TG6002

Annex II

ENVIRONMENTAL RISK ASSESSMENT

20 February 2018

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FIGURE

TABLE

LIST OF ABBREVIATIONS

5-FC	Flucytosine (INN)
5-FU	5-fluorouracil
5-FUMP	5-fluorouridine 5'monophosphate
AIDS	Acquired immune deficiency syndrome
BSL	Biosafety level
CDase	cytosine deaminase
CDC	US Centers for Disease Control and Prevention
DLT	Dose limiting toxicity
DNA	Deoxyribonucleic acid
EU	European union
FCU1	*Saccharomyces cerevisiae* cytosine deaminase uracil phosphoribosyltransferase fusion protein
GI	Gastro-intestinal
GMO	Genetically modified organism
hGM-CSF	Human granulocyte macrophage-colony stimulating factor
HIV	Human immunodeficiency virus
I4L	Ribonucleotide reductase coding gene
IL2	Interleukin-2
IMPD	Investigational medicinal product dossier
IV	Intravenous
J2R	Thymidine kinase coding gene
JX-594	Pexastimogene devacirepvec (INN), recombinant VV, Wyeth strain, expressing hGM-CSF and β-galactosidase (also called Pexa-Vec)
MUC1	Mucin-1 tumor antigen
MTD	Maximum tolerated dose
ORR	Overall response rate
PFS	Progression free survival
pfu	Plaque forming unit
RP2D	Recommended phase 2 dose
RR	Ribonucleotide reductase
TG1031	Vaccinia virus of the Copenhagen strain deleted in TK gene and coding for mucin-1 tumor antigen and human interleukin-2
TG6002	Vaccinia virus of the Copenhagen strain deleted in *TK* and *RR* genes and coding for FCU1
TK	Thymidine kinase
UPRTase	Uracil phosphoribosyltransferase
USA	United States of America
VV	Vaccinia virus

A. INTRODUCTION

TG6002 is a replicative vaccinia virus (VV) of the Copenhagen strain in which three genetic modifications have been performed: 1) deletion of the thymidine kinase (*TK*) gene, 2) deletion of the ribonucleotide reductase (*RR*) gene, and 3) insertion of the chimeric yeast *FCU1* gene in the TK locus.

TG6002 is a genetically modified organism (GMO) which is developed as a therapeutic candidate to treat patients with solid tumors. In the proposed trial, patients with advanced gastro-intestinal (GI) tumors will be treated with the combination of TG6002 and the prodrug flucytosine (5-FC). Patients will receive three weekly intravenous (IV) injections of TG6002 and oral 5-FC.

B. CHARACTERISTICS OF THE GMO AND RELEASE

B1. Characteristics of the GMO

The parental virus of TG6002 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 TG6002:

- VV is a genetically stable DNA virus,
- VV has no known host in nature but grows well under experimental conditions in a wide host range,
- VV infects a wide range of human tissues but does not cause any known human disease except for vaccination complications,
- It has a natural tropism for tumors (Shen Y. and Nemunaitis J., 2005; Zeh H.J. and Bartlett D.L., 2002),
- Its DNA does not integrate the host chromosome since it remains in the cytoplasm, thus eliminating the risk of chromosomal integration (Moss B., 2007),
- VV encodes its own transcriptional enzymes. It does not require the host machinery for DNA synthesis and is therefore not dependent upon host cell replication[1],
- VV has a quick, efficient lifecycle, forming mature virions in about 6 hours after infection,
- It replicates and lyses cells rapidly compared with other virus species,
- It spreads efficiently cell-to-cell, thus increasing the efficacy of *in vivo* infection. VV also moves unhindered through the bloodstream, which allows a systemic IV route of administration,
- It is highly efficient at spreading to distant tumors,
- VV has a large genome that can accept several kilobases of foreign DNA without viral genome rearrangements (Jolly D., 1994) (Smith G.L. and Moss B., 1983),

[1] Of note, TG6002, due to its deficiency in *TK* and *RR* genes, is dependent on host cell replication.

- VV possesses its own strong promoters capable of achieving very high levels of transgene expression,
- VV is the virus which has the longest and most extensive history of use in humans through smallpox vaccination. Adverse effects post VV inoculation are very well known as well as people who are at higher risk to develop these adverse effects (see section B3).

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 have been attenuated through genetic engineering. The resulting GMO, TG6002, consists of the following elements (Table 1):

- The genome of the parental VV organism.
- The *I4L* gene which was inactivated by deleting 141 amino acids of the central domain of the RR protein including the active site.
- The *TK* (or *J2R*) gene which was inactivated by insertion of the p11K7.5/FCU1 cassette that separates the regions encoding ATP/Mg^{2+} binding and the nucleoside binding domains of the TK protein. Moreover, the p11K7.5/FCU1 cassette insertion induced the deletion of 29 nucleotides of *J2R* and the insertion of a stop codon at the end of the first part of *J2R*.
- The vaccinia early/late p11K7.5 promoter which drives the expression of the *FCU1* transgene[2].
- The *FCU1* transgene which was inserted in the *TK* locus located in the highly conserved *Hin*dIII fragment J of VV genome (*Hin*dIII fragment J, GenBank accession No. X01078).

Table 1: Genetic elements of TG6002

Nature of the genetic element	**GenBank accession No**	**Purpose**
VV Copenhagen strain	M35027.1	Complete viral genome, backbone of TG6002
I4L	AAA48059	Deletion of vaccinia RR activity
J2R	AAA48082	Deletion of vaccinia TK activity
p11K7.5	CS054492	Vaccinia synthetic early/late promoter
FCU1	AF312392	Gene of interest, yeast cytosine deamina-se/uracil phosphoribosyltransferase fusion gene

The deletion of part of the *TK* and *RR* genes turns off the activities of the respective enzymes and conditions TG6002 replication to 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).

[2] Transcription of genes encoded by the VV genome occurs within the host cell cytoplasm and requires virally transported and encoded transcription machinery. Therefore, any foreign genes like *FCU1* must be under the control of vaccinia promoters in order to be transcribed by this machinery

The FCU1 protein expressed by the *FCU1* transgene is a bifunctional enzyme with cytosine deaminase (CDase) and uracilphosphoribosyltransferase (UPRTase) activities. FCU1 catalyzes the conversion of the non-toxic anti-fungal flucytosine (5-FC) into the toxic chemotherapeutic 5-fluorouracil (5-FU) and 5-fluorouridine monophosphate (5-FUMP) agents (Cordier P. *et al.*, 2002; Erbs P. *et al.*, 2000).

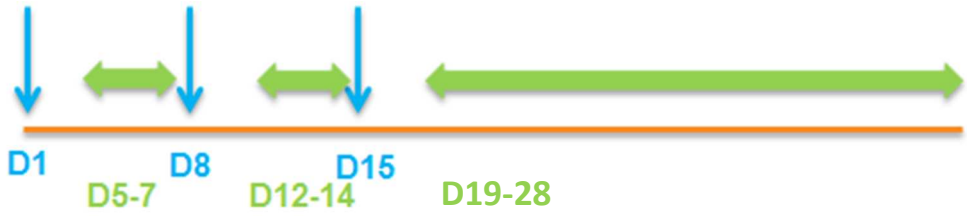
TG6002 has been designed to have a multi-mechanistic mode of action consisting of infection and selective lysis of tumor cells through direct viral replication (also called oncolysis) and targeted chemotherapy through *in situ* conversion of 5-FC into 5-FU and 5-FUMP.

B2. Characteristics of the release

TG6002 is a therapeutic GMO that will be administered in the proposed release to patients with advanced GI tumors. This study will be conducted in hospitals in Belgium, France, Germany and Spain to evaluate the safety of the combination of TG6002 with 5-FC and to identify the maximum tolerated dose (MTD) and recommend a phase 2 dose (RP2D) of TG6002 when combined with 5-FC in patients with GI tumors.

Approximately 59 patients are planned to participate in the study with up to 24 patients in the phase I part and 35 patients in the phase IIa part of the study. The phase I part is a dose-escalation. In this phase, patients will receive 3 weekly IV infusions of TG6002 at one of the following doses: 1 x 10^6 pfu (or 6.0 log10 pfu), 1 x 10^7 pfu (or 7.0 log10 pfu), 1 x 10^8 pfu (or 8.0 log10 pfu) or 3 x 10^8 pfu (or 8.5 log10 pfu) on days 1, 8 and 15 (Figure 1). One additional dose might be considered i.e. 3 x 10^7 pfu (or 7.5 log10 pfu). Of note, in case the first-in-human ONCOVIRAC phase I study (EudraCT number 2015-004452-21) using the same TG6002 regimen demonstrates the safety of the 1 x 10^7 pfu dose level, then the study will skip the 1 x 10^6 pfu cohort and will start with the 1 x 10^7 pfu dose level.

Patients will also receive the prodrug 5-FC on days 5-7, 12-14 and 19-28 at the dose of 200 mg/kg/day, which can be adjusted on day 19. Two weeks after the last intake of 5-FC, the cycle of TG6002/5-FC treatment can be repeated if there is evidence of benefit for the patient. TG6002/5-FC cycles will be continued until evidence of disease progression, occurrence of toxicity or patient decision to discontinue the treatment.



Blue arrows represent TG6002 infusions, green arrows correspond to 5-FC treatment schedule.

Figure 1: TG6002 and 5-FC treatment schedule in TG6002.02 study

Based on the outcome of the phase I, the study will be extended to a phase IIa part. In this part of the study, patients will receive TG6002 at the RP2D identified in phase I and 5-FC according to the schedule of administration used in phase I (Figure 1). In addition to safety, the efficacy of the combination TG6002/5-FC will be assessed in this part of the study. The efficacy will be

measured by the overall response rate (ORR) and the progression free survival (PFS) in patients with advanced GI tumors who have received the combination of TG6002 with 5-FC.

The patients will be recruited at several clinical sites located in Europe and in the US. The patients will be injected with TG6002 in conventional hospital rooms. The patients will be hospitalised overnight after the first TG6002 infusion and for at least 4 hours after the second and third infusions. Patient safety will be closely monitored by physical examinations, adverse event reporting, clinical laboratory assessments and vital signs and ECG recording throughout the study.

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

Biosafety considerations

According to the Directive 2000/54/EC (2000/54/EC), VV is considered as a group 2 biological agent. The group 2 designation applies to agents that can cause human disease and might be a hazard to workers, that are unlikely to spread to the community and for which there is usually effective prophylaxis or treatment available. Examples of other group 2 biological agents include the measles virus, salmonellae, and the influenza viruses (types A, B and C). VV is also classified as a Biosafety Level 2 (BSL-2) infectious substance by the US Centers for Disease Control and Prevention (CDC, 2009) and as a risk group 2 organism by the US National Institutes of Health Guidelines (NIH).

TG6002 must be used in clinical/laboratory/research activities in accordance with national and/or local regulatory containment level requirements: L1 or L2 containment level, depending on countries, according to site's standard operating procedures and all applicable laws and regulations.

In addition to national and/or local regulatory containment level requirements, site's standard operating procedures and applicable laws and regulations:

- It is requested for the study staff listed hereafter to wear a personal protective equipment (i.e. **waterproof gloves**, **gown**, **surgical/procedure mask** and **safety goggles with side shields**):
    - Staff involved in the preparation of TG6002 injection
    - Laboratory staff handling and performing analyses on biological samples of patients exposed to TG6002
    - Site personnel involved in TG6002 administration to the patients
    - Site personnel involved in patients' care or in direct contact with patients exposed to TG6002
    - Staff involved in the decontamination/cleaning of materials/surfaces/etc which have been in contact with TG6002

- It is requested for the study staff listed hereafter to work under a **class 2 microbiological safety cabinet**:
    - Staff involved in the preparation of TG6002 injection

- Laboratory staff handling and performing analyses on biological samples of patients exposed to TG6002

All transfers of TG6002 contaminated material will be done within a sealed plastic transport bag or other sealed, leak-proof secondary container displaying a clearly marked biohazard symbol. All materials in contact with TG6002 will be decontaminated and/or destroyed according to the instructions described below. Detailed technical descriptions on the handling and preparation of TG6002 will be given to investigators, pharmacists and any other personnel involved in the handling and preparation of TG6002.

Cleaning/decontamination/destruction recommendations

Lipid enveloped viruses, like the vaccinia-derived viruses such as TG6002, are sensitive to inactivation by either physical or chemical methods of disinfection. Heat is the most effective antimicrobial agent (viable counts of a vaccinia virus are reduced 10^7 fold by exposure to 60ºC at ambient pressure within an hour or less). Vaccinia is rendered non-infectious following treatment in an autoclave. Hospital-grade chemical disinfectants containing bleach, aldehydes, alcohols, hydrogen peroxide, phenols, and quaternary ammonium compounds are also effective against lipophilic viruses such as vaccinia. Standard chemical germicides such as Aseptanios Terminal Spore®, Amphospray 41 IP stérile®, Anios Oxy'Floor®, Aniospray SF IP stérile®, Surfa'Safe Premium®, Aniosurf®, Aniosurf Premium®, Rivascop®, Surfanios Premium®, Surfa'Safe®, Surfa'Safe SH® (non-exhaustive list) are adequate for routine cleaning of work areas, when used according to the manufacturers' instructions.

The area used to prepare TG6002 for injection must be cleaned with a standard disinfectant active on vaccinia virus (see above) before and after manipulation.

All disposable material and equipment (e.g. syringes, catheters, needles, gauze, tubing, gloves, bandages, used or unused vials, containers, etc.) that come in contact with TG6002 must be disposed 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 sodium hypochlorite solution before incineration).

Non-disposable material contaminated by TG6002 (e.g. labcoat, goggles, patient gown, bedding, linens, towels) 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).

All patient-care equipment and medical devices (e.g. bedpans, commodes, blood pressure cuffs, oximeters, glucose meters) will be cleaned with a hospital-grade disinfectant before use on another patient, as per universal precautions/routine practices.

Cleaning items such as dishes and utensils with hot water (≥ 71°C) and detergent will be adequate for decontamination.

B3. Risk assessment for the public health

The wild type VV is non-integrative (cytoplasmic localization), replicative, lytic and propagative (able to spread locally to adjacent cells and systematically by release into the blood stream and lymphatic system of infectious particles (Smith G.L. *et al.*, 2002)). The genetic modifications performed on the wild type virus to obtain TG6002 (i.e. *TK* and *RR* deletions and insertion of *FCU1* in the *TK* locus) have limited the replication capability of the virus to actively dividing cells like tumor cells but are not expected to have an impact on the other characteristics of the virus.

Since the safety data available to date with TG6002 is limited, this section provides a risk assessment based on the experience of VV use in the smallpox eradication program, the non-clinical data with TG6002 and the clinical experience of TRANSGENE with two other recombinant VV, JX-594 and TG1031.

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).

As a consequence, patients in the following groups or patients in close physical contact with a person in the following groups must not receive TG6002:

- Severely immunocompromised individuals (e.g. organ transplant recipients, HIV-positive individuals, or those receiving chronic immunosuppressive medication)
- Individuals with history of severe exfoliative skin conditions (e.g. eczema or psoriasis requiring systemic treatment, etc.)
- Pregnant women
- Breastfeeding women
- Children <12 months of age.

Healthcare workers or housekeeping personnel in the following “at risk” groups will be excluded from direct physical contact with TG6002 during drug preparation, should not administer TG6002 or provide direct care to study patients:

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

Vaccination with VV is known to result in the invasion of keratinocytes, causing areas of necrosis and papule/vesicule/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)).

TG6002 non-clinical data: The spreading character of TG6002 is mostly limited to the tumor, the injection site and the skin as demonstrated by the non-clinical data. The *in vitro* evaluation of TG6002 selectivity has evidenced a preference of the virus for tumor cells (see non-clinical part of the IMPD, section 1.2.4). The evaluation of TG6002 biodistribution in immunodeficient mice bearing subcutaneous tumors after a single IV injection has shown that TG6002 had almost exclusive distribution in the tumors (see non-clinical part of the IMPD, sections 2.3 and 2.4). In immunocompetent healthy rabbits which received 3 weekly IV injections, the presence of viral DNA in blood has been limited to a very few number of samples at only one early time point indicating a rapid clearance of the virus (see non-clinical part of the IMPD, section 2.5). In this experiment, there has also been very low and limited spreading from bloodstream to organs with only a few target organs including the injection site, the skin distal to the injection site and the spleen. The major finding of repeated systemic administrations of TG6002 up to 5×10^7 pfu/kg in healthy monkeys has been the induction of dose-related infectious skin papules/vesicles (see non-clinical part of the IMPD, sections 3.5.2 to 3.5.4). These signs have been more pronounced after the first administration than subsequent ones likely due to protective immune responses. In addition, combination with 5-FC had resulted in a better tolerance than TG6002 alone at the same dose level. Quantification of viral DNA in blood has been performed in one of the experiments in the monkeys. Viral DNA could only be quantified in the blood of 2 out 4 monkeys on days 5, 9 and 12 after the first TG6002 IV injection.

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 JX-594 (or Pexa-Vec) : JX-594 is a VV from the Wyeth strain (i.e. the most widely used strain during smallpox vaccination) deleted in TK region and expressing hGM-CSF. It has been administered in over 330 patients with advanced cancer (Breitbach C.J. *et al.*, 2011; Burke J.M. *et al.*, 2012; Heo J. *et al.*, 2012; Heo J. *et al.*, 2011; Heo J. *et al.*, 2013; Hwang T.H. *et al.*, 2011; Mastrangelo M.J. *et al.*, 1998; Park B.H. *et al.*, 2008; Park Y.S. *et al.*, 2012). In its clinical program, this recombinant virus has been administered by single or multiple intratumoral or IV injections at doses up to 3 x 10^9 pfu. JX-594 has shown an acceptable safety profile with the most common side effects being flu-like symptoms, gastrointestinal disorders mainly nausea and vomiting, anorexia, headache, asthenia, pain, dyspnoea, hypotension, injection site reaction, cutaneous viral infection, hypertension, leukocytosis and leukopenia, thrombocytopenia, anemia, hyponatremia, hyper- or hypoglycemia, hyperbilirubinemia and papulo-pustular rashes. None of the serious complications which has occurred during the smallpox vaccination has been observed following treatment with JX-594. In addition, no reports of virus transmission from JX-594 recipients to health care personnel or patient contacts have been made.

In approximately 20% – 25% of patients treated with JX-594 (less than 7% of total treatment administrations) spontaneous skin pustule formation has occurred following treatment. JX-594 related pustules have typically occurred only after the first IV dose. These pustules were vesicular in appearance and similar in size (<5 mm in diameter) to pustules that have been observed after administration of standard (non-attenuated) vaccinia vaccine. These blister-appearing pustules contained JX-594 and usually evolved towards drying, scab formation and fall within 3 weeks. As no ulceration is usually observed, contrary to smallpox vaccine, complete healing is observed without remaining scar. All pustules to date have been self-limited and resolved without complications or the need for specific anti-viral treatment. Onset has been typically within 1 week after an initial IV infusion with resolution within the following 2–3 weeks (consistent with the usual course following intentional vaccination with standard [non-attenuated] vaccinia vaccine).

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

Prevention of virus spread: Efficient measures to prevent spread of the virus in humans comprise frequent hand washing with soap and water or disinfecting agents, proper dressing (e.g. with a non-occlusive bandage or with a gauze and long-sleeved clothing) of the vaccination site and of the skin lesions if any and proper disposal of contaminated dressings (e.g.

contaminated bandages should be placed in sealed plastic bags before trash disposal and contaminated clothes and linen should be decontaminated with routine laundering in hot (≥71°C) water with detergent) (Cono J. *et al.*, 2003; Rotz L.D. *et al.*, 2001; Stark J.H. *et al.*, 2006; Talbot T.R. *et al.*, 2004). These measures will be applied in the proposed clinical trial. A summary of these measures in lay language will be provided to the patients as part of their informed consent process. These measures are conservative and are based on the Centers for Disease Control and Prevention (CDC) recommendations/guidelines[3] for wild type VV including: BSL-2 precautions, contact precautions for in- and out-patient care, and local injection site and/or pustule care (in the event of pustule formation).

The measures taken in the proposed clinical trial which are the basis of the prevention of contamination with TG6002 are:

- Skin pustules will be covered with a non-occlusive bandage. If appropriate, clothing will be worn over the bandaged skin, day and night, to provide a second barrier of protection.
- Frequent hand washing for people who have been in direct contact with TG6002 or contaminated material or skin pustules.
- Working under a class 2 microbiological safety cabinet for the staff involved in the preparation of TG6002 infusion and the laboratory staff handling and performing analyses on biological samples of patients exposed to TG6002.
- Wearing a personal protective equipment (i.e. waterproof gloves, gown, surgical/procedure mask and safety goggles with side shields) for the staff involved in the preparation of TG6002 infusion, the laboratory staff handling and performing analyses on biological samples of patients exposed to TG6002, the site personnel involved in TG6002 administration to the patients, the site personnel involved in patient care or in direct contact with patients exposed to TG6002 and the staff involved in the decontamination/cleaning of materials/surfaces/etc. which have been in contact with TG6002.
- Disposal of contaminated material in accordance with the hospital standard procedure for infectious waste.
- Decontamination/inactivation/cleaning of the non-disposable material.
- The patients treated with TG6002 and study staff in contact with TG6002 must avoid physical contact with people at risk of developing a severe adverse reaction following exposure to VV.
- The patients must use a barrier method of contraception (e.g. condom for either male, female patients or partners of female patients) during TG6002 treatment period and for a minimum of 3 months following the last treatment with TG6002. In addition, to minimize the risk of pregnancy, female patients or female partners of male patients who are of childbearing potential must use an additional effective method of contraception (e.g. one of the following: hormonal contraception, intrauterine device or intrauterine system, or male sterilization).

[3] http://www.bt.cdc.gov/agent/smallpox/vaccination/live-virus.asp
http://www.cdc.gov/mmwr/preview/mmwrhtml/rr5010a1.htm

- The patients will be hospitalized overnight after the first TG6002 injection and at least 4 hours after the 2nd and 3rd TG6002 injections as a precaution to ensure prompt treatment of potential acute toxicities.
- In the extremely unlikely case of a clinically-significant, progressive toxicity that, in the opinion of the investigator could be related to TG6002, the use of vaccinia immune globulin (VIG) should be considered.

To conclude, the deletion of the TK and RR activities, which restricts TG6002 replication to highly dividing cells such as cancer cells, should considerably reduce the pathogenicity and the dissemination of the recombinant virus compared to its parental virus. It is therefore expected that TG6002 safety profile will be acceptable and its transmission potential 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 TG6002. Patients who are administered TG6002 are requested to avoid direct physical contact with anyone in an "at risk" group. In addition, they are asked to use a barrier method of contraception during the study treatment and for at least 3 months after the last TG6002 injection. To prevent spread of the virus, the aforementioned measures will be applied. These precautionary measures should minimize any potential risk for the public health. Furthermore, poxviruses are not endemic in the population. It is therefore unlikely that TG6002 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, nor has there been any virus-induced tumor formation.

B4. Risk assessment for the environment

Therapy with a replicating virus can theoretically lead to shedding of the virus into the environment. VV does not produce a latent infection, so after an initial period with some viral shedding in biological fluids, the virus becomes completely cleared from the host.

In order to investigate routes by which infectious vaccinia could be shed to the environment, urine and oral swab samples were obtained from subjects with different advanced cancers treated with JX-594 by different routes of administration (either IT or IV) and with different schedules of administration (single or repeated administrations). Testing of throat swabs (which sample both cellular and acellular material) demonstrated low-level JX-594 in 30 of 93 patients (32%) between days 4–8 after IV treatment; patients tested were relatively asymptomatic at the time of sampling. It is unclear if the low level of JX-594 detected was the result of swab collection of shed tumor cells and/or epithelial cells infected with JX-594, or free JX-594 itself. The number of infectious units detected was low (<1% of the standard dose). There was no VV detected in throat swab samples assayed at later time points, even in case of additional administrations of JX-594 after the first time point. This confirms that VV does not produce a persistent infection. Testing of patient urine samples (a total of 62 patients at multiple time points) after treatment with JX-594 by either IV or IT route revealed no positive samples.

TG6002 shedding through urine and feces was assessed in the rabbits treated with 3 weekly injections of TG6002 by the IV route on days 1, 8 and 15 (Pablo M.J., 2013). Samples were collected before treatment (baseline), on days 2 and 16 post treatment and on day 90 of the recovery period. No positive samples were detected in feces at any time-point and only 1.3% of urine samples contained quantifiable viral genome on day 16.

The monitoring of TG6002 presence in blood, saliva, urine and feces will be performed in the proposed clinical trial and will help to design tailored instructions to prevent dissemination of the virus and contamination.

In the proposed clinical trial, spills of potentially contaminated biological fluids will be handled according to standard institutional procedures for handling spills of potentially infectious material.

In case shedding or spill would occur and the virus would not be immediately destroyed by decontamination, TG6002 survival in the environment would be extremely low. Indeed viability data obtained with a recombinant VV, Raboral V-RG® (TK-inactivated VV of the Copenhagen strain expressing the glycoprotein G of the rabies virus) in liquid formulation demonstrated that the viral titer decreased within days at 20°C (Pastoret P.P. *et al.*, 1996) and that this viral titer decreased dramatically if the liquid medium in which the VV was stored was not preserved from air (Mahnel H., 1987).

Of note, Raboral V-RG® 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 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.

In the proposed clinical trial, TG6002 will be released at the hospital, in a restricted environment and with specific measures to avoid dissemination of the virus. Instructions will be given to the patient once he/she is discharged from the hospital in order to prevent dissemination at home. These instructions are described in lay language in their informed consent.

In conclusion, the likelihood of TG6002 becoming persistent and invasive in natural habitats is extremely low for the following reasons:

- Due to the inactivation of its *TK* and *RR* genes, TG6002 replicates preferentially in actively dividing cells. TG6002 is therefore expected to propagate mostly in cancer cells.
- TG6002 bringing back its genome up to the structure of its parent would mean that the recombinant virus would eliminate the FCU1 expression cassette inserted in the *TK* gene, recover the *TK* deleted sequence and recover the *RR* deleted sequence. These represent several spontaneous recombination events which are highly improbable. Current genetic stability studies on TG6002 have not detected spontaneous revertants of TG6002. 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 TG6002 with the wild type 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, dispositions have been taken in the proposed 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 the non-attenuated parental virus of TG6002.

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

Replicative and propagative characteristics of VV have been attenuated in TG6002 with the disruption of the *TK* and *RR* genes. VV is non-integrative in human and this phenotype is preserved in TG6002.

Therapy with a replicating virus can theoretically lead to shedding of the virus into the environment, and potentially to the public. TG6002 has been designed to target cancer cells and non-clinical experiments show that it replicates preferentially in actively dividing cells. Virus presence was documented at very low levels in the tails, ovaries, kidneys, lymph nodes, lungs and bone marrow of tumor bearing mice and at very low level and in only a few samples in the urine, at the injection site, in the skin distal to injection site and in the spleen of healthy rabbits. In healthy monkeys, the major toxicology findings were infectious skin lesions, weight loss, hematology and clinical chemistry changes the first two weeks of treatment.

There is no data yet with TG6002 presence in human tissues and excreta. 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 virus-associated toxicity, therapy could be initiated with VIG to circumvent any public health risk.

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

In conclusion, with the preventive measures which will be applied in the proposed clinical trial, the GMO TG6002 is not considered to represent a risk for the environment and for the public health.

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