BT-001

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

Final - 12 August 2020

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LIST OF ABBREVIATIONS

AE	Adverse Event
CDC	Centers for Disease Control and Prevention
CEF	Chicken embryo fibroblasts
cDNA	Complementary deoxyribonucleic acid
CTLA-4	Cytotoxic T-lymphocyte- antigen 4
DNA	Deoxyribonucleic acid
DP	Drug product
EEC	European Economic Community
GMO	Genetically modified organism
GM-CSF	Granulocyte-Macrophage Colony stimulating factor
GFP	Green Fluorescent Protein
irAE	Immune related Adverse Event
IT	Inrtatumoral
mAb	Monoclonal Antibody
PCR	Polymerase chain reaction
PFU	Plaque Forming Unit
SC	Subcutaneous
VV	Vaccinia virus
VV-COP	Vaccinia virus of the Copenhagen strain

A. GENERAL INFORMATION

1. Details of notification

a) Member State of notification Belgium

b) Notification number pending

c) Date of acknowledgement of notification pending

d) Title of the project

A Phase I/IIa study of intra-tumoral BT-001 (TG6030) administered alone and in combination with pembrolizumab in patients with cutaneous or subcutaneous lesions or easily injectable lymph nodes of metastatic/advanced solid tumors.
Study code: BT-001.01

e) Proposed period of release

From 30 October 2020 to 30 September 2024

2. Notifier

Name of institution or company

Sponsor: Transgene
400 Boulevard Gonthier d'Andernach
Parc d'Innovation
CS80166
67405 Illkirch Graffenstaden cedex
FRANCE

Co-developer: BioInvent International AB
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Fax: +46 (0)46-211 08 06
www.bioinvent.com

3. GMOs characterization

1. 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)

2. Identity of the GMO (genus and species)

Genus: *Orthopoxvirus*
Species: *Vaccinia virus (VV)*

The genetically modified organism (GMO) is a viral suspension of the recombinant virus BT-001 (TG6030), a non-integrative, conditionally replicative, recombinant thymidine kinase and ribonucleotide reductase-double deleted vaccinia virus of Copenhagen strain (VV-COP TK- RR-) carrying DNA sequences encoding the human Granulocyte-Macrophage Colony stimulating factor (hGM-CSF) and 4-E03, a human monoclonal antibody of the IgG1 isotype targeting the human Cytotoxic T-Lymphocyte-Antigen 4 (anti-hCTLA-4 mAb).

For the purposes of this document:

- The Parenteral virus is defined as the vaccinia virus of the Copenhagen strain (VV-COP)
- The Recipient is defined as COPTG19156, a thymidine kinase and ribonucleotide reductase-double deleted vaccinia virus of Copenhagen strain (VV-COP TK- RR-) carrying DNA sequences encoding the green fluorescent protein (GFP) and the mCherry genes at the TK and RR loci respectively.
- The Vectors are defined as the transfer plasmids pTG19367 and pTG19384.
- The name for the Genetically Modified Organism (GMO) is BT-001 [Drug Product], COPTG19384 [Drug Substance] or VV-COP TK- RR- / 4-E03 anti-hCTLA-4 mAb / hGM-CSF.

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

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

The genetic stability study demonstrated 94 % stability of BT-001 final research virus stock after 5 passages on CEF.

Sequencing of the entire genome of BT-001 drug substance demonstrated that vector genomic integrity was maintained at a passage level comparable to production batch.

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

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 FR
- 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 BT-001 becoming persistent and invasive in natural habitats is very low for the following reasons:

- Due to the inactivation of its TK and RR genes, BT-001 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 4-03 mAb and hGM-CSF transgene, BT-001 is comparable to its non-recombinant parental virus. The genetic modifications introduced in BT-001 are therefore not expected to increase dissemination and survival capacity of the GMO compared to the parental virus.
- BT-001 remains exclusively in the cytoplasm of infected cells thus eliminating any risk of integration of the viral DNA into the host genome.
- 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 BT-001 will not result in virus viability over a few hours or days. Furthermore, BT-001 is a lipid encapsulated virus and is consequently sensitive to many classical disinfectants. So an accidental spill during BT-001 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 ☒
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 ☐

The parental VV-COP organism is not naturally found in the environment.

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

Yes ☐ No ☒

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

Yes ☐ No ☒

4. Natural habitat of the organism

a) *If the organism is a microorganism*

Water ☐
Soil, free-living ☐
Soil in association with plant-root systems ☐
In association with plant leaf/stem systems ☐
In association with animal ☐

other, specify

The parental VV-COP 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

COPTG19156 recipient (VV-COP TK- RR- double deleted with GFP and mCherry genes inserted) can be identified by two techniques:

- Identification can be done by PCR with specific set of primers.
- Detection of green and red fluorescent plaques allow to identify the presence of COPTG19156 recipient due to the activity of the mCherry and GFP genes which is lost by the BT-001 GMO.

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 (Directive 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.

This classification considers the wild type strain of the vaccinia virus. The double deleted form of the vaccinia virus (i.e.: VV-COP TK- RR-) used in COPTG19146 recipient which significantly decrease its virulence has not been classified.

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

VV has a long and extensive history of use in humans. (Note: vaccinia virus and smallpox are distinct viruses from the same poxviridae family. Upon vaccination, the immune response induced against vaccinia virus cross-reacts and neutralizes smallpox)

Following injection into the skin, the virus typically establishes only a brief and limited subcutaneous (SC) infection. Indeed, VV does not produce latent infection and once the infection arises, the virus is rapidly cleared from the host. As VV contains antigens that stimulate an immune response that are cross-reactive with smallpox antigens, the vaccine thereby confers protection from the human smallpox disease. Vaccination with VV is associated with known adverse effects that range from mild to severe. Mild vaccine reactions include formation of skin lesions, fever, muscle aches, regional lymphadenopathy, fatigue, headache, nausea, rashes, and soreness at the vaccination site (Belongia E.A. and Naleway A.L., 2003). Serious vaccination complications are extremely rare and include death (1 per million vaccinated), progressive vaccinia (1.5 per million vaccinated), eczema vaccinatum (39 per million vaccinated), postvaccinal encephalitis (12 per million vaccinated) and generalized vaccinia (241 per million vaccinated) (Lane J.M. *et al.*, 1970). A statistically significant increased risk of myo/pericarditis (1-2 par 10,000 vaccinees) was demonstrated more recently (Arness M.K. et al., 2004). It was clearly shown that the great majority of the serious adverse events occurred in defined subsets of what are referred to as “at risk” groups including:

• Children <12 months of age

• Severely immunocompromised individuals (e.g. organ transplant recipients, HIV-positive individuals, or those receiving chronic immunosuppressive medication)
• Patients with inflammatory skin conditions (e.g. eczema requiring previous treatment, atopic dermatitis, etc.).
In addition, vaccination was not recommended during pregnancy (due to the exceedingly rare risk of fetal vaccinia) or for breastfeeding women (because of the theoretical risk of transmission to the nursing infant).

The parental vaccinia virus of the Copenhagen strain and COPTG19156 recipient differentiate by the 2 genes inactivation in the VV-COP genome. This inactivation of the TK and RR activities have been performed to restrict BT-001 GMO replication to highly proliferative cells such as tumor cells containing high concentration of nucleotides. They considerably reduce the pathogenicity of the recombinant virus compared to its parental virus. 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 vaccinia virus (Foloppe J. *et al.*, 2019) or herpes simplex virus mutants compared to non RR-mutated viruses (Brandt C.R. *et al.*, 1991) (Mineta T. *et al.*, 1995). It was also evidenced in pre-clinical *in vitro* experiments that insertions of the transgenes in COP TK- RR- viral vector did not impair BT-001 high selectivity toward tumor cells.

Lastly, a number of approved antiviral agents are available in case of infectious complications due to vaccinia virus vaccination, the following antiviral therapies may be considered:
- Vaccinia Immune Globulin (VIG)
  VIG is an FDA-licensed therapeutic indicated for the treatment of complications due to smallpox vaccination, including eczema vaccinatum, progressive vaccinia, severe generalized vaccinia, vaccinia infections in individuals who have skin conditions, and aberrant infections induced by vaccinia virus, except in cases of isolated keratitis and post-vaccinal encephalitis
- TPOXX (tecovirimat)
  TPOXX is an inhibitor of the orthopoxvirus VP37 envelope wrapping protein, which is highly conserved in all members of the Orthopoxvirus genus. The effectiveness of TPOXX for the treatment of smallpox disease was established based on animal studies in non-human primates and rabbits infected with non-variola orthopoxviruses. TPOXX is approved by the FDA for the treatment of human smallpox disease.

In the proposed project BT-001.01, VIG and TPOXX tecovirimat will be made available as rescue medications in case of a serious complication following BT-001 final GMO administration.

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°C 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

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 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 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) (Stark J.H. *et al.*, 2006) (Talbot T.R. *et al.*, 2004).
Other containment and protective measures planned following BT-001 administration and during the post discharge phase are detailed in section F.4.(c)

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)

BT-001 GMO is part of a platform from the notifier TRANSGENE using the same viral vector and differing by the nature of the transgenes inserted.
A genetic modification using a double deleted vaccinia virus recipient (VV-COP TK- RR-) was then already notified in Belgium for the GMO TG6002 with reference number B/BE/18/BVW1.

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 resulting GMO, BT-001 will be administered to patients by intratumoral (IT) injections.

Once injected to the patient, it is hypothesized that the oncolytic vaccinia virus and the vectorized therapeutic proteins i.e., 4-E03 and hGM-CSF cytokine act together to inhibit the tumor growth through a multi-pronged antitumoral mechanism of action. BT-001 replicates preferentially in tumor cells, induces cell death and local inflammation, primes adaptative immune responses and delivers in situ its 2 immunomodulatory proteins i.e., 4-E03 and hGM-CSF.

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 were used to generate BT-001: pTG19367 and pTG19384.

c) Host range of the vector

The plasmid vectors replicate in *Escherichia coli* bacteria

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

Not relevant.

e) Constituent fragments of the vector

The plasmid pTG19367 contains the heavy chain (HC) gene of the 4-E03 monoclonal antibody under the control of the p7.5K promoter as well as BRD and BRG flanking sequences surrounding the J2R locus (TK gene) of the Vaccinia Virus.

The plasmid pTG19384 contains the light chain (LC) gene of the 4-E03 monoclonal antibody under the control of the p7.5K promoter and the human GM-CSF gene under the control of the pSE/L promoter as well as BRD and BRG flanking sequences surrounding the I4L locus (RR gene) of the Vaccinia Virus.

f) Method for introducing the vector into the recipient organism

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

The nucleotide sequences of interest are inserted by two successive homologous recombination in Chicken Embryo Fibroblasts (CEF) between COPTG19156 recipient with transfer plasmid pTG19367 then with transfer plasmid pTG19384 without insertion of non-coding sequence

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

Constituent of the insert	Intended function
Insert in J2R locus	
p7.5K	Early/ late promoter
4-E03 mAb HC	Anti-CTLA-4 monoclonal antibody
Insert in I4L locus	
p7.5K	Early/ late promoter
4-E03 mAb LC	Anti-CTLA-4 monoclonal antibody
pSE/L	Early/ late promoter
hGM-CSF	Cytokine

.

b) Source of each constituent part of the insert

Gene sequences encoding HC of 4-E03, LC of 4-E03 and hGM-CSF are from DNA synthesis origin. The protein sequences of the transgenes correspond to the human GM-CSF and a human IgG1.

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

The hGM-CSF cytokine is a strong stimulator of systemic anti-tumor immunity (Dranoff G. *et al.*, 1993). It promotes the differentiation of hematopoietic precursors into dendritic cells (Pardoll D.M., 1995), the professional antigen presenting cells that mediate the cellular immune response, by processing and presenting tumor antigens released as the tumor cells are killed by the vaccinia virus.

The CTLA-4 is upregulated following T-cell receptor (TCR) engagement, CTLA-4 dampens TCR signaling through competition with the costimulatory molecule CD28 for the B7 ligands: B7-1 (CD80) and B7-2 (CD86). 4-E03 binds to hCTLA-4 and inhibits hCTLA-4/B7 interactions.

Additionally, CTLA-4 regulates T-cell activation by modulating the activity of regulatory T cells (Tregs) which constitutively express on their surface high levels of CTLA-4. 4-E03 has Treg depleting activity.

In both inserts, the promoters p7.5K and pSE/L allow to initiate transcription of the genes of interest.

d) Location of the insert in the host organism

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

The insert encoding the heavy chain (HC) of the 4-E03 mAb is fully integrated in the J2R locus of the Vaccinia Virus genome.
The insert encoding the light chain (LC) of the 4-E03 mAb and the hGM-CSF is fully integrated in the I4L locus of the Vaccinia Virus genome

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:

for p7.5K	Viroid	☐
	RNA virus	☐
	DNA virus	☒ Vaccinia virus
	bacterium	☐
	fungus	☐
	animal	☐
mAb, pSE/L	- mammals	☒ hGM-CSF, 4-E03 IgG1
	- insect	☐
	- fish	☐
	- other animal	☐ specify phylum, class

other, specify: Two DNA fragments coding for the two inserts named “Fragment HC” and “plasmid LC_GMCSF” were synthesized. The sequences coding for the transgenes were optimized for human codon usage. They encode both a monoclonal human IgG1 recognizing the CTLA- antigen under the control of the p7.5K promoter and for the human GM-CSF cytokine under the control of the pSE/L promoter.

2) Complete name

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

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

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

The 2 gene inactivation in VV-COP genome restrict BT-001 GMO replication to highly proliferative cells such as tumor cells containing high concentration of nucleotides and considerably reduces the pathogenicity of the recombinant virus compared to its parental virus.

The inactivation of the TK and RR activities is already present in the recipient COPTG19156. The GMO BT-001 is then considered similar to the recipient COPTG19156 for 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

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

Yes ☐ No ☒ Not known ☐

Specify

As introduced in item E.1.(a), the TK and RR genes inactivation in VV-COP genome restrict BT-001 GMO replication to highly proliferative cells such as tumor cells containing high concentration of nucleotides and considerably reduces the pathogenicity of the recombinant virus compared to its parental virus. 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).

The inactivation of the TK and RR activities is already present in the recipient COPTG19156. The GMO BT-001 is then considered in all aspects similar to the recipient COPTG19156 for pathogenicity.

2. Genetic stability of the genetically modified organism

The genetic stability study demonstrated 94 % stability of BT-001 final research virus stock after 5 passages on CEF cells.

The BT-001 drug substance was also analysed by sequencing of both expression cassettes. Alignment of sequencing results showed 100 % homology with the theoretical expected sequences.

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)

a) Potential toxic risks associated with BT-001 viral particles active substance:

- Pre-clinical toxicity profile:

The toxicity profile of BT-001 was investigated in primate toxicity studies following single and repeated administrations by IV route.
Doses from 10^4 to 10^6 PFU/kg in primate (i.e. about $6x10^5$ to $6x10^7$ PFU human equivalent dose) were well tolerated after IV administration representing a maximal systemic exposure compared to IT route planned in patients.

- Clinical toxicity profile:

In BT-001.01, BT-001 GMO will be administered for the first time in Humans. There is then no data available in terms of adverse reactions related to BT-001.

The clinical experience cumulated from vaccination campaigns with the wild type non-attenuated parental vaccinia virus and from clinical trials sponsored by TRANSGENE assessing other products of its VV-based vectors platform can allow to anticipate some probable safety events related to BT-001. The Copenhagen strain which is used for the TRANSGENE COP platform and BT-001 construct led to an intermediate/high rate of adverse events (AEs). Of note, severe AEs were extremely rare even with the strains displaying the highest rate of AEs (Kretzschmar M. *et al.*, 2006). The most common AEs related to TG6006 or TG6002 (single TK- deleted VV of the Wyeth strain and TK- and RR- double deleted VV of the Copenhagen strain respectively) reported have been transient flu-like symptoms, including pyrexia, chills, nausea, fatigue, headache, and vomiting which generally develop and resolve shortly.

Immune-related reaction (irAEs) that is associated with anti-CTLA-4 treatments should also be considered as BT-001 carries 4-E03 anti-CTLA-4 mAb transgene. Viral vectorization of an anti-CTLA-4 monoclonal antibody should narrow its expression to the tumor microenvironment and is then expected to decrease irAEs associated with anti-CTLA4.

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

BT-001 GMO can be detected and its identity can be confirmed by PCR with a set of primers hybridizing with the genetic inserts and with corresponding flanking viral sequences of the recombinant VV-COP.

F. INFORMATION RELATING TO THE RELEASE

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

The release will be the administration of the BT-001 GMO investigational medicinal product, in a hospital or clinic setting, by IT injection to patients as a part of the international multicentric clinical trial BT-001.01.

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 BT-001 and its parental virus VV-COP are not naturally found in the environment.

3. Information concerning the release and the surrounding area

a) Geographical location (administrative region and where appropriate grid reference):
In the scope of BT-001.01 clinical trial, BT-001 is administered at the following clinical site located in Belgium:

Cliniques universitaires Saint-Luc (UCLouvain)
Avenue Hippocrate 10
1200 Bruxelles

Principal investigator: Jean-François BAURAIN

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 release area at clinical site. However all zones in which BT-001 is handled and administered to the patients and in which the patients are hospitalized after dosing with BT-001 must have restricted access (cf section F.4.c).

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

Not applicable.
Given the route of administration (intratumoral) for BT-001 investigational medicinal product, release area and procedures for waste treatment, the exposure to significant biotopes, protected areas and drinking water supplies is expected to be negligible to null.

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 maximum dose administered per administration is 4 x 10^8 PFU per IT administration. Patients will have IT administration of BT-001 every 3 weeks or every 2 weeks or weekly according to the cohort they are taking part in. They will receive up to 4 administrations (Phase I, Part A) or until documented confirmed disease progression, unacceptable toxicity or patient refusal (Phase I, part B or Phase IIa).

In the Phase I of the BT-001.01 clinical trial, up to 48 patients will participate. The maximum quantity of GMO expected to be released during the Phase I of the study should not exceed $2x10^{11}$ PFU in the European Union and 4 x 10^{10} PFU in Belgium.
The number of patients needed in Phase IIa will be defined based on Phase I results, statistical hypothesis as well as the number of open cohorts and statistical design.

b) Duration of the operation

See F.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 in a hospital setting, according to the clinical protocol and in respect of the Good Clinical Practices. The product must be prepared in aseptic conditions compliant with injectable solutions. BT-001 is prepared in a laboratory or pharmacy under the direction of an accredited pharmacist.

a) *The containment and protective measures planned for BT-001 preparation and administration operations:*

- Study documentation and clinical staff training:

Before being able to participate in any BT-001 operation (i.e.: preparation or administration) and in the care of patients receiving BT-001, the worker must attend training and a set of documents is provided by the sponsor to all personnel involved in handling of the BT-001 product.

These documents give information related to the clinical lot of BT-001, the conditions and precautions of BT-001 use, instructions in case of incident or inadvertent exposure including accidental spillage and for waste management, step by step instructions for preparation and administration operations. These documents then detail the different containment and protective measures listed below.

- Exclusion of "at risk" group in BT-001 operations:

Healthcare workers or housekeeping personnel in the following "at risk" groups should not have direct physical contact with BT-001, should not administer BT-001 or provide direct care to study patients:

- People with severe active exfoliative skin conditions (e.g. eczema or psoriasis requiring systemic treatment)
- Immunocompromised individuals (severe deficiencies in cell-mediated immunity, including patients with acquired immune deficiency syndrome (AIDS), organ transplant recipients, hematologic malignancies)
- Pregnant or breastfeeding women

- Personal protective equipment (PPE) requirements:

All staff involved in handling of BT-001 or any material or linen potentially contaminated with BT-001 must wear personal protective equipment (PPE): waterproof gloves (conform with EN374, EN420, EN455 and ISO 16604 with an AQL of 0.65 or lower), gown, surgical/procedure mask (conform with the norm NBN EN 529, a FFP2 type (EN149:2001) and with a P2 filter (EN 143:2000), safety goggles with side shields and needle stick resistant shoes.

- Protection of BT-001 area from intrusion by unauthorized individuals:

All zones in which BT-001 will be handled and administered to the patients and in which the patients will be hospitalized after BT-001 administration have restricted access (i.e., access to these zones will be controlled and limited to authorized hospital staff who has received training on measures to control infection).

It will be also asked to store BT-001 in an alarmed and temperature-monitored freezer with restricted access to authorized staff only, under the supervision of the study Pharmacist / Investigator (or his/her delegate). The international biohazard label will be affixed.

- BT-001 transfer operations:

All transfers of BT-001 (in primary vial from the pharmacy or in syringes after preparation procedure completion) must be done using a leak-proof packaging (i.e.: hermetic transport box containing absorbent paper towels) displaying a clearly marked biohazard symbol.

- Preparation and administration area cleaning instructions:

All surfaces and floor of the preparation area (i.e.: class 2 microbiological safety cabinet) as well as the ones of the administration area will be wiped down with a disinfectant active on vaccinia virus after each use. Lipid-encapsulated viruses such as BT-001 are sensitive to many classical hospital-grade chemical disinfectants containing bleach [sodium hypochlorite is known to inactivate VV within 10 minutes at a concentration as low as 0.02% (Klein M. and DeForest A., 1965)] or other chemical substances. A list of standard hospital-grade disinfectants active on vaccinia viruses that could be considered is provided in the study documentation.

The surfaces and floors of hospital rooms and other patient care areas used by the patient 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.

- Procedures in case of incident with BT-001:

Detailed instructions to follow are provided in the BT-001.01 study documentation technical in case one of the following incidents happens:

- BT-001 accidental spillage
- Skin contamination with BT-001
- Eye contamination with BT-001
- BT-001 ingestion
- BT-001 inhalation

In addition, presence of a spill kit will be requested in the BT-001 handling facility. This spill kit should contain appropriate disinfectant, personal protective equipment, tongs or forceps in order to take broken vials, absorbent paper towels and biohazard waste bags.

b) *The protective measures planned following BT-001 administration and during the post discharge phase are:*

- Patient monitoring at clinical site after BT-001 administrations:

After a BT-001 IT administration, patients will not be immediately discharged home. All patients must be monitored after BT-001.01 according to protocol specifications. While it is in place for safety monitoring, it will also drop the potential risk of dissemination to the environment and to the patient’s household contacts.

The patient will be hospitalized during those observation periods in a private hospital room with as far as possible dedicated bathroom and toilet. The access to the patient’s room will be limited to the study staff. Any person in an “at risk group” (i.e. individual with severe active exfoliative skin conditions, immunocompromised individual, pregnant or breastfeeding woman, child <12 months of age) will not be allowed to enter in the patient’s room. The study staff entering in the patient’s room will be equipped with personal protective equipment (PPE): waterproof gloves, gown, surgical/procedure mask and safety goggles with side shields.

- Prevention of potential dissemination from the injection site

A dry occlusive dressing will be put on each injection site and will have to be kept for at least 6 hours after BT-001 administration. Once removed, the dressing(s) must be placed in a small waterproof plastic bag marked with the "biological hazard" symbol provided by the sponsor before being brought back to the hospital for destruction.

- Study documentation and BT-001.01 outpatient observation period:

When discharging home, the patient will be asked by the study staff to report adverse events and to be attentive to some situation as described in the BT-001.01 study protocol, the BT-001.01 Patient Information Note and the BT-001.01 patient card. The two last documents will be provided to the patient and he/she take them back at home.

Patients will be instructed to contact the Investigator immediately in case of:

- the development of skin pustules, for adequate management and care,
- the development of skin pustules in a household member,
- any medical emergency, if the patient seeks care or visits a different hospital, the patient should notify the staff immediately of his/her participation in the BT-001 study, so that proper precautions can be taken,
- the development of any serious symptoms or of any event, unusual or different from what the patient has been told to expect.

- Specific precautions for pustule management and hygiene kit provision:

Specific precautions for pustule management are set-up to be applied to patients treated with BT-001 in whom the occurrence of pustules is observed, to prevent potential contamination from spread of the virus in the environment:

These measures are applicable up to seven days after the last BT-001 injection or, in case of the occurrence of pustules, up to the scab has fallen off, i.e. about 3 weeks after the pustule appearance:

- Wash hands frequently with soap and hot water, or with a hand sanitizer containing at least 60% alcohol (hydro-alcoholic solution)
- Avoid sharing personal items: toiletries, eating tableware items
- Avoid intimate contacts (kissing, sex)
- Avoid contact with pets

If the patient notices the appearance of a pustule in the skin or in the mouth for herself/himself or a household member, he will be asked to contact the trial staff as soon as possible to receive the guidelines to be followed and must follow the instructions below immediately, until complete healing of the lesion (falling-off of the scab):

“1. Cover the lesion with the protective dressing included in the kit provided to you. In case of a pustule in the mouth, wear a mask in the presence of other people.

2. Do not allow other persons or pets to have a direct contact either with the pustule or with potentially contaminated surfaces (e.g., the bandage covering the pustule, a piece of cloth, or linen). If you need care (e.g., change of bandage), gloves should be worn, and hands be washed with soap and hot water, or with a hand sanitizer containing at least 60% alcohol.

3. Avoid any contact of the pustule(s) or contaminated surface(s) with:

- children less than 12 months old
- pregnant or breastfeeding women
- people with chronic active inflammatory skin diseases (eczema, psoriasis, atopic dermatitis, etc.)
- immunocompromised persons (e.g., organ transplant recipients, patients with AIDS or chronically treated with immunosuppressive drugs)

4. Avoid any contact with other parts of your body (e.g., the eyes, the nose, etc.) after having touched a pustule or any other potentially contaminated surface (e.g., after having changed your bandage).

5. Dispose all non-washable contaminated materials (bandages, gauze) in a hermetically closed container or in the waterproof pocket with a zip-lock included in the hygiene kit supplied by the hospital and bring it back to the hospital at the next visit. Laundry (e.g., sheets, clothes, towels) can be washed with hot water and detergent. You may also use bleach (one cup per wash load) for decontamination."

After each hospitalization for treatment with BT-001, the patient will receive a hygiene kit to be used in case of appearance of pustules, in which there are:

- antiseptic
- sterile compress
- protective dressings
- a mask
- a waterproof pocket with a zip-lock to put in contaminated material

In addition to these immediate measures, the study staff should ensure:

- The injection sites and any skin lesions should be covered loosely with a dry occlusive bandage (e.g., gauze) held in place with first-aid tape for at least 1 week after treatment. If a blister forms, bandaging of the infusion sites or skin lesions should be continued either until healed or the scab falls off.

- Dry occlusive bandage should be changed at least every 3 days or every time the bandage becomes wet.

- Infectious wastes returned by the patient in the waterproof pocket with a zip-lock provided in the hygiene kit should be disposed of according to BT-001 disposable waste material instructions.

Recommendation to wear personal protective equipment when changing the bandage on the injection/infusion sites and skin lesions, to remove the gloves and clean hands immediately after changing the bandage and before touching any other part of the patient's body, another person, or objects in the environment.

- Contraception measures:

To prevent any risk of potential viral dissemination through semen as well as potential vertical transmission, BT-001.01 patients will be asked to use an appropriate precaution against pregnancy while on study. Both men and women of childbearing potential must use a highly effective contraception method (i.e., with a failure rate of ≤1 % per year) combined with a barrier method (e.g. condom) during and after the BT-001 treatment period as per BT-001.01 protocol instructions. Furthermore, pregnant and breastfeeding women will not be allowed to take part in the BT-001.01 study.

- Rescue medications in case of serious complication due to the VV-COP

In case of a serious infectious complication following BT-001 administration, the VIG and TPOXX rescue medication antiviral therapies are made available to investigator by TRANSGENE.

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 BT-001.01 clinical trial is the first-in-man use of the GMO BT-001 so there is no data regarding previous releases carried out with the same GMO.

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)
(b) Family name (for plants)
(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)

Following intratumoral administration, BT-001 armed oncolytic therapeutic vaccinia virus and the vectorized therapeutic proteins i.e. 4-E03 and GM-CSF cytokine act together to inhibit the tumor growth through a multi-pronged antitumoral mechanism of action. BT-001 replicates preferentially in tumor cells, induces cell death and local inflammation, primes adaptative immune responses and delivers its 2 immunomodulatory therapeutic proteins i.e. 4-E03 that inhibits CTLA-4/B7 interactions and induces Treg depletion and hGM-CSF that promotes the differentiation of hematopoietic precursors into dendritic cells, the professional antigen presenting cells.

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

There is very low potential for gene transfer to other species under the proposed release of the GMO. As mentioned in section F, the GMO will be 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.

BT-001 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

The replicative and propagative characteristics of vaccinia virus have been attenuated in BT-001 with the inactivation of the TK and RR genes which renders the modified organism dependent of highly dividing cells such as cancer cells. Therefore BT-001 should have reduced competitiveness and invasiveness compared to parenteral 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

BT-001 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 BT-001 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

Potential unlikely secondary transmission may occur 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 will be released in a hospital examination room and is unlikely to come in contact with other animal species. Furthermore BT-001 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 or from rabies vaccination campaigns.

Rabbies vaccination campaigns experience is of interest when considering potential consequence of potential highly unlikely release in the ecosystem of other species in the scope of BT-001.01 clinical trial. Raboral V-RG® (TK-inactivated VV of the Copenhagen strain expressing the glycoprotein G of the rabies virus liquid vaccine packaged inside edible baits) is also based on an attenuated form of the VV-COP as BT-001 and has been placed on the market in the EU (93/572/EEC) and the USA. It is used in these continents to vaccinate wild animals since 1987 and is spread in baits over the zones of rabies contamination. At the time of the marketing authorization assessment, the European Commission considered the exposure to this TK-inactivated VV as a low safety risk for human health and the environment. More than 250 million doses have been distributed globally by public health officials since 1987 without any reports of adverse reactions in n wildlife or domestic animals. (Maki et al., 2017) The United States Department of Agriculture Animal and Plant Health Inspection Service (APHIS) published in 2018 an update of its Raboral V-RG® Environmental Assessment and still concluded that it release has no significant impact on the quality of the human environment.

(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 BT-001 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 on patients will be achieved using the following clinical assessments: physical examinations, vital signs, adverse event reporting, clinical laboratory assessments and immunomonitoring throughout the BT-001.01 clinical trial 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 BT-001 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 BT-001 GMO is administered to patients by IT route in conventional 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. Moreover, as per the guideline on follow-up of patients administered with gene therapy medicinal products (reference EMEA/CHMP/GTWP/60436/2007), long term follow-up observations have been implemented in BT-001.01 for a duration of five years after the last study visit.

6. Frequency of the monitoring

Patients will be monitored for safety from the first treatment dose to the end-of-study visit according to time-points specified in the BT-001.01 clinical protocol. For the long term follow-up, monitoring is performed yearly.

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 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 an active disinfectant.

2. Post-release treatment of the GMOs

Material in contact with BT-001 must be considered as contaminated by infectious material. It must be stored in appropriate biohazard containers and decontaminated prior to disposal.

Decontamination and disposal of all contaminated materials and unused or partially used product should occur as per methods described in section I.3.(b).

3. (a) Type and amount of waste generated

Two types of material and equipment are likely to be in contact and contaminated by BT-001. They can then be waste to treat:

- Disposable material and equipment contaminated by BT-001 like used and unused vials, empty and non-empty dilution vials, syringe, needles, gauze, dressings, gloves, gown, masks, bandages, etc
- Non-disposable material and equipment contaminated by BT-001 like labcoat, goggles, patient gown, bedding, linens, towels, etc

The maximum quantity of BT-001 GMO waste per administration will be generated if the highest dose is prepared and not injected to the patient. In this case, the quantity of waste could be up to 4×10^8 PFU.

3. (b) Treatment of waste

Disposable material and equipment contaminated by BT-001 must be discarded according to regular hospital procedure for infectious waste:

• Autoclaving/incineration

or

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

Non-disposable material and equipment contaminated by BT-001 must be cleaned/treated according to regular hospital procedure for infectious material (e.g. hot water ≥ 71°C washing with detergent and hot air drying).

J. INFORMATION ON EMERGENCY RESPONSE PLAN

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

It will be recommended to personnel involved in handling of the GMO investigational medicinal product to act as recommended below in case of incident with the use of BT-001. The complete instructions are given to clinical sites in the "BT-001 Technical Sheet" document.

- **Accidental shedding**:
  Contaminated area must be cleaned with a standard disinfectant active on vaccinia virus (e.g., bleach at 0.6% of active chlorine in contact for at least 30 minutes or any other active disinfectant used according to manufacturers' instructions to ensure adequate contact time and to confirm the ability of the equipment to withstand the disinfectant used).

- **Skin contamination without injury:**

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.

- **Cuts or punctures:**
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). The injured person should receive counselling 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 follow up of the exposed individual are required until an active infection is ruled out, or as required by institutional policies.

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 BT-001.01 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 BT-001 with the inactivation 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 BT-001 outside patients' tumors is very low.

There is no clinical information on BT-001 to date as BT-001.01 is its First in Human use. The clinical data from other recombinant VVs suggest that TK-deficient VVs do not spread to caregivers or household contacts in the environment of the treated patients.

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 BT-001 within the proposed clinical trial conditions would result in any other environmental effect.

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