



TG4010

**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**

May 2017

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LIST OF ABBREVIATIONS

AmpR	Ampicillin resistance
BHK	Baby hamster kidney
CDC	Centers for Disease Control and Prevention
CEF	Chicken embryo fibroblasts
cDNA	Complementary deoxyribonucleic acid
DNA	Deoxyribonucleic acid
DP	Drug product
EEC	European Economic Community
GMO	Genetically modified organism
IL2	Interleukin 2
MHC	Major human histocompatibility
MUC1	Mucine 1
MVA	Modified vaccinia virus Ankara
MVATG9931	Recombinant vector
MVS	Master virus seed
PCR	Polymerase chain reaction
PMVS	Pre master virus seed
pTG9931	Transfer plasmid
SC	Subcutaneous
TG4010	Final GMO, viral suspension of MVATG9931
VV	Vaccinia virus
WVS	Working virus seed

A. GENERAL INFORMATION

1. Details of notification

- a) Member State of notification Belgium
- b) Notification number
- c) Date of acknowledgement of notification
- d) Title of the project

The project, TG4010.24 clinical trial, is entitled “A phase II study evaluating the efficacy and the safety of first-line chemotherapy combined with TG4010 and nivolumab in patients with advanced non-squamous Non-Small-Cell Lung Cancer (NSCLC)”.

- e) Proposed period of release
From January 2018 until Quarter 1 2020

2. Notifier

Name of institution or company

Sponsor: Transgene SA
400 Boulevard Gonthier d’Andernach
Parc d’Innovation
CS80166
67405 Illkirch Graffenstaden cedex - France

3. GMOs characterization

- a) Indicate whether the GMO is a:

- | | |
|----------------|--|
| viroid | <input type="checkbox"/> |
| RNA virus | <input type="checkbox"/> |
| DNA virus | <input checked="" type="checkbox"/> |
| bacterium | <input type="checkbox"/> |
| fungus | <input type="checkbox"/> |
| animal | <input type="checkbox"/> |
| - mammals | <input type="checkbox"/> |
| - insect | <input type="checkbox"/> |
| - fish | <input type="checkbox"/> |
| - other animal | <input type="checkbox"/> specify phylum, class |

other, specify (kingdom, phylum and class)

- b) Identity of the GMO (genus and species)

The final genetically modified organism (GMO) is TG4010 and consists of a non-replicative, recombinant vaccinia vector consisting of the modified vaccinia virus Ankara (MVA) genome containing inserted transgenes that encode two proteins: the human mucin 1 (MUC1) and the human interleukin-2 (IL2).

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

A genetic stability program was designed to assess the genetic stability of TG4010 at several steps of the production process: Pre Master Virus Seed 1 (PMVS1), Master Virus Seed (MVS), final Drug Product (DP) and DP + 3 passages. In addition, an accelerated study was performed by sub-passing 6 times the PMVS1 at laboratory scale.

A working Virus Seed (WVS) was produced in 2007, to be used as virus seed for future clinical and commercial lots. Production of lots from the WVS involved an additional viral passage and thus the genetic stability study was updated with additional data.

The vector MVATG9931 was shown to be genetically homogeneous from the MVS through the DS or DP lots and at a passage corresponding to 3 passages beyond the passage intended to be used in clinical applications.

4. Is the same GMO release planned elsewhere in the Community (in conformity with article 6 (1)), by the same notifier?

Yes

No

If yes, insert the country code(s): **FR, DK, HU**

Please use the following country codes:

Austria AT; Belgium BE; Bulgaria BG; Cyprus CY; Czech Republic CZ; Denmark DK; Estonia EE; Finland FI; France FR; Germany DE; Greece GR; Hungary HU; Ireland IE; Italy IT; Latvia LV; Lithuania LT; Luxembourg LU; Malta MT; Netherlands NL; Poland PL; Portugal PT; Romania RO; Slovak Republic SK; Slovenia SI; Spain ES; Sweden SE; United Kingdom GB.

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

Yes

No

If yes:

- Member State of notification
- Notification number

FOR CLINICAL TRIAL TG4010.14:

Member State of notification Notification number	BE B/BE/11/BVW1
Member State of notification Notification number	DE B/DE/11/PEI1419
Member State of notification Notification number	HU B/HU/15/01
Member State of notification Notification number	ES B/ES/11/28
Member State of notification Notification number	FR Not published on the Eur. Com. GMO register
Member State of notification Notification number	UK Not published on the Eur. Com. GMO register
Member State of notification Notification number	IT Not published on the Eur. Com. GMO register
Member State of notification Notification number	PO Not published on the Eur. Com. GMO register

Other dossiers not for clinical trial:

Member State of notification	BE
Notification number	B/BE/01/B7
Member State of notification	DE
Notification number	B/BE/02/B7

6. Has the same GMO been notified for release or placing on the market outside the Community by the same or other notifier?

Yes

No

If yes:

- Member State of notification Israel and the United States of America
- Notification number Not applicable

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

The likelihood of TG4010 becoming persistent and invasive in natural habitats is very low for the following reasons:

- There is no known human poxvirus able to complement MVA (parent of TG4010) to generate a replication competent virus.
- No spontaneous reversion of MVA to replication competent vaccinia virus (VV) has ever been documented.
- TG4010 is unable to produce progeny vector particles in primary human cells, in addition, in human studies, TG4010 appeared to remain localized at the injection site as vector deoxyribonucleic acid (DNA) could not be detected by polymerase chain reaction (PCR) in the urine or blood of patients (n=94). Based on these observations it is considered unlikely that any significant shedding of infectious particles occurs.

B. INFORMATION RELATING TO THE RECIPIENT OR PARENTAL ORGANISMS FROM WHICH THE GMO IS DERIVED

1. Recipient or parental organism characterization:

a) Indicate whether the recipient or parental organism is a:

- viroid
- RNA virus
- DNA virus
- bacterium
- fungus
- animal

- mammals
- insect
- fish
- other animal

specify phylum, class

other, specify

2. Name

- (i) Order and/or higher taxon (for animals) **Poxviridae**
- (ii) Genus **Orthopoxvirus**
- (iii) Species **Vaccinia virus**
- (iv) Subspecies
- (v) Strain **Modified Vaccinia virus Ankara**
- (vi) Pathovar (biotype, ecotype, race, etc.)
- (vii) Common name **MVA**

3. Geographical distribution of the organism

a) Indigenous to, or otherwise established in the country where the notification is made:

Yes No Not known

b) Indigenous to, or otherwise established in other EC countries:

(i) Yes

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

Atlantic
Mediterranean
Boreal
Alpine
Continental
Macaronesian

(ii) No

(iii) Not known

The parental organism is not naturally found in the environment.

c) Is it frequently used in the country where the notification is made?

Yes No

d) Is it frequently kept in the country where the notification is made?

Yes 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
- In association with animal

other, specify

The parental organism is not naturally found in the environment.

b) If the organism is an animal: natural habitat or usual agroecosystem:

Not applicable.

5. (a) Detection techniques

See 5.(b).

5. (b) Identification techniques

The identity of the vector can be confirmed by Polymerase Chain Reaction (PCR) technique.

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

Yes

No

If yes, specify

In terms of classification of hazard, the human vaccinia virus is classified as a group 2 biological agent according to the European Economic Community (EEC) classification for the protection of workers with biological agents (Directive 2000/54/EC).

The MVA strain has not been classified. However MVA is a highly attenuated vaccinia virus strain obtained after several passages on primary chicken embryo fibroblasts (CEF). It replicates within the cytoplasmic compartment of the cell and cannot propagate in humans.

Laboratory and other health-care personnel who work with highly attenuated strains of vaccinia virus (e.g., MVA) do not require routine vaccinia vaccination. Furthermore, no reports of transmission to health-care personnel from vaccine recipients have been published.

Although no formal surveillance system has been established to monitor laboratory workers, no laboratory-acquired infections resulting from exposure to this highly attenuated strain or from exposure to recombinant vaccines derived from this strain have been reported in the scientific literature or to Centers for Disease Control and Prevention (CDC) (Vaccinia (Smallpox) Vaccine: Recommendations of the Advisory Committee on Immunization Practices (ACIP), June 22, 2001 / 50(RR10);1-25 (<http://www.cdc.gov/mmwr/preview/mmwrhtml/rr5010a1.htm>)).

7. Is the recipient organism significantly pathogenic or harmful in any other way (including its extracellular products), either living or dead?

Yes

No

Not known

If yes:

a) to which of the following organisms:

Humans	<input type="checkbox"/>
Animals	<input type="checkbox"/>
Plants	<input type="checkbox"/>
Other	<input type="checkbox"/>

b) give the relevant information specified under Annex IIIA, point II. (A)(11)(d) of Directive 2001/18/EC

MVA is severely host cell restricted with efficient replication in CEF and baby hamster kidney (BHK) cells but not in human and most other mammalian cells tested. In non-permissive cells, there is therefore no production of virions which could propagate and infect other cells. There is also no risk of integration in host cell genome because MVA remains in the cytoplasm.

MVA is not an animal pathogen as it was administered in several species (mice, piglets, calves, dogs, cats, macaques and elephants) without significant side effects. MVA is also not pathogenic in adult birds.

MVA was also shown to be safe in humans during Smallpox vaccination campaigns in Germany in the 1970s. The most frequent adverse reactions reported in patients administered with MVA based vaccines have been injection site reactions, headache, fatigue, malaise, and fever.

8. Information concerning reproduction

a) Generation time in natural ecosystems:

Not relevant as MVA is not naturally found in the environment. Furthermore, as explained above, MVA is severely host-cell restricted and replicates efficiently in CEF and BHK cells but not in human and other mammalian cells.

b) Generation time in the ecosystem where the release will take place:

Not relevant.

c) Way of reproduction: Sexual Asexual

Not relevant.

d) Factors affecting reproduction:

Not relevant.

9. Survivability

a) ability to form structures enhancing survival or dormancy:

(i) endospores	<input type="checkbox"/>
(ii) cysts	<input type="checkbox"/>
(iii) sclerotia	<input type="checkbox"/>
(iv) asexual spores (fungi)	<input type="checkbox"/>

- (v) sexual spores (fungi)
- (vi) eggs
- (vii) pupae
- (viii) larvae
- (ix) other, specify...

Not relevant.

b) Relevant factors affecting survivability:

MVA vector is destroyed with bleach at 0.5% of active chlorine (i.e. 5 g/l of active chlorine) or autoclaving at 121°C for 20 minutes.

10.(a) Ways of dissemination

The GMO as the parental MVA remains localized in the cytoplasm until the cell destruction. Viral shedding was not observed in the previous clinical trial performed with the GMO. The GMO is expected to stay localized at the injection site.

Similar observations were reported with other recombinant MVA vectors developed by the Sponsor.

10. (b) Factors affecting dissemination

Not relevant.

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)

Not applicable.

C. INFORMATION RELATING TO THE GENETIC MODIFICATION

1. Type of the genetic modification

- i. Insertion of genetic material
- ii. Deletion of genetic material
- iii. Base substitution
- iv. Cell fusion
- v. Other, specify

2. Intended outcome of the genetic modification

The intended outcome of the genetic modification is a therapeutic purpose. The GMO, TG4010, a recombinant MVA encoding humans MUC1 and IL2, will be delivered to patients by subcutaneous (SC) injections. In the SC space, the GMO can transduce cells including dendritic cells and, in the lymph node draining the injection site, which is away from the tolerogenic local milieu of the lesion itself, express and present MUC1 and IL2 epitopes. In this context, the development of a targeted cell mediated immune response should be allowed.

TG4010 (MVA-MUC1-IL2) is meant to induce both adaptive and innate immune responses. The components of the product have been shown to act on several aspects of the immune response, adaptive and innate, cellular and humoral: the full-length MUC1 protein expressed by TG4010 in the cytoplasm of cells infected by the MVA vector shares epitopes associated with tumoural MUC1 against which it induces a cellular immune response. This immunization is potentiated by danger signals related to the viral nature of the product inducing both cellular (interferon IFN γ -associated Th1) and humoral responses and co-expression of IL2 at the injection site which optimizes presentation of the antigen to T cells and stimulate T cells (especially in the draining lymph nodes) and natural killer (NK) cells.

3. (a) Has a vector been used in the process of modification

Yes No

If no, go straight to question 5.

3. (b) If yes, is the vector wholly or partially present in the modified organism?

Yes 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	<input checked="" type="checkbox"/>
Bacteriophage	<input type="checkbox"/>
Virus	<input type="checkbox"/>
Cosmid	<input type="checkbox"/>
Transposable element	<input type="checkbox"/>

Other, specify

b) Identity of the vector

pTG9931

c) Host range of the vector

Escherichia coli

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

Yes No

Antibiotic resistance

Other, specify

GPT marker gene encoding for Xanthin-Guanine Phosphoribosyl Transferase (used as a selection marker for recombinant MVA).

Indication of which antibiotic resistance gene is inserted

Ampicillin resistance (AmpR) gene: AmpR sequence is finally not contained in the DNA fragment which is inserted in the recipient.

a) Constituent fragments of the vector

The plasmid pTG9931 contains DNA sequences coding for the human MUC1 protein and for the human IL2. These sequences are flanked by 2 MVA genomic regions (BRD2, BRG2) that allow homologous recombination between the plasmid pTG9931 and the recipient organism (i.e. the MVA).

b) Method for introducing the vector into the recipient organism

- i. transformation
- ii. electroporation
- iii. macroinjection
- iv. microinjection
- v. infection
- vi. other, specify

Homologous recombination between MVA and pTG9931 in CEF.

5. If the answer to 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 contains the two donor genes: MUC1 and IL2. The insert also contains vaccinia virus promoters for transgenes expression (i.e., pH5R, p7.5).

b) Source of each constituent part of the insert

The primary donor sequences are the MUC1 gene (DONOR 1) and the human IL2 gene (DONOR 2).

MUC1 complementary DNA (cDNA) was isolated from a human breast carcinoma cell line T47D cells.

The human IL2 cDNA was isolated from mitogen activated peripheral blood lymphocytes.

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

TG4010 is a MUC1 targeted immunotherapy derived from a replication defective strain of VV (MVA) engineered to express MUC1 protein as well as un-modified human IL2. TG4010 is designed to help the body's immune system identify cancerous cells carrying the MUC1 tumor-associated antigen as a target to be destroyed.

The components of the product have been shown to act on several aspects of the immune response, adaptive and innate, cellular and humoral:

- The full-length MUC1 protein expressed by TG4010 in the cytoplasm of cells infected by the MVA vector shares epitopes associated with tumoural MUC1 against which it induces a cellular immune response.
- This immunization is potentiated by danger signals related to the viral nature of the product TG4010 inducing both cellular (interferon IFN γ -associated Th1) and humoral responses.
- The co-expression of IL2 at the injection site optimizes presentation of the antigen to T cells and stimulate T cells (especially in the draining lymph nodes) and natural killer (NK) cells. This cytokine is thus included in the product to act as an adjuvant in the immune response.

The ultimate objective is to specifically destroy tumors cells expressing MUC1.

d) Location of the insert in the host organism

- on a free plasmid
- integrated in the chromosome
- other, specify

Following transfection, the insert remains in the cytoplasm as part of the viral vector genome. The insert is fully integrated in the MVA genome by homologous recombination.

e) Does the insert contain parts whose product or function are not known?

Yes No

If yes, specify

D. INFORMATION ON THE ORGANISM(S) FROM WHICH THE INSERT IS DERIVED

MUC1

1. Indicate whether it is a:

Viroid
RNA virus
DNA virus
bacterium
fungus
animal

- mammals
- insect
- fish
- other animal specify phylum, class

other, specify

2. Complete name

i. Order and/or higher taxon (for animals)

- ii. Family name (for plants)
- iii. Genus Homo
- iv. Species Sapiens
- v. Subspecies
- vi. Strain
- vii. Cultivar/breeding line
- viii. Pathovar
- ix. Common name Human

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

Yes No Not known

If yes, specify the following

a) To which of the following organisms?

Humans	<input type="checkbox"/>
Animals	<input type="checkbox"/>
Plants	<input type="checkbox"/>
Other	<input type="checkbox"/>

b) Are the donated sequences involved in any way to the pathogenic or harmful properties of the organism?

Yes No 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 related to exposure to biological agents at work?

Yes No

If yes, specify

5. Do the donor and recipient organism exchange genetic material naturally?

Yes No Not known

IL2

1. Indicate whether it is a:

Viroid

- RNA virus
- DNA virus
- bacterium
- fungus
- animal
- mammals
- insect
- fish
- other animal specify phylum, class

other, specify

2. Complete name

- x. Order and/or higher taxon (for animals)
- xi. Family name (for plants)
- xii. Genus **Homo**
- xiii. Species **Sapiens**
- xiv. Subspecies
- xv. Strain
- xvi. Cultivar/breeding line
- xvii. Pathovar
- xviii. Common name **Human**

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

Yes No Not known

If yes, specify the following

c) To which of the following organisms?

- Humans
- Animals
- Plants
- Other

d) Are the donated sequences involved in any way to the pathogenic or harmful properties of the organism?

Yes No 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 related to exposure to biological agents at work?

Yes No

If yes, specify

5. Do the donor and recipient organism exchange genetic material naturally?

Yes No 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 No Not known

Specify

(b) is the GMO in any way different from the recipient as far as mode and/or rate of reproduction is concerned?

Yes No Unknown

Specify

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

Yes No Not known

Specify

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

Yes No Not known

Specify

2. Genetic stability of the genetically modified organism

A genetic stability program was designed to assess the genetic stability of TG4010 at several steps of the production process: Pre Master Virus Seed 1 (PMVS1), Master Virus Seed (MVS), final Drug Product (DP) and DP + 3 passages. In addition, an accelerated study was performed by sub-passing 6 times the PMVS1 at laboratory scale.

A working Virus Seed (WVS) was produced in 2007, to be used as virus seed for future clinical and commercial lots. Production of lots from the WVS involved an additional viral passage and thus the genetic stability study was updated with additional data.

The vector MVATG9931 was shown to be genetically homogeneous from the MVS through the DS or DP lots and at a passage corresponding to 3 passages beyond the passage intended to be used in clinical applications.

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

Yes No 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)

Not relevant.

4. Description of identification and detection methods

a) Techniques used to detect the GMO in the environment

Identity of MVA strain: This test is done to confirm by PCR the identity of the test article. DNA is extracted from the test sample using a commercially available kit. PCR is then performed with a set of primers designed in the genetic insert and in the flanking viral sequences. Amplification products are separated by agarose gel electrophoresis and sized by comparison with a DNA size marker.

b) Techniques used to identify the GMO

The identity of the GMO can be confirmed by controlling the genomic integrity by restriction enzyme mapping or by PCR as described above.

F. INFORMATION RELATING TO THE RELEASE

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

The release in this context will be the administration of the product, in a hospital or clinic setting, by SC injection to patients as a part of a multinational, multicenter clinical trial protocol. There are no foreseen problems of this release.

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 No

If yes, specify

Not applicable. The GMO and the MVA are not naturally found in the environment. The current release can be compared to the use of MVA during Smallpox eradication campaign.

3. Information concerning the release and the surrounding area

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

TG4010 will be administered in the following clinical sites:

Investigator	Institution
Dr Frédéric Forget	Centre Hospitalier de l'Ardenne - Site de Libramont Avenue de Houffalize 35 6800 Libramont

b) Size of the site (m²):

i. Actual release site (m²):

See below.

ii. Wider release area (m²):

No specific size is required for the site. The room where the 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 applicable.

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

Not applicable.

4. Method and amount of release

a) Quantities of GMOs to be released

A total of 39 patients are planned to be enrolled in the proposed clinical study in Europe and in other regions of the world. Patients will be injected with the GMO up to disease progression or premature discontinuation. Each patient is anticipated to receive an average of 13 injections at the dose of 1×10^8 plaque-forming units (PFU).

b) Duration of the operation

The duration of the operation lasts from the first study treatment administration until the last study treatment administration.

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

The GMO is released for clinical use only, supplied in closed vials and labeled appropriately. The administration is under the responsibility of the investigator, according to the clinical protocol and in respect of the Good Clinical Practice. The product must be prepared in aseptic conditions compliant with injectable preparations.

The area used to prepare the GMO before injection must be cleaned with a standard disinfectant active on vaccinia virus before and after manipulation. Lipid-encapsulated viruses such as TG4010 are sensitive to physical treatment such as steam sterilisation (autoclaving) and to chemical treatment with hospital-grade disinfectants containing bleach, aldehydes, alcohols, hydrogen peroxide, phenols, and quaternary ammonium compounds. Standard chemical germicides such as Aseptanios Terminal Spore®, Amphospray 41 IP sterile®, Anios Oxy'Floor®, Aniospray SF IP stérile®, Aniosurf®, Aniosurf Premium®, Rivascop®, Surfanios Premium®, Surfa'Safe®, Surfa'Safe SH® (non-exhaustive list) are adequate for routine cleaning of work areas, when used according to the manufacturers' instructions to ensure adequate contact time and to confirm the ability of the equipment to withstand the disinfectant used.

During product manipulations, labcoat, goggles, gloves and mask must be used. All transport of product (vial or syringe containing the dose to be injected) must be done using a leakproof container/bag with a biohazard sign. Prior to the administration of the product, the product must be prepared under conditions compliant with parenteral preparations. Furthermore, the staff will follow the standard hospital policy recommended for the manipulation of live virus vaccines.

In case of accidental shedding of TG4010, every contaminated surface area will be treated according to the conventional hospital procedures for infectious product. All personnel involved in handling the product is informed that in case of skin contamination, the skin must be immediately washed thoroughly with water and disinfected locally with 4% iodine and, in case of eyes contamination, it is recommended to wash and rinse thoroughly with water only, and an examination by an ophthalmologist must take place as soon as possible.

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

Not applicable.

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

Since 1999 this product has been released in the context of 8 clinical trials. As of today, a total of 380 patients have been treated with at least one dose of TG4010. TG4010 has been found to be generally safe and well tolerated during these trials with the main adverse events related to the GMO being injection site reactions.

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 organisms (if applicable)

- xix. Order and/or higher taxon (for animals)
- xx. Family name (for plants)
- xxi. Genus **Homo**
- xxii. Species **Sapiens**

- xxiii. Subspecies
- xxiv. Strain
- xxv. Cultivar/breeding line
- xxvi. Pathovar
- xxvii. Common name **Human**

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

TG4010 is expected to induce a MUC1 specific cellular immune response and to produce a non-specific activation of several components of the immune system of patients administered with TG4010.

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

There is minimal potential for gene transfer to other species under the proposed release of the GMO. As mentioned above, the GMO will be released in a conventional clinic examination room and is unlikely to come in contact with other animal species. In order for the viral genes encoded by TG4010 to transfer into the genome of other species of poxviruses, susceptible cells would need to be simultaneously infected with pox virus and co-infected by vector which is extremely unlikely.

4. Is post-release selection such as increased competitiveness, increased invasiveness for the GMO likely to occur?

Yes No Not known

Give details

No selective advantage or disadvantage has been conferred to TG4010 and the parental MVA is not endemic in the human population.

5. Types of ecosystems to which the GMO could be disseminated from the site of release and in which it could become established

TG4010 is not predicted to interact with non-target organisms because of its highly restricted host range and because of the manner of its proposed release. In the unlikely event of inadvertent administration to non-target organisms further spread would be unlikely as several studies have demonstrated that MVA is non-virulent in immunocompetent and immunodeficient laboratory animals and in primary human cell cultures.

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 nature

Not applicable.

7. Likelihood of genetic exchange in vivo

- (a) from the GMO to other organisms in the release ecosystem:

There is minimal potential for gene transfer to other species under the proposed release of the GMO. The GMO will be released in a hospital examination room and is unlikely to come in contact with other animal species. Furthermore TG4010 as the parental MVA virus remains localized in the cell cytoplasm up to the lysis of the infected cell. It is partially replicative (can replicate its DNA including the transgene coding sequence), non-integrative (cytoplasmic localization) and non-propagative in mammalian cells (no longer able to generate infectious particles). There is no possible genetic exchange with other human poxviruses as they are not endemic in humans. In animals susceptible to infection by the virus (even with being non permissive for its propagation), few opportunity for genetic recombination with animal poxviruses could occur, since the level of replication that the vector DNA undergoes in vivo is low, and limited to cells infected by the inoculum (no generation of infectious particles).

- (b) from other organisms to the GMO:

See 7 (a).

- (c) Likely consequences of gene transfer:

No data are available.

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 simulated natural environments (e.g. microcosms, etc.):

No data are available.

9. Possible environmentally significant interactions with biogeochemical process (if different from the recipient or parental organism)

Not applicable.

H. INFORMATION RELATING TO MONITORING

1. Methods for monitoring the GMOs

Monitoring of the direct and indirect effects of the GMO on patients will be achieved using the following clinical assessments: e.g. physical examinations, vital signs, adverse event reporting, assessment of injection site reactions, complete blood cells count, biochemistry analyses, tumor evaluation, immunological assessments as per protocol requirements.

2. Methods for monitoring ecosystem effects

No viral shedding was shown in humans injected with the GMO so far and no significant dissemination of the GMO outside the injection site was observed in animal studies providing

evidence for the non-spreading character of the GMO which appears to remain localized to the injection site.

These data along with the animal biodistribution results outline the non-spreading character of the MVA vector that stays localized to the injection site up to the lysis of the infected cells. MVA virus is also known to be a non-propagative virus and highly attenuated.

3. Methods for detecting transfer of the donated genetic material from the GMO to other organisms

Not applicable as TG4010 is not predicted to interact with non-target organisms because of its highly restricted host range, the manner of its proposed release and the expected transient nature of its gene expression

4. Site of the monitoring area (m2)

Not applicable: the GMO will be administered to patients by SC injections in conventional hospital or clinic rooms.

5. Duration of the monitoring

According to the protocol, a safety follow up visit will be performed at least 28 days after the last administration of the GMO.

6. Frequency of the monitoring

Monitoring visits, during which safety and efficacy will be assessed, are planned on a regular basis according to clinical study protocol.

I. INFORMATION ON POST-RELEASE AND WASTE TREATMENT

1. Post-release treatment of the site

The place where the product will be prepared for injection will be decontaminated before and after the manipulation with a standard disinfectant based solution.

2. Post-release treatment of the GMOs

The place where the product will be prepared for injection will be decontaminated before and after the manipulation with a standard disinfectant based solution.

3. (a) Type and amount of waste generated

The waste generated during the clinical trial are: vials of TG4010 product, syringes, needles, gauze, dressings, gloves, labcoats, goggles, patient gown, bedding, linens, towels, etc.

The exact amount of waste is not possible to estimate considering that patients have not a fixed number of TG4010 injections.

3. (b) Treatment of waste

Disposable material contaminated by TG4010, e.g.:

- used and unused vials,
- injected and non-injected syringes,
- other used material such as needles, gauze, dressings, gloves, etc.,

must be disposed according to country-specific requirements and/or regular hospital procedure for infectious waste (e.g. autoclaving or treatment with sodium hypochlorite solution before incineration).

Non-disposable material contaminated by TG4010, e.g.:

- labcoat, goggles, patient gown, bedding, linens, towels, etc.

must be cleaned/treated according to country-specific requirements and/or regular hospital procedure for infectious material (e.g. hot water $\geq 71^{\circ}\text{C}$ washing with detergent and hot air drying).

J. INFORMATION ON EMERGENCY RESPONSE PLAN

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

It will be recommended to personnel involved in TG4010 handling to act as recommended below in case of incident with the use of TG4010. The complete instructions are given to clinical sites in the “Technical Sheet” and “IMP Guidelines” documents.

- Accidental shedding:

Contaminated area must be cleaned with a standard disinfectant active on TG4010 (e.g., bleach at 0.6% of active chlorine or any other active disinfectant). Leave in contact for at least 30 minutes.

- Skin contamination:

The skin must be immediately washed thoroughly with water and disinfected locally with a solution of bleach at 0.45 % of active chlorine or with a solution of 4% iodine. Leave in contact for at least 5 minutes.

- Needle stick injury:

Bleeding from the wound should be allowed before flushing under a running stream of clean, and preferably sterile, water. Then, the injured skin area must be covered with a sterile gauze dressing, which should be discarded according to hospital standard procedure for infectious material when removed. The exposed individual should be referred to and medically monitored by a physician knowledgeable in the care and treatment of patients with vaccinia virus infections. Medical evaluation and follow up of the exposed individual is required until an active infection is ruled out, or as required by institutional policies.

- Eyes contamination:

The affected eye(s) should be rinsed immediately and for 3 minutes with tap water or ideally physiological saline solution (NaCl 0.9 %) making the liquid flow laterally into the affected eye(s). If a single eye is affected, avoid contaminating the other one (the affected eye must be below the other eye while rinsing). The eyelids should be kept opened and movement of eye(s) should be made in all directions. If available, one drop of a solution of trifluridine 1 % is to be instilled. The injured person should receive counseling from an ophthalmologist as soon as possible.

- Ingestion:

Vomiting must not be induced. The investigator or a doctor is to be called immediately. Medical evaluation and follow up of the exposed individual is required until an active infection is ruled out, or as required by institutional policies.

- Inhalation:

This product is an aqueous solution. In case of splash or droplet inhalation of TG4010, the person should consult a physician immediately and be followed for a period of at least 2 weeks in order to ensure that the subject is asymptomatic and no unexpected serious adverse event has resulted from this intake.

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

See J.1, accidental shedding.

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 will be monitored for the occurrence of adverse events and serious adverse events according to the clinical protocol. Each serious adverse event will be recorded and assessed by the hospital staff and the study sponsor, and Health Authorities will be notified when applicable.

The probability of propagation is very low based on characteristics of the MVA viral vector. As mentioned earlier, the MVA vector is poorly replicative and non-propagative. Thus, any propagation is unexpected. Besides, a complementing propagation-competent poxvirus should be necessary to generate the vector propagation. This event is unlikely since no wild poxvirus is currently endemic in the human population. Moreover it is unlikely that several independent mutations occur, including restorations of the deleted regions of the genome, in order to bring back this genome up to the structure of its parent: the smallpox virus. This phenomenon has never been observed during smallpox vaccination in humans, and a mechanism able to cause and select for an event of such a magnitude is hardly conceivable. Furthermore, studies have shown that repair of multiple genes is required to fully restore the ability of MVA to replicate efficiently in human cells. That is consistent with the inability to detect spontaneous revertants and supports the safety of MVA as a vaccine vector.

Furthermore, viral propagation has never been reported during the previous clinical experience with TG4010 and with other recombinant MVA vectors.