TG6002

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

BELGIUM

20 February 2018

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LIST OF ABBREVIATIONS

AmpR	Ampicillin resistance gene
BSC	Best supportive care
CDC	US Centers for Disease Control and Prevention
DNA	Deoxyribonucleic acid
E. coli	*Escherichia coli*
GI	Gastro-intestinal
GMO	Genetically modified organism
HED	Human equivalent dose
hGM-CSF	Human granulocyte macrophage-colony stimulating factor
IL2	Interleukin-2
IT	Intratumoral
IV	Intravenous
JX-594	Recombinant VV, Wyeth strain, expressing hGM-CSF and β-galactosidase (also called Pexa-Vec)
MUC1	Mucin-1 tumor antigen
lacZ	β-galactosidase coding gene
PCR	Polymerase chain reaction
PPE	Personal protective equipment
TG1031	Vaccinia virus of the Copenhagen strain deleted in thymidine kinase gene and coding for mucin-1 tumor antigen and human interleukin-2
TK	Thymidine kinase
TK_L	ATP-binding domain sequence of TK
TK_R	Nucleoside-binding domain sequence of TK
VV	Vaccinia virus
X-gal	5-Bromo-4-chloro-3-indolyl galactopyranoside

A. GENERAL INFORMATION

1. *Details of notification*

a) Member State of notification *BE*

b) Notification number B/BE/18/BVW1

c) Date of acknowledgement of notification 18/04/2018

d) Title of the project

The clinical study to be conducted with TG6002 is entitled:
"A Phase I/IIa study of TG6002 (VV TK-RR-FCU1) administered by intravenous (IV) infusions in combination with oral flucytosine (5-FC) in patients with advanced gastro-intestinal (GI) tumors".
The clinical study code is ***TG6002.02****.*

e) Proposed period of release *Q4 2018 to Q3 2021*

2. *Notifier*

Name of institution or company

TRANSGENE S.A.
Parc d'Innovation, CS80166
400 boulevard Gonthier d'Andernach
67405 Illkirch-Graffenstaden cedex
France

3. *GMOs characterization*

a) Indicate whether the GMO is a:

viroid ☐
RNA virus ☐
DNA virus ☒ *Vaccinia 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)

Genus: Orthopoxvirus
Species: Vaccinia virus (VV)

The GMO is a viral suspension of the recombinant virus TG6002. TG6002 is a non-integrative, conditionally replicative, recombinant VV derived from the Copenhagen strain. TG6002 differs by three genetic modifications from the Copenhagen strain: 1) deletion of the viral thymidine kinase (TK) gene, 2) deletion of the viral ribonucleotide reductase (RR) gene and 3) insertion of the chimeric yeast FCU1 suicide gene in the TK locus.

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

Double-stranded DNA viruses, such as VV, typically have very low rates of mutation from one passage to the next (Nalca A. and Zumbrun E., 2010). Furthermore, the FCU1 gene is inserted in the TK locus which is located in the highly conserved HindIII fragment J of VV genome.

Reversion of TK- to TK+ virus, which readily occurs with point mutations, is unlikely when large insertions are made into the body of the TK gene. Buller et al did not detect any revertants to TK+ virus after extensive tissue culture passage of several Western Reserve TK-recombinant viruses (Buller R. et al., 1985).

The genetic stability study has demonstrated 100 % stability after 10 passages on the production cell (Foloppe J., 2009). In addition, for each production lot, the virus identity is confirmed by PCR and restriction site analysis. Indirectly this testing provides verification of the genetic stability.

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, 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
- Notification number

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

The likelihood of TG6002 becoming persistent and invasive in natural habitats is low for the following reasons:

- *Due to the inactivation of its TK and RR genes, TG6002 replicates preferentially in actively dividing cells such as cancer cells. This limits the propagation of the recombinant virus. Apart from this difference and the insertion of the FCU1 transgene, TG6002 is comparable to its non-recombinant parental virus. The genetic modifications introduced in TG6002 are therefore not expected to increase dissemination and survival capacity of the GMO compared to the parental virus.*

- *TG6002 remains exclusively in the cytoplasm of infected cells thus eliminating any risk of integration of the viral DNA into the host genome.*

- *Shedding of infectious particles into the environment and potentially to the public can occur during the proposed release. However, dispositions are taken in this clinical trial to minimize dissemination and inadvertent transmission.*

- *The risk of contact transmission is rare as demonstrated with vaccinia-based smallpox vaccine (occurrence of secondary transmission of VV from a vaccinated recipient was shown as rare as 0.0054%) (Wertheimer E.R. et al., 2011). The risk of transmission in the proposed clinical trial is reduced by the use of universal precautions by healthcare workers and the education of patients in meticulous hand hygiene and appropriate dressing of the injection site.*

- *VV persistence is affected by temperature and air and except if it happens at freezing temperature, an accidental spill of TG6002 will not result in virus viability over a few hours or days. Furthermore, TG6002 is a lipid encapsulated virus and is consequently sensitive to many classical disinfectants. So an accidental spill during TG6002 handling will be easily decontaminated and will not result in environmental spread and persistence.*

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	☒ *Vaccinia 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 *Copenhagen*

(vi) Pathovar (biotype, ecotype, race, etc.)

(vii) Common name

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 ☐

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*

Not applicable. There is no detection or identification technique able to discriminate the parental VV from the GMO except by:

- *Restriction mapping of the HindIII region*
- *Sequencing of the TK gene*
- *Sequencing of the RR gene*
- *Sequencing of the FCU1 gene.*

5. *(b) Identification techniques*

See 5. (a).

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, VV is considered as a group 2 biological agent as per the European Economic Community classification for the protection of workers with biological agents (2000/54/EC). The group 2 designation applies to agents that can cause human disease and might be a hazard to workers, that are unlikely to spread to the community and

for which there is usually effective prophylaxis or treatment available. Examples of other group 2 biological agents include the measles virus, salmonellae, and the influenza viruses (types A, B and C).

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	☐

VV has the longest and most extensive history of use in humans acquired during the smallpox vaccination campaign up to the early 1980s with hundreds of millions of people vaccinated. The fact that VV was massively used in the smallpox eradication campaign means that there is unprecedented information on its behaviour in humans, including the identification of populations which are at risk for rare adverse events (Cono J. et al., 2003; Kretzschmar M. et al., 2006). Very rare, but serious and potentially life-threatening, complications included eczema vaccinatum, disseminated vaccinia rash, progressive vaccinia and encephalitis (Fields B.N., 1996). The individuals who have been identified with an increased risk of serious adverse effects are:

- *Children less than 12 months old*
- *Pregnant or breastfeeding women*
- *People with exfoliative skin conditions (e.g. severe eczema, ectopic dermatitis or similar skin disorder) that requires systemic therapy*
- *People with significant immunodeficiency due to an underlying illness (e.g. HIV/AIDS) and/or medication (e.g. systemic corticosteroids or other immune-suppressive medications including cortisone, dexamethasone, hydrocortisone, prednisone, prednisolone, interferon, cisplatin, doxorubicin, fluorouracil, etc.).*

VV causes a transient infection, with elimination of viral components over several weeks. Host cells infected with VV are short lived (days) and die by a mixed form of apoptosis/necrosis. VV replicates in the cytoplasm of infected cells, and viral DNA does not integrate into the host cell DNA. Thus, VV is incapable of colonizing the host organisms that it infects.

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

There is no known natural reservoir host of VV. Humans, cows, buffaloes, camels, foxes, raccoons, pigs, etc. have already been infected by VV. However VV does not produce latent infection and once the infection arises, the virus is rapidly cleared from the host.

VV can infect a wide range of human tissues but does not cause any known human disease except for vaccination complications (see section 7. a). A number of approved or

experimental antiviral agents are available to treat poxvirus infections in case of a serious adverse response. Due to their availability on the market and supporting preclinical data, the use of cidofovir and/or ribavirin should be considered in the proposed clinical trial.

VV replication exclusively occurs in the cytoplasm thus eliminating any risk of integration of the viral DNA into the host genome (Moss B., 2007).

8. *Information concerning reproduction*

a) Generation time in natural ecosystems:

Not relevant as VV is not naturally found in the environment.

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:

VV survivability is dependent upon the ability to replicate within a host cell. Poxviruses have also the capacity to survive for long periods in dried material and are relatively stable when stored frozen or lyophilized under carefully controlled conditions. However stability decreases significantly with increasing temperature. Under normal environmental conditions, poxviruses lose viability within days or weeks.

VV viruses are sensitive to inactivation by either physical or chemical methods of disinfection. Heat is the most effective antimicrobial agent (viable counts of a VV are reduced 10^7 fold by exposure to 60^oC at ambient pressure within an hour or less). VV is rendered non-infectious following treatment in an autoclave. Hospital-grade chemical disinfectants are also effective against lipophilic viruses such as VV.

10. (a) Ways of dissemination

The non-recombinant VV has the ability to widely disseminate in human body and is known not to produce latent infection. So, after an initial period with some viral shedding in biological fluids, the virus becomes completely cleared from the host.

Secondary transmission post-vaccination with VV is a rare occurrence, but has been described in household, sexual contacts (CDC, 2007; MMWR, 2004; MMWR, 2010; Vora S. et al., 2008) and sport partners (Hughes C.M. et al., 2011; Young G.E. et al., 2011). A recent paper reports that there were 5.4 cases of vaccinia secondary transmission per 100.000 vaccinees with non-recombinant VV (Wertheimer E.R. et al., 2011). Contamination occurs by physical contact (e.g. the virus can be transmitted by touching a vaccinee's unhealed vaccination site or by touching bandages or clothing that have become contaminated with live virus from the vaccination site or by touching spontaneously occurring pustules).

10. (b) Factors affecting dissemination

Efficient measures to prevent spread of VV to another person comprise frequent hand washing with soap and water or disinfecting agents, proper dressing of the vaccination site (e.g. with a non-occlusive bandage or with a gauze and long-sleeved clothing) and proper disposal of contaminated dressings (e.g. contaminated bandages should be placed in sealed plastic bags which have to be returned to the hospital site for destruction. Contaminated clothes and linen should be decontaminated with routine laundering in hot (≥71°C) water with detergent) (Cono J. et al., 2003; Rotz L.D. et al., 2001; Stark J.H. et al., 2006; Talbot T.R. et al., 2004).

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

TG6002 is a GMO developed as a therapeutic candidate to treat patients with cancer. The vaccinia TK and RR genes are inactivated in TG6002 to enhance the in vivo tropism of the virus for cancer cells versus normal cells. Once TG6002 has infected the cancer cells, it replicates and destroys them. This represents the oncolytic activity of TG6002. TG6002 has another claimed antitumor activity relying on the insertion of the therapeutic transgene FCU1. FCU1 encodes for an enzyme which is able to convert in situ the prodrug 5-FC into

the cytotoxic 5-FU and 5-FUMP agents. This represents the chemotherapeutic activity of TG6002.

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

Two transfer plasmids are used to generate TG6002: pTG15466 and pTG17137.

c) Host range of the vector

pTG15466 and pTG17137: 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 :

e) Constituent fragments of the vector

The plasmid pTG15466 is generated from the plasmid pRS306-FCU1 (Erbs P. et al., 2000) and contains the FCU1 gene which is under the control of the vaccinia synthetic early/late p11K7.5 promoter and surrounded by portions of the vaccinia TK gene. The plasmid pTG17137 carries the GFP/GPT gene which is under the control of the pHR5 promoter and surrounded by portions of the vaccinia RR gene (Foloppe J., 2009).

f) Method for introducing the vector into the recipient organism

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

An intermediate recombinant virus (VVTK⁻/FCU1 or VVTG15466) is generated by homologous recombination between the plasmid pTG15466 and the parent VV (Foloppe J. et al., 2008). The final recombinant VV (i.e. TG6002, also named VVTK-RR-/FCU1 or VVTG17137) is obtained by infection of production cells with VVTK⁻/FCU1 in the presence of pTG17137. Two successive homologous recombinations between the shuttle plasmid pTG17137 and the viral genome of VVTK⁻/FCU1 leads to the final recombinant virus.

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 gene which encodes for FCU1. The insert also contains a VV promoter for transgene expression (i.e., synthetic p11K7.5).

b) Source of each constituent part of the insert

Constituent of the insert	***Source***	***Intended function***
I4L	*VV*	*Deletion of vaccinia RR activity*
J2R	*VV*	*Deletion of vaccinia TK activity*
p11K7.5	*VV*	*Vaccinia synthetic early/late promoter*
FCU1	*Yeast*	*Gene of interest, yeast cytosine deamina-se/uracil phosphoribosyltransferase fusion gene*

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

The therapeutic transgene encoding FCU1 was inserted in order to convert in situ the 5-FC prodrug into the cytotoxic 5-FU and 5-FUMP agents.

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 VV genome by homologous recombination in the TK gene.

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	☒	*Vaccinia virus for p11K7.5*
bacterium	☐	
fungus	☒	*FCU1*
animal	☐	
- mammals	☐	
- insect	☐	
- fish	☐	
- other animal	☐	specify phylum, class

other, specifiy

2. Complete name

	FCU1	*p11K7.5*
i. Order and/or higher taxon (for animals)	*Eukaria*	*Poxviridae*
ii. Family name (for plants)	*Saccharomycetaceae*	
iii. Genus	*Saccharomyces*	*Orthopoxvirus*
iv. Species	*S. cerevisiae*	*Vaccinia virus*
v. Subspecies		
vi. Strain		*Copenhagen*
vii. Cultivar/breeding line		
viii. Pathovar		
ix. Common name	*Saccharomyces cerevisiae*	

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	☐

VV: see sections B.7.a) & B.7.b).

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
VV: see section B.6.

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
The deletion of the TK and RR activities in TG6002 conditions its replication to highly dividing cells such as cancer cells and considerably reduces its survivability.

(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

The TK and RR genes in TG6002 are inactivated. It has already been shown in preclinical experiments that TK inactivation decreases VV virulence (Buller R. et al., 1985) by restricting viral replication to proliferating cells. This also targets dissemination of the virus to tumors (Puhlmann M. et al., 2000).

In humans, TG6002 could disseminate from biological fluids, the injection site and vaccine pustules.

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

Yes ☐ No ☐ Not known ☒

Specify

In preclinical models, reduced virulence had already been demonstrated in a TK-deleted Western Reserve (WR) VV compared to the wild-type virus (Buller R. et al., 1985) as well as in RR-deleted herpes simplex virus mutants compared to non RR-mutated viruses (Brandt C.R. et al., 1991), (Mineta T. et al., 1995).

2. *Genetic stability of the genetically modified organism*

A genetic stability study has been performed and demonstrated 100 % stability of the final research virus stock after 10 passages on the production cell (Foloppe J., 2009).

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)

Non-clinical toxicity:

The toxicity profile of TG6002 was investigated alone in the rabbit (Kaiser S., 2010; Pablo M.J., 2013) and in combination with 5-FC in the monkey (Xanxo S., 2014a; Xanxo S., 2014b; Xanxo S., 2014c). Three weekly systemic administrations of TG6002 up to 5 x 10^7 pfu/kg induced dose-related infectious skin papules/vesicles, inappetence resulting in weight loss and decreased locomotor activity. These signs were more pronounced after the first administration than subsequent ones likely due to protective immune responses. Treatment of

rabbits and monkeys with TG6002 resulted in a similar clinical picture, but less severe in rabbits. Indeed, both species developed skin papules-vesicules which were mild in rabbits but severe in monkeys at the dose of 5 x 10^7 pfu/kg corresponding to a human equivalent dose (HED) of 1 x 10^9 pfu in a 60 kg person. In monkey, dose-dependent scattered skin lesions ranging from 1 mm to 10 mm appeared after the first administration corresponding to the viral acute phase. The lesions progressed through the following stages: papulovesicles surrounded by a red areola, wounds (hemorrhagic or not hemorrhagic), crusted scabs and scars with pigmentation. These findings were consistent with known effects of VV and were reduced in a large extent when TG6002 was combined with 5-FC.

Biodistribution:
Viral DNA distribution in blood and organs (bone marrow, brain, gastrointestinal tract, heart, skin, spleen, kidneys, liver, lungs, lymph nodes, skeletal muscle, spinal cord, urinary bladder, ovaries, testes and injection site) of rabbits was studied after 3 weekly IV injections of TG6002 (days 1, 8 and 15) (Pablo M.J., 2013). TG6002 biodistribution showed very low and limited spreading from bloodstream to tissues or organs [see further details in Annex IIIA, paragraph II.C.2.(i)(i)]. The target organs for biodistribution included injection site, skin distal to injection site and spleen, consistent with the toxicity profile.

Clinical toxicity:
The safety data set of TG6002 in human is limited to date with one patient in ONCOVIRAC study who was fully treated at the 1 x 10^5 pfu dose level without any study-medication related serious adverse reaction. Risks and adverse drug reactions can be anticipated for TG6002 based on the experience of VV use in the smallpox eradication program as well as TRANSGENE clinical experience with TG1031 and published data of JX-594, both TG1031 and JX-594 being recombinant VV with TK inactivated gene.

For the adverse reactions identified during smallpox vaccination, see paragraph B.7.a.

With JX-594, a VV from the Wyeth strain (i.e. the most widely used strain during smallpox vaccination) deleted in TK region and expressing hGM-CSF, there is clinical experience in over 250 patients with advanced cancer (Breitbach C.J. et al., 2011; Burke J.M. et al., 2012; Heo J. et al., 2012; Heo J. et al., 2011; Heo J. et al., 2013; Hwang T.H. et al., 2011; Mastrangelo M.J. et al., 1998; Park B.H. et al., 2008; Park Y.S. et al., 2012). JX-594 was administered by single or multiple intratumoral or IV infusions at doses up to 3 x 10^9 pfu. It has shown an acceptable safety profile with the most common side effects being flu-like symptoms, gastrointestinal disorders mainly nausea and vomiting, anorexia, headache, asthenia, pain, dyspnoea, hypotension, injection site reaction, cutaneous viral infection, hypertension, leukocytosis and leukopenia, thrombocytopenia, anemia, hyponatremia, hyper- or hypoglycemia, hyperbili-rubinemia and papulo-pustular rashes. None of the serious complications which occurred during the smallpox vaccination was observed following treatment with JX-594. In addition, no reports of virus transmission from JX-594 recipients to health care personnel or patient contacts have been made.

With TG1031, TK-inactivated VV from the Copenhagen strain carrying the sequences coding for MUC1 and IL2, TRANSGENE performed 3 clinical trials in a total of 56 patients with breast or prostate cancer. TG1031 was administered by single or repeated intramuscular injections at doses ranging from 5 x 10^5 to 5 x 10^7 pfu. The administration of TG1031 was generally well tolerated. The most frequently observed adverse events were injection site conditions (inflammation, erythema and pruritus), headache, fatigue, pyrexia, arthralgia, myalgia, weakness, hypertension and gastrointestinal disorders. Abnormal lymphocyte count and perturbation of liver parameters were also observed but were not clinically significant.

One patient developed thyroiditis and the instructions were to further monitor auto antibodies including anti thyroid antibodies.

The deletion of the TK and RR activities, which restricts TG6002 replication to highly dividing cells such as cancer cells, should considerably reduce the pathogenicity of the recombinant virus compared to its parental virus. It is therefore expected that the safety profile of TG6002 will be acceptable. Furthermore, individuals from the "at risk" groups identified during smallpox vaccination (see paragraph B.7.a) are not eligible for entry in the proposed clinical trial. In the extremely unlikely case of a clinically-significant, progressive toxicity that, in the opinion of the investigator could be related to TG6002, the use of cidofovir and/or ribavirin which is supported by preclinical data should be considered.

4. Description of identification and detection methods

a) Techniques used to detect the GMO in the environment

See 4.b)

b) Techniques used to identify the GMO

The virus identity can be confirmed by Polymerase Chain Reaction (PCR) with a set of primers designed in the genetic insert and in the flanking viral sequence of the recombinant VV.

F. INFORMATION RELATING TO THE RELEASE

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

The release is the administration of the product by intravenous injections to patients in the hospital setting as part of an international multicentric clinical trial.

In the proposed phase I/II trial, patients with advanced gastro-intestinal (GI) tumors are treated with the combination of TG6002 and the prodrug flucytosine (5-FC). Patients receive three weekly IV injections of TG6002 and oral 5-FC. A maximum of 59 patients are planned to participate in the study with 24 patients in the dose escalation phase I part of the study and 35 patients in the phase II part. All patients receive TG6002 infusions. After study completion, all patients will be followed up for survival.

The release is performed by dedicated and trained medical and pharmacy personnel. The potential for viral shedding from patients biological fluids is closely monitored. Detailed instructions on how to prevent contamination by the virus have been written on the basis of the medical knowledge acquired during the smallpox eradication campaign and TRANSGENE's experience with 2 other recombinant VVs. These instructions are provided to all personnel involved in handling of the product and the patients.

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 VV are not naturally found in the environment. The current release corresponds to a clinical trial and could possibly be compared to the use of VV during smallpox eradication campaign.

3. Information concerning the release and the surrounding area

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

TG6002 is administered at the following clinical site located in Belgium:

Institut Jules Bordet
Boulevard de Waterloo 121,
1000 Bruxelles

Principal investigator : Dr Ahmad Awada

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 clinical site. However all zones in which TG6002 is handled and administered to the patients and in which the patients are hospitalized after dosing with TG6002 must have restricted access (i.e. access to these zones are controlled and limited to authorized hospital staff who has received training on measures to control infection). The international biohazard symbol is affixed at each entrance of the restricted zones. It is also affixed on the freezer in which TG6002 is stored. No other specific measure to protect the site from intrusion by unauthorized individuals is planned. The site pharmacy and laboratory must be equipped with a vertical biological safety cabinet in accordance with biosafety level 2 handling guidelines. The patient stays into a hospital room overnight after the first TG6002 infusion and for at least 4 hours after the second and third infusions.

Environmental surfaces, hospital rooms, patients' care areas, patients-care equipment and medical devices should be routinely cleaned with a hospital-grade disinfectant. Following the patient's discharge home, all surfaces of the room and bathroom should be wiped down with a hospital grade disinfectant. Items such as dishes, utensils, textiles and fabrics are decontaminated with hot water (>70°C) and detergent. Any and all waste should be autoclaved, incinerated, or treated with sodium hypochlorite solution by personnel who are trained to dispose of biohazard waste.

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 3 x 10^8 pfu (or 8.5 log10 pfu) per IV infusion. If there is evidence of benefit for the patient, he/she could receive several cycles of 3 weekly infusions of TG6002, with a period of 2 to 4 weeks between each cycle.

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 principal investigator of the clinical site, according to the clinical protocol and in accordance with the Good Clinical Practice.

The product must be prepared in aseptic conditions compliant with injectable solutions. TG6002 is prepared under a vertical biological safety cabinet of type II in a laboratory or pharmacy under the direction of an accredited pharmacist. The safety cabinet is cleaned before and after manipulation with an active disinfectant. Lipid-encapsulated viruses such as TG6002 are sensitive to many classical hospital-grade chemical disinfectants containing bleach, aldehydes, alcohols, hydrogen peroxide, phenols, and quaternary ammonium compounds. Standard chemical germicides such as Aseptanios Terminal Spore®, Amphospray 41 IP stérile®, Anios Oxy'Floor®, Aniospray SF IP stérile®, Surfa'Safe Premium®, Aniosurf®, Aniosurf Premium®, Rivascop®, Surfanios Premium®, Surfa'Safe®, Surfa'Safe SH® (non-exhaustive list) are adequate for routine cleaning of work areas, when used according to the manufacturers' instructions.

All staff involved in handling of TG6002 or any material or linen potentially contaminated with TG6002 must wear personal protective equipment (PPE) (i.e. waterproof gloves, gown, surgical/procedure mask and safety goggles with side shields). All transfers of TG6002 must be done using a sealed plastic transport bag or other sealed, leak-proof secondary container displaying a clearly marked biohazard symbol. Furthermore, the site staff follows the standard hospital policy recommended for the manipulation of live virus vaccines.

In case of an accidental spill, the contaminated area must be secured. Personnel involved in the clean-up of the spill must wear the PPE. Aerosols should be allowed to settle before paper towels or lab diapers are placed carefully over the spill. The spill should be absorbed with paper towels and an active disinfectant (e.g. bleach solution at 0.6% of active chlorine or any other active disinfectant) should be applied. The contact with the disinfectant is allowed for 30 minutes. Contaminated paper towels are then replaced by fresh paper towels soaked in disinfectant. The contact between the area and the disinfectant should be reduced to 10 minutes. Then the cleaned area should be left to dry. Finally, all waste items should be disposed in accordance with the hospital standard procedure for infectious waste.

All personnel involved in handling TG6002 is informed that in case of:

- *Eye splash: the eyes should be rinsed with clean water or physiological saline solution (NaCl 0.9%) for 3 minutes and then, if available, one drop of trifluridine 1% should be instilled.*
- *Intact skin splash: the contaminated clothes should be removed and an absorbent tissue should be placed immediately on the affected area. After removing the tissue, the skin should be washed with mild soap thoroughly and rinsed abundantly with water. Then the skin should be covered for 5 minutes with a pad soaked with either a solution of bleach at 0.45% of active chlorine*

or a solution of 4% iodine. Then the skin should be rinsed again abundantly with water. The contaminated clothes, absorbent tissue and pad should be treated as infectious material as per hospital standard procedure.

- *Cuts or punctures: the wound should be allowed to bleed before it is flushed under a running stream of clean, and preferably sterile, water. Then the injured skin area should be covered with a sterile gauze dressing, which should be discarded according to hospital standard procedure for infectious material when removed.*
- *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 TG6002, 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 (SAE) has resulted from this intake.*

The exposed individual should be referred to and medically monitored by a physician knowledgeable in the care and treatment of patients with vaccinia infections.

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*

The proposed clinical trial with TG6002 will be run in parallel of ONCOVIRAC study, the first-in-human trial with TG6002 conducted in France and sponsored by Assistance Publique – Hôpitaux de Paris. This study is currently recruiting. To date one patient has been treated with TG6002 without any report of accidental release of the GMO.
Secondary transmission and shedding to the environment have never been reported with TG1031 and JX-594.

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)*

i. Order and/or higher taxon (for animals)
ii. Family name (for plants)
iii. Genus
iv. Species

v. Subspecies
vi. Strain
vii. Cultivar/breeding line
viii. Pathovar
ix. Common name

The target organisms are the human beings.

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

TG6002 is an armed oncolytic therapeutic vaccinia virus designed to selectively replicate in and destroy cancer cells. Tumor destruction by TG6002 is achieved through a multi-mechanism of action, encompassing infection and selective lysis of tumor cells through direct viral replication (i.e. oncolysis) and targeted chemotherapy through in situ conversion of 5-FC into 5-FU and 5-FUMP.

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 is released in a hospital operating room and is unlikely to come in contact with other animal species in this environment.

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

TG6002 remains exclusively in the cytoplasm of infected cells thus eliminating any risk of integration of the viral DNA into the host genome.

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

Yes ☐ No ☒ Not known ☐

Give details

Not applicable. The replicative and propagative characteristics of vaccinia virus have been attenuated in TG6002 with the deletions of the TK and RR activities which renders the modified organism dependent of highly dividing cells such as cancer cells. Therefore TG6002 should have reduced competitiveness and invasiveness compared to vaccinia virus.

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

TG6002 is anticipated not to interact with non-target organisms due to the conditions of the proposed release. Indeed, the GMO is confined to the hospital site, including the operating room, pharmacy, clinical laboratory, and autoclaving/incineration area. In the unlikely event of inadvertent administration to non-target organisms, further spread would be unlikely as there were only rare cases of secondary transmissions during the smallpox vaccination campaign with vaccinia virus and the pathogenicity of TG6002 is reduced compared to vaccinia virus.

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

It is possible that hospital staff may be injected by accident and that secondary transmission occurs in patients' family members. Infection would be harmful in at risk populations (see section B.7.b) but patients who cannot avoid direct physical contact with people in those at risk groups as well as healthcare personnel in those at risk groups are excluded from study participation.

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 is released to be administered to patients in hospital rooms and is unlikely to come in contact with other animal species. Furthermore TG6002 remains localized in the cell cytoplasm up to the lysis of the infected cell. There is no possible genetic exchange with other human poxviruses as they are not endemic in humans. In animals susceptible to infection by the vaccinia virus, the opportunity for genetic recombination with animal poxviruses is probably low since, to our knowledge, this has never been reported during the smallpox eradication campaign.

(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 TG6002 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

Monitoring of the direct and indirect effects of the GMO in patients is 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 vaccinia 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 TG6002 has no nuclear localization and there is no known human endemic virus able to complement, to recombine or to exchange genetic material with the vaccinia genome.

4. Site of the monitoring area (m2)

Not applicable: the GMO is administered to patients by intravenous injections in hospital rooms.

5. Duration of the monitoring

Safety assessments are performed all along the patient's participation in the clinical trial and up to 4 weeks after treatment discontinuation.

6. Frequency of the monitoring

Patients will be evaluated for safety from the first treatment dose to the end-of-study visit according to time-points specified in the clinical protocol.

I. INFORMATION ON POST-RELEASE AND WASTE TREATMENT

1. Post-release treatment of the site

The biological safety cabinet where the product is prepared for injection is decontaminated before and after the manipulation with any active disinfectant.

All material dedicated to the clinical trial is disposed of after use and is then autoclaved, incinerated, or treated with sodium hypochlorite solution by personnel who are trained to dispose of biohazard waste.

The material not dedicated to the clinical trial is sterilized or cleaned with an active disinfectant.

Following the patient's discharge home, the hospital room (surfaces and floor) and the bathroom are cleaned in a standard way using a hospital grade disinfectant.

2. Post-release treatment of the GMOs

For clinical waste treatment, see I.3.(b).

3. (a) Type and amount of waste generated

The virus titer of the clinical batch which is used for the proposed clinical trial is 2.0 x 10^8 pfu/mL (or 8.3 log10 pfu/mL). The virus is suspended in an extractable volume of 0.5 mL. As a consequence, the maximum quantity of waste is generated if the dose is prepared and not injected to the patient. In this case, the quantity of waste is 1.0 x 10^8 pfu (or 8.0 log10 pfu).

3. (b) Treatment of waste

The waste is to be deactivated by:

- *Autoclaving/incineration*

or

- *Use of a disinfectant [e.g. bleach solution at 0.6% of active chlorine or any other active disinfectant].*

J. INFORMATION ON EMERGENCY RESPONSE PLAN

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

It is recommended to personnel involved in TG6002 handling to act as described in paragraph F.4.c) in case of incident with the use of TG6002.

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 are monitored for the occurrence of adverse events and serious adverse events (SAE) according to the clinical protocol. Each SAE is recorded and assessed by the hospital staff and the study sponsor, and Health Authorities are notified when applicable.

Replicative and propagative characteristics of vaccinia virus have been attenuated in TG6002 with the deletions of the TK and RR genes which makes the virus replication dependent on actively dividing cells such as cancer cells. Therefore the probability of propagation of TG6002 outside patients' tumors is very low.

The clinical information on TG6002 is limited to date with only one patient treated with TG6002/5-FC in the ONCOVIRAC trial. The clinical data from 2 other recombinant VVs suggest that TK-deficient VVs do not spread to caregivers in contact with the treated patients. Should virus shedding occur, the level of exposure would be predicted to be low compared to the doses received by the patients in the proposed clinical study. In the unlikely event that an exposed individual were to demonstrate virus-associated toxicity, therapy could be initiated with vaccinia immune globulin (VIG) to circumvent any public health risk.

No adverse effect on the environment had been reported further to the massive use of the non-attenuated virus during the smallpox eradication program. It is therefore not expected that the release of TG6002 within the proposed clinical trial conditions would result in any other environmental effect.

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