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

25 May 2011

TABLE OF CONTENT

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, TG4010.14 clinical trial, is entitled "A Phase IIb/III randomized, double-blind, placebo-controlled study comparing first-line therapy with or without TG4010 immunotherapy product in patients with stage IV non-small cell lung cancer (NSCLC)".

e) Proposed period of release *From December 2011 until December 2015 (date of study completion)*

2. *Notifier*

Name of institution or company

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

3. *GMOs characterization*

a) Indicate whether the GMO is a:

viroid	☐
RNA virus	☐
DNA virus	☒
bacterium	☐
fungus	☐
animal	☐
- mammals	☐
- insect	☐
- fish	☐
- other animal	☐ 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 poorly replicative, recombinant vaccinia vector consisting of the modified vaccinia virus Ankara (MVA) genome containing inserted transgenes that encode two proteins: the human mucine 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.
The results of the genetic stability study performed on vector MVATG9931 derived from the MVS are in agreement with the acceptance criteria and do allow the use of the vector in clinical studies. Today a Working Virus Seed (WVS) has been produced and the analyses are planned on new lots of DP and on further viral passages (3 passages after DP lots produced from the WVS).

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): *BE, BG, FR, DE, HU, IT, PL, ES and GB*

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

Study	Country	Authority	Reference number	Submission year
TG4010.02	**Switzerland**	SKBS	None	1999
		OICM	1999S01266	
		OFSP	GT-1999 014	
TG4010.04	**Switzerland**	Swissmedic	2002GT2001	2001
		OICM / OFSP	Notification only	2002
	France	CGB	B/FR/01.10.01	2001
		CGG	3739	
	Belgium	SBB	B/BE/01/B7	2001
TG4010.05	**Switzerland**	Swissmedic	2002GT2002	2001
	France	CGB	B/FR/01.10.02	2001
		CGG	3740	
	Belgium	SBB	SBB : B/BE/01/B7	2001

TG4010.06	**France**	CGB	BR/FR/02.05.01	2002
		CGG	3795	
	Belgium	SBB	SBB : B/BE/02/B7	2002
TG4010.09	**France**	CGB	B/FR/05.07.01	2005
		CGG	4437	
	Poland	CRCT	344/UR/CEBK/10/06	2005
		Environment Ministry	01-14/2006	2006
	Hungary	NIP	3125/40/2006	2005
	Germany	PEI	231	2006

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 *Notification planned*

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, specifiy

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 PCR. DNA is extracted from the test sample using a commercially available kit. PCR is then performed with a specific set of primers for MVATG9931, 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.

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 ☐
Animals ☐
Plants ☐
Other ☐

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*	☐
(ii)	*cysts*	☐
(iii)	*sclerotia*	☐
(iv)	*asexual spores (fungi)*	☐
(v)	*sexual spores (fungi)*	☐
(vi)	*eggs*	☐
(vii)	*pupae*	☐
(viii)	*larvae*	☐
(ix)	*other, specify…*	

Not relevant.

b) Relevant factors affecting survivability:

MVA vectors are 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 assumed to stay localized at the injection site.
Similar observations were reported with other recombinant MVA vectors developed by Transgene.

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.

The TG4010 strategy is to induce MUC1 antigen expression in a non-tumor environment, i.e. where the immune system is fully functional, in order to induce:

- *A specific immunity: MUC1 tumor antigen presentation to T cells through the major histocompatibility complex (MHC) molecules (Class I and II) that can induce specific cellular and humoral immune responses.*
- *A non-specific activation of immune system via vaccinia virus infection and activation by IL2.*

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	☒
Bacteriophage	☐
Virus	☐
Cosmid	☐
Transposable element	☐

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.

The MUC1 protein is a highly glycosylated mucin normally found at the apical surface of mucin secreting epithelial cells in many types of tissue including breast, lung, pancreas, stomach, ovaries, fallopian tubes, intestine, and kidney. Cancer in lung, breast, prostate, pancreas, ovaries, uterus, and other malignancies is often accompanied by an over expression of the MUC1 antigen by tumor cells. This MUC1 protein over expressed by tumor cells is less glycosylated than the normal form of MUC1, revealing new peptide and carbohydrate antigenic epitopes. These immunological differences between MUC1 in normal cells and in tumors make it a target for immunotherapy. Further, the oncoprotein MUC1 appears to be positively selected during tumor progression and for this reason therapeutic vaccination against MUC1 may be efficient even in advanced disease.

The human IL2 is a cytokine that has been shown to be an essential factor in the manifestation of cell mediated and humoral immunity as well as for primary and secondary immune responses. This cytokine is thus included to act as an adjuvant in the immune response.

d) Location of the insert in the host organism

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

. 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, specifiy

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	☐
Animals	☐
Plants	☐
Other	☐

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, specifiy

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: PMVS1, MVS, DP and DP + 3 passages.

The results of the genetic stability study performed on vector MVATG9931 derived from the MVS are in agreement with the acceptance criteria and do allow the use of the vector in clinical studies. Today a WVS has been produced and the analyses are planned on new lots of TG4010 and on further viral passages (3 passages after TG4010 lots produced from the WVS).

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

***Vector identity**: DNA is extracted from the test sample using a commercially available kit. PCR is then performed with a specific set of primers for MVATG9931, 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 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. Léon Bosquee	*C. H. U. Sart-Tilman*
Dr. Frédérique Bustin	*C. H. R. de la Citadelle*
Dr. Danny Galdermans	*ZNA Middelheim*
Dr. Frederic Forget	*Centre Hospitalier de l'Ardenne*

b) Size of the site (m^2):

i. Actual release site (m^2):

See below.

ii. Wider release area (m^2):

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

Patients of the treatment arm will receive SC injections of TG4010 at the dose of 10^8 pfu every week for 6 weeks and then once every 3 weeks until progression. Considering the number of patients planned in the clinical trial and the mean number of injection per patient, the estimated quantity of GMO to be released across all clinical sites in all countries of the study is $1.5x10^{12}$ PFU.

b) Duration of the operation

The duration of the operation lasts from the first study treatment administration until the last study treatment administration, according to the schedule of administration depicted above.

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 TG4010 for injection will be decontaminated before and after manipulation with a standard disinfectant based solution (e.g., bleach > 0.5% Cl; i.e. 5 g active chlorine per liter of water or any other active disinfectant).

For the manipulations, goggles and laboratory coat must be worn, gloves are recommended. All transfers of the preparation must be done using a closed container. Furthermore, the site staff will follow the standard hospital or clinic 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 (wheather, 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 7 clinical trials. A total of 270 patients have been treated with at least 1 injection. TG4010 has been found to be generally safe and well tolerated during these trials with the main adverse event reported 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.

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 poorly replicative (replication aborts at a late stage of the virus life cycle, after DNA replication including the transgene coding sequence; virion morphogenesis is interrupted), 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: physical examinations, vital signs, adverse event reporting, assessment of injection site reactions, complete blood cells count, biochemistry analyses, cardiac enzyme, immunological assessments and viral safety / dissemination evaluation by swabbing.

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.

However, some swabbing samples will be collected and analyzed at the first injection site of the GMO, before (negative control) and 6 hours after the first GMO injection and at later time points, i.e., on D8 and D15 of Cycle 1 and D22 (i.e., D1 of Cycle 2) after the first injection.

The samples will always be collected in 30 treated patients of the Phase IIb part (in order to get samples from at least 10 patients under TG4010 treatment). The samples will be analyzed by quantitative PCR.

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 will be assessed, are planned every week during 4 weeks then every 4 weeks during 20 weeks and then every 12 weeks up to the end of the follow up.

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.

Following the patient's discharge home, the clinic or hospital room (surfaces and floor) and the toilets will be cleaned in a standard way using an active 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.
Following the patient's discharge home, the clinic or hospital room (surfaces and floor) and the toilets will be cleaned in a standard way using an active disinfectant based solution.

3. (a) Type and amount of waste generated

Considering:

- *the dose administered per patient, i.e. 10^8 pfu per injection,*
- *the total number of patients planned to be treated with TG4010 in the whole phase IIb/III study, i.e. 1018 patients,*
- *the average number of TG4010 injections per patients, i.e. 15,*

the maximum quantity of GMO to be released across all countries involved in the proposed study is $1.5x10^{12}$ pfu.

3. (b) Treatment of waste

See I.2.

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.

- ***Accidental shedding****:*
Contaminated area must be cleaned with a standard disinfectant active on TG4010 (e.g., bleach at 0.5%Cl; i.e. 5 g/l 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 4% iodine.

- ***Needle stick injury:***
Wash immediately and abundantly under tap water. Then treat the area as follows:
- *Wash with mild soap for 5 minutes, having removed contaminated clothes which will be treated as contaminated material. Rinse abundantly with water. Then treat the area with a disinfectant (e.g., bleach at 0.45% Cl; i.e. 4.5 g/l of active chlorine) for at least 5 minutes. Rinse abundantly with water.*

or:
- *Wash with a solution of 4% iodine for 5 minutes. Rinse abundantly with water. Then treat the area with a solution of 10% iodine for 5 minutes. Rinse abundantly with water.*

In addition, cover the injury with an occlusive, dry dressing, which should be appropriately discarded when removed. The injured person should be seen by a physician and should be closely followed for at least 2 weeks.

- ***Eyes contamination****:*
Rinse immediately and for 15 minutes the affected eye or eyes with physiological saline solution making the water flow laterally into the affected eye. If a single eye is affected, avoid contaminating the other one (the affected eye must be below the other one). Maintain the eyelids opened and move the eye in all ways. If available, instil one drop of a solution of trifluridine 1%. The injured person should undergo an ophthalmological examination as soon as possible.

- ***Ingestion:***
Do not induce vomiting and consult a physician immediately. The person should be closely followed for at least 2 weeks.

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

See J.1.

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.