TG4001

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

Nº Notificación: B/ES/18/29

29 January 2019

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
MVA	Modified vaccinia virus Ankara
MVATG8042	Recombinant vector
MVS	Master virus seed
PCR	Polymerase chain reaction
PMVS	Pre-master virus seed
pTG8042	Transfer plasmid
SC	Subcutaneous
TG4001	Final GMO, viral suspension of MVATG8042
VV	Vaccinia virus
WVS	Working virus seed

A. GENERAL INFORMATION

1. *Details of notification*

a) Member State of notification Spain

b) Notification number B/ES/18/29

c) Date of acknowledgement of notification 10.10.2018

d) Title of the project

A phase Ib/II trial evaluating the combination of TG4001 and avelumab in patients with HPV-16 positive recurrent or metastatic malignancies and expansion cohort to oropharyngeal squamous cell carcinoma of the head and neck (SCCHN).
Study code: TG4001.12

e) Proposed period of release

From 01 March 2019 to 31 December 2020 (Phase II part)

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 ☐
- 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 TG4001 and consists of a poorly replicative, recombinant vaccinia vector consisting of the modified vaccinia virus Ankara (MVA) genome containing inserted transgenes that encode for the modified forms of the human papillomavirus type 16 (HPV16) E6 and E7 proteins (delE6 and delE7) and the human cytokine IL2 (hIL2).

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

A genetic stability program was designed to assess the genetic stability of TG4001 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

The genetic stability of the TG4001 genome was assessed in a series of tests. Testing of the expression, functionality, characterization and the nucleotide sequences of the genetic inserts as well as the immunoplaquing assays were performed. TG4001 still has its expected characteristics 3 passages beyond the passage intended for the production of clinical material.

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):

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 BE, FI, FR,
- Notification number B/BE/09/BVW1

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 Mexico, Switzerland, United States of America
- Notification number

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

The likelihood of TG4001 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 TG4001) to generate a replication competent virus.
- No spontaneous reversion of MVA to replication competent vaccinia virus (VV) has ever been documented.
- TG4001 is unable to produce progeny vector particles in primary human cells, in addition, in human studies, TG4001 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 more than 150 subjects. 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 MVA strain is determined by PCR. DNA is extracted from the test sample using primers complementary to the sequences flanking the deletion II excision site in MVA.

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 Europe and in the USA, the manipulation of recombinant MVA or vectors derived from MVA requires Biosafety Level 1 or 2 when used in clinical/research and is dependent on the country and biosafety committees.
Examples of classifications:

- French Biosafety Commission (Haut Conseil des Biotechnologies): Class 1
- US NIH: Biosafety Level 2 recommended
- Swiss classification: Risk Group 1, Biosafety Level 1 recommended
- German classification (Statement of the ZKBS on Handling of Recombinant Vaccinia Viruses): Risk Group 1, Safety Level 1 recommended.

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

. MVA is not naturally found in the environment, it is a laboratory strain thus its dissemination into the environment is not expected expect in case of accident resulting of spillage.

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, TG4001, a recombinant MVA encoding the modified forms of the HPV16 E6 and E7 proteins (delE6 and delE7) and the human IL2, will be delivered to patients by subcutaneous (SC) injections.

TG4001 is a targeted active immunotherapy product designed for first-line treatment of HPV-16 related diseases by inducing a specific immune response against HPV16 E6 and E7 and a non-specific activation of the immune system via the vaccinia virus infection and expression of IL2 at the injection site.

When injected into a patient, it is hypothesized that TG4001 transduces specialized antigen presenting cells that present the modified E6/E7 antigen epitopes through the MHC class I pathway to CD8 + effector T-cells. In turn, this will initiate a killer T-cell response against the transformed epithelial cells that express the HPV E6/E7 antigen epitopes and enable the eradication of the tumor lesion

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

pTG8042

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

E. coli UidA marker gene encoding for beta glucuronidase (used as a selection marker for colorimetric assay).

Indication of which antibiotic resistance gene is inserted

Not relevant.

e) Constituent fragments of the vector

The plasmid vector contains DNA sequences encoding HPV16 E6 and E7 mutated antigens and human IL2 together with promoters. These gene sequences are flanked by genomic regions that allow homologous recombination between the transfer plasmid and the MVA genome. The transfer plasmid also includes a selection marker, IudA gene, coding for the β-glucuronidase. This marker gene is excised during the second homologous intragenic recombination to obtain the final GMO.

f) Method for introducing the vector into the recipient organism

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

Co-transfection (MVA and pTG8042) in chicken embryo fibroblasts.

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 3 donor genes: delE6, delE7, and IL2. Other elements (secretory and transmembrane sequences) used in the constructs were derived from the measles and rabies viruses and non-transcribed promoter sequences were derived from vaccinia virus.

b) Source of each constituent part of the insert

The primary donor sequences are the HPV16 E6 and E7 genes (DONOR 1) and the IL2 gene (DONOR 2). The E6 and E7 coding sequences were derived from HPV16 viral DNA. The IL2 gene was derived from human peripheral blood mononuclear cells.

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

TG4001 is an HPV16 targeted immunotherapy derived from a replication defective strain of vaccinia virus (Modified Vaccinia Ankara, MVA) engineered to express modified forms of the HPV16 early proteins E6 and E7(delE6 and delE7) as well as un-modified human interleukin 2 (IL2).

The E6 and E7 genes of HPV16 were modified by deletion to eliminate the interaction of their encoded proteins with the respective tumor suppressor proteins pRb and p53, while retaining their immunogenicity. The safety and immunogenicity of these two proteins has potentially been further enhanced by use of heterologous (measles and rabies virus) viral sequences to direct the translated proteins away from the nucleus and anchor them on the cell membrane, thus avoiding nuclear localization and limiting their interaction with pRb and p53.

IL2 is a cytokine that has been shown to be an essential factor in cell-mediated and humoral immune responses. IL2 therapy has been recognized as effective for patients with metastatic renal adenocarcinoma and in metastatic melanoma. Secretion of this cytokine locally produces very low levels in the circulation while presumably augmenting immune activation.

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

(1) E6 and E7 modified genes (delE6 and delE7) derived from human papillomavirus genotype 16

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)	Papillomaviridae
ii.	Family name (for plants)	
iii.	Genus	Alphapapillomavirus
iv.	Species	Human papillomavirus 16
v.	Subspecies	
vi.	Strain	
vii.	Cultivar/breeding line	
viii.	Pathovar	
ix.	Common name	HPV16

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): The oncogenic potential of high-risk HPVs lies in the E6 and E7 oncoproteins, which are responsible for disturbing the control of the cell cycle and initiating the series of alterations associated with cellular transformation. HPV antigens E6 and E7 inserted in the GMO have been modified to remove their oncogenic potential.

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
Classification 2

Note: As indicated in section D.(1).3.b), in TG4001, the E6 and E7 genes have been modified to abolish (E7) or to significantly reduce (E6) interactions with the pRb and p53 tumor suppressor genes, respectively.

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

Yes ☐ No ☒ Not known ☐

(2) Secretory and transmembrane anchoring sequences from rabies virus linked to delE7.

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)	Mononegavirales
ii.	Family name (for plants)	Rhabdoviridae
iii.	Genus	Lyssavirus
iv.	Species	Rabies
v.	Subspecies	
vi.	Strain	
vii.	Cultivar/breeding line	
viii.	Pathovar	
ix.	Common name	Rabies virus

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
Classification 2

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

Yes ☐ No ☒ Not known ☐

(3) Secretory and transmembrane anchoring sequences from measles virus linked to delE6.

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)	Mononegavirales
ii.	Family name (for plants)	Paramyxoviridae
iii.	Genus	Morbillivirus
iv.	Species	Measles virus
v.	Subspecies	
vi.	Strain	
vii.	Cultivar/breeding line	
viii.	Pathovar	
ix.	Common name	Measles virus

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
Classification 2

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

Yes ☐ No ☒ Not known ☐

(4) 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*

i.	Order and/or higher taxon (for animals)	Primate
ii.	Family name (for plants)	Hominidade
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 ☐

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

d) Genetic stability of the genetically modified organism

A genetic stability program was designed to assess the genetic stability of TG4001 at several steps of the production process: from the Master Virus Seed (MVS #Y186) till 3 passages beyond the passage number of the Drug Substance (DS) lots issued from the MVS and used for clinical studies.

The genetic stability of the TG4001 genome was assessed in a series of tests: expression and functionality of the inserted transgenes, characterization assays, nucleotide sequencing of the inserted coding sequences and of the non-coding regions of the inserted cassette as well as immunoplaquing assays were performed. TG4001 still has its expected characteristics 3 passages beyond the passage intended for the production of clinical material.

e) 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.

f) Description of identification and detection methods

a) Techniques used to detect the GMO in the environment

The GMO can be detected in the environment by PCR method. DNA is extracted from the vectors 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 for each recombinant vector. Amplification products are electrophoresed through an agarose gel 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 will consist in the administration of the investigational medicinal product TG4001, in a hospital or clinic setting, by SC injection to patients as a part of a multicenter clinical study protocol.

g) *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.

h) *Information concerning the release and the surrounding area*

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

- **Hospital Universitario Virgen de la Victoria**
  Campus de Teatinos, S/N, 29010 Málaga
- **Hospital Universitario 12 de Octubre**
  Av. Cordoba, s/n, 28041 Madrid
- **Hospital Clinico Universitario San Carlos**
  Calle del Prof Martín Lagos, s/n, 28040 Madrid
- **Hospital Universitario Virgen de las Nieves**
  Avenida de las Fuerzas Armadas, 2, 18014, Granada
- **Hospital General Universitari Valencia**
  Avenida Tres Cruces, 2, 46014 Valencia
- **Institut Català d'Oncologia- Badalona**
  Carretera de Canyet, s/n, 08916 Badalona, Barcelona

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

i. Actual release site (m^2):

See below.

ii. Wider release area (m^2):

The size of each site will vary but it is important to note that contamination of the site at which the administration is performed is expected to be minimal, when suitable precautions are adhered to.

TG4001 will be administered at licensed healthcare facilities where there are standard facility controls in place for administration of GMO medicinal products, collection and processing of blood and serum samples, and all equipment and staff required for the clinical evaluation of study subjects.
The size of preparation and administration rooms is detailed for each clinical site on the Technical Study document.

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

Given the nature of the product administration and procedures for waste treatment, the exposure to significant biotopes, protected areas and drinking water supplies is expected to be minimal.

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

Since the parental organism is not naturally found in the environment, is severely host-cell restricted and does not replicate in human and other mammalian cells, proximity to other flora and fauna is not a concern.

i) Method and amount of release

a) Quantities of GMOs to be released

The GMO product will be supplied to study site pharmacies in a 4-mL glass vial as a single 0.5 mL dose, containing $5x10^7$ PFU of the GMO.
The maximum volume of TG4001 administered to a patient is 0.5 mL.

In the Phase Ib part of the study, 3 patients received the GMO at the dose of $5x10^6$ PFU and 6 patients at the dose of $5x10^7$ PFU, according to the following schedule of administration: TG4001 administration weekly for 6 weeks and then once every 2 weeks until month 6 (from the start of study treatment) thereafter once every 3 months until disease progression or premature discontinuation due to any reason, or a maximum of 2 years of study treatment, whichever occurs first.

In the Phase II part of the study, 12 patients evaluable are planned to be enrolled in Spain and administered with the GMO at the $5x10^7$ PFU dose defined as the maximum tolerated dose, according to the same schedule of administration as in the Phase Ib part.

b) Duration of the operation

The complete administration procedure from preparation of the dosing syringe to completing the injection procedure is expected to take 2 hours.

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 TG4001 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).

During all GMO manipulations labcoat, goggles, gloves and mask must be used. All transport of GMO (vial or syringe containing the dose to be injected) must be done using a leak-proof container/bag. The sponsor will provide, if needed, DIAGNOBAG A4 E3 CORTEX bags to the clinical sites. Prior to the administration, the product must be prepared under conditions compliant with injectable preparations. Furthermore, the staff will follow the standard hospital policy recommended for the manipulation of live virus vaccines.

In case of accidental shedding of the GMO, 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.

j) Short description of average environmental conditions (wheather, temperature, etc.)

Not applicable.

k) 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 9 clinical trials conducted in different stages of cervical lesions related to HPV16 infection. A total of 322 patients have been treated with at least one injection of the GMO. TG4001 has been found to be generally safe and well tolerated during these trials with the main adverse event reported being injection site reactions.

The shedding of TG4001 was investigated in a total of 48 subjects as part of the first phase I studies. No viral DNA was found in any sample tested (blood and urine). These data along with the animal biodistribution results confirm the non-spreading character of the MVA vector. MVA virus is also known to be a non-propagative virus and highly attenuated.
Thus the likelihood for persistent or systemic infection of personal contacts of the patients and health care personnel is considered low. If this were to occur it is not expected that any significant clinical manifestations would be evident as effects in non-tumor tissue in TG4001 treated subjects have not been noted.

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)

(a) Order and/or higher taxon (for animals) Primate
(b) Family name (for plants) Hominidae
(c) Genus Homo

(d) Species Sapiens
(e) Subspecies
(f) Strain
(g) Cultivar/breeding line
(h) Pathovar
(i) Common name Human

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

Local expression of IL2, modified E6 and modified E7 (delE6 and delE7) gene products.

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 TG4001 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 TG4001 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*

TG4001 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 TG4001 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, complete blood cells count, biochemistry analyses, immunomonitoring, etc.

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. Thus, no viral shedding study is contemplated for the proposed clinical study.

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

Not applicable as TG4001 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*

Monitoring will occur throughout the patient's participation in the study, including a period of safety follow-up, as defined in the study protocol: patients who discontinue the study treatment will be evaluated for safety 30 days after the last study treatment administration.

6. *Frequency of the monitoring*

Clinical assessments will be made according to the predefined schedule detailed in the study protocol. The study period during which all adverse events (AEs) and serious adverse events (SAEs) must be reported begins after informed consent is obtained from the patient and ends 30 days following the last administration of any study treatment. After this period, investigators should only report SAEs that are considered as related to study treatment.

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*

Disposable material contaminated by the GMO (used and unused vials, injected and non-injected syringes) 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).

3. *(a) Type and amount of waste generated*

Waste generated from administration of TG4001 will be limited to:

- Used vials and needles
- Used swabs and items used to clean injected area
- Used dressings applied to the injection sites if any

• Personal Protective Equipment (e.g. labcoat, goggles, patient gown, bedding, linens, towels).

Based on maximum TG4001 treatment duration of 2 years, a total of 24 vials could be administered to each subject in this timeframe. Thus, the maximum total dose for an individual patient administered at $5x10^7$ PFU is up to $1.2x10^9$ PFU.
Each administration will result in the waste identified above.

3. (b) Treatment of waste

See I.2.

Disposable material contaminated by TG4001, 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 TG4001, 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 ≥ 71°C washing with detergent and hot air drying).

Lipid-encapsulated viruses such as TG4001 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.

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 TG4001 handling to act as recommended below in case of incident with the use of TG4001. The complete instructions are given to clinical sites and are available in TG4001 Investigator Brochure, section 6.12.4.

- **Accidental shedding**:
  Contaminated area must be cleaned with a standard disinfectant active on TG4001 (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 5 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 TG4001, 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.

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 TG4001 and with other recombinant MVA vectors.