TG4010

Annex II

ENVIRONMENTAL RISK ASSESSMENT

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LIST OF ABBREVIATIONS

CTL	Cytotoxic T lymphocyte
CVA	Chorioallantois Vaccinia virus Ankara of donkey
DNA	Deoxyribonucleic acid
GMO	Genetically modified organism
ID	Intradermal
IL2	Interleukin-2
IM	Intramuscular
IP	Intraperitoneal
IT	Intratumoral
IV	Intravenous
MUC1	Mucine 1
MVA	Modified Vaccinia virus of Ankara
MVATG9931	Recombinant vector
PFU	Plaque forming unit
SC	Subcutaneous
TG4010 or MVA-MUC1-IL2	Viral suspension of MVATG9931

A. INTRODUCTION

The final product, TG4010, is a viral suspension of the recombinant vector MVATG9931, considered as the active ingredient and the genetically modified organism (GMO). MVATG9931 is a recombinant vaccinia virus, from the highly attenuated Modified Vaccinia virus Ankara (MVA) strain, containing nucleotide sequences coding for human mucin 1 (MUC1) and interleukin-2 (IL2).

TG4010 is developed to treat cancer patients whose tumors express the MUC1 antigen. The MUC1 protein is a highly glycosylated mucin normally found at the apical surface of mucin-secreting epithelial cells in many types of tissue. In cancer cells several modifications occur. MUC1 is over expressed by tumor cells and is less glycosylated than in normal cells. The other protein whose sequence is coded by the vector is human IL2, an immune modulator that can stimulate immune response and particularly can activate cytotoxic T lymphocyte (CTL).

B. CHARACTERISTICS OF THE GMO AND RELEASES

B1. CHARACTERISTICS OF THE GMO

The parental virus of the MVATG9931 vector, the MVA, is an attenuated vaccinia virus developed by Mayr and Stickl during the course of the smallpox eradication campaign. MVA (Mayr A. *et al.*, 1975), derived from CVA (Chorioallantois Vaccinia virus Ankara of donkey), was specifically developed to immunize high risk patients (children under the age of three and individuals with a history of eczema) against smallpox. It has been tested in a variety of animal species and been used in primary vaccination in more than 120,000 children and adults (Mayr A. *et al.*, 1975; Mayr A. *et al.*, 1978; Stickl H. *et al.*, 1974).

MVA is a member of the Poxviridae family (genus Orthopoxvirus). The attenuated phenotype of the MVA results from alterations of the MVA genome, which has been entirely sequenced (Antoine G. *et al.*, 1998). In comparison with the parental virus (CVA strain) or the Western Reserve strain, structural analysis of the MVA genome reveals 6 major deletions resulting in the loss of 30 kb or 16% of its genetic information (Altenburger W. *et al.*, 1989; Meyer H. *et al.*, 1991).

The advantages of a vaccinia virus combined with reduced pathogenicity make MVA an attractive expression vector (Goossens M. *et al.*, 2013). These advantages include:

- Host cell restriction and low replicativity in human cells: MVA grows well in avian cells but is unable to replicate in human and most other mammalian cells tested (Carroll M.W. and Moss B., 1997) (Verheust C. *et al.*, 2012). The strain is highly attenuated due to 6 major deletions in its DNA as described above. Revertance of MVA to a replicative phenotype is expected to be highly unlikely because MVA's replication restriction and attenuation is most probably based on a multitude of missing or only partly functional gene products (Meisinger-Henschel C. *et al.*, 2010). Repair of multiple genes is required to fully restore the ability of MVA to replicate efficiently in human cells. This results in the inability to detect spontaneous revertants (Wyatt L. *et al.*, 1998).
- High yield of protein expression: Despite its non-replicative phenotype, the MVA can achieve high levels of protein expression in human host cells. The block in non-permissive cells occurs at a late stage of the replication cycle. Biochemical and electron microscopic analyses of HeLa cells infected with MVA indicated that viral late gene expression occurs but that virion morphogenesis is interrupted (Sutter G. and Moss B., 1992). In contrast with other host-range vaccinia virus mutants where viral protein

synthesis is inhibited soon after infection of non-permissive cells, recombinant vaccinia viruses of the MVA strain are able to produce high levels of non-host proteins in human cells (Chang D.Y. *et al.*, 1995; Gillard S. *et al.*, 1985; Njayou M. *et al.*, 1982; Ramsey-Ewing A.L. and Moss B., 1996). For reference, the expression levels of recombinant proteins obtained with MVA are similar to those yielded by recombinant vaccinia viruses of the WR strain (Sutter G. and Moss B., 1992).

- Non-integrative: Virus genome expression does not require host cell genome integration. As the virus remains in the cytoplasm (Moss B., 1990; Schramm B. and Locker J.K., 2005), there is no risk of insertional mutagenesis. This characteristic of the MVA vector eliminates the risk of host cell transformation, such as the acquisition of a malignant phenotype, resulting of ectopic chromosomal insertion of DNA.

- Non-propagative: MVA is no longer able to generate infectious particles (Meyer H. *et al.*, 1991) (Sutter G. and Moss B., 1992). Numerous studies have documented that MVA propagates poorly in all human continuous cell lines tested (Blanchard T.J. *et al.*, 1998), (Mayr A., 1966) and does not propagate in primary human fibroblasts or in primary peripheral blood mononuclear cells (PBMC), dendritic cells, and monocytes (Drexler I. *et al.*, 1998). It has also been shown in primary macrophages that wild-type vaccinia virus (strain WR) is blocked in several late stages of viral infection, including replication of viral DNA and production of infectious progeny virions (Broder C.C. *et al.*, 1994). Thus, it is likely that MVA is also defective in these stages of its viral lifecycle in this cell type.

- Low pathogenicity: MVA is not an animal pathogen. MVA was successfully administered by different routes (SC, IM and intraperitoneal [IP] routes) in different species (mice, piglets, calves, dogs, cats, macaques and elephants) without significant side effects (Hochstein-Mintzel V. *et al.*, 1972; Mayr A. and Danner K., 1979) (Mayr A. *et al.*, 1975; Mayr A. *et al.*, 1978). In animal studies in rabbits, mice and monkey, the virulence of the MVA strain was clearly reduced in comparison with the Elstree strain used for the production of smallpox vaccine (Hochstein-Mintzel V. *et al.*, 1975). It is also not virulent in immunosuppressed animals (irradiated mice and rabbits) (Mayr A. *et al.*, 1978) (Werner G.T. *et al.*, 1980). Besides, MVA is not pathogenic in adult birds (Mayr A., 1966). More recently, animal studies have shown that MVA is both safe and immunogenic in healthy and immunosuppressed mouse and macaque models (Earl P.L. *et al.*, 2004) (McCurdy L.H. *et al.*, 2004).

- Good safety profile and high immunogenicity: In the literature, multiple human clinical trials using Vaccinia derived vectors have been reported, and MVA alone has been administered to >100,000 volunteers. MVA was successfully used without significant side effects in human in the context of the German smallpox vaccination campaign in the 70's (Mayr A. et al., 1975) (Stickl H. et al., 1974) (Mayr A. et al., 1978). Only mild or moderate side effects were associated with the use of this vaccine: local reaction (redness) which demonstrated that subjects responded to the immunization, fever (in ~2 % of vaccinees), 'flu-like' symptoms (in ~ 4 % of vaccinees) (Mahnel H. and Mayr A., 1994) (Mayr A. *et al.*, 1978) (Stickl H. *et al.*, 1974). More recently, MVA safety and immunogenicity were evaluated at different doses and different routes of administration in vaccinia-naïve and vaccinia-immune volunteers. MVA was safe, and the immune response was dose-dependent (Vollmar J. et al., 2006). The vector was also evaluated as an alternative to Dryvax® in vaccinia-naïve and immune adult volunteers. Subjects received MVA or placebo by the IM route followed by Dryvax® challenge at 3 months.

MVA vaccination was shown to be safe and immunogenic and improved the safety and immunogenicity of subsequent Dryvax® vaccination (Parrino J. et al., 2007), even in immunocompromised individuals such as HIV-subjects (Greenberg R.N. *et al.*, 2013). The safety and immunogenicity of MVA as a smallpox vaccine was also investigated in a controlled phase I clinical trial in healthy subjects (n = 15) and in subjects with either mild allergic rhinitis, an history of atopic dermatitis (AD) or presenting with mild AD (von Sonnenburg F. *et al.*, 2014). MVA was given by SC injections at day 0 and week 4. MVA was shown to be equally well tolerated and immunogenic in all enrolled subjects with mild to moderate pain and redness at the injection site being the most frequent adverse reactions. There were no differences in the safety and immunogenicity profile of MVA in healthy subjects or those with AD or allergic rhinitis. The cardiac safety of one or two doses of MVA compared to placebo was recently assessed in 745 healthy subjects (Zitzmann-Roth E.M. *et al.*, 2015). Vaccinia-naïve subjects received either one dose of MVA and one dose of placebo, two doses of MVA, or two doses of placebo by SC injection four weeks apart; vaccinia-experienced subjects received a single dose of MVA. In this clinical trial, the vaccination with MVA was shown to be safe, well tolerated and did not increase the risk for development of myo-/pericarditis. Finally, a similar MVA construct developed by TRANSGENE (MVA-HPV-IL2) was administered to a total of 313 subjects and overall no safety concerns were raised. Cardiac safety was specifically monitored in 202 subjects included in a phase I and in a Phase II and no clinically significant safety concern was raised.

- No pre-immunity impact: Attenuated MVA can be used as an immunizing agent under conditions of pre-existing immunity to the vector (Ramirez J.C. *et al.*, 2000).
- Large vectorization capacity: MVA is able to carry up to 10 kb of insert DNA (Jolly D., 1994) and to produce efficiently and in large quantity recombinant viruses.

The resulting GMO TG4010 consists of the elements listed in Table I.

Table I: Genetics elements of the expression cassette of MVATG9931

Nature of the elements	**Scientific Name**	**Taxonomy**	**Comment**
Parental organism	BRG2	Pox virus MVA strain	Flanking sequences (left part) surrounding the deletion II of the MVA genome
Promoter	p7.5	Pox virus MVA strain	p7.5 vaccinia virus early late promoter
Gene of interest	Hu-IL2	Human gene	Human IL2 cDNA
Gene of interest	MUC1 synth (orETAtm.5repsynt.)	Synthetic gene	Sequence coding for the synthetic MUC1 anchored human mucin
Promoter	pH5R	Pox virus MVA strain	pH5R vaccinia virus early late promoter
Parental organism	BRD2	Pox virus MVA strain	Flanking sequences (right part) surrounding the deletion II of the MVA genome

Since no viral sequences are eliminated during assembly of the passenger gene with the MVA strain to make up the MVATG9931, and no essential viral functions are destructed at the insertion site, it is likely that the overall MVA viral phenotype is preserved in the MVATG9931 vector constituting the product TG4010.

B2. CHARACTERISTICS OF THE RELEASE

TG4010 is a therapeutic GMO and will be released through the administration of the product, in a hospital or clinic setting, by subcutaneous (SC) injection to patients as a part of the proposed multinational, multicenter clinical trial. There are no foreseen products of this release.

The TG4010.24 clinical trial is planned to enroll 39 patients in the EU as well as non-EU countries.

All transfers of the preparation will be done using a closed container. For the manipulations, goggles, laboratory coat, gloves and mask must be worn. All materials in contact with TG4010 will be decontaminated and/or destroyed according to regular hospital procedure for infectious wastes.

All transport of product (vial or syringe containing the dose to be injected) must be done using a leakproof container/bag. During product manipulations, labcoat, goggles, gloves and mask must be used.

B3. RISK ASSESSMENT FOR THE PUBLIC HEALTH

As the parental MVA virus, the GMO TG4010 is poorly replicative (can replicate its DNA to express the transgene coding sequence), non-propagative (no longer able to generate infectious particles) and non-integrative (cytoplasmic localization). The GMO presents a host range severely restricted. It grows well in avian cells as well as in baby hamster kidney cells but is unable to propagate in human and most other mammalian cells tested (Carroll M.W. and Moss B., 1997).

- Non-spreading character of MVA related vectors:

Once the patient's cells are infected, the GMO TG4010 as the parental MVA virus is expected to stay localized at the injection site. Non-clinical biodistribution data of recombinant MVA vectors developed by Transgene confirmed its non-spreading character with the absence of significant internal dissemination after IM or SC injection, the MVA vector being mainly localized at the injection site (internal study report). Furthermore, the viral shedding data obtained so far from more than 100 patients treated with TG4010 (n=94) or other Transgene MVA related vectors (n=60) showed the absence of MVA viral DNA in blood and urine. Swabbing of the injection site was also performed in a subset of patients (n=10) exposed to TG4010 in study TG4010.14. It showed in the worst-case presence of quantifiable MVA DNA at low dose short time after the injection (maximum: 0.6% of the injected dose 6 h after the injection) which was non-persistent (i.e.: no sample with quantifiable MVA DNA several days after injection) (internal study report). Besides, data from another MVA-based product developed by Transgene (TG4001 – MVA-HPV-IL2) showed that viral DNA concentration in tissue compartments (biopsies) after a single SC administration at $5x10^7$ PFU is very low (internal study report).

- Acceptable safety profile of TG4010 and MVA related vectors:

The toxicity profile of TG4010 was investigated in mice, rat and in rabbit following single and/or repeated administrations. TG4010 was well tolerated in a single intravenous (IV) dose

toxicity study in rat at a dose level representing 25-fold the human dose. A repeated dose toxicity study in rat at two doses levels covering respectively 1.5 and 15 times the maximal human dose by IM route did not evidence target organs for potential toxicity. The local tolerance at the injection site was assessed in a study in rabbits. Minor local reactions, which are frequently observed following administration of vaccines, were reported at the injection sites. An additional SC toxicity study using an improved manufacturing process (CF10 Process 4) at a dose level corresponding to 1000-fold the human dose and given over a 3-month period did not reveal any additional toxicity.

Based on these observations, TG4010 is considered to have a favorable safety profile.

More generally, the safety profile of SC injections of vaccinia virus is well-known. Wild-type vaccinia virus and MVA may cause local reactions including erythema, edema and systemic reactions such as fever and malaise, as has been observed with conventional vaccination to smallpox (Vollmar J. *et al.*, 2006), (Mahnel H. and Mayr A., 1994) (Mayr A. *et al.*, 1978) (Stickl H. *et al.*, 1974). Studies of smallpox vaccines have identified cardiac injury including pericarditis and myocarditis as a potential risk (Eckart R.E. *et al.*, 2004). However, this complication has not been observed with MVA or MVA-based vaccines in many thousands of individuals.

More specific to recombinant MVA vectors, safety data have been collected in the previous clinical trials performed with TG4010 and several clinical trials conducted with other MVA-based vectors developed by Transgene. A total of 380 patients received IM or SC injections of TG4010 and more than 800 patients were administered by SC, IM or intratumoral (IT) route with Transgene MVA based vectors with acceptable safety profiles. To date, the most frequent observed adverse events considered by investigators as possibly or probably related to TG4010 are injection site reactions (including injection site erythema, pain, induration, inflammation), fatigue and pyrexia. Those are generally of mild to moderate intensity.

Also, because TG4010 is produced in chicken embryo fibroblasts and its manufacturing process involves the use of gentamicin, there is a potential risk of hypersensitivity reaction in individuals exposed to this product who have a history of allergy to eggs and to gentamicin.

To conclude, due to the observed low dissemination of vaccinia virus vectors, a contamination of healthcare staff and patient's family is unlikely. Should it occur, and considering that only a fraction of the administered dose would participate in this event, its effect is not expected to be toxic given the safety of such viral vectors already observed from the clinical studies conducted in patients.

For the people in charge of the manipulation of TG4010 GMO, some precautions are to be taken:

- Less attenuated productively replicating vaccinia virus strains have led to permanent eye damage after topical cornea or conjunctiva contact. There is no evidence that MVA or TG4010 could induce similar lesions. Nonetheless, when conducting operations with the risk of formation of aerosols or splashes of TG4010, safety goggles must be worn.
- Exposure of irritated skin with TG4010 vector may result in contamination with the vector, but as explained above, no systemic clinical sequelae are expected as the vector is not replication competent in human cells. Nonetheless, when conducting operations with the risk of formation of aerosols or splashes, a standard laboratory coat and gloves should be worn.

B4. RISK ASSESSMENT FOR THE ENVIRONMENT

Considering the characteristics of the MVA virus, the risk of dissemination in the environment is unlikely. Viral shedding data collected up to now from previous clinical studies with TG4010 and other Transgene's recombinant MVA vectors confirmed the absence of dissemination. More than 100 patients (n=154) were monitored for the presence of viral DNA in blood and urine after the injection of the recombinant MVA vectors administered either by IM or SC routes at equivalent dose levels. There was no detection of viral DNA in the samples. The spreading of the vector remains confined to the injection site.

As mentioned above, MVA replicates poorly in most mammalian cells but well in avian cells such as chicken embryo fibroblasts. Its propagation is restricted to avian cells, virus replication being blocked late in morphogenesis in non-permissive cells. Even though MVA virus is able to replicate in avian cells, it is not pathogenic in adult birds (Mayr A., 1966). Besides, MVA is a potent inducer of type I interferon from primary human cells which may restrict virus spread *in vivo* (Blanchard T.J. *et al.*, 1998).

There are no known or predicted environmental conditions which may affect survival, multiplication and dissemination of the GMO TG4010. The wild type vaccinia virus is not naturally found in the environment and therefore, recombination events cannot occur with the GMO.

Should wild type vaccinia virus be present in the environment, genetic recombination events allowing MVA virus to bring back its genome up to the structure of its parental itself are unlikely because it requires several independent mutations, including restorations of the deleted regions of the genome. This phenomenon has never been observed during smallpox vaccination in humans, and a mechanism able to cause and select for an event of such a magnitude is hardly conceivable. Furthermore, the findings of L. Wyatt *et al* (Wyatt L.S. *et al.*, 1998) have shown that repair of multiple genes is required to fully restore the ability of MVA to replicate efficiently in human cells. That is consistent with the inability to detect spontaneous revertants and supports the safety of MVA as a vaccine and gene therapy vector.

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

The foundation of the risk analysis for this type of product is the fact that the MVA strain is poorly replicative, non-propagative and non-integrative in human. This phenotype is preserved in TG4010 vector. Furthermore, such recombinant MVA vectors are known for their non-spreading character and their acceptable safety profile.

Therefore, the TG4010 GMO (viral suspension of MVATG9931) is considered not to represent a risk for the environment and for the public health.

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