NOTIFICATION FOR DELIBERATE RELEASE OF THE THERAPEUTIC DRUG ADXS11-001 (GMO)

Clinical Trial Title:

PHASE 3 STUDY OF ADXS11-001 ADMINISTERED FOLLOWING CHEMORADIATION AS ADJUVANT TREATMENT FOR HIGH RISK LOCALLY ADVANCED CERVICAL CANCER: AIM2CERV

SPONSOR:
ADVAXIS, INC, USA

NOTIFIER**:**
INVENTIV HEALTH CLINICAL ROMANIA

ADXS11-001

13 JUNE 2017

List of abbreviations

Ab	antibody
ADXS11-001	Lm-LLO-E7 immunotherapy for HPV-associated cancers
APCs	antigen presenting cells
Bp	base pair
BSL-2	biosafety level 2
BMBL	Biosafety in microbiological and biomedical laboratories
CAT	Chloramphenicol acetyl transferase
CBC	Complete Blood Count
CFU	Colony forming units
CMP	Comprehensive metabolic panel
CRP	C-reactive Protein
ESR	Erythrocyte sedimentation rate
FDA	Food and Drug Administration
GMO	Genetically Modified Organism
GMP	Good Manufacturing Practice
HIV	Human immunodeficiency virus
HPV	Human Papilloma Virus
HRLACC	High risk locally advanced cervical cancer
LLO	Listeriolysin O
IND	Investigational New Drug
IV	Intravenously
Lm	Listeria monocytogenes
MHC	Major Histocompatibility Complex
NaClO	sodium hypochlorite
NCI	National Cancer Institute
PCR	polymerase chain reaction
PPE	personal protective equipment
SAE	serious adverse events
tLLO	Truncated LLO
WT	wild type

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	ROMANIA
(b)	Notification number	B/RO/17/02
(c)	Date of acknowledgement of notification	03/04/2017
(d)	Title of the project	PHASE 3 STUDY OF ADXS11-001 ADMINISTERED FOLLOWING CHEMORADIATION AS ADJUVANT TREATMENT FOR HIGH RISK LOCALLY ADVANCED CERVICAL CANCER: AIM2CERV (Advaxis IMmunotherapy 2 prevent CERVical recurrence)
(e)	Proposed period of release	From 01/09/2017 until 31/12/2023

2. Notifier

Name of institution or company:

INVENTIV HEALTH CLINICAL ROMANIA SRL Bvd. Eroilor, no.22, county 5, 050513 Bucharest, Romania

Sponsor:

Advaxis, Inc.
305 College Road East
Princeton, NJ 08540, USA

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)

The GMO is *an attenuated Listeria monocytogenes strain which has been engineered to express the tLLO-E7 fusion protein. The E7 protein sequence was derived from Human Papilloma Virus 16. The Listeria monocytogenes strain is named axalimogene filolisbac, and is also known as ADXS11-001. Axalimogene filolisbac is a clinical drug product administered to patients for cancer treatment.*

(c) Genetic stability – according to Annex IIIa, II, A(10)

The recombinant plasmid that maintains the attenuation in axalimogene filolisbac as well as expressing the tumor antigen E7 is stably maintained *in vitro* and *in vivo*. The *in vitro* plasmid stability has been assessed by culturing the bacteria in the presence and absence of selection pressure using the antibiotic chloramphenicol. The plasmid is stable for up to 70 generations in the bacteria when cultured in the absence of selection. In order to assess potential plasmid stability *in vivo*, mice were injected with axalimogene filolisbac and bacteria were subsequently isolated from removed spleens for plating and assessment of colony forming units (CFUs) with and without selection for the plasmid. No significant differences in the bacterial viability with or without selection was observed, indicating that the axilimogene filolisbac plasmid is retained *in vivo* until the bacteria are cleared by the immune system.

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) NL, PL, RO, ES

5. Has the same GMO been notified for release elsewhere in the Community by the same notifier?

Yes (X) No (.)

If yes:

- Member State of notification ES
- Notification number B/ES/17/02

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: United States (Used in the US for clinical trials under US IND 13712)
- Notification number: N/A

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

The environmental risk caused by vial breakage is low. Axalimogene filolisbac is an attenuated *L. monocytogenes* and the product is easily killed and removed by using standard antimicrobial cleaning agents. When patients are treated with Axalimogene Filolisbac in the clinical trials, they will be given a 7-day supply of antimicrobial agents beginning approximately 72 hours following the completion of the study treatment administration. Also, the healthcare providers in the clinical study are adequately trained in the safe handling of GMOs and have biosafety practices implemented in order to minimize any accidental exposure to the environment.
Patients are treated with antibiotics after dosing with the product. Bacteria should be eliminated by the antibiotic treatment.
The health personnel who handle the product have been trained prior to use of the drug product.
Thus, the environmental risk through patients is very low too.

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)	…
(ii)	genus	Listeria
(iii)	species	monocytogenes
(iv)	subspecies	…
(v)	strain	…
(vi)	pathovar (biotype, ecotype, race, etc.)	serovar 1/2 a
(vii)	common name	XFL7 (avirulent *L. monocytogenes* strain)

3. Geographical distribution of the organism

(a) Indigenous to, or otherwise established in, the country where the notification is made:
Yes (X) No (.) Not known (.)

(b) Indigenous to, or otherwise established in, other EC countries:
(i) Yes (X)

If yes, indicate the type of ecosystem in which it is found:

Atlantic	X..
Mediteranean	X..
Boreal	X..
Alpine	X..
Continental	X..
Macaronesian	X..

(ii) No ()
(iii) Not known (.)

(c) Is it frequently used in the country where the notification is made?
Yes (.) No (x)

(d) Is it frequently kept in the country where the notification is made?
Yes (.) No (x)

4. Natural habitat of the organism

(a) If the organism is a microorganism

water	(x)
soil, free-living	(x)
soil in association with plant-root systems	(.)
in association with plant leaf/stem systems	(.)
other, specify	Animals including poultry and cattle

(b) If the organism is an animal: natural habitat or usual agroecosystem:
Not Applicable

5. (a) Detection techniques
Listeria can be detected by plating on bacteria growth media.
(b) Identification techniques
Listeria can be detected by plating on bacteria growth media and identified by specific biochemical methods, such as Listeria identification strips.

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

8. Information concerning reproduction
Listeria is reproduced by binary fission.

(a) Generation time in natural ecosystems:
40 minutes for one generation.

(b) Generation time in the ecosystem where the release will take place:
Not applicable since the Listeria strain is not released in the ecosystem

(c) Way of reproduction: Sexual .. Asexual ..
Not relevant

(d) Factors affecting reproduction:
Growth 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 (funghi) (.)
(vi) eggs (.)
(vii) pupae (.)
(viii) larvae (.)
(ix) other, specify … (.)
Not applicable

(b) relevant factors affecting survivability:
Growth conditions

10. (a) Ways of dissemination

Listeria are generally soil-derived bacteria; however, *L. monocytogenes*, can also be found in raw foods, such as unpasteurized milk, raw vegetables, and in raw or under-cooked poultry. It has the ability to grow at low temperatures, thus allowing for growth in refrigerated storage conditions.

(b) Factors affecting dissemination
Growth conditions

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/../../…
ADXS11-001 is a mutant strain of L. monocytogenes created by the insertion of a recombinant plasmid (specifically the precursor strain named XFL7 which contains the plasmid pGG55) lacking the essential virulence gene prfA. PrfA is a transcription factor acting on a number of genes including all of the virulence genes but not required for in vitro culture of Listeria. Thus, XFL7 is avirulent but can be maintained in broth cultures. The plasmid pGG55 carries a copy of mutated prfA (point mutation D133V). The mutated prfA is impaired in its ability to activate expression of prfA-dependent genes that results in the attenuation of ADXS11-001.

C. **Information relating to the genetic modification**

1. Type of the genetic modification

(i)	insertion of genetic material	(.)
(ii)	deletion of genetic material	(x)
(iii)	base substitution	(x)
(iv)	cell fusion	(.)
(v)	others, specify	

2. Intended outcome of the genetic modification
The bacterial strain used in the ADXS11-001 is mutant strain XFL7 lacking the essential virulence gene prfA. This gene is a transcription factor acting on a number of genes including all of the virulence genes such as actA and LLO but it is not required for in vitro culture of Listeria. Thus, XFL7 is avirulent but can be maintained in broth cultures.

The recombinant vaccine protein is expressed from plasmid pGG55 containing a fusion of inactive LLO and HPV16 E7 under the control of the LLO promoter. The plasmid also carries a copy of mutated prfA (D133V) for in vivo retention by complementation of prfA-negative strain XFL7. These genes were introduced into Gram-positive / Gram-negative bacteria shuttle plasmid pAM401 (Wirth, An et al. 1986) which can be amplified in E. coli as well as in Listeria since genetic manipulations cannot be readily carried out in gram-positive organisms. Therefore plasmid genes include replication factors for gram-positive and gram-negative bacteria as well as antibiotic selection markers (Chloramphenicol) for Gram-positive and Gram-negative bacteria. The plasmid is retained in vitro by Chloramphenicol selection while the prfA complementation is inactivated. In vivo, the plasmid is retained by the prfA complementation system without Chloramphenicol selection pressure.

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 (x) No (.)

If no, go straight to question 5.

4. If the answer to 3(b) is yes, supply the following information

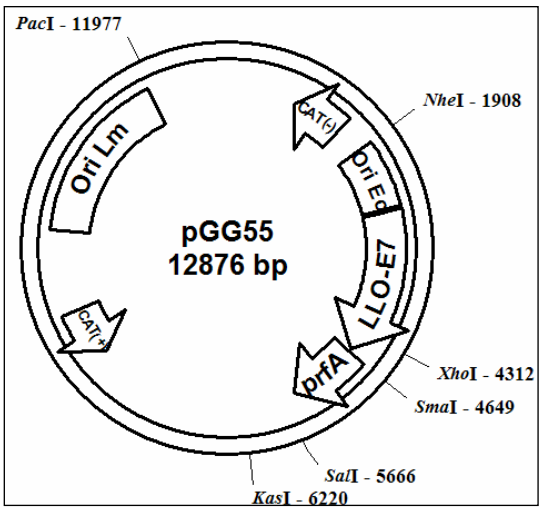
(a) Type of vector

plasmid	(x)
bacteriophage	(.)
virus	(.)
cosmid	(.)
transposable element	(.)
other, specify	…

(b) Identity of the vector

.

The schematic plasmid map of pGG55 displays selected restriction sites and all active genes as follows: OriLM: replication genes for *L. monocytogenes*, OriEc: Replication genes for *E. coli*, *prfA*: *L. monocytogenes prfA* including its promoter, tLLO-E7: fusion protein of *L. monocytogenes hly* and HPV16 E7 including the *hly* promoter, Cat(+): Chloramphenical resistance gene for *L. monocytogenes*, Cat(-): Chloramphenical resistance gene for *E. coli*. Plasmid pGG55 has been sequenced entirely by the NCI.



(c) Host range of the vector

Replicates in both Gram-positive and Gram-negative bacteria.

(d) Presence in the vector of sequences giving a selectable or identifiable phenotype

Yes (x) No (.)

antibiotic resistance	(x)
other, specify	…

Indication of which antibiotic resistance gene is inserted

- Chloramphenicol resistance gene in the plasmid pGG55

(e) Constituent fragments of the vector
Explained in 1b above

(f) Method for introducing the vector into the recipient organism

(i)	transformation	(.)
(ii)	electroporation	(x)
(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	(.)
(ii)	microinjection	(.)
(iii)	microencapsulation	(.)
(iv)	macroinjection	(.)
(v)	other, specify …	

6. Composition of the insert

(a) Composition of the insert

The insert coding for the fusion protein tLLO-HPV16E7 is indicated in the schematic. The expression of the fusion protein is under the control of bacterial transcriptional promoter and translational sequences. The functional HPV16E7 protein cannot be expressed by any eukaryotic cell.

(b) Source of each constituent part of the insert

Shuttle plasmid pAM401 served as a backbone into which two expression cassettes were introduced: (1) The gene encoding transcription factor prfA including its promoter in order to complement the deficient mutant strain XFL7 and (2) the gene encoding the vaccine antigen HPV16 E7 fused to Listeria gene hly (LLO) including the hly promoter upstream of LLO-E7. All recombinant sequences were generated by PCR amplification prior to ligation for creating pGG55.

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

The plasmid pGG55 contains genes that include replication factors for gram-positive and gram-negative bacteria as well as antibiotic selection markers (Chloramphenicol) for both Gram-positive and Gram-negative bacteria. The Gram-negative portion of pAM401 / pGG55 consists of commercial pACYC184 which has been well characterized (New England Biolabs). The prfA gene encodes for mutated PrfA to ensure plasmid retention by ADXS11-001 in vivo. The fusion protein tLLO-E7 secreted by axalimogene filolisbac is responsible for generation of immune responses specific for E7 protein.

(d) Location of the insert in the host organism

-	on a free plasmid	(x)
-	integrated in the chromosome	(.)

- 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		(x)
bacterium		(.)
fungus		(.)
animal		
-	mammals	(.)
-	insect	(.)
-	fish	(.)
-	other animal	(.)
	(specify phylum, class)	…
other, specify		…

2. Complete name

Human Papilloma Virus 16

(i)	order and/or higher taxon (for animals)	…
(ii)	family name for plants	…
(iii)	genus	…
(iv)	species	…
(v)	subspecies	…
(vi)	strain	*human papillomavirus* Strain 16
(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 (x) No (.) Not known (.)

If yes, specify the following:.

(a) to which of the following organisms:

humans	(x)
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):
No, because the E7 gene is cloned in Listeria-specific plasmid to express a fusion protein tLLO-HPV16E7 that is non-functional and non-pathogenic.

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 (x) No (.)

If yes, specify BSL2 organism.

The handling of ADXS11-001 in accordance with Biosafety Level 2 practices, safety equipment and facility requirements is based on CDC classification of bacterial agents and its weblink is provided below.

http://www.cdc.gov/biosafety/publications/bmbl5/index.htm.

From this website, on page 143 to 144 of the "Biosafety in Microbiological and Biomedical Laboratories", the CDC suggested that BSL-2 practices, containment equipment, and facilities are recommended when working with the wild type of *Listeria monocytogenes*. For ADXS11-001, although it has been attenuated comparing to the wild type of *Listeria monocytogenes*. it is still recommended to follow BSL-2 for its handling

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 (.)

Specify: as the GMO is attenuated, the survivability decreases.

(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) Unknown (.)

Specify …

(c) is the GMO in any way different from the recipient as far as dissemination is concerned?

Yes (.) No (x) Not known (.)

Specify …

(d) is the GMO in any way different from the recipient as far as pathogenicity is concerned?

Yes (X) No () Not known (.)

Specify as the GMO is attenuated, the pathogenicity decreases.…

2. Genetic stability of the genetically modified organism

The recombinant plasmid pGG55 is stably maintained by axalimogene filolisbac *in vitro* and *in vivo*. The stability has been previously explained (refer to Section A 3c).

3. Is the GMO significantly pathogenic or harmful in any way (including its extracellular products), either living or dead?

Yes (.) No (x) Unknown (.)

(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)

…

4. Description of identification and detection methods

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

Axalimogene filolisbac can be detected by plating on the growth medium selective for *L. monocytogenes* containing antibiotics chloramphenicol and streptomycin.

(b) Techniques used to identify the GMO

Axalimogene filolisbac can be identified using a polymerase chain reaction (PCR)-based method. Positive identity for Axalimogene filolisbac contains a specific deletion in the *prfA* gene (XFL7) and tLLO-E7 secretion by Western Blot. Wild-type *L. monocytogenes* does not contain the specific deletion in the *prfA* gene and E7 secretion can be easily distinguished.

F. Information relating to the release

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

Axalimogene filolisbac is a clinical drug product administered through intravenous infusion to patients for cervical cancer treatment.

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 sites of release are the clinical trial sites participating in this clinical trial.

3. Information concerning the release and the surrounding area

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

Hospital/Department	Address	Investigator
Clinical Hospital Filantropia, Medical Oncology Department	Bucharest, Romania	Dr. Dragos-Mircea Median
SC Radiotherapy Centre Cluj SRL, Medical Oncology Department	Floresti, county Cluj, Romania	Dr. Ioan-Catalin Iacob
Oncology Centre Sf. Nectarie SRL	Craiova, county Dolj, Romania	Dr. Michael Schenker
Clinical County Emergency Hospital "Sf. Ioan Cel Nou" Suceava, Medical Oncology Department	Suceava, county Suceava, Romania	Dr. Doina-Elena Ganea

(b) Size of the site (m^2):
(i) actual release site (m^2): … m^2
(ii) wider release site (m^2): … m^2

Not specified. No specific size is required for the site. The room where patients will be treated is a conventional hospital room.

(c) Proximity to internationally recognized biotopes or protected areas (including drinking water reservoirs), which could be affected:

Not relevant as there is no shedding of this drug product.

(d) Flora and fauna including crops, livestock and migratory species which may potentially interact with the GMO

Not relevant

4. Method and amount of release

(a) Quantities of GMOs to be released:
Each vial contains $\geq 1 \times 10^9$ cfu

(b) Duration of the operation:

ADXS11-001 administered IV over approximately 60 minutes every 3 weeks (Weeks 1, 4 and 7 only) for 3 doses (Prime Phase), thereafter, subjects will receive an additional dose every 8 weeks (Weeks 15, 23, 31, 39, and 47) for 5 doses (Maintenance Phase). The total treatment period will be approximately 1 year.

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

Axalimogene filolisbac is transported to the receiving site in sealed cryogenic vials. Each vial is individually labelled identifying the contents and quantity of material. The vials stored on dry ice or equivalent shipping conditions inside an insulated shipping container. The shipping containers are insulated to prevent vial breakage and vial thaw. The main hazard for the shipment of these vials relates to vial breakage. If a vial were to be received cracked it is possible for the product to leak out from the vial and contaminate the shipping components. Axalimogene filolisbac is an attenuated L. monocytogenes commonly found as a soil bacterium. The product is easily removed from surfaces using standard antimicrobial cleaning agents. The probability for vial breakage is low for several reasons. The product is shipped within insulated shipping containers, designed to prevent vial breakage. The detectability of the vial breakage is high, because vial cracks would be visible. The environmental risk caused by vial breakage is low.

5. Short description of average environmental conditions (weather, temperature, etc.)

Not relevant. Used in clinical studies.

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.

The product was released for clinical trials in the USA under US IND 13712. There is no impact reported to the environmental and human health.

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)

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

2. Anticipated mechanism and result of interaction between the released GMOs and the target organism (if applicable)

The mechanism of action of axalimogene filolisbac is indicated below.

- Infection and intracellular growth of the axalimogene filolisbac strain in antigen presenting cells
- Expression and processing of the tLLO-E7 antigen in antigen presenting cells via the cellular proteasome mechanism

- Expression of processed peptides on Class-I major histocompatibility scaffolding on the cell surface of the antigen presenting cells

- Generation of the appropriate immune response from antigen presentation and cognate recognition

3. Any other potentially significant interactions with other organisms in the environment
None, based on shedding studies performed with axalimogene filolisbac

4. Is post-release selection such as increased competitiveness, increased invasiveness for the GMO likely to occur?
Yes (.) No (x) Not known (.)
Give details
The 4-5 logs attenuation of ADXS11-001 when compared to wild-type L. monocytogenes supports that release of GMO will likely not increase invasiveness of this bacteria (Verch et al., 2004).

5. Types of ecosystems to which the GMO could be disseminated from the site of release and in which it could become established
The bacteria could be possibly disseminated through both soil and water.

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) …
(ii) family name for plants …
(iii) genus …
(iv) species …
(v) subspecies …
(vi) strain …
(vii) cultivar/breeding line …
(viii) pathovar …
(ix) common name …
Not relevant

7. Likelihood of genetic exchange in vivo

(a) from the GMO to other organisms in the release ecosystem:
Not observed when axalimogene filolisbac has been co-cultured with other bacteria such as *E. coli* in the absence of antibiotic chloramphenicol. The horizontal transfer of plasmid via conjugation or other means to other Listeria stains has not been investigated. The likelihood of gene transfer to other bacteria or eukaryotic cells is low due to lack to gene transfer mechanisms by axalimogene filolisbac.

(b) from other organisms to the GMO:
The transfer of genes from other microbes to axalimogene filolisbac has not been investigated.

(c) likely consequences of gene transfer:

Due to lack of data, it is not applicable.

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.):
Not applicable

9. Possible environmentally significant interactions with biogeochemical processes (if different from the recipient or parental organism)
Not applicable

H. Information relating to monitoring

1. Methods for monitoring the GMOs
The axalimogene filolisbac management and surveillance monitoring phase will consist of a 6-month course of oral Trimethoprim/sulfamethoxazole, ampicillin or matching placebo, obtaining a blood sample to monitor CBC, CMP, including CRP and ESR, and blood cultures at regular intervals. This testing will be performed on all subjects who have received at least one dose of Study treatment. Any subject who receives at least one dose of ADXS11-001 will be managed and monitored for safety, including post-treatment long-term (6 months) antibiotics and long-term safety surveillance. Extended treatment with an oral antibiotic is intended to increase the likelihood that Lm will be eradicated. A visit to the investigative site will be conducted every 3 months (±2 weeks) beginning on the 3rd month after the last dose of study treatment or immediately at the time of study discontinuation unless a subject withdraws her consent, for 3 years. Subjects will receive the first dose of oral trimethoprim/sulfamethoxazole, ampicillin or matching placebo approximately 72 hours following the last dose of study treatment or immediately following study discontinuation. Trimethoprim/sulfamethoxazole will be the drug of first choice for all subjects unless a subject is intolerant or allergic to this drug. In this case, ampicillin will be administered. The dose of trimethoprim/sulfamethoxazole consists of 80 mg trimethoprim/400 mg sulfamethoxazole tablet administered once daily for 7 consecutive days. The dose of ampicillin consists of 500 mg four times daily over a 7-day consecutive period. Review the approved product labeling for trimethoprim/sulfamethoxazole or ampicillin and monitor antibiotic tolerance as dosing adjustments may be necessary.
Previous clinical studies have not shown shedding of these bacteria.

2. Methods for monitoring ecosystem effects
Not applicable

3. Methods for detecting transfer of the donated genetic material from the GMO to other organisms
Not relevant

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

Not applicable

5. Duration of the monitoring
3 years. Please see details for item H.1. above.

6. Frequency of the monitoring
Every three months. Please see details for item H.1. above.

I. Information on post-release and waste treatment

1. Post-release treatment of the site
All investigational product can be discarded in accordance with the Institution's policies for Biohazard Waste Disposal or by using the steps below for ADXS11-001.
ADXS11-001 can be treated with a 0.5 % sodium hypochlorite / NaClO / bleach for disinfection. A 70 % isopropyl alcohol solution can be used as an alternative.
Unopened or opened vial(s) and residual IV preparation materials are to be treated in accordance with the site's biohazard waste requirements. The materials that cannot be treated as biohazard should be disinfected with a 0.5 % sodium hypochlorite (NaClO) solution to disinfect the materials. The diluted 10 % bleach solution should not be harmful to a hood, and cleaning with bleach can be followed by cleaning with 70% alcohol. If the pharmacy cannot use a bleach solution for disinfection, they will need to treat accidental spills for 10 min with 70 % isopropanol.

2. Post-release treatment of the GMOs
Antibiotics treatment for the patients

3. (a) Type and amount of waste generated
Biohazard, one product vial/ IV infusion set per patient per dose.

3. (b) Treatment of waste
Standard Biohazard waste disposal practices. Unopened or opened vial(s) and residual IV preparation materials are to be treated in accordance with the site's biohazard waste requirements. The materials that cannot be treated as biohazard should be disinfected with a 0.5 % sodium hypochlorite (NaClO) solution to disinfect the materials. The diluted 10 % bleach solution should not be harmful to a hood, and cleaning with bleach can be followed by cleaning with 70% alcohol. If the pharmacy cannot use a bleach solution for disinfection, they will need to treat accidental spills for 10 min with 70 % isopropanol.

J. Information on emergency response plans

1. **Methods and procedures for controlling the dissemination of the GMO(s) in case of unexpected spread**

Axalimogene filolisbac is transported to the receiving site in sealed cryogenic vials. Each vial is individually labelled identifying the contents and quantity of material. The vials stored on dry ice or equivalent shipping conditions inside an insulated shipping container. The shipping containers are insulated to prevent vial breakage and vial thaw. The main hazard for the shipment of these vials relates to vial breakage. If a vial were to be received cracked it is possible for the product to leak out from the vial and contaminate the shipping components. Axalimogene filolisbac is an attenuated *L. monocytogenes* commonly found as a soil bacterium. The product is easily removed from surfaces using standard antimicrobial cleaning agents. The probability for vial breakage is low for several reasons. The product is shipped within insulated shipping containers, designed to prevent vial breakage. The detectability of the vial breakage is high, because vial cracks would be visible. The environmental risk caused by vial breakage is low.

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

In case of an accidental spill or exposure to axalimogene filolisbac during handling, personnel should act as recommended below.

Accidental Spills

All accidental spills shall be handled in compliance with applicable site safety procedures or following the guidance below.

1. In the event there is an accidental spill of axalimogene filolisbac, isolate the area and notify others in the vicinity. Put on appropriate personal protective equipment (PPE) if not already worn (e.g. gown or lab coat, gloves and loose fitting mask with eye shield or goggles). Remove any broken glass or sharps and place them into sharps container.

2. Decontaminate the area of the axalimogene filolisbac spill by placing paper towel over the spill. Saturate the paper-towel(s) with a 0.5 % sodium hypochlorite (NaClO) solution and starting at the outside of the spill and working towards the center wipe up the spill. Allow the 0.5 % sodium hypochlorite (NaClO) solution to remain on the area for approximately 10 min. Dispose the paper towel(s) in a biohazardous waste container. Discard all materials including PPEs, in the designated biohazardous waste container(s).

Exposure to axalimogene filolisbac

All exposure incidences shall be handled in compliance with applicable site safety procedures or following the guidance below.

1. In the event of an accidental exposure remove and dispose of contaminated PPEs or clothing into the designated biohazardous waste containers.

    a. For skin contamination: thoroughly wash the effective area immediately with soap and water.

    b. For needle stick injury: wash the affected area thoroughly with soap and water and cover the area with a sterile gauze dressing. Notify PI who will determine appropriate medical actions to be taken.

    c. For eye contamination: immediately and thoroughly rinse the affected area for up to 15 minutes using an eyewash; making the water flow across the affected eye from the

nose to the outer corner of the eye. If only one eye is contaminated, avoid contaminating the other eye (position your head so the affected eye must be below the other eye).

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

Not Applicable

4. **Plans for protecting human health and the environment in the event of an undesirable effect**

Patients who receive the treatment with the product will be monitored for the occurrence of adverse events and serious adverse events (SAE) according to the clinical protocol. Each SAE will be recorded and assessed by the hospital staff and the study sponsor, and Health Authorities will be notified when applicable.

Each patient receiving the GMO will be given a 7-day supply of trimethoprim/sulfamethoxazole, Ampicillin to be taken beginning approximately 72 hours following the completion of the study treatment administration, in order to clear the Listeria bacteria in the pateint's body.

In the case of accidental spills, procedures as described in above J.1 section will be followed to protect environment.