Bioveiligheidsraad
Conseil de Biosécurité

05 September 2017

Secretariaat
Secrétariat

O./ref.: WIV-ISP/41/BAC/17_0676

Title: Advice of the Belgian Biosafety Advisory Council on the notification B/BE/17/BVW1 of the company Transgene for deliberate release in the environment of genetically modified organisms other than higher plants for research and development

Context

The notification B/BE/17/BVW1 has been submitted by Transgene to the Belgian Competent Authority in June 2017 for a request of deliberate release in the environment of genetically modified organisms other than higher plants for research and development according to Chapter II of the Royal Decree of 21 February 2005.

The planned activity concerns a clinical trial and the title of the notification is: **"A Phase II study evaluating the efficacy and the safety of first-line chemotherapy combined with TG4010 and nivolumab in patients with advanced non-squamous Non-small Lung Cancer".** The primary objective of the study is to evaluate the anti-tumor activity and the safety profile in patients treated with TG4010, nivolumab and first-line chemotherapy. TG4010 is a highly attenuated strain of the *Vaccinia* virus genetically modified to express the genes of the human mucin 1 antigen (MUC1) and of the human interleukin 2 (hIL2).

The product TG4010 is administered sub-cutaneously weekly for 6 weeks then every 3 weeks thereafter until progression of the disease. Nivolumab will be administered every three weeks until disease progression. The GM virus is deemed unable to replicate in human cells but the virus may be found on the wound dressing covering the injection site up to the lysis of the infected cells. Given the dose schedule it is not possible for the subjects to remain within contained facilities for the duration of the study. In Belgium, one clinical site in Wallonia is planning to recruit 5 patients. The national territory is considered as the potential release area of TG4010.

The dossier has been officially acknowledged by the Competent Authority on 04 July 2017 and forwarded to the Biosafety Advisory Council for advice.
Within the framework of the evaluation procedure, the Biosafety Advisory Council, under the supervision of a coordinator and with the assistance of its Secretariat, contacted experts to evaluate the dossier. Two experts from the common list of experts drawn up by the Biosafety Advisory Council and the Biosafety and Biotechnology Unit (SBB) answered positively to this request. The SBB also took part in the evaluation of the dossier. It is noted that TG4010 has

already been assessed by the BAC in the framework of a previous notification (B/BE/11/BVW1)[1].
The experts and the SBB assessed whether the information provided in the notification was sufficient and accurate in order to state that the deliberate release of the genetically modified organism for its intended use, would not raise any problems for the environment, animal health or human health (people coming in contact with the treated patient and/or with the GMO).

For the purpose of this evaluation, the following legal basis has been considered:
- Annex II (principles for the risk assessment) and annex III (information required in notifications) of the Royal Decree of 21 February 2005.
- Commission Decision 2002/623/EC of 24 July 2002 establishing guidance notes supplementing Annex II to Directive 2001/18/EC.
The pure medical aspects concerning the efficacy of the medicinal product and its safety for the treated patient, as well as aspects related to social, economical or ethical considerations, are outside the scope of this evaluation.

In parallel to the scientific evaluation of the notification, the Competent Authority also made the dossier available on its website for the one-month public consultation foreseen in the abovementioned Royal Decree. The Competent Authority didn't receive any reaction of the public relevant for the environmental and/or public health safety of the GMO.

Summary of the Scientific evaluation

1. The characteristics of the donor, the recipient or parental organism

The donor, recipient and parental organisms have been adequately described in the dossier. It is noticed that the notifier adequately implemented the remarks of the BAC that were issued in the framework of the previous dossier (B/BE/11/BVW1).

2. Information related to the characteristics of the GMO and of the medication

Referring to its previous advice on the notification B/BE/11/BVW1, the Biosafety Advisory Council is of the opinion that the MVA vector may be classified as risk class 1 but the inserted genes – MUC-1 and IL-2 – take it to risk class 2. The adverse effects observed in patients treated with subcutaneous TG4010 are injection site and/or skin reactions (erythema, pain, induration, and inflammation), fatigue, pyrexia, influenza-like illness, erythema and myalgia. These side effects are probably related to IL2. In addition potential autoimmune toxicity from TG4010 related to cross reactivity with natural MUC1 protein cannot be excluded. This justifies measures to prevent accidental vaccination of health care personnel handling and administering the product.
Based on the information provided by the applicant, the Biosafety Advisory Council has no further remarks.

[1] Advice on the notification B/BE/11/BVW1 of 30 September 2011 (ref BAC_2011_0906) – see Annex 2

3. The condition of release

The primary hazards when manipulating MVA vectors consist in ingestion, droplet or aerosol exposure of mucous membrane or broken skin.
In the framework of the evaluation of the notification B/BE/11/BVW1 the BAC had several criticisms relating to the measures to prevent inadvertent release of the GMO and the instructions prepared for the personnel. These remarks were taken into account by the notifier in the current notification. For example, the wearing of gloves, a google and a mask is mandatory according to to preparation procedure for personnel. In addition, the applicant provided instructions for the GMO product (vial or syringe containing the dose to be injected) to be transported in leakproof container/bag labelled with a biohazard sign. Furthermore, personnel involved in the handling of the GMO will be informed about the procedure to implement in case of skin or eye contamination.

4. Potential risks for the environment, animal or human health associated with the release of the GMO

One expert remarked that the applicant did not discuss the impact of the additional application of nivolumab compared to the application of TG4010 alone with respect to the risk for the environment and human health. Nivolumab, a monoclonal anti-PD-1 antibody, works as a checkpoint inhibitor, blocking a signal that would have prevented activated T cells from attacking malingnant cells. The use of PD-1 inhibitors has been approved by the US FDA as novel immunotherapy to aid the immune system to clear a variety of malignancies. TG4010 is a recombinant modified Vaccinia Ankara virus, which is a highly attenuated strain of vaccinia virus that has lost about 10% of the vaccinia genome and with it the ability to replicate efficiently in primate cells. MVA strain is poorly replicative, non-propagative (spreading from the injection site is unlikely) and non-integrative in human. Along these considerations, the Biosafety Advisory Council is of the opinion that there are no evidences pointing to an impact of the monoclonal anti-PD-1 antibody on the ability of replication of TG4010 in human cells.

It was further noted that the notifier took into account the remarks of the BAC addressed in the context of the notification B/BE/11/BVW1 in regards biodistribution studies. In its risk assessment, the applicant also included new viral dissemination data performed during the TG4010.14 study investigating the presence of viral DNA at the injection site.

5. The monitoring, control, waste treatment and emergency plans proposed by the applicant

Compared to previous notification (B/BE/11/BVW1), no further blood samples will be collected nor additional viral dissemination data will be collected during the clinical trial as animal studies confirmed non-spreading character of the GMO and no viral shedding outside the injection site was shown in human injected with the GMO. The Biosafety Advisory Council further agrees with the proposed procedures, including those described in the technical sheet (fiche technique).

Conclusion

Based on the scientific assessment of the notification made by the Belgian experts, taking into account the previous advice on notification B/BE/11/BVW1 and considering the data presently available the Biosafety Advisory Council concludes that it is unlikely that the genetically modified *Vaccinia* virus (TG4010) genetically modified to express MUC1 and hIL2 and developed as a therapeutic vaccination for the treatment of lung cancer, here administered in combination with nivolumab, will have any adverse effects on human health or on the environment in the context of the intended clinical trial.

Therefore, the Biosafety Advisory Council issues a **positive advice with the following conditions**:

- The notifier and the investigators must strictly apply the clinical trial protocol and all the safety instructions as described in the dossier.
- Any protocol amendment has to be previously approved by the Competent Authority.
- The notifier is responsible to verify that each study centre has qualified personnel experienced in handling infectious material and that the investigator has the required authorisations to perform the clinical trial activities inside the hospital (laboratory, pharmacy, hospital room, consultation room...) according to the Regional Decrees transposing Directive 2009/41/EC on Contained use of genetically modified micro-organisms.
- For the transport of the IMP the notifier should conform to the transportation rules regarding transport of GMOs
- The Biosafety Advisory Council should be informed within 2 weeks when the first patient starts the treatment and the last subject receives the last treatment.
- At the latest six months after the last visit of the last patient included in the trial, the notifier must send to the competent authority at the attention of the Biosafety Council a report with details concerning the biosafety aspects of the project. This report will at least contain:
    - the total number of patients included in the trial and the number of patients included in Belgium;
    - a summary of all adverse events marked by the investigators as probably or definitely related to the study medication;
    - a report on the accidental releases, if any, of the recombinant *Vaccinia* virus.

Prof. M. De Proft
President of the Belgian Biosafety Advisory Council

Annex 1: Compilation of comments of experts in charge of assessing the dossier B/BE/17/BVW1 (ref: BAC_2017_0639)

Annex 2 : Advice on the notification B/BE/11/BVW1 of 30 September 2011 (ref BAC_2011_0906)

Bioveiligheidsraad
Conseil de Biosécurité

23 August 2017

Secretariaat
Secrétariat

O./ref.: WIV-ISP/41/BAC_2017_0639
Email: bac@wiv-isp.be

Compilation of comments of experts in charge of assessing the dossier B/BE/17/BVW1

Mandate for the Group of Experts: Mandate of the Biosafety Advisory Council (BAC) of 28 June 2017
Coordinator: Prof. Jozef Anné (KUL)
Experts: Jean-Claude Twizere (ULg), Anton Roebroek (KUL), Aline Baldo (WIV-ISP, SBB)
Domains of expertise of experts involved: Molecular genetics, virology, biosafety, contained use
Secretariat (SBB): Didier Breyer, Fanny Coppens, Katia Pauwels

INTRODUCTION

Dossier **B/BE/17/BVW1** concerns a notification of the company Transgene for deliberate release in the environment of genetically modified organisms other than higher plants according to Chapter II of the Royal Decree of 21 February 2005.
The notification has been officially acknowledged on 4 July 2017 and concerns a clinical trial entitled: **"A Phase II study evaluating the efficacy and the safety of first-line chemotherapy combined with TG4010 and nivolumab in patients with advanced non-squamous Non-small Lung Cancer".** The primary objective of the study is to evaluate the anti-tumor activity and the safety profile in patients treated with TG4010, nivolumab and first-line chemotherapy. TG4010 is a highly attenuated strain of the *Vaccinia* virus genetically modified to express the genes of the human mucine 1 antigen (MUC1) and of the human interleukin 2 (hIL2).
The product TG4010 is administered subcutaneously weekly for 6 weeks then every 3 weeks thereafter until progression of the disease or death or premature discontinuation due to any reason whichever occurs first. Nivolumab will administered every three weeks until disease progression or death or premature discontinuation due to any reason or for a maximum of 24 months whichever occurs first
The use of TG4010 has already been notified and assessed by the Biosafety Advisory Council in the framework of a previous dossier (B/BE/11/BVW)[1].

♦ INSTRUCTIONS FOR EVALUATION

Depending on their expertise, the experts were invited to evaluate the genetically modified organism considered in the notification as regards its molecular characteristics and its potential impact on human health and the environment. The pure medical aspects concerning the efficacy of the medicinal product and its safety for the treated patient are outside the scope of this evaluation.

[1] Advice on the notification B/BE/11/BVW1 of 30 September 2011 (ref BAC_2011_0906)

The comments of the experts are roughly structured as in
- Annex II (principles for the risk assessment) of the Royal Decree of 21 February 2005
- Annex III (information required in notifications) of the Royal Decree of 21 February 2005
- Commission Decision 2002/623/EC of 24 July 2002 establishing guidance notes supplementing Annex II to Directive 2001/18/EC.

List of comments received from the experts

1. INFORMATION RELATED TO THE CHARACTERISTICS OF THE DONOR, THE RECIPIENT OR PARENTAL ORGANISM

(e.g. possibility of natural transfer of genetic material to other organisms, pathological, ecological and physiological characteristics, indigenous vectors ...)

Comment 1
No questions/comments

Comment 2
Minor comment: Please verify the format of the restriction map (annexe III, page 21, figure 1).

Comment 3
No questions/comments

2. INFORMATION RELATED TO THE VECTOR

(e.g. description, sequence, mobilisation ...)

Comment 1
No questions/comments

Comment 2
No questions/comments

3. INFORMATION RELATED TO THE CHARACTERISTICS OF THE GMO

3.1. Information related to the genetic modification

(e.g. methods used for the modification, description of the insert/vector construction ...)

Comment 1
No questions/comments

Comment 2
No questions/comments

Comment 3
No questions/comments

3.2. Information on the molecular characteristics of the final GMO

(e.g. number of copies of the transgenes, phenotypic and genetic stability of the transgenes, expression of the new genetic material, re-arrangements in the genome, inclusion or suppression of genetic material ...)

Comment 1
No questions/comments

Comment 2
No questions/comments

3.3. Considerations for human, animal or plant health

(e.g. invasiveness and virulence, toxic or allergenic effects, possibility of survival outside of receiving host, other product hazards ...)

Comment 1
No questions/comments

Comment 2
No questions/comments

Comment 3
No questions/comments

4. INFORMATION RELATING TO THE CONDITION OF RELEASE

(e.g. description of the activity, quantities of GMO to be released, workers protection measures, elimination of any contaminating material in the preparation of the GMO stock, elimination of the GMO at the end of the experiment ...)

Comment 1
No questions/comments

Comment 2
No questions/comments

Comment 3
The medical staff should wear closed shoes in order to be protected against sharp and syringes that fall. The internal transport of the vials containing the TG4010 should be performed in a hermetic transport box containing absorbent paper towels.

Comment coordinator
In the technical sheet and in other parts of the dossier it is mentioned that for all TG4010 handlings, lab coat, goggles, gloves and mask must be worn and that all transport of TG4010 (vial or syringe containing the dose to be injected) must be done using a leakproof container/bag with on the outside a biohazard sign.

5. INFORMATION RELATED TO THE RISKS FOR THE ENVIRONMENT AND HUMAN HEALTH

5.1. Information on spread ("shedding") of the GMO from the treated patient/animal to other persons/animals or to the environment (including indirect/delayed effects due to vertical transmission to offspring).

(e.g. genetic transfer capability, routes of biological dispersal, target organisms ...)

Comment 1 **(Roebroek)**
No questions/comments

Comment 2 **(Twizere)**
No questions/comments

Comment 3 **(Baldo)**
No assessment is made concerning the potential impact of the combined application of TG4010 and nivolumab compared to application of TG4010 alone with respect to the risk for the environment and human health. The additional application of nivolumab should be discussed and evaluated in comparison with application of TG4010 alone. It should be discussed that additional application of nivolumab is not expected to have any impact at all on the replication of TG4010 in human cells and subsequent shedding etc. and subsequently on the risk for the environment and human health in comparison to TG4010 alone.

Comment coordinator :
Nivolumab works as a checkpoint inhibitor, blocking a signal that would have prevented activated T cells from attacking the cancer, thus allowing the immune system to clear the cancer. On the other hand *Modified Vaccinia Ankara* (*MVA*') virus, is a highly attenuated strain of vaccinia virus that has lost about 10% of the vaccinia genome and with it the ability to replicate efficiently in primate cells. MVA strain is poorly replicative, non-propagative and non-integrative in human. MVA is no longer able to generate infectious particles.
Therefore I do not see how nivolumab could have an impact on the ability of replication of the virus.

5.2. Information on possible effects on human health resulting from interactions of the GMO and persons working with, coming into contact with or in the vicinity of the GMO release (carekeepers, patient relatives, immunocompromised people ...).

Comment 1
No questions/comments

Comment 2
No questions/comments

Comment 3
No questions/comments

5.3. Information on possible effects on animal health or on the environment.

Comment 1
No questions/comments

Comment 2
No questions/comments

Comment 3
No questions/comments

5.4. Information on selective advantages or disadvantages conferred to the GMO compared to the parental organism.

Comment 1
No questions/comments

Comment 2
No questions/comments

5.5. Information on the possibility of the GMO to reconvert to his wild type form and possible consequences for human health or the environment.

Comment 1
No questions/comments

Comment 2
No questions/comments

Comment 3
No questions/comments

5.6. Information on the possibility of the GMO to exchange genetic material with other micro-organisms and possible consequences for human health or the environment.

Comment 1
No questions/comments

Comment 2
No questions/comments

Comment 3
No questions/comments

5.7. Information on the possibility of gene transfer to other organisms and about the selective advantages or disadvantages conferred to those resulting organisms (possible consequences for human health or the environment).

Comment 1
No questions/comments

Comment 2
No questions/comments

Comment 3
No questions/comments

6. INFORMATION RELATED TO THE MONITORING, SURVEILLANCE AND CONTROL, WASTE TREATMENT AND EMERGENCY PLANS PROPOSED BY THE APPLICANT

6.1. Monitoring plan proposed by the notifier and possibility to identify the occurrence of non-anticipated adverse effects.

(adequation between the monitoring plan and risks identified during the risk assessment, when appropriate measures to minimize the potential risks to offspring ...)

Comment 1
No questions/comments

Comment 2
No questions/comments

6.2. Surveillance and control of the release

(adequation between the procedures to avoid and/or minimise the spread of the GMO and risks identified during the risk assessment...)

Comment 1
No questions/comments

Comment 2
No questions/comments

6.3. Information on the waste generated by the activity and its treatment.

(e.g. type of waste, amount ...)

Comment 1
No questions/comments

Comment 2
No questions/comments

6.4. If applicable, information on the emergency plan(s) proposed by the notifier.

Comment 1
No questions/comments

Comment 2
In case of accidental spills or breakage of a vial containing the GMO, the medical staff should alert people in the area of the spill, remove contaminated clothes and leave the area for 30 min. He should close the area and post “DO NOT ENTER”. After 30 min, he must wear a clean lab coat and wear gloves, glasses and a mask in order to clean the area.

Comment coordinator :

A procedure for handling accidental spills is described in the technical sheet and elsewhere in the dossier.

6.5. Information related to the identification of the GMO and the detection techniques
(e.g. identification methods and detection techniques, sensitivity, reliability and specificity of the proposed tests ..)

Comment 1
No questions/comments

Comment 2
No questions/comments

7. OTHER INFORMATION

7.1 Do you have any other questions/comments concerning this notification that are not covered under the previous items?

Comment 1
The risk assessment for clinical trial TG4010.24 is in fact an update of the risk assessment for clinical trial TG4010.14. This risk assessment for clinical trial TG4010.14 regarding the use of TG4010 was in general considered as adequate and clinical trial TG4010.14 obtained a positive advice with only a limited number of additional conditions from the Biosafety Advisory Council. The risk assessment for clinical trial TG4010.24 incorporated these conditions together with addition of new viral dissemination data from the TG4010.14 study and data of viral DNA concentration in skin tissues from TG4001 (MVA-HPV-IL2) supporting the safety profile of TG4010 further. Therefore, the conclusion is that use of TG4010 in clinical trial TG4010.24 can be considered not to represent a risk for the environment and for public health.

Comment 2

General comment: All the scientific documents relate only to the TG4010 (MVA overexpressing MUC1 and IL2). Nivolumab is a monoclonal antibody not considered as a "GMO", therefore I don't see any additional advice that the Biosafety Advisory Council could issue on the use of TG4010.24.

Comment 3

No questions/comments

Bioveiligheidsraad
Conseil de Biosécurité

30-09-2011

Secretariaat
Secrétariat

O./ref.: WIV-ISP/41/BAC/2011_0906

Title: Advice of the Belgian Biosafety Advisory Council on the notification B/BE/11/BVW1 of the company Transgene for deliberate release in the environment of genetically modified organisms other than higher plants for research and development

Context

The notification B/BE/11/BVW1 has been submitted by Transgene to the Belgian Competent Authority in June 2011 for a request of deliberate release in the environment of genetically modified organisms other than higher plants for research and development according to Chapter II of the Royal Decree of 21 February 2005.

The planned activity concerns a clinical trial and the title of the notification is: **"A Phase IIb/III randomized, double-blind, placebo-controlled study comparing first-line therapy with or without TG4010 immunotherapy product in patients with stage IV nonsmall cell lung cancer (NSCLC)."** The purpose of the study is to determine whether the therapeutic vaccine TG4010, a highly attenuated strain of the *Vaccinia* virus genetically modified to express the genes of the human mucine 1 antigen (MUC1) and of the human interleukin 2 (hIL2), improves the benefit of the standard treatment of lung cancer.

The product TG4010 or matched placebo is administered sub-cutaneously and each subject in the study will receive it weekly for 6 weeks and then once every 3 weeks until progression of the disease. The GM virus is deemed unable to replicate in human cells but virus can sometimes be found on the wound dressing covering the injection site. Given the dose schedule it is not possible for the subjects to remain within contained facilities for the duration of the study. As the trial centres are located in Brussels and in Wallonia and as the patients will be treated ambulatory, the national territory is considered as the wider potential release area of the GM *Vaccinia* virus.

The dossier has been officially acknowledged by the Competent Authority on 05 July 2011 and forwarded to the Biosafety Advisory Council for advice.
Within the framework of the evaluation procedure, the Biosafety Advisory Council, under the supervision of a coordinator and with the assistance of its Secretariat, contacted experts to evaluate the dossier. Three experts from the common list of experts drawn up by the Biosafety Advisory Council and the Biosafety and Biotechnology Unit (SBB) answered positively to this request. The SBB also took part in the evaluation of the dossier while the Platform for Molecular Biology and Biotechnology of the Scientific Institute of Public Health evaluated the analytical procedure for the detection of TG4010 submitted by the notifier.
The experts and the SBB assessed whether the information provided in the notification was sufficient and accurate in order to state that the deliberate release of the genetically modified organism for its intended use, would not raise any problems for the environment, animal health or human health (people coming in contact with the treated patient and/or with the GMO).

On 22 August 2011, based on a list of questions prepared by the Biosafety Advisory Council, the Competent Authority requested the notifier to provide additional information about the notification. The answers from the notifier to these questions were received by the Competent Authority on 15 September 2011 and transmitted to the secretariat of the Biosafety Council on the same day. This complementary information was reviewed by the coordinator and the experts.

For the purpose of this evaluation, the following legal basis has been considered:
- Annex II (principles for the risk assessment) and annex III (information required in notifications) of the Royal Decree of 21 February 2005.
- Commission Decision 2002/623/EC of 24 July 2002 establishing guidance notes supplementing Annex II to Directive 2001/18/EC.

The pure medical aspects concerning the efficacy of the medicinal product and its safety for the treated patient, as well as aspects related to social, economical or ethical considerations, are outside the scope of this evaluation.

In parallel to the scientific evaluation of the notification, the Competent Authority also made the dossier available on its website for the one-month public consultation foreseen in the abovementioned Royal Decree. The Competent Authority didn't receive any reaction of the public relevant for the environmental and/or public health safety of the GMO.

Summary of the Scientific evaluation

1. The characteristics of the donor, the recipient or parental organism

No major risks were identified but the Biosafety Council asked to the notifier to update its dossier with more recent data regarding the effects of MUC1 and IL2 on cells or tissues.
This information was received and the notifier has adequately updated his technical dossier with more references.

2. Information related to the vector

In order to exclude any presence of MVA particles able to replicate in human cells, the Biosafety Council requested more information concerning the homogeneity of the parental MVA strain and of the final TG4010 vector.
This information was received and the new data do not confirm the homogeneity of the viral population. However the Biosafety Council agrees that the TG4010 vector is attenuated and unable to replicate efficiently *in vitro* and hence *in vivo*.

3. Information related to the characteristics of the GMO

The notifier was asked to clarify the construction process and to complete his dossier with the MUC1 and hIL2 sequences.
The notifier has adequately answered this question: sequences and their references are provided, alignments have been done and the schematic constructions are more detailed making clear that no additional sequences are present.

The notifier was also asked to update his technical dossier regarding the safety of MUC-1 and IL-2 in the context of this planned clinical trial.
This information was received and judged satisfactory.
The Biosafety Council agrees with the notifier that the MVA vector may be classified as risk class 1 but the inserted genes – MUC-1 and IL-2 – take it to risk class 2. The adverse effects observed in patients treated with subcutaneous TG4010 are injection site and/or skin

reactions (erythema, pain, induration, and inflammation), fatigue, pyrexia, influenza-like illness, erythema and myalgia. These side effects are probably related to IL2. In addition potential autoimmune toxicity from TG4010 related to cross reactivity with natural MUC1 protein cannot be excluded. Therefore, measures have to be taken to prevent accidental vaccination of health care personnel handling and administering the product (see below).

4. The condition of release

Concerning the proposed worker protection measures and the measures to avoid accidental exposure to TG4010, the Biosafety Council noticed several discrepancies in the dossier. It also made suggestions for proper management measures and asked to the notifier to submit amended instructions for the people who will handle the product.

The working practices proposed by the notifier in his answer are not optimal neither for the protection of the workers nor to avoid unnecessary release of the vector into the environment.

The primary hazards when manipulating MVA vectors consist in ingestion, droplet or aerosol exposure of mucous membrane or broken skin. Some precaution should be taken:

- The use of a Biosafety Cabinet is not mandatory. However, it has to be noted that the puncture of the flask containing the vector with a needle is certainly a source of aerosolisation. This makes the wearing of goggle but also of a mask mandatory.
- The use of gloves is an absolute requirement to avoid any skin contamination.
- To avoid unnecessary release of the vector into the environment in case of skin contamination, an absorbent tissue should be placed on the affected area in order to absorb all viral vectors. The disinfectant should then directly be applied to the tissue. After removing this tissue (which must be disposed as contaminated material), the skin should be washed thoroughly. In case of eye contamination wash the eyes over a closed basin where the wash water can be decontaminated with active chlorine bleach before being released into the sewer system.

The Biosafety Council is also concerned about possible needle stick injury during the preparation of the syringe as described in the technical sheet of the notifier. The instructions should stipulate that the needle has to be removed in a hands free operation (i.e. hands do not touch the needle) into a closed container.

5. The risks for the environment and human health

With the exception of the risks for health care personnel handling and administering the product (see above), no major risks were identified for the general population or people coming into contact with the treated patients.

The Biosafety Council requested further information on the biodistribution studies referred to in the dossier to support the non-spreading character of TG4010.
This information was received and judged satisfactory.

6. The monitoring, control, waste treatment and emergency plans proposed by the applicant

The notifier was asked to consider the analysis of the concentrations of TG4010 in local and sequestered tissue microenvironments.
The arguments and explanation given by the notifier were judged satisfactory.

7. The analytical procedure proposed by the notifier to accompany the control samples that will be send to the Scientific Institute of Public Health after the start of the clinical trial

The notifier was questioned about the specificity of the detection method and requested to submit a clearer and more detailed analytical procedure.
The information and needed document were provided by the notifier and deemed acceptable.

8. Additional points considered by the experts of the Belgian Biosafety Advisory Council

Although out of the scope of the Directive 2001/18, the Biosafety Advisory Council drew the attention of the notifier on one point concerning the safety of the product.
The question of the Council and the answer received from the notifier are given in annex 1.

Conclusion

Based on the scientific assessment of the notification made by the Belgian experts, the Biosafety Advisory Council concludes that it is unlikely that the genetically modified *Vaccinia* virus (TG4010) genetically modified to express the genes of the human mucine 1 antigen and of the human interleukin 2 and developed as a therapeutic vaccination for the treatment of lung cancer, will have any adverse effects on human health or on the environment in the context of the intended clinical trial.

Therefore, the Biosafety Advisory Council issues a **positive advice with the following conditions**:

- The notifier and the investigators must strictly apply the clinical trial protocol, as described in the dossier. In addition, the technical sheet has to be amended according to the recommendations described under point 4 (The condition of release) and summarized below:
    - the wearing of goggles, mask and gloves is mandatory when preparing and administering the product;
    - in case of skin contamination, an absorbent tissue should be placed on the affected area in order to absorb all viral vectors. The disinfectant should then directly be applied to the tissue. After removing this tissue, the skin should be washed thoroughly;
    - in case of eye contamination wash the eyes over a closed basin where the wash water can be decontaminated with active chlorine bleach before being released into the sewer system;
    - The instructions for preparation of the syringe should stipulate that the needle has to be removed in a hands free operation into a closed container.

- As already agreed by the notifier in his answer of 15 September, the technical sheet as to be amended regarding the presence of a biohazard sign during transport of the product and the need to mark the injection sites subject to swabbing with an indelible felt-tipped pen.

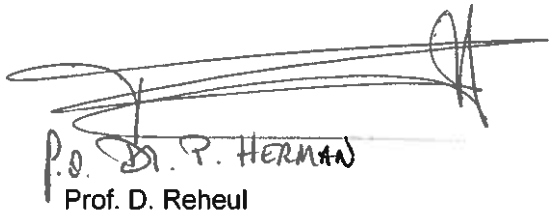
- Any protocol amendment has to be previously approved by the Competent Authority.

- The notifier is responsible to verify that each study centre has qualified personnel experienced in handling infectious material and that the investigator has the required authorisations to perform the clinical trial activities inside the hospital (laboratory, pharmacy,

hospital room, consultation room...) according to the Regional Decrees transposing Directive 2009/41/EC on Contained use of genetically modified micro-organisms.

- The Biosafety Advisory Council should be informed within 2 weeks when the first patient starts the treatment and the last subject receives the last treatment.

- At the latest six months after the last visit of the last patient included in the trial, the notifier must send to the competent authority at the attention of the Biosafety Council a report with details concerning the biosafety aspects of the project. This report will at least contain:
  - the total number of patients included in the trial and the number of patients included in Belgium;
  - a summary of all adverse events marked by the investigators as probably or definitely related to the study medication;
  - a report on the accidental releases, if any, of the recombinant *Vaccinia* virus.



Prof. D. Reheul
President of the Belgian Biosafety Advisory Council

Annex 1: Additional comments related to the safety and efficacy of the product and answers given by the applicant.
Annex 2: Compilation of comments of experts in charge of assessing the dossier B/BE/11/BVW1 (ref: BAC_2011_0780)

Annex 1: Additional comment related to the safety of the product and answer given by the applicant.

Although out of the scope of the Directive 2001/18/EC, the Biosafety Advisory Council would like to draw the attention of the notifier on the following point :

MVA production may contain traces of egg antigens. As allergy against egg antigens is quite frequent in the human population, the applicant should make sure that anti-choc treatments are available when the drug is administered.

Response:

Patients presenting allergy to eggs antigens should not be included in the study, according to the protocol exclusion criteria (see Section 5.2 of the clinical protocol).
The study is exclusively conducted in hospital sites specialized in oncology and having emergency and resuscitation procedures in place. Even if the probability of occurrence is low, patients experiencing allergic shocks will appropriately be taken into care.

Bioveiligheidsraad
Conseil de Biosécurité

22-08-2011

Secretariaat
Secrétariat

O./ref.: WIV-ISP/41/BAC_2011_0780
Email: BAC@sbb.ihe.be

Compilation of Comments of Experts in charge of assessing the dossier B/BE/11/BVW1

Mandate for the Group of Experts: mandate of the Biosafety Advisory Council (BAC) of 22 June 2011
Coordinator: Prof. Philippe Hermans
Experts: Jean-Claude Twizere (ULg), Viggo Van Tendeloo (UZ Antwerpen), Céline Verheust (WIV/ISP), Karen Willard-Gallo (ULB), Nicolas Willemarck (WIV/ISP)
Domains of expertise of experts involved: Human medicine, oncology, virology, therapeutic vaccination, design of vectors, clinical trials, biosafety viral vectors, risk assessment, workers protection
Secretariat (SBB): Didier Breyer, Martine Goossens, Philippe Herman, Katia Pauwels

INTRODUCTION

Dossier **B/BE/11/BVW1** concerns a notification of the company Transgene for deliberate release in the environment of genetically modified organisms other than higher plants according to Chapter II of the Royal Decree of 21 February 2005.
The notification has been officially acknowledged on 05 July 2011 and concerns a clinical trial with TG4010 a recombinant viral vector MVATG9931 derived from an attenuated strain of the modified vaccinia virus Ankara (MVA). The MVA has been genetically modified to express the human mucine 1 (MUC1) antigen and the human interleukin-2 (IL2). This GM-medication is developed for use as an immunotherapy in patients with stage IV non-small cell lung cancer (NSCLC).

♦ INSTRUCTIONS FOR EVALUATION

Depending on their expertise, the experts were invited to evaluate the genetically modified organism considered in the notification as regards its molecular characteristics and its potential impact on human health and the environment. The pure medical aspects concerning the efficacy of the medicinal product and its safety for the treated patient are outside the scope of this evaluation.
The comments of the experts are roughly structured as in
- Annex II (principles for the risk assessment) of the Royal Decree of 21 February 2005
- Annex III (information required in notifications) of the Royal Decree of 21 February 2005
- Commission Decision 2002/623/EC of 24 July 2002 establishing guidance notes supplementing Annex II to Directive 2001/18/EC.

List of comments received from the experts

1. INFORMATION RELATED TO THE CHARACTERISTICS OF THE DONOR, THE RECIPIENT OR PARENTAL ORGANISM

(e.g. possibility of natural transfer of genetic material to other organisms, pathological, ecological and physiological characteristics, indigenous vectors ...)

Comment 1

Has evaluated this item and has no questions/comments.

Comment 2

Has evaluated this item and has no questions/comments.

Comment 3

Has evaluated this item and has no questions/comments.

Comment 4

Major Comments:
-The information on phenotypic and genetic markers is missing in the dossier for both MUC1 and IL-2. AnnexIIIA, sections II.A.a) 4. : the texts below "Phenotypic and genetic markers" should be placed under the section II.A.a) 6. "description of identification…". In the context of MUC1 and IL-2 (donors in the dossier), "Phenotypic and genetic markers" sections should at least include observed properties of MUC1 and IL-2 proteins on cells or tissues. Some recent references for MUC1 phenotypes include Abnet et al. Nat Genet. 2010 Sep; 42(9):764-7; Meyer et al. Plos Genet. 2010 Aug 5;6(8); for IL-2 phenotypes Nat Genet. 2007 Jul 39(7):827-9; Nat Genet. 2011 Jun 19;43(7):699-705;…. or http:omim.org/entry /147680. Genetic markers also exist for both MUC1 and IL-2 and can be found on the NCBI web site (www.ncbi.nlm.nih.gov; examples: D1S3411 and SHGC-12710 for MUC1 ; PMC138391P1 and SHGC4-74 for IL-2). Similar remarks could be made for the recipient. I believe that *Transgene* misinterpreted "phenotypic and genetic markers".
-The references concerning the donors should be updated by including more recent papers on the roles of MUC1 and IL-2. (Examples: Saeland et al., Int J Cancer. 2011 Aug 5; Byrd and Bresalier, Cancer Metastasis Rev. 2004 Jan-Jun 23(1-2)77-99; Xiong et al., J. Surg Oncol.2011 Aug 3)
- The information on IL2 pathogenicity is not well documented in the dossier. The dossier should include information on the roles of IL2 on activation of latent viruses. IL-2 has been closely linked, from its discovery, to HIV-1 and other human retroviruses and their expression in target cells (there are several references on the subject, see for example: Jeeninga et al. Retrovirology. 2008 Apr 25; 5:37, Krichbaum-Stenger et al., Blood 1987 Nov; 70(5); Higuchi et al, J Virol 2007 Nov;81(21):11900-7).

Additional comment from the coordinator:
As HIV positive patients are excluded from the planned trial, the question regarding the possible role of IL-2 in activation of human retro-viruses is regarded as not pertinent in the context of this application.

Comment 5

The European vaccinia virus strain MVA is considered the strain of choice for the design of novel and safe pox-virus based vectors and thus MVA based vectors are quite suitable for clinical use. Its restricted host range, avirulence in animals and extensive safety testing in humans greatly reduces any potential hazard to health care workers and transmission to non-target environments. Recombinant MVA can be considered an effective and safe viral vector. Concerning the MUC1 and IL2 sequences inserted, there is not the same long-term experience with their administration to humans in a viral vector, but given the self-limiting expression of MVA there is no reason to suspect that these genes will be integrated in the patient. Concern for adverse effects by these genes is principally limited to health care workers inadvertently exposed through an incident (more below).

2. INFORMATION RELATED TO THE VECTOR

(e.g. description, sequence, mobilisation ...)

Comment 1

Has evaluated this item and has no questions/comments.

Comment 2

According to the dossier:

- the MVA genome of the isolate (parental vector MVATGN33.1) obtained at Transgene has been sequenced from position 6354 to position 171730: the sequence was found 99.9% homologous to the published sequence (GenBank/U94848) (page 14 of Annex IIIA).
- the genetic stability of the final vector TG4010 has been undertaken by PCR and sequencing (page 25 of Annex IIIA).

However, it has been shown that some MVA strains appear to be heterologous (such as the deposited strains MVA-572 and MVA-I721). Indeed, as described in Suter *et al.* (2009), some strains contain viral populations or variants that have an altered genotype compared to the original parental MVA strains and that are actually able to replicate in some human cell lines. Since they only represent a minority of the viral populations, these variants can not be detected by PCR or sequencing alone. *In vitro* or *in vivo* studies must be performed in order to assess the homogeneity of the strain.

The notifier should comment on the homogeneity of the parental MVA TGN33.1 strain and/or the final vector TG4010. The potential presence of such replicative variants should be assessed.

Comment 3

Has evaluated this item and has no questions/comments.

Comment 4

Minor comment:
The restriction map of the vector pTG9931 may indicate cloning positions for MUC1.

Comment 5

The MUC1 and IL2 sequences inserted were carefully controlled at each stage through to the final recombinant TG4010 to insure that no mutations, deletions or insertions occurred in the original sequence. In addition, potentially troubling regulatory sequences have been eliminated from the recombinant virus or through homologous recombination in the final TG4010 preparation.

3. INFORMATION RELATED TO THE CHARACTERISTICS OF THE GMO

3.1. Information related to the genetic modification

(e.g. methods used for the modification, description of the insert/vector construction ...)

Comment 1

Has evaluated this item and has no questions/comments.

Comment 2

Has evaluated this item and has no questions/comments.

Comment 3

Has evaluated this item and has no questions/comments.

Comment 4

Minor comments:

- Does the sequence of the MUC1 cDNA clone isolated from T47D cells correspond to any referenced sequence ID? Please provide the gene ID (ex: human MUC1 gene ID is 4582) or other sequence references in your text for each of your donor sequences.
- The amino acid change between the two tandem repeats of MUC1 should be indicated as it may affect protein expression and functions.
- It is not clear how the MUC1 expression block was assembled. Schematic restriction map and ligation representations of the experiments should be provided. For example, a scheme starting with the pPolyETAtm restriction map and showing the two mentioned triple ligations with M13TG6131 and a scheme showing the digestion of M13TG4061 and insertion of the repeats into M13TG8172, are needed to ensure that no additional sequences from different vectors are present in the MUC1 expression block.
- Similar suggestions for the construction of the IL2 expression block: -How the sequence of the IL2 clone relate to the reference sequence in NCBI databases? – Detailed Schematic representations of the construction of the expression block will also ensure that no additional sequences are present.

Comment 5

Standard molecular biology techniques were used to design and insert specific sequences in the vectors. The descriptions provided are detailed and indicate that these procedures were carefully controlled at each step.

3.2. Information on the molecular characteristics of the final GMO

(e.g. number of copies of the transgenes, phenotypic and genetic stability of the transgenes, expression of the new genetic material, re-arrangements in the genome, inclusion or suppression of genetic material ...)

Comment 1

Has evaluated this item and has no questions/comments.

Comment 2

See comment under “2.Information related to the vector”

Comment 3

Has evaluated this item and has no questions/comments.

Comment 4

Comments:
-Even if the native and antigen forms of MUC1 present a high degree of polymorphism, in the context of this GMO, genetic stability of the cloned MUC1 sequence should be examined by infecting MRC5 cells with the test product. PCR and sequence verification of the transgene, which has been modified (ex. Tandem repeats) and thus distinct to the native form, should assess the genetic stability of the synthetic form of MUC1.

Comment 5

TG4010 is an MVA based vector whose expression is self limited in human cells. The MVA proteins and the donor sequences (MUC1 and IL2) will therefore only be expressed (in principal) until the MVA infection kills the infected cells (most infected cells usually die within 24-48 hours). Our collective >50 years of experience indicates that new mature virus particles with the potential to infect other cells or be transmitted to another host are not produced during MVA infection in humans.

3.3. Considerations for human, animal or plant health

(e.g. invasiveness and virulence, toxic or allergenic effects, possibility of survival outside of receiving host, other product hazards ...)

Comment 1

Has evaluated this item and has no questions/comments.

Comment 2

Has evaluated this item and has no questions/comments.

Comment 3

Has evaluated this item and has no questions/comments.

Comment 4

Potential pathological effects of lower or over expression of MUC1 are not well documented in the AnnexIIIA of the dossier. The dossier should include recent references describing different effects of MUC1 on cancer, intestinal or immunological cells. See for example Hasegawa et al. Leuk Lymphoma 2011 Jun.; Li et al. Plos One. 2011 Apr 20; Every et al. Infect Immun 2011 Aug 1; Scholz et al., Arch Gynecol Obstet 2011 Jul 30). Does MUC1 overexpression induce allergenic or inflammatory effects? How about IL-2?

Comment 5

MVA infection is self-limiting and therefore not a human health problem when used as a vaccine. Transmission of TG4010 to plants or animals from the vaccinated patients is unlikely since MVA infection does not produce infectious particles.

4. INFORMATION RELATING TO THE CONDITION OF RELEASE

(e.g. description of the activity, quantities of GMO to be released, workers protection measures, elimination of any contaminating material in the preparation of the GMO stock, elimination of the GMO at the end of the experiment ...)

Comment 1

Has evaluated this item and has no questions/comments.

Comment 2

Has evaluated this item and has no questions/comments.

Comment 3

MINOR_technical sheet_precautions: All transport of TG4010/TG0008 (vial or syringe containing the dose to be injected) must be done using a leak-proof container/bag with on the outside a biohazard sign.

Comment 4

Has evaluated this item and has no questions/comments.

Comment 5

I have many criticisms concerning the Technical Sheet (it lacks critical details) and the information provided in Annexes II and IIIA.
Concerning worker protection measures, nowhere in the information provided is the issue of a needle stick injury specifically addressed. Needle stick injuries are actually relatively common in hospitals, with doctors injured at a higher frequency than nurses. The TG4010 protocol calls for removal of the virus containing solution from its original glass vial with needle #1 attached to a syringe, exchange of this needle with a luer lock cap, and then placement of needle #2 prior to administration at the patient bedside. It is well known that removing needles from syringes is risky and dramatically increases the probability of a needle stick injury. If this needle exchange is absolutely necessary to insure sterility for the patient, then there must be a more detailed protocol for how to safely remove and dispose of needle #1. In addition, qualified personnel ONLY who are **experienced in handling infectious material** (and not just routine live virus vaccines but materials considered to be biohazardous) must be responsible for preparing the injection syringes. This vaccine could have serious consequences if it is injected into a healthy individual - this includes the potential induction of an autoimmune reaction to MUC1 and immune effects from the IL2. While the levels of IL-2 produced by the TG4010 vaccine are well below the amount infused in late stage renal carcinoma and metastatic melanoma patients, the quantity their studies show are produced by TG4010 are still quite significant (0.4 mg = 7.1×10^6 IU from a s.c. vector dose of 5×10^7 PFU - maximum trial dose). This amount of IL2 in a healthy individual could induce serious consequences – a study where ultra low doses of IL2 were injected in healthy humans (s.c injections of 1000-10000 IU/kg, which is 10-100 fold lower their estimated dose for a 70 kg person) had demonstrated effects on immune cells, similar to what was observed as an adverse event in some patients treated with TG4010 in Phase II trials (neutropenia, leucopenia, etc)(the reference for the study in healthy humans is PubMed ID 12480497).
In addition, the Technical Sheet details what to do in case of an Incident. For skin contamination with/without injury, they say the first thing to do is “wash immediately and abundantly under tap water”. Everywhere else they specify the use of decontaminants and to leave them in contact for a specified amount of time, to treat the contaminated clothes as contaminated material, etc. BUT they want to wash the most concentrated virus down the sink immediately. UNBELIVEABLE!!!!!!!!! What needs to be done immediately is to place an absorbent tissue or gauze on the affected area, allow it to absorb all the virus containing liquid, apply disinfectant directly to this tissue or gauze in place before removing and disposing of it as contaminated material. Then follow procedures to wash, etc. The most concentrated solution of TG4010 must be adsorbed and disposed of as infectious material, not washed away into the public water supply!

Additional comment from the coordinator:
On page 49 of Annex III it is said that in case of skin contamination, the skin must be immediately disinfected locally with hydrogen peroxide and washed thoroughly with water and soa. This is contradictory with the statement on page 50 of the same document.

Concerning elimination of any contaminating material in the preparation of the GMO stock it is absolutely critical that all used materials are disposed of correctly. They should not be prepared in the patient's room or in an area used to prepare other patient medications unless there is physical separation (i.e. laminar flow hoods).
Concerning elimination of the GMO at the end of the experiment they have detailed relatively well the disinfectants to use except in reference to cleaning the patient's room afterward he/she leaves – this is just stated as standard hospital disinfectant – the specific and effective disinfectants known to kill MVA should also be used to clean the room. In addition, if these treatments are given in the "hospital de jour", then the patient should be in a private room, since it is neither necessary nor desirable to expose another cancer patient, potentially in an immunodepressed state, to any, even a very small risk, of exposure (for example if the syringe was dropped thereby creating an aerosol).

5. INFORMATION RELATED TO THE RISKS FOR THE ENVIRONMENT AND HUMAN HEALTH

5.1. Information on spread ("shedding") of the GMO from the treated patient/animal to other persons/animals or to the environment (including indirect/delayed effects due to vertical transmission to offspring).

(e.g. genetic transfer capability, routes of biological dispersal, target organisms ...)

Comment 1

Has evaluated this item and has no questions/comments.

Comment 2

1) Biodistribution:
In Annex II, page 7, it is written that "the evaluation of the biodistribution of recombinant MVA vectors in animal confirmed the non spreading character of such viral vector with the absence of significant internal dissemination after IM or SC injection, the MVA vector being mainly localized at the injection site". No reference is cited to support this statement.
However, a study done by Ramirez and co-workers has shown that MVA vectors (expressing luciferase) are able to reach target tissues other than the site of administration.
Since these data are contradictory, the notifier should comment on this and provide further information on the biodistribution studies performed (data on the study design, the strain used, and references) to support the non-spreading character of TG4010.

2) Shedding:
Shedding analyses are planned for the PhaseIIb part of the study: swabbing will be performed at the injection site before, 6 hours after injection, on D8, D15 and D22 and samples will be analyzed to assess the potential shedding.

Considering the fact that MVA is a non replicative vector, it is expected to be rapidly cleared (after 48 hours according to Ramirez et al, 2000). The swabbing should therefore be focused on the first hours/days after injection. The notifier should explain the reason behind the design of the shedding studies.

Comment 3

Patients will be administrated with the IMP by the subcutaneous route. Although most studies suggesting no significant shedding of infectious particles, there is a chance of spreading / leakage of the IMP (as infectious virus particles) from the injection site. The ultimate goal of the IMP is to induce specific cellular and humoral immune response to Muc-1 (epitope H23) expressing cells. It is not clear if the serial injections (in time) with the IMP are boosting the immune response during the study. In this case, the chance of dissemination of the GGO by blistering of the skin or by unintentional scratching of the patient increases after each injection. In this view it is maybe better to monitor / control the shedding/spreading of the GMO from all injection sites and this until the end of the study? Additionally, monitoring by (q)PCR makes no differences between non-infectious/inactive and still infectious GMO virus particles (what is the infectivity of the viral vector?). The procedure to swap at the injection detects possible spreading of the GMO at a certain time moment, it is a snapshot, but doesn't give an idea of the spreading/leakage during the period when patients are out of the hospital. Therefore, it is maybe better to collect and check also the used wound plasters on the presence of infectious viral particles or viral DNA.

Comment 4

Comments + Questions:
-Section IV.B.5. "Measures employed to ensure and to verify genetic stability…." . Why is the possibility to check for genetic stability of the vector TG4010 in patients limited? Samples collected as described in V.A.1. could be used to verify genetic stability of the vector.
-Section IV.B.5. "Measures employed to ensure and to verify genetic stability…." . The last sentence of the paragraph is very speculative: How will the evaluation of humoral and cellular responses to MUC1 allow *Transgene* to indirectly control genetic stability of the vector? To my opinion, genetic stability can be assessed by PCR and sequence analysis of the vector.

Additional comment from the coordinator:
The sample collected are, according to the notifier, negative for the presence of the vector: the PCR will be negative….this is why the notifier said "the possibility to check the stability is limited"

Comment 5

Follow up tests are planned by swabbing the first injection site just prior to the injection (negative control) and a several time points over the first 3 weeks following this injection. It has not been indicated in the protocol or on technical sheet that this region needs to be marked somehow so that the subsequent swabs are taken from the true injection site and not an area adjacent to it, which would nullify the test results.

5.2. Information on possible effects on human health resulting from interactions of the GMO and persons working with, coming into contact with or in the vicinity of the GMO release (carekeepers, patient relatives, immunocompromised people ...).

Comment 1

Has evaluated this item and has no questions/comments.

Comment 2

Has evaluated this item and has no questions/comments.

Comment 3

Has evaluated this item and has no questions/comments.

Comment 4

Has evaluated this item and has no questions/comments.

Comment 5

The risk of exposure and health effects to people in contact with the treated patient (once at home) are relatively small. The real and present risk of exposure is in the preparation of TG4010 for injection and during its administration to the patient. In addition to a potential needle stick injury detailed above, it is possible that the health care worker could drop the syringe during its preparation or administration. This has the potential to create an aerosol that would likely contaminate any individual in the room at the time. MVA vaccines can be administered nasally. Therefore, it should be recommended that TG4010 is loaded into the syringe in a laminar flow hood. Furthermore wearing a lab coat and goggles is stipulated but gloves are made optional. Gloves should be **mandatory**, both during the preparation and administration of TG4010 (>70% of needle stick injuries occur to people not wearing gloves) - it is absurd to wear goggles but not protect the hands – the most vulnerable to physical injury. A mask worn by the health care worker should also be recommended if not stipulated. In addition, when the syringe is removed from the leak-proof container/bag in the patient's room, the Technical Sheet should indicate that only the patient and the person administering the shot are in the room, family, friends and other personnel can be asked to leave for this short intervention. The Technical sheet should also detail how to deal with an incident involving an aerosol (and as mentioned above, a needle stick injury).

5.3. Information on possible effects on animal health or on the environment.

Comment 1

Has evaluated this item and has no questions/comments.

Comment 2

Has evaluated this item and has no questions/comments.

Comment 3

Has evaluated this item and has no questions/comments.

Comment 4

Has evaluated this item and has no questions/comments.

Comment 5

It is unlikely that TG4010 will be released into the environment except in the case of an incident in the preparation room or patient's room at the clinical trial setting. Thus, once again it would be wise to prepare TG4010 for injection in a laminar flow hood that can be easily decontaminated. If an incident occurs in the patient's room, a procedure needs to be in place for immediately closing off access until the room is properly disinfected.

5.4. Information on selective advantages or disadvantages conferred to the GMO compared to the parental organism.

Comment 1

Has evaluated this item and has no questions/comments.

Comment 2

Has evaluated this item and has no questions/comments.

Comment 3

Has evaluated this item and has no questions/comments.

Comment 4

Has evaluated this item and has no questions/comments.

Comment 5

Has evaluated this item and has no questions/comments.

5.5. Information on the possibility of the GMO to reconvert to his wild type form and possible consequences for human health or the environment.

Comment 1

Has evaluated this item and has no questions/comments.

Comment 2

Has evaluated this item and has no questions/comments.

Comment 3

Has evaluated this item and has no questions/comments.

Comment 4

Has evaluated this item and has no questions/comments.

Comment 5

MVA has been passaged more than 500 times to achieve this attenuated virus and lacks genes critical in the pathogenicity of the wild-type vaccinia virus. For MVA there is sufficient distance from the first human injections to determine that the virus cannot revert to wild-type in the human host.

5.6. Information on the possibility of the GMO to exchange genetic material with other micro-organisms and possible consequences for human health or the environment.

Comment 1

Has evaluated this item and has no questions/comments.

Comment 2

Other animal poxviruses that are also able to infect humans exist (cowpox, monkeypox, buffalopox, …). Could they recombine with TG4010?

Comment 3

Has evaluated this item and has no questions/comments.

Comment 4

Has evaluated this item and has no questions/comments.

Comment 5

Has evaluated this item and has no questions/comments.

5.7. Information on the possibility of gene transfer to other organisms and about the selective advantages or disadvantages conferred to those resulting organisms (possible consequences for human health or the environment).

Comment 1

Has evaluated this item and has no questions/comments.

Comment 2

See comment under 5.6

Comment 3

Has evaluated this item and has no questions/comments.

Comment 4

Has evaluated this item and has no questions/comments.

Comment 5

Has evaluated this item and has no questions/comments.

6. INFORMATION RELATED TO THE MONITORING, SURVEILLANCE AND CONTROL, WASTE TREATMENT AND EMERGENCY PLANS PROPOSED BY THE APPLICANT

6.1. Monitoring plan proposed by the notifier and possibility to identify the occurrence of non-anticipated adverse effects.

(adequation between the monitoring plan and risks identified during the risk assessment, when appropriate measures to minimize the potential risks to offspring ...)

Comment 1

Has evaluated this item and has no questions/comments.

Comment 2

See comment under **5.1.** concerning the shedding analysis which is planned for the phase II b part.

Comment 3

Has evaluated this item and has no questions/comments.

Comment 4

Comments:
-V.A.1: Blood samples from untreated patients should also be collected as negative controls.

Comment 5

Has evaluated this item and has no questions/comments.

6.2. Surveillance and control of the release

(adequation between the procedures to avoid and/or minimise the spread of the GMO and risks identified during the risk assessment...)

Comment 1

Has evaluated this item and has no questions/comments.

Comment 2

Has evaluated this item and has no questions/comments.

Comment 3

Minor_ Although there is chance of leakage of the GMO from the injection place, there are no details given how to minimise the spread of the GMO from the injection site during the first hours/days post injection. A wound plaster or bandages to cover the injection site is sufficient to minimise the spread of the GMO?

Additional comment from the coordinator:
The procedure foreseen by the applicant i.e. "disinfection of the skin at the injection site" should minimise the spread. However if for any reason a bandage or plaster is required, the patient should receive a a biohazard box to eliminate any material in contact with the skin and asked to return it at the next visit.

Comment 4

Has evaluated this item and has no questions/comments.

Comment 5

Other than leakage at the injection site and the materials associated with the preparation and administration of the vaccine (vials, syringes, needles, swabs, bandages, etc), which place the health care workers involved at risk exposure to TG4010 should remain limited to the injected individual. Correct biohazard disposal of ALL the materials used should be done immediately at the trial site.

6.3. Information on the waste generated by the activity and its treatment.

(e.g. type of waste, amount ...)

Comment 1

Has evaluated this item and has no questions/comments.

Comment 2

Has evaluated this item and has no questions/comments.

Comment 3

Has evaluated this item and has no questions/comments.

Comment 4

Has evaluated this item and has no questions/comments.

Comment 5

Correct biohazard disposal of ALL the materials used should be done immediately at the trial site by a team trained and certified to work with these materials.

6.4. If applicable, information on the emergency plan(s) proposed by the notifier.

Comment 1

Has evaluated this item and has no questions/comments.

Comment 2

Has evaluated this item and has no questions/comments.

Comment 3

Has evaluated this item and has no questions/comments.

Comment 4

Has evaluated this item and has no questions/comments.

Comment 5

As stated above, they need to revise the advice on the technical sheet for what to do if there is an incident that inadvertently exposes the health care worker or someone else to the vaccine

6.5 Information related to the identification of the GMO and the detection techniques

(e.g. identification methods and detection techniques, sensitivity, reliability and specificity of the proposed tests ..)

Comment 1

Has evaluated this item and has no questions/comments.

Comment 2

Has evaluated this item and has no questions/comments.

Comment 3

See comment under 5.1: monitoring by (q)PCR makes no differences between non-infectious/inactive and still infectious GMO virus particles.

Additional comment from the coordinator:
(q)PCR is regarded as adequate. However if shedding is demonstrated by this method further investigation will maybe be needed to evaluate if the shedding concerns still infectious viral particles.

Comment 4

Comments:
-To my opinion, V.A.2. is applicable: molecular biology techniques (DNA extraction, PCR, restriction digestion, sequencing) should allow to distinguish TG4010 from MUC1, IL2 and the empty vector.
- I agree with the notifier that genetic transfer is unlikely. However, the notifier should state that PCR and sequence analysis techniques are able to detect potential transfer of the donated genetic material to other organisms. For example, recombination could occur with another MVA-based vector already present in treated patients.

Additional comment from the coordinator:
Questions about the specificity of the detection technique have also been raised by the GMO-lab of the Scientific Institute of Public Health.

Comment 5

While PCR and other immunological and molecular biological techniques are highly sensitive, the level of detection is not an absolute and for humans usually the tissue available for testing the presence of residual DNA is limited to blood, serum, urine, etc. Concentrations in local and sequestered tissue microenvironments may be much higher than anticipated and need to be considered.

7. OTHER INFORMATION

7.1 Do you have any other questions/comments concerning this notification that are not covered under the previous items?

Comment 1

No, the risk assessment of this dossier is adequate, complete and complies in my opinion to all regulations.

Comment 2

I am concerned about the Centre Hospitalier de l'Ardenne as one of the four centers in Belgium. The other three centers are large hospitals with infectious disease departments, and therefore presumably have personnel trained in preparing, administering and disposing of infectious material. However, the Ardenne center appears as inexperienced and not competent to handle these materials. In all four centers the indicated principal investigators are oncologists and therefore it seems very unlikely that they are trained in working with GMO viral vectors. I think that restrictions should be placed on the use of this vaccine at a qualified center since a needle stick injury is not without precedent and TG4010 expressed in a healthy individual would likely have medical consequences (autoimmune reactions to MUC1 and unwanted effects by IL2 on the immune system).

The list of bibliographic references is a total waste – providing the first author name and year of publication does not allow one to find the article!

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