BT-001

Annex II

ENVIRONMENTAL RISK ASSESSMENT

Belgium

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TABLE OF CONTENT

LIST OF ABBREVIATIONS

AE	Adverse Event
CEF	Chicken embryo fibroblast
CTLA-4	Cytotoxic T-lymphocyte-antigen 4
DNA	deoxyribonucleic acid
GM-CSF	Granulocyte-Macrophage Colony stimulating factor
GMO	Genetically modified organism
HC	Heavy Chain
IMP	Investigational medicinal product (BT-001)
IT	IntraTumoral
irAE	immune-related adverse events
LC	Light Chain
mAb	Monoclonal Antibody
PFU	Plaque forming unit
RDPB	recommended dose for Part B
VV	Vaccinia virus
VV-COP	Vaccinia virus of the Copenhagen strain

A. INTRODUCTION

BT-001 (TG6030) is a viral suspension of the 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).

BT-001 is a genetically modified organism (GMO) which is co-developed by Transgene and Bioinvent Inc as a therapeutic candidate to treat patients with solid tumors.

B. CHARACTERISTICS OF THE GMO AND RELEASE

B1. Characteristics of the GMO

The parental virus of BT-001 is the VV, Copenhagen strain. VV is a member of the *Poxviridae* family (genus Orthopoxvirus). It is a double-stranded deoxyribonucleic acid (DNA) virus. The inherent biological properties of the wild type VV make it ideal as a vector for expression of transgenes of therapeutic interest as well as an oncolytic agent. These properties have been exploited to design BT-001:

- VV are genetically stable DNA viruses,
- VV have no known host in nature,
- VV infect a wide range of human tissues but do not cause any known human disease,
- VV have a natural tropism for tumors (Shen Y. and Nemunaitis J., 2005) (Zeh H.J. and Bartlett D.L., 2002),
- VV DNA does not integrate the host chromosomes since they remain in the cytoplasm, thus eliminating the risk of chromosomal integration (Moss B., 2007),
- VV encode their own replication and transcriptional enzymes. They do not require the host machinery for DNA synthesis and are therefore not dependent upon host cell replication,
- VV have a quick, efficient lifecycle, forming mature virions in about 6 hours after infection,
- VV replicate and lyse cells rapidly compared with other virus species,
- VV spread efficiently cell-to-cell, thus increasing the efficacy of in vivo infection. They also move unhindered through the bloodstream, which allows a systemic IV route of administration,
- VV are highly efficient at spreading to distant tumors,
- VV are able to accommodate multiple large transgenes (at least 25 kb) (Jolly D., 1994),
- VV possess their own strong promoters capable of achieving very high levels of transgene expression.

Multiple strains of VV exist. The Copenhagen strain was used for smallpox vaccination in Denmark and the Netherlands in the 1950s (Kretzschmar M. *et al.*, 2006). This strain is one of the most lytic strains which is of upmost interest in the oncolytic application.

Replicative and propagative characteristics of VV-COP have been attenuated through genetic engineering through inactivation of its TK and RR genes. BT-001 was indeed generated by two successive homologous recombination between COPTG19156 recipient which is a sub-clone of the vaccinia virus Copenhagen strain with TK and RR genes inactivated and carrying the mCherry gene and the green fluorescent protein gene (GFP), and two transfer plasmids: pTG19367 encoding the heavy chain (HC) of 4-E03 mAb and pTG19384 encoding the GM-CSF and the light chain (LC) of 4-E03 mAb.

The homologous recombination between the transfer plasmids and the vaccinia virus recipient enabled the generation of BT-001.

The resulting GMO, BT-001, consists in a recombinant VV-COP TK- RR- vaccinia virus which lost the GFP and the mCherry expression cassettes and gained the 4-E03 monoclonal antibody and GM-CSF expression cassettes.

The deletion of the *TK* and *RR* genes turns off the expression of the respective enzymes and conditions BT-001 replication to highly proliferating cells. The TK deletion also enhances VV inherent selectivity for tumors (Puhlmann M. *et al.*, 2000; Zeh H.J. and Bartlett D.L., 2002).

BT-001 has been designed a multi-pronged antitumoral mechanism of action. Once administered to patient by intratumoral (IT) route, BT-001 replicates preferentially in tumor cells, induces cell death and local inflammation, primes adaptative immune responses and delivers its 2 immunomodulatory therapeutic proteins:

- The 4-E03 mAb that inhibits CTLA-4/B7 interactions and induces Treg depletion.
- The hGM-CSF cytokine that promotes the differentiation of hematopoietic precursors into dendritic cells, the professional antigen presenting cells.

B2. Characteristics of the release

Release description

The release will be the administration of BT-001 GMO, in a hospital or clinic setting, by IT injections to patients as part of the Phase I/IIa multinational, multicenter clinical trial BT-001.01.

The objectives of the BT-001.01 clinical trial and then of BT-001 deliberate release in this context are to:

- Assess the local and systemic safety and tolerability of BT-001 as a single agent and in combination with pembrolizumab

- Evaluate tumor response of injected and non-injected lesions

- Assess BT-001 blood pharmacokinetics

- Perform BT-001 viral shedding analyses in different biological samples

- Assess immunogenicity of BT-001 and immune changes in the tumor microenvironment

The BT-001.01 clinical trial will include 3 parts:

- Phase I, Part A: It will be a dose escalation in patients with metastatic/advanced solid tumors. BT-001 will be tested as a single agent with repeated BT-001 IT administrations.

- Phase I, Part B: Repeated BT-001 IT administrations in combination with intravenous (IV) infusions of pembrolizumab in patients with metastatic/advanced solid tumors. BT-001 will be administered at the recommended dose for Part B (RDPB) meaning that BT-001 regimen will be defined according to the results of Phase I, Part A.

Up to 48 patients will take part in the Phase I (Part A and Part B) of the study.

- Phase IIa: Repeated BT-001 IT administrations in combination with IV infusions of pembrolizumab in patients with metastatic/advanced soft tissue sarcoma (STS), Merkel cell carcinoma (MCC), melanoma, triple negative breast cancer (TNBC) or non-small cell lung cancer (NSCLC). BT-001 will be administered at the RDPB.

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.

Details of the trial design and its objectives are given in the BT-001.01 study protocol.

Sites of the release

In the scope of BT-001.01 clinical trial, BT-001 is administered in one clinical site located in Belgium (Cliniques universitaires Saint-Luc [UCLouvain], Brussels).

Release conditions

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 Practice.

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.

B3. Risk assessment for the public health

VV has a long and extensive history of use in humans for the worldwide smallpox eradication program. 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. The genetic modifications performed on the wild type virus to obtain BT-001 (i.e. *TK* and *RR* gene inactivation) limit the replication capability of the virus to actively dividing cells like tumor cells while they have not impacted the other characteristics of the virus.

Since there are no human safety data available to date with BT-001, this section provides a risk assessment based on the experience of VV use in the smallpox eradication program, the BT-001 pre-clinical toxicology profile, the clinical experience of TRANSGENE with two other

recombinant VV constructs and the transgenes safety profile. It also provides the protective measures taken to mitigate identified risks.

Potential risks for public health

VV use in the worldwide smallpox eradication program: Wild type VV was administered in hundreds of millions of people during the smallpox eradication program. Clinical safety of VV used in vaccination conditions (skin scarification with $1 \times 10^5 – 1 \times 10^6$ pfu) is therefore very well known (Cono J. *et al.*, 2003; Kretzschmar M. *et al.*, 2006). Wild type (non-attenuated) VV is being considered a minor pathogen for humans (Dumbell K.R., 1985). VV replication exclusively occurs in the cytoplasm thus eliminating any risk of integration of the viral DNA into the host genome (Moss B., 2007). VV does not produce a latent infection, so once the infection arises, the virus is rapidly cleared from the host. VV does not cause any known human disease. However, 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).

Vaccination with VV is known to result in the invasion of keratinocytes, causing areas of necrosis and papule/vesicle/pustule formation at the injection site. The skin lesion resolves with the formation of a scab which heals 14-21 days after vaccination. The skin lesion is infectious until the scab heals. During that time, care must be taken to prevent spread of the virus to another area of the body or to another person.

Inadvertent self-inoculation is a common adverse event of vaccination with VV. It usually occurs when a person transfers VV from the vaccination site to another location on their body, usually the eyes, mouth, nose or genitalia. An eye infection by vaccinia, referred to as ocular vaccinia, can be clinically mild to severe and can lead to vision loss. If ocular vaccinia is suspected, the event will be managed in consultation with an ophthalmologist (Lewis F.M. *et al.*, 2006).

Secondary transmission (i.e. transmission of the virus to another person) is a rare occurrence. It has been described in household contacts, 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).

No data exist that would indicate airborne transmission of vaccinia (Centers for Disease Control and Prevention: frequently asked questions about smallpox vaccine; (Lane J.M. and Fulginiti V.A., 2003)).

BT-001 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.

VV strains: Multiple strains of VV exist that have different levels of virulence. Kretzschmar *et al.* compared the frequency of adverse events which occurred after smallpox vaccination with different VV strains (Kretzschmar M. *et al.*, 2006). Overall, vaccination with the Wyeth strain caused the lowest rate of adverse effects, whereas vaccination with the Lister strain led to an intermediate rate of adverse effects. The Copenhagen strain led to an intermediate/high rate of adverse events and the Bern strain accounted for the highest rate of adverse reactions. Of note, severe adverse effects were extremely rare even with the strains displaying the highest rate of adverse events.

Clinical experience with other recombinant VV constructs : TRANSGENE has two recombinant VV constructs in clinical development: TG6006 which is based on a single TK-deleted VV of the Wyeth strain and TG6002 which is based on the TK- and RR- double deleted VV of the Copenhagen strain. The most common adverse events (AEs) related to these two products reported have been transient flu-like symptoms, including pyrexia, chills, nausea, fatigue, headache, and vomiting which generally develop and resolve shortly.

Transgenes safety profile: Immune-related reaction (irAEs) that is associated with anti-CTLA-4 treatments should also be considered as BT-001 carries 4-E03 anti-hCTLA-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-CTLA-4.

Risk mitigation protective measure for public health

To prevent potential risks for public health with the use of BT-001, including for participating patients, healthcare workers, housekeeping personnel and household contacts some protective measures are set-up in the BT-001.01 clinical trial.

a) 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" groups 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 technical documentation 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) 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 the injection site(s) 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 each 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 until complete healing of the lesion (falling-off of the scab):

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 immediate measures, the study staff should ensure that:

- The injection sites and any skin lesions are 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 injection sites or skin lesions should be continued either until healed or the scab falls off.

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

- Infectious wastes are 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 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 following rescue medication antiviral therapies are made available to investigator by TRANSGENE: Vaccinia Immune Globulin (VIG) as a first option and TPOXX (tecovirimat) as a second line option.

- 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.

Risk assessment for public health conclusion

The inactivation of the TK and RR expression, which restricts BT-001 replication to highly dividing cells such as cancer cells, considerably reduces the pathogenicity and the dissemination of the recombinant virus compared to its parental virus. It is therefore expected that BT-001 safety profile will be acceptable and its potential transmission will be attenuated compared with the wild type VV. Furthermore, individuals from the "at risk" groups are not eligible for entry in clinical trials with BT-001. Patients who are administered BT-001 are requested to avoid direct physical contact with anyone in an "at risk" group.

Poxviruses are not endemic in the population. It is therefore unlikely that BT-001 recombines with a wild type virus to produce a more virulent strain (Sandvik T. *et al.*, 1998). Despite worldwide use of the non-attenuated virus during smallpox vaccination, no adverse events due to mutation to a more aggressive phenotype have ever been reported.

To prevent dissemination of the virus to close contacts (e.g.: healthcare workers, housekeeping personnel and household contacts) and potential subsequent adverse events in these subjects, the aforementioned protective measures will be applied in the BT-001.01 study.

Lastly, in the unlikely occurrence of a serious complication due to BT-001 use for a patient or a close contact, antiviral rescue medication is made available by the sponsor.

Therefore, BT-001 is not considered to represent a risk for the public health.

B4. Risk assessment for the environment

Therapy with a replicating virus can theoretically lead to shedding of the virus into the environment. BT-001 can theoretically be disseminated through human fluids and secreta. As VV does not produce a latent infection, after an initial period with some viral shedding in biological fluids, the virus becomes completely cleared from the host.

The monitoring of BT-001 presence in blood, saliva, urine and feces will be performed in the proposed BT-001.01 clinical trial and will help to design tailored instructions to prevent dissemination of the virus and contamination.

In the meantime, protective measures to prevent potential virus dissemination in the environment will be applied in the proposed BT-001.01 clinical trial as described in section B3. In the proposed clinical trial, BT-001 will then be released at the hospital, in a restricted environment and with specific measures to avoid dissemination of the virus.

In the event of a spill, people in the immediate contaminated area should be alerted and access must be limited to the persons entitled to handle BT-001. Decontamination of the affected area should then be conducted as requested in the study documentation using either bleach or a standard disinfectant active on vaccinia virus according to manufacturers' instructions to ensure adequate contact time and to confirm the ability of the equipment to withstand the disinfectant used.

After BT-001.01 operations, all waste generated should be autoclaved, incinerated, or treated with sodium hypochlorite solution by personnel who are trained to dispose of biohazard waste.

o Disposable material and equipment contaminated by BT-001 (e.g. used and unused vials, empty and non-empty dilution vials, syringe, needles, gauze, dressings, gloves, gown, masks, bandages, etc) must be discarded according to regular hospital procedure for infectious waste (e.g. the disposable material will be placed in containers and will then be autoclaved or treated with bleach solution for inactivation before incineration or disposal through the DASRI pathway).

o Non-disposable material and equipment contaminated by BT-001 (e.g. labcoat, goggles, patient gown, bedding, linens, towels, etc) 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).

Instructions will be given to the patient by study staff once he/she is discharged from the hospital in order to prevent dissemination at home. A written summary of these instructions in lay language will be provided to the patients as part of their informed consent process.

While highly unlikely in the scope of BT-001.01 clinical trial, absence of impact is anticipated in case of a potential BT-001 environment exposure based on Raboral V-RG® experience. Raboral V-RG® (TK-inactivated VV of the Copenhagen strain expressing the glycoprotein G of the rabies virus liquid vaccine packaged inside edible baits) 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 wildlife or domestic animals. (Maki J. *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.

In conclusion, the likelihood of BT-001 becoming persistent and invasive in natural habitats is extremely low for the following reasons:

- Due to the inactivation of its *TK* and *RR* genes, BT-001 replicates preferentially in actively dividing cells. BT-001 is therefore expected to propagate mostly in cancer cells.
- BT-001 bringing back its genome up to the structure of its parenteral virus would mean that the recombinant virus would eliminate the 4-E03 mAb HC expression cassette inserted in the J2R locus, eliminate the 4-03 mAb LC and hGM-CSF sequence inserted in the I4L locus, recover the *TK* deleted sequence and recover the *RR* deleted sequence. These represent several spontaneous events which are highly improbable. Current genetic stability studies have not detected spontaneous revertants of BT-001. Furthermore, VV biology prevents co-infection of the same cell with another VV (Doceul V. *et al.*, 2010). There is therefore no risk of recombination of BT-001 with the wild type VV.
- VV is not naturally found in the environment and there is no known natural reservoir of VV.
- Poxviruses cannot reproduce in the absence of a susceptible host cell.
- Poxviruses remain 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, preventive measures are in place in the proposed BT-001.01 clinical trial to minimize dissemination and inadvertent transmission.
- No environmental concern was raised during the smallpox vaccination campaign during which hundreds of millions of people were administered with VV wild type, the non-attenuated parental virus of BT-001.
- No environmental concern or any reports of adverse reactions in wildlife or domestic animal has emerged from oral rabies vaccine bait campaigns in US and European regions using a TK-inactivated VV of the Copenhagen strain (Maki J. et al, 2017).

C. CONCLUSIONS ON THE POTENTIAL ENVIRONMENTAL IMPACT FROM THE RELEASE OR THE PLACING ON THE MARKET OF GMOS

Propagative and pathogenic characteristics of VV have been attenuated in BT-001 with the inactivation of the *TK* and *RR* genes.

Therapy with a replicating virus can theoretically lead to shedding of the virus into the environment, and potentially to the public. BT-001 has been designed to target cancer cells and non-clinical experiments demonstrated that it replicates preferentially in actively dividing cells like cancer cells.

Dispositions will be taken in this clinical trial to minimize dissemination and inadvertent transmission. 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 was to demonstrate serious virus-associated toxicity, therapy could be initiated with antiviral rescue medication 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 and to the use of an attenuated virus in oral rabies vaccination campaigns delivered by edible bait. It is therefore not expected that the release of BT-001 within the proposed clinical trial conditions would result in any other environmental effect.

In conclusion, with the preventive measures which will be applied in the proposed clinical trial, the BT-001 GMO is not considered to represent a risk for the public health and for the environment.

D. REFERENCES

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