	5	6[a]		7[b]	8[c]
Type of Visit	C	C	C	C	C	C		C	P
Study Procedure Day	-7	1	8	15	22	29	D7PT	W4PT	W40PT
Permitted Intervals (days)	±4		±2	±2	±2	±4	+4	±7	±28
Informed Consent [d]	X								
Assess Inclusion/Exclusion	X[e]								
Confirm Inclusion/Exclusion		X							
Randomization		X							
Demographics	X								
Medical/Surgical History	X								
Comprehensive Physical Exam	X								
Brief Physical Exam						X			
Vital Signs		X	X	X	X				
Hematology	X	X	X	X	X	X		X	
Serum Chemistry	X	X	X	X	X	X		X	
Urinalysis	X	X	X	X	X	X		X	
Serum pregnancy test	X	X	X	X	X	X		X	
Urine pregnancy test						X			
Stool Samples		X	X	X	X				
- Cultures	X								
- Ova & Parasites	X								
- *C. difficile*	X								
- Biological Containment Assessment		X	X				X[f]		
Biomarkers [g] (Belgium only)	X					X			
hIL-10 in plasma		X				X			
Anti-hIL-10 antibodies in plasma		X				X			
Flexible Sigmoidoscopy	X[h]					X			
Colonic Biopsies	X					X			
Mayo Clinic Disease Activity Score	X					X			
Ulcerative Colitis Clinical Score	X	X	X	X	X	X		X	
Investigator and Subject Global Rating		X	X	X	X	X		X	
CRP		X		X		X		X	
Fecal Calprotectin		X		X		X		X	
Dispense Study Drug		X	X	X	X				
Dispense and Review Directions for Use		X							
Dispense Stool Diary	X	X	X	X	X				
Dispense Study Medication Diary		X	X	X	X				
Drug Accountability & Compliance			X	X	X	X			
Concomitant Medications		X	X	X	X	X			
Adverse Event Assessment		X	X	X	X	X			
Serious Adverse Event Assessment		X	X	X	X	X		X	X

C = Clinic Visit; P = Phone Visit;
D7PT = Day 7 Post-Treatment; W4PT = Week 4 Post-Treatment; W40PT = Week 40 Post-Treatment

a. Perform Visit 6 procedures when subject completes Day 29 or withdraws from the study early.
b. Perform Visit 7 procedures 4 weeks after last study drug dose.
c. Perform Visit 8 procedures 40 weeks after last study drug dose.
d. Informed consent process can begin prior to Visit 1, for example, if washout from medications is required.
e. Notify Randomization centre to request shipment of study drug for potential randomization.
f. Obtain stool sample for fecal excretion of AG011 and biological containment assessment 7 days after stopping treatment.
g. Biomarkers will be performed in a subset of subjects (Belgian subjects only).
h. Colonoscopy may be performed instead of sigmoidoscopy if clinically indicated.

MATERIAL SAFETY DATA SHEET

AG011

1. Product and company identification

Product name:
AG011

Identification of the substance or preparation :

sAGX0037 is a genetically modified *Lactococcus lactis* expressing human IL-10.
AG011 corresponds to the oral and rectal formulation of a Freeze-dried recombinant *Lactococcus lactis* sAGX0037 expressing human IL-10.

Use of the substance or preparation

The substance is used in the framework of a clinical trial.

Product owner:

ActoGeniX N.V.
Technologiepark 4
9052 GENT-Zwijnaarde
Belgium
Telephone: +32 9 261 06 00
Fax: +32 9 261 06 19

2. Composition / Information on ingredients

Composition of AG011-capsules	Composition of AG011-Enema
AG011-DS-01:	**AG011-DS-01**:
Freeze-dried bacteria sAGX0037	*Freeze-dried bacteria sAGX0037*
Dextrin (from maize starch)	*Dextrin (from maize starch)*
Sorbitol	*Sorbitol*
Sodium glutamate	*Sodium glutamate*
Talc	**Colloidal silica**
Anhydrous dicalcium phosphate	**Mannitol**

3. Hazards information

Hazardous components: NONE

Non hazardous components:
Dextrin (from maize starch)
Sorbitol
Sodium glutamate
Talc
Mannitol
Colloidal silica
Anhydrous dicalciumphosphate
Freeze dried bacteria sAGX0037

4. First aid measures

After inhalation:	Consult a physician
After skin contact:	Wash off with plenty of water.
After eye contact:	Rinse out with plenty of water with eyelid held wide open. Consult a physician if necessary.
After swallowing:	Drink plenty of water. Consult physician if feeling unwell

5. Fire-fighting measures

Suitable extinguishing media:
The choice is to be determined by materials which are stored in the vicinity of the product.
Special risks: Not Applicable

6. Accidental release measures

Person related precautionary measures:
Wear protective gloves when removing spilled material

Procedures for cleaning:
In the case of an accidental spillage, apply a standard detergent (soap) or bleach to inactivate the GMOs and decontaminate the affected area.
Mop up spilled material with tissue and dispose of waste in accordance with local guidelines for GMO's.
The freeze dried *Lactococcus lactis* is short-lived when dissolved in water at room temperature. Any additional cleaning treatment will ensure that additional spread is prevented. It is expected that the biological containment system will limit the spread in space and time, as described in paragraph 12 "Ecological information".

7. Handling and storage

Handling: No further requirements
Storage: at 2-8°C

8. Exposure controls / personal protection

Personal protective equipment: Clothing designed for working in GMP class C/D area's should be worn when working with substance.

Respiratory protection:	Mouth mask is recommended when handling the powder
Eye protection:	Required
Hand protection:	Use of appropriate gloves is recommended
Industrial hygiene:	Change contaminated production clothing. Wash hands after working with substance

9. Physical and chemical properties

Form: Lyophilized powder
Colour: Off white/light yellow
Odour: Odourless

pH value (dissolved at 2 mg/ml in water): 6 to 8

Melting point: Not applicable
Boiling point: Not applicable

Ignition temperature:		Not available
Flash point:		Not available
Explosion limits:	Lower:	Not available
	Upper:	Not available

Density (20°C): ≈ 0.6 g/ml

Solubility in water (20°C):

Dispersible in water 1 in 20 resulting in a white homogeneous suspension

10. Stability and reactivity

Conditions to be avoided: Heating, contact with humidity.

Substances to be avoided: Not applicable

Hazardous decomposition products: None

11. Toxicological information

Acute toxicity LC_{50}: Not available
Further toxicological information: Not available
NOAEL (13-week mouse toxicology study, oral only): 2.3×10^{12} CFU per kg
NOEL (4-week monkey toxicology study, oral and rectal): 1.6×10^{12} CFU per kg

12. Ecological information

L. lactis is a poor competitor, only capable of multiplication in special ecological niches such as raw milk. Dispersal is passive. While survival and metabolic activity are possible outside of the specific ecological niche, the bacteria are susceptible to a range of environmental factors resulting.
Survival, multiplication and dispersal require the presence of a carbon source (not lactose), simple amino acid source and thymidine. The absence of any of these (which would also occur following exhaustion of a nutrient source) makes survival, multiplication and dispersal impossible.
Biological containment of sAGX0037 is based on its thyA negative genotype.. As a result, growth of sAGX0037 is strictly dependent on the addition of thymidine to the culture broth. Furthermore, survival of the strain is strictly dependent on the presence of thymidine.

13. Disposal considerations

Product:
GMO products generally count as special waste. The disposal of the latter is regulated in the EC member countries through corresponding laws and regulations. We recommend that you either contact the authorities in charge or approved waste disposal companies who will advise you on how to dispose of special waste.

Packaging:
Disposal will be performed in compliance with official regulations. Handle contaminated packaging in the same way as the product itself. If not officially specified differently, non contaminated packaging may be treated like household waste or recycled.

14. Transport information

Product	UN 3245 GENETICALLY MODIFIED MICRO-ORGANISMS
	Class 9: UN 3245

Packing method: material is packed in either:

- Alu/alu blisters enclosed in a child resistant carton box or
- Plastic powder containers in sealed aluminum sachet.

Both items are enclosed within a cardboard surrounding box with other items requested for the trial (Solution for reconstitution, reconstitution set, canula and gloves).

Cartagena Protocol on Biosafety:
sAGX0037, the specific *Lactococcus lactis* strain expressing human IL-10, is a "Living Modified Organism (LMO)" as defined in the Cartagena Protocol on Biosafety. Transboundary movements are subject to the requirements of the Protocol.

Keep product in original packaging between 2 - 8 °C.

15: Regulatory Information

Risk Group Classification
L. lactis is a food-grade micro-organism and has a long history of safe use in the food industry. *L. lactis* is not classified under a risk group, is not a quarantine organism and does not appear under the list of organisms for which a specific certificate is required.

Belgium	Belgian competent authorities allow the use of the specific strain at containment level 1.
Canada	Health Canada included *L. lactis* in the list of non-pathogenic organisms, for which no import permit is required under the HPIR. Health Canada confirmed that the strain can be used in BSL1 containment.
EU	The European Food Safety Authority introduced the concept of "Qualified Presumption of Safety" to establish a generic safety assessment of micro-organisms used in food production. As the first evaluations of candidate micro-organisms are ongoing, *L. lactis* bacteria have been included in the list of Gram-positive, non-sporulating bacteria.
Germany	The German "Zentrale Kommission für die Biologische Sicherheit" lists *L. lactis* subsp. cremoris as a Level 1 organism
UK	The organism is not listed in "The Approved List of biological agents" published by the Advisory Committee on Dangerous Pathogens. UK HSE

	confirmed that the strain can be used in BSL1 containment.

Regulations for the use of GMOs
sAGX0037, the specific *Lactococcus lactis* strain expressing human IL-10, is a genetically modified organism ("GMO") which are also known as "recombinant" organism. The use of GMOs or recombinant organisms may be subject to specific legislation. In the EU the national implementations of Council Directive 90/219/EEC on the contained use of genetically modified micro-organisms, as last amended by Council Directive 98/81/EC and of Directive 2001/18/EC of the European Parliament and of the Council on the deliberate release into the environment of genetically modified organisms, should be observed as applicable.

16. Other information

Reason for alteration: Version n° 1.0
Date of issue: 11 June 2008

07 April 2009

Occupational Health and Safety

Re: Biohazardous Agents Registry Form
Dr. Barnett – University Hospital

Hello:

I have included the BARF along with the following:
Emails regarding the status of the product with Health Canada
Commencement of Import letter from Robarts Research Institute
Chemical properties of L. Lacis
Protocol synopsis for AG011-MDUC-201
Material Safety Data Sheet for AG011

Please let me know if you require any more documentation. Thanks!

Barb

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