TG4040

PART 1 (COUNCIL DECISION 2002/813/EC)

SUMMARY NOTIFICATION INFORMATION FORMAT FOR THE RELEASE OF GENETICALLY MODIFIED ORGANISMS OTHER THAN HIGHER PLANTS IN ACCORDANCE WITH ARTICLE 11 OF DIRECTIVE 2001/18/EC

TABLE OF CONTENT

A. General information 4
B. Information relating to the recipient or parental organisms from which the GMO is derived 6
C. Information relating to the genetic modification 11
D. Information on the organism(s) from which the insert is derived 13
E. Information relating to the genetically modified organism 15
F. information relating to the release 16
G. Interactions of the GMO with the environment and potential impact on the environment, if significantly different from the recipient or parent organism 19
H. Information relating to monitoring 21
I. Information on post-release and waste treatment 22
J. Information on emergency response plan 22

LIST OF ABBREVIATIONS

BHK	Baby hamster kidney
CEF	Chicken embryo fibroblasts
DNA	Deoxyribonucleic acid
GMO	Genetically modified organism
HCV	Hepatitis C virus
IMP	Investigational medicinal product
MHC	Monochain human histocompatibility
MVA	Modified Vaccinia virus of Ankara
MVATG16643	Recombinant vector
MVS	Master virus seed
NS3, NS4 and NS5B	Non structural proteins of hepatitis C virus
PCR	Polymerase chain reaction
PMVS	Pre master virus seed
pTG16643	Transfer plasmid
RNA	Ribonucleic acid
SC	Subcutaneous
TG4040 or MVA-HCV	Final GMO, viral suspension of MVATG16643
WVS	Working virus seed

A. GENERAL INFORMATION

1. Details of notification

a) Member State of notification *Romania*

b) Notification number B/RO/10/08

c) Date of acknowledgement of notification 04/06/2010

d) Title of the project

The project, TG4040.02 clinical trial, is entitled "A phase II randomized, multicenter, open-label study of TG4040 (MVA-HCV) in combination with pegylated interferon alfa-2a and ribavirin versus pegylated interferon alfa-2a and ribavirin in treatment-naïve patients with chronic genotype 1 hepatitis C".

e) Proposed period of release *From 01 May 2010 until 31 March 2013 (date of study completion)*

2. Notifier

Parexel International Romania SRL on behalf of

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 GMO is TG4040 and consists of recombinant MVA vector particles (MVATG16643) in suspension in a formulation solution. MVATG16643 is a non integrative, poorly replicative, non propagative, recombinant vaccinia vector consisting of the modified vaccinia virus of Ankara (MVA) genome containing nucleotide sequences encoding Hepatitis C virus (HCV) non structural proteins NS3, NS4 and NS5B.

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

In reference to Annex IIIa, section II. A. a) 10, a major feature of HCV is the heterogeneity of its genome. Mutations are responsible for the fact that HCV circulates in the host as a complex viral population referred to as quasispecies. Extensive phylogenetic analysis has permitted classification of HCV isolates into 6 major genotypes (1 to 6) containing at least 70 different subtypes (a, b, c).

The entire central coding region of the MVA genome of the Transgene's isolate has been sequenced and was shown to be homologous to the published sequence in GenBank (section II. A. b) 10 of Annex IIIa).

A genetic stability program was designed to assess the genetic stability of MVATG16643 at several steps of the production process: Pre Master Virus Seed 1 (PMVS1), PMVS1 + 6 passages, Master Virus Seed (MVS), Working Virus Seed (WVS), Investigational Medicinal Product (IMP) and IMP + 3 passages. Testing of the expression, characterization and the nucleotide sequences of the genetic inserts as well as the immunoplaquing assays were performed. MVATG16643 still has its expected characteristics 3 passages beyond the passage intended for the production of the clinical lot.

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; DE; PL; RO; ES]*

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

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

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

The likelihood of TG4040 becoming persistent and invasive in natural environment is very low for the following reasons:

- *TG4040 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). The available biodistribution data of TG4040 including viral shedding monitored in blood and urine of patients (n=3) demonstrate the non spreading character of the GMO which remains localized at the injection site. These observations were also reported from biodistribution and viral shedding studies with other recombinant MVA vectors developed by Transgene (n=94).*

- *The wild type vaccinia virus and the GMO are not naturally found in the environment and by consequent, recombination events cannot occur. Should wild type vaccinia virus be present in the environment together with the GMO, genetic recombination events allowing the MVA virus (parent of the GMO) to bring back its genome up to the structure of its parent are unlikely because it requires several independent mutations, including restorations of the deleted regions of the genome. 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, it was 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 and gene therapy vector.*

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 of 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 MVA strain can be confirmed by polymerase chain reaction (PCR). It is based on the presence of MVA deletion II, characteristics encountered only in the MVA strain of vaccinia virus.

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 1.6°Cl (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, TG4040, a recombinant MVA encoding NS3, NS4 and NS5B non-structural proteins of HCV, will be delivered to patients by subcutaneous injections. In the subcutaneous 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 NS3, NS4 and NS5B epitopes. In this context, the development of a targeted cell mediated immune response should be allowed. A hypothesis is that the GMO will transduce specialized antigen presenting cells that will present epitopes from the 3 HCV antigens through the monochain human histocompatibility (MHC) class I pathway to CD8+ effector T cells and/or to MHC class II pathway to CD4+ effector T cells. This will initiate a killer T cell response, and/or help to initiate this response, against HCV infected cells and will enable the elimination of the infected 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 ☒
Bacteriophage ☐

Virus ☐
Cosmid ☐
Transposable element ☐

Other, specify

b) Identity of the vector
pTG16643

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
Indication of which antibiotic resistance gene is inserted
Ampicilline resistance (AmpR) gene. However, the AmpR sequence is finally not contained in the DNA fragment which is inserted in the recipient.

e) Constituent fragments of the vector
The vector pTG16643 contains DNA sequences coding for the HCV NS3, NS4 and NS5B proteins derived from a prototypic genotype 1b viral isolate and regulation sequences (promoters). In addition, the HCV sequences are flanked by 2 MVA genomic regions (BRD3, BRG3) that allow homologous recombination between the transfer plasmid and MVA.

f) 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 pTG16643 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 donor genes which encode for 3 HCV proteins: NS3, NS4 and NS5B. The insert also contains vaccinia virus promoters for transgenes expression (i.e., pH5R, p7.5K).

b) Source of each constituent part of the insert

The sequences of interest (NS3, NS4 and NS5B genes) were derived from a prototypic genotype 1b viral isolate of the Hepatitis C Virus (HCV-JA strain).
The other elements, pH5R and p7.5K, are early late promoters of vaccinia virus.

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

There are no pathological traits of the non structural proteins NS3, NS4 and NS5B of HCV. The non-structural HCV antigens, in particular NS3, are the targets of T cell responses associated with natural viral clearance.

d) Location of the insert in the host organism

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

Following co-transfection of MVA and pTG16643, the insert is fully integrated in the MVA genome by homologous recombination in the deletion III region of the MVA DNA.

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. 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)	*Flaviridae*
ii.	Family name (for plants)	
iii.	Genus	*Hepacivirus*
iv.	Species	*Human Hepatitis C virus*
v.	Subspecies	*HCV genotype 1b*
vi.	Strain	*JA strain*
vii.	Cultivar/breeding line	
viii.	Pathovar	
ix.	Common name	*HCV*

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	☐

Hepatitis C virus (HCV) is a common infectious agent worldwide, affecting about 170 million people. HCV is a ribonucleic acid (RNA) virus belonging to the family of Flaviviridae; thus, it is not integrated into the host genome. WHO estimates that about 3% of the world's population is infected with HCV and that some 170 millions are chronic carriers. Severe complications with cirrhosis may develop in 20% of infected persons with a risk of carcinoma of 1-4% per year which makes hepatitis C the leading cause of liver transplantation in the US and EU.

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
HCV is classified as a group 3 biological agent according to the European Economic Community (EEC) classification for the protection of workers with biological agents {Directive 2000/54/EC}.

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 TG4040 at several steps of the production process: Pre Master Virus Seed 1 (PMVS1), PMVS1 + 6 passages, Master Virus Seed (MVS), Working Virus Seed (WVS), Investigational Medicinal Product (IMP) and IMP + 3 passages. Testing of the expression, characterization and the nucleotide sequences of the genetic inserts as well as the immunoplaquing assays were performed. TG4040 still has its expected characteristics 3 passages beyond the passage intended for the production of the clinical lot.

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)

There are no pathological and ecological traits of the insert (i.e. NS3, NS4 and NS5B antigens of HCV). Those HCV antigens, in particular NS3, are the targets of T cell responses associated with natural viral clearance.

Non clinical (mice and rabbits) and clinical studies (2 phase I trials with 30 and 9 patients recruited respectively) performed with the GMO until now have shown no major toxic effect which could be related to the GMO.

4. Description of identification and detection methods

a) Techniques used to detect the GMO in the environment

The presence of MVA strain can be detected by Polymerase Chain Reaction (PCR). It is based on the presence of MVA deletion II, characteristics encountered only in the MVA strain. The PCR test was validated according to ICH Q2 (R1) guideline. It was shown to be specific for the identification of the MVA strain.

b) Techniques used to identify the GMO

The identity of the GMO can be confirmed by controlling the genomic integrity by restriction enzyme mapping.

F. INFORMATION RELATING TO THE RELEASE

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

The release corresponds to the clinical trial TG4040.02 entitled « A phase II randomized, multicenter, open-label study of TG4040 (MVA-HCV) in combination with pegylated interferon alfa-2a and ribavirin versus pegylated interferon alfa-2a and ribavirin in treatment-naïve patients with chronic genotype 1 hepatitis C ».
The main purpose is to determine whether the therapeutic vaccine TG4040 (MVA-HCV) improves the efficacy of the current reference treatment for chronic hepatitis C, that is pegylated interferon alpha-2a associated with ribavirine. During the trial, the efficacy will be assessed by measuring the quantity of HCV in the blood of patients (viral load).
Currently, the reference treatment for hepatitis C combines interferon alpha (conventional and pegylated) (IFNα) with ribavirine (RBV). This bi-therapy tries to prevent the evolution of the disease and obtain a cure by reaching a sustained virologic response (SVR), defined as

the absence of viral RNA 6 months after the end of the treatment. However, these treatments may involve frequent and major adverse reactions such as:
depression, flu symptoms, irritability, fatigue, fever, neutropenia due to the IFNα, as well as: haemolytic anaemia, itchiness, rash due to the RBV. The adverse haematological effects may be controlled by regular monitoring, the administration of growth factors, or reductions in the dose of RBV and/or Peg-IFNα. All these adverse events lead to a deterioration in the quality of life.
In addition, the rate of response to treatment combining Peg-IFNα and RBV varies according to the genotype of the virus. It only reaches 40 to 50% for genotype 1 and 70 to 80% for genotypes 2 and 3.
The partial efficacy of current anti-HCV treatments and their major adverse reactions demonstrate that the development of an effective vaccine against the virus remains necessary.

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):
TG4040 will be administered in the following clinical sites:

Hospital / department	Adress	Investigator	
Spitalul Clinic Judetean de Urgenta Timisoara	Bd. Iosif Bulbuca Nr. 10, Timisoara, 300736, Romania	Prof.Dr.	**Sporea** Ioan
Mediclass Sananova SRL	Str. Sfantul Elefterie nr. 47-49 Complexul Comercial Cotroceni, Bucuresti, 050524, Romania	Dr.	**Manuc** Mircea
Private practice. Policlinica Algomed SRL	Str. Lucian Blaga Nr. 4, Timisoara, 300002, Romania	Prof.Dr.	**Goldis** Adrian
Gastromedica SRL,	Str. Garabet Ibraileanu nr.4B, et. 2 ap.5-8, Iasi, 700506, Romania	Prof.Dr.	**Stanciu** Carol
Spitalul Clinic Colentina Bucuresti	Sos. Stefan cel Mare nr. 19-21, Bucuresti, 020125, Romania	Prof.Dr.	**Tanasescu** Coman

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

The maximal dose administered is 10^7 pfu per injection. A given patient will receive a maximum of 17 injections over a 49-week period.

b) Duration of the operation

See 4.a)

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 TG4040 for injection will be decontaminated before and after manipulation with a standard disinfectant based solution (e.g., bleach > 1.6° Cl; i.e. 5 g active chlorine per liter of water).

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 TG4040, 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.

No specific biological analysis in the personnel handling the product is planned.

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 its entry in clinical development (i.e. in 2006), TG4040 has been released in a clinical setting closed to the proposed one in 2 prior occasions. A total of 39 patients have been injected with TG4040 so far. TG4040 was shown to be generally safe and well tolerated during these trials with the most frequent adverse events reported being injection site reaction (induration, inflammation, erythema, oeadema, pain), fatigue, lymphadenopathy, nausea and headache.

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)

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

The target organisms are the human beings.

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

TG4040 will be administered to patients by subcutaneous injections in the thighs and the arms. In the subcutaneous space, TG4040 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 NS3, NS4 and NS5B epitopes. In this context, the development of a targeted cell mediated immune response should be allowed. This response should enable the elimination of the HCV infected cells.

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

There is extremely low potential for gene transfer to other species under the proposed release. As mentioned in section F, the GMO will be released in a conventional hospital examination room and is unlikely to come in contact with other animal species.

Recombination events with other organisms are unlikely since this would require the presence of other poxviruses which are not naturally found in the environment.

Furthermore, there are no known pathological and ecological traits of the viral proteins encoded by TG4040 (i.e. NS3, NS4 and NS5B antigens of HCV). These HCV antigens, in particular NS3, are the targets of T cell responses associated with natural viral clearance in humans.

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 TG4040 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

TG4040 is anticipated not to interact with non-target organisms due to its severely restricted host range and due to the conditions of the 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 not 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 TG4040 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 regarding the behaviour and characteristics of TG4040 in the mentioned environments.

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
No viral shedding was shown in human injected by subcutaneous route with TG4040 or other MVA based products (n~100) and no significant dissemination of TG4040 outside the injection site was observed in animal studies confirming the non spreading character of TG4040 which remains localized at the injection site up to the lysis of the infected cells. Based on this information, no specific viral detection relative to TG4040 is scheduled in the present proposal.
Monitoring of the direct and indirect effects of the GMO in patients will be achieved using the following clinical assessments: physical examinations, adverse event reporting, clinical laboratory assessments throughout the clinical study for all patients.

2. Methods for monitoring ecosystem effects
Not planned as the GMO and the parental MVA virus are not naturally found in the environment.

3. Methods for detecting transfer of the donated genetic material from the GMO to other organisms
Method not available - The probability for a transfer of the donated genetic material to other organisms is unlikely since TG4040 has no nuclear localization and there is no known human endemic virus able to complement, to recombine or to exchange genetic material with the MVA genome.

4. Site of the monitoring area (m2)
Not applicable: the GMO will be administered to patients by subcutaneous injections in conventional hospital or clinic rooms.

5. Duration of the monitoring

Safety assessments will be performed all along the patient's participation in the clinical trial and up to 8 months after last study injection.

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. Additional monitoring visits will be performed at each GMO injection.

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

In hospital units where patients are treated with TG4040, a detailed procedure for the preparation of the product is provided to the personnel involved in the product handling. Adata sheet describing the procedure for the injection, the conditions for the elimination of wastes and the procedure to follow in case TG4040 is accidentally disseminated are available in the places where the product is handled. All wastes related to the use of the product should be stored in a special closed container that is labelled and eliminated according to the standard hospital procedures for infectious materials.

3. (a) Type and amount of waste generated

TG4040 titer in the clinical batch will be within the required specifications (i.e., 7.5 x 10^6 to 5.0 x 10^7 pfu/mL). TG4040 is suspended in a total volume of 0.725 mL. As a consequence, the quantity of waste per injection is limited and will not be more than 2.625 x 10^7 pfu.

The total number of patients in this clinical trial will be 123 evaluable, meaning that approximately 140 patients will have to be included. In this aim, it has been estimated that a given clinical site would recruit approximately 7 patients over the recruitment period. Forty percent of the patients per site will receive a maximum of 17 injections of 10^7 pfu of TG4040. Another forty percent of the patients will receive no more than 7 injections of 10^7 pfu of TG4040 while the last twenty percent of the patients might receive a maximum of 13 injections. The overall quantity of waste per site is therefore considered limited.

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 TG4040 handling to act as recommended below in case of incident with the use of TG4040.

- ***Accidental shedding**:*
  Contaminated area must be cleaned with a standard disinfectant active on TG4040 (e.g., bleach at 1.6°Cl; i.e. 5 g/l of active chlorine). 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 1.4°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 (SAE) according to the clinical protocol. Each SAE will be recorded and assessed by the hospital staff and the study sponsor, and Health Authorities will be notified when applicable.

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 TG4040 and with other recombinant MVA vectors.