Adviesraad voor Bioveiligheid
Conseil consultatif de Biosécurité

Advice of the Belgian Biosafety Advisory Council on the notification B/BE/20/BVW2 of the company Vaccitech Limited for deliberate release in the environment of genetically modified organisms other than higher plants for research and development

05/06/2020
Ref. SC/1510/BAC/2020_0511

Context

The notification B/BE/20/BVW2 has been submitted by Vaccitech limited to the Belgian Competent Authority in February 2020 for a request of deliberate release in the environment of genetically modified organisms (GMO) other than higher plants for research and development according to Chapter II of the Royal Decree of 21 February 2005 modified by the Royal Decree of 19 February 2020.

The planned activity concerns a clinical trial and the title of the notification is: ***"A Phase 1b/2, Randomised, Placebo-controlled, Dose-ranging Study to Evaluate Safety, Tolerability and Immunogenicity of a modified Chimpanzee Adenovirus (ChAdOx1)-vectored Multigenotype High Risk Human Papillomavirus (hrHPV) Vaccine and Modified Vaccinia Ankara (MVA)-vectored Multigenotype hrHPV Vaccine in Women with Low-grade HPV-related Cervical Lesions".***

The proposed vaccination regimen comprises the use of two viral vaccines with Human Papillomavirus (HPV) surface antigens developed as a novel therapeutic HPV prime-boost vaccination strategy for the treatment of persistent HPV infection. The aim is to induce an antigen-specific robust T-cell response comprising both CD4+ and CD8+ T-cells. The prime investigational therapeutic HPV vaccine consist of a recombinant replication-defective Chimpanzee Adenovirus vector (ChAdOx1) harbouring 59 gene segments from HPV proteins E1, E2, E4, E5, E6 and E7 from genotypes 16, 18, 31, 52, 53 and 58. Segments range from 9-55 amino acids in length are joined end to end. The boost investigational therapeutic HPV vaccine is a highly attenuated orthopoxvirus Modified Vaccinia Virus Ankara (MVA), replication-deficient in humans and other mammals, with the same antigens-encoding transgene resulting in the viral vaccine MVA-HPV.
Three different doses of ChAdOx1-HPV (2×10^8 vp, 2×10^9 vp and 2×10^{10} vp) and two different doses of MVA-HPV (1×10^7 pfu and 1×10^8 pfu) will be administered by intramuscular route (IM) to study the safety, tolerability and immunogenicity of the investigational vaccine candidates.

It is planned to conduct this first in human study in four clinical sites located in Brussels and the Flemish Region. A total of 32 patients will be enrolled in the study conducted in Belgium, of which 11 will receive placebo.

The dossier has been officially acknowledged by the Competent Authority on 28 February 2020 and forwarded to the Biosafety Advisory Council (BAC) for advice.

Within the framework of the evaluation procedure, the BAC, 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 BAC and the Service Biosafety and Biotechnology (SBB) of Sciensano answered positively to this request.
The experts 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 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) in the context of its intended use. See Annex I for an overview of all the comments from the experts.

The scientific evaluation has been performed considering following legislation:
- Royal Decree of 21 February 2005 (Belgian Official Journal of 24.02.2005, p. 7129) modified by the Royal Decree of 19 February 2020 (Belgian Official Journal of 02.03.2020, p. 12666).
- 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, economic or ethical considerations, are outside the scope of this evaluation.

On 14 April 2020, based on a list of questions prepared by the BAC, 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 13 May 2020 and transmitted to the secretariat of the BAC on the same day. This complementary information was reviewed by the coordinator and the experts and resulted in a second request of additional information, which was sent to the notifier on 26 May 2020. The answers from the notifier to these questions were received by the Competent Authority on 3 June 2020 and transmitted to the secretariat of the BAC on the same day for review by the coordinator.

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 received no reactions from the public.

Summary of the scientific evaluation

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

Upon a question of the BAC, the applicant expanded on the information relative to the assay to detect replication competent adenovirus (RCA) in the context of release testing of the drug substance of all batches of ChAdOx1-HPV manufactured.
The BAC had no other remarks with respect to the description of the donor, recipient and parental organisms.

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

The applicant was requested to further substantiate its statement on the properties of the transgene by experimental data or supportive publications as the provided confidential data showing the DNA sequence coding for the HPV antigen expression cassette, without any further details on the protein sequence or its function in the HPV replication/life cycle, was deemed insufficient to support the applicant's statement on the properties of the transgene in terms of its lack of toxicity or capacity to alter the clinical vector (replication, survival) or the transmission route.
In its answer the applicant complemented its assessment by referring to Hancock *et al*., 2019[1]. For instance, it is specified that the newly created 5GHPV3 immunogen lacks sequences of L1 and L2 proteins that encode viral capsid and that it is not expected to form functional protein (nor conformational correct protein). The applicant also provided the results of a GLP toxicology study in CD-1 mice thereby documenting the lack of toxicity associated to the immunogen.
The BAC had no other remarks with respect to the molecular characteristics of ChAdOx1-HPV and MVA-HPV including phenotypic and genetic stability of the transgenes.

3. The conditions of the release

The Biosafety Advisory Council is of the opinion that the information provided is sufficient and does not raise safety concerns.

4. The risks for the environment or human health

The BAC questioned the impact of deletion of the full ChAdY25 E4Orf6/7 and its replacement with the Human Adenovirus type 5 E4Orf6/7 with respect to the potential of recombination and any possible risk should such an event occur. Also because the applicant recognized that the human Adenovirus type 5 E4Orf4,6/7 gene sequence could be a partner in homologous recombination events, the BAC asked the applicant to provide an improved evaluation of the probability and outcome of events leading to the generation of replication-deficient chimeric vector harbouring HPV sequences and replication competent ChAdOx1/AdHu5 chimeric vectors harbouring the E1 gene and thus lacking HPV sequences. The BAC further took note of the findings of Youil *et al*.,2002[2] indicating a decreased replication capacity of adenovirus chimeras from different species and also acknowledges Wold and Toth, 2013[3] concluding that recombination events between replication-deficient adenoviral vectors have not been reported and if these were to occur, these would not lead to replication-competent viruses expressing the transgene.
Given the replication-defective properties of ChAdOx1-HPV, its low probability of shedding and low likelihood of co-infection with naturally occurring respiratory virus such as AdHu5 associated to the intramuscular route of injection, the fact that no recombination events have been reported so far with E1/E4-deleted replication-defective vector, and the absence of indication that the 5GHPV3 immunogene could influence the shedding behavior of ChAdOx1-HPV, the BAC agrees that the risk of exposure for the environment and human health associated to the use ChAdOx1-HPV is low.

[1] Hancock *et al.,* A Multi-Genotype Therapeutic Human Papillomavirus Vaccine Elicits Potent T Cell Responses to Conserved Regions of Early Proteins. Sci Rep. 2019 Dec 10;9(1):18713. doi: 10.1038/s41598-019-55014-z

[2] Youil et al., 2002, Hexon Gene Switch Strategy for the Generation of Chimeric Recombinant Adenovirus. Hum Gene Ther 13(2): 311-20.

[3] Wold, W. S. and K. Toth (2013). "Adenovirus vectors for gene therapy, vaccination and cancer gene therapy." Curr Gene Ther 13(6): 421-433.

The BAC had no remarks with respect to MVA-HPV.
The BAC further acknowledges the history of use of both viral vectors for vectored vaccines in clinical trials.

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

The BAC had a few remarks with respect to disinfection procedures for needle-stick injuries for personnel, exposure of skin and eye and the disposal of the dressing after injection, after which the applicant satisfactorily amended the procedures in the updated application form (including the ERA).

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 investigational therapeutic ChAdOx1-HPV and MVA-HPV vaccines will have any adverse effects on human health or on the environment in the context of the intended clinical trial provided that all the foreseen safety measures are followed.

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

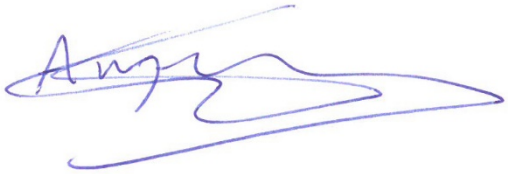
- The notifier and the investigators must strictly apply all the control, personal protection, decontamination and disinfection measures during handling or administration of the investigational therapeutic ChAdOx1-HPV and MVA-HPV vaccines as described in the updated application form (version 3) (including ERA) as submitted on 02 June 2020.

- 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 authorizations 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 two weeks when the first patient starts the treatment and the last patient 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 ChAdOx1-HPV and MVA-HPV.



Prof. Dr. ir. Geert Angenon
President of the Belgian Biosafety Advisory Council

Annex I: Compilation of comments of experts in charge of evaluating the dossier B/BE/20/BVW2 (ref. SC/1510/BAC/2020_0324)

Adviesraad voor Bioveiligheid
Conseil consultatif de Biosécurité

Compilation of comments of experts in charge of evaluating the dossier B/BE/20/BVW2
And comments submitted to the notifier

14 April 2020
*Ref. SC/1510/BAC/2020_0324_*

Mandate for the Group of Experts: Mandate of the Biosafety Advisory Council (BAC) of 26 February 2020.
Coordinator: Anton Roebroek (KUL)
Experts: Rik Gijsbers (KUL), Nicolas van Larebeke-Arschodt (UGent, VUB), Willy Zorzi (ULiège)
SBB: Katia Pauwels

INTRODUCTION

Dossier **B/BE/20/BVW2** concerns a notification of the company Vaccitech limited 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 modified by the Royal Decree of 19 February 2020.
The notification has been officially acknowledged on 28/02/2020 and concerns a clinical trial with two viral vaccines with Human Papillomavirus (HPV) surface antigens developed as a novel therapeutic HPV prime-boost vaccination strategy for the treatment for persistent HPV infection. The prime investigational therapeutic HPV vaccine consist of a recombinant replication-defective Chimpanzee Adenovirus vector (ChAdOx1) carrying gene segments from HPV proteins and the boost investigational therapeutic HPV vaccine is a highly attenuated orthopoxvirus Modified Vaccinia Virus Ankara (MVA), which is replication-deficient in humans and other mammals, with the same antigens-encoding transgene resulting in the viral vaccine MVA-HPV.

♦ 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

Remark: The comments below have served as basis for a list of questions that the Competent authority forwarded on 14-04-2020 to the notifier with a request to provide additional information. The comments or remarks highlighted in grey correspond to the questions addressed to the notifier.

List of comments/questions 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 **(Gijsbers)**

PartA1-2.5 - p9; second half third paragraph

The applicant states that replication of the MVA-virus is very poor in most cells. First, it is not clarified whether this is true for the target cells envisioned here in the trial and second, as indicated in Goosens et al (Curr Gene Ther 2013) and Verheust et al. (Vaccines 2012), only a very limited number of cell lines and types have been tested in literature in my opinion. The statement that most cell types only poorly support replication is in my opinion not correct.
I would suggest to indicate the cell lines tested, or rephrase the sentence.

Coordinator comment:

The expert is in principle right with his remark. It is, however not so relevant in the context of the evaluation of the dossier, that a remark on this issue should be communicated to the applicant. MVA was used in the past successfully to eradicate small pox.

PartA1-2.6 – p10; last paragraph

The study for the 120.000 people in Germany should be referenced.

Coordinator comment:

A remark concerning the omission of a reference should be added to a list of 'typos and other errors/omissions'

PartA1-2.7 – ChAdOx1 paragraph

Throughout the text the word 'non-integrative' is used. This should be replaced with 'non-integrating'

Coordinator comment:

A remark concerning the incorrect use of 'non-integrative' should be added to a list of 'typos and other errors/omissions'

PartA1-2.9 – p13; last paragraph

See also first remark: The applicant indicates that the host range is restricted and that the targeted human host cell does not support replication. Please indicate the host cell(s) that will be targeted. Are there supportive data or publications for this statement?
Altenburg et al (Sci Reports, 2017) reported that despite frequent testing in clinical trials, the cellular tropism of MVA, particularly in relevant animal models, has been studied only to a limited extent.

Coordinator comment:
The expert is in principle right with his remark. It is, however not so relevant in the context of the evaluation of the dossier, that a remark on this issue should be communicated to the applicant. MVA was used in the past successfully to eradicate small pox.

Comment 2 **(van Larebeke)**
Has evaluated this item and has no questions/comments.

Comment 3 **(Zorzi)**
Has evaluated this item and has no questions/comments.

2. INFORMATION RELATED TO THE VECTOR
(e.g. description, sequence, mobilisation ...)

Comment 1 **(Gijsbers)**

PartA1-2.10 – p16
CEF is not explained. I guess chicken embryonic fibroblasts is referred to.

Coordinator comment:
In this brief description of the manufacturing process of the clinical vector, it should be accepted that not all details are presented.

PartA1-2.10 – p17; table
Embryo digestion: this should be 'chicken' embryo digestion.
Cell seeding: are all cells seeded, or fibroblasts only?

Coordinator comment:
In this brief description of the manufacturing process of the clinical vector, it should be accepted that not all details are presented.

PartA1-2.12 – p19; (vi)
Please indicate the 'well-established *in vitro* assay' that is used.

PartA1-2.16 – p25; third paragraph
Throughout the text the words 'virus' and 'infect/infection' are used when 'viral vector' and 'transduction/transduce' should be used. For example in third paragraph. But also further on in the application.

Coordinator comment:
A remark concerning the incorrect use of 'virus' and 'infect/infection' should be added to a list of 'typos and other errors/omissions'

PartA1-2.16 and 2.17 – p26/27; last paragraph
MVA is very resistant to drying. In addition it does not replicate in human cells (at least if extrapolation of the results obtained in a limited number of human cells is valid), however, would bird cells support replication of the vaccine? If so, this should be considered in the ERA.

Coordinator comment:
Considering the only use of the product as vaccine in human, exposure of birds to the vaccine can be excluded. See also first SBB comment in **3.3. Considerations for human, animal or plant health.**

PartA1-2.18 – p28-30; MVA
In this section only mouse studies have been referenced and cited. I wonder how relevant mouse data are to get a view on human biodistribution. Studies are available online that assessed other models, both cell and animal models (ferret, non-human primates). For example, Altenburg et al (Sci Reports, 2017) systematically assessed tissue and cell tropism of the vaccine vector MVA in vitro, ex vivo and in vivo using an rMVA expressing a fluorescent reporter protein. Even though different animal species and administration routes were used, they demonstrated consistent predominant infection of MHC class II+ APCs. In addition, in the different species local myocytes and local epithelial cells were infected after either IM injection or respiratory administration, respectively.
I think this would add to the file.

Coordinator comment:
The information provided by the expert is indeed relevant information, but addition of this information seems not to be essential for this dossier. The paper does not add any new information on the shedding of the vector, which is a very important aspect of biodistribution with respect to environmental risk assessment.

Comment 2 **(van Larebeke)**
Has evaluated this item and has no questions/comments.

Comment 3 **(Zorzi)**
Has evaluated this item and has no questions/comments.

Coordinator comment:

PartA1-2.12 – p19;
(v) The recombination with a wild-type human adenovirus is negligible, since the degree of DNA sequence homology is too low to allow for this event to occur.

PartA1-2.17 – p27;
(ii) The likelihood of recombination with a wild-type human adenovirus is negligible, since there is not enough DNA sequence homology to allow for this event to occur.

What about the Human Adenovirus type 5 E4Orf6/7 gene sequence in the ChAdOx1 virus?
In PartA1-2.14 – p21) it is stated that the full ChAdY25 E4Orf6/7 sequence was deleted and replaced with the Human Adenovirus type 5 E4Orf6/7 gene to facilitate packaging of the ChAdOx1 virus in the HEK293 cell line. No further information is given on the size of this Human Adenovirus type 5 E4Orf6/7 gene sequence, which of course could be a partner in a homologous recombination event with Human

Adenovirus type 5 resulting in a chimeric Human/Chimpanzee adenovirus. The outcome and risk of occurrence of such a recombination should evaluated.

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 **(Gijsbers)**
Has evaluated this item and has no questions/comments.

Comment 2 **(van Larebeke)**
Viral replication will be blocked, but the presence of the modified E4 region is intended to optimise growth rate and yield in human cells, I assume by increasing transcription. Can we be sure that this increased transcription of viral genes does not harm human cells *in vivo*?

SBB comment *:*
In regards to this question, no further information was found in the scientific literature referenced by the applicant. For your information, beside effect on virus yield, data obtained with several E4 modifications indicate that E4 modification has no effect on immunogenicity (Dicks et al, 2012).

Coordinator comment:
The coordinator agrees with the SBB comment.

Comment 3 **(Zorzi)**
Has evaluated this item and has 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 **(Gijsbers)**

PartA1-2.15 – p23
The applicant indicates that the HPV transgene expressed is synthetic and derived from consensus regions from thousands of strains of HPV. It is indicated that the transgene is not toxic and does not confer advantage to the clinical vector (replication, survival). Also transmission route is not supposed to be altered.
Although I do not directly see an issue, no experimental data or supportive publications have been provided for these statements. Have tests been performed to assess toxicity and cell growth in cell culture experiments for example? If so, what were the results.
In my opinion section 2.15 is not sufficiently detailed and not sufficient info is provided on the role of the transgene expressed.

Coordinator comment:

The coordinator assumes that this is not discussed in detail further, because it would potentially reveal the identity of the HPV protein to be expressed by the vector. The confidential data shows only the DNA sequence coding for the antigen expression cassette without any further details on the protein sequence or its function in the HPV replication/life cycle.

Page 24, however, states “the origin of the sequence for the HPV synthetic antigen is derived from consensus regions from thousands of strains of HPV. Although HPV viruses are known to be pathogenic, the gene sequences extracted from them which are compiled into the MVA vector do not encode any known harmful, pathological, oncogenic or allergenic products. Furthermore, they are not known to alter the infectivity, toxicity, virulence or antigenicity of the MVA vector.”
The question, however, for additional experimental information, if existing, is legitimate. Thus, the applicant should provide additional information to support the argument that the transgene is not toxic and does not confer advantage to the clinical vector (replication, survival) and that the transmission route is not supposed to be altered. These arguments could be based on experimental data or otherwise. Just stating that it is not known to be toxic etc. is not sufficient. Was it ever investigated?

Comment 2 **(van Larebeke)**

On p9 following statement is made : "*Replication-incompetent adenoviruses have been administered to thousands of clinical trial participants and there have been no reports of recombination.* " It seems important that we are very sure about this. Can evidence be provided?
That on p 11 mention is made of "During growth of ChAdOx1 up to 1014 viral particles, no replication-competent virus has been identified, despite the presence of the E1 gene in the actual cell line used (IMPD-ChAdOx1-HPV)." Is however quite reassuring.
On p 18 one reads;" The supplemented E1 region is a human adenoviral region and the cells do not host any wild-type Chimpanzee adenovirus, this precludes recombination of the ChAdOx1-HPV back to the original ChAdY25 virus." I think that the possible presence of human adeno virus sequences in addition to E1 might allow recombination with ChAdOx1-HPV , but I assume that this would not suffice to cause a reversion to the original ChAdY25 virus. Is this indeed so?

SBB comment:
It can be noted that Wold & Toth *et al*., 2013 (also referenced by the applicant) provides a comprehensive review based on clinical experience with replication deficient adenovirus vectors and states that ‘*Vector recombination events with wild-type Ad have not been reported; and even if they did happen, they would not generate an RC vector expressing the transgene (consider the recombination events that could occur between the vector genomes shown in Figs. 1a and 1b)*.’

Coordinator comment:
The coordinator agrees with the SBB comment.

Comment 3 **(Zorzi)**
Has evaluated this item and has no questions/comments.

Coordinator comment:

PartA1-2.15 – p24;
The confidential data on the DNA sequence coding for the antigen expression cassette (only one sequence) suggests that both vaccine vectors code for the same HPV synthetic antigen. From the text

on page 24 it seems contradictory, that only for the MVA-HPV vector it is stated, that a highly efficient leader sequence was added. Is this leader sequence also present in the ChAdOx1-HPV vector? If not, explain the rationale behind this difference.

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 **(Gijsbers)**

PartA1-2.11 – p18; last paragraph

The applicant indicates that MVA-HPV has a host range restriction to avian cells and consequently does not require transcomplementation by the cells for growth. This makes the vector safe for human use (since it does not replicate). However, does this mean that MVA-HPV replicates on all avian cells?
What is the chance of shedding to birds in the environment? This is not discussed in the application.

SBB comment :

As pointed out by the applicant and the expert MVA is replication-deficient due to a failure in virus particle assembly (except in avian cells) and therefore only replication--deficient particles can conceivably be shed by human. Also, because it is unlikely that new viral particles will be assembled in the human hosts upon administration of the candidate vaccine, transfection of cells (even if permissive for MVA replication) with shed MVA DNA will not necessarily result in viral infection.
Preclinical studies performed on mice and macaques suggests that MVA is able to reach target tissues other than the site of administration (reviewed in Goossens, 2013). MVA is also rapidly cleared from the tissues, which is consistent with the replication defective properties of the MVA strain. Only a few data about MVA biodistribution in humans are available in published literature and shedding studies have been rarely reported in publications on clinical trials or often have been limited to the first 2 weeks. The applicant indicate that biodistribution studies in mice indicate that viral DNA was not detected in excretory organs (kidneys, rectum and bladder). However, as pointed out by the expert (see 2 – comment 1 of this document) , it is not yet clear whether these findings can be extrapolated to humans. Noteworthy, based on biodistribution studies in rabbits of MVA-BN and a clinical shedding study with another MVA vector, both submitted in the context of a marketing authorization application EMEA/H/C/005343 evaluated by the BAC earlier this year, it can be considered that shedding from vaccinated individuals is not expected upon intramuscular administration of the MVA-HPV.
Vascular leakage from the vaccination site would be another possible source of dissemination into the environment. However, if the proposed measures for disinfection after administration can be improved as suggested by two experts (see 3.3 comment 1 and 6.2 comment 2 of this document), the exposure to avian species due to vascular leakage would be low to very low.
It should also be noted that there is no evidence pointing to an adverse effect associated to the transgene should avian species be exposed to the candidate vaccine. In terms of overall risk to the environment, the weight of evidence points to the fact that the risk for avian species would be negligible.

Coordinator comment:

The coordinator agrees with the SBB comment.

PartA1-3.6a – p35; last paragraph

The applicant indicates that a dressing is applied for 10 minutes to absorb any 'virus' (should be 'viral vector') that may leak. Next, the dressing is removed and disposed of as GMO waste, the injection site is left to dry for 20 more minutes. After that the patient can go.

In my opinion it would be best that after this procedure, the injection site is again decontaminated using the appropriate disinfectant. If vector is leaking, the vector will be in the dressing, but also on the skin of the patient. In order to limit the spread to the environment, an additional disinfection will wipe the residual vector and inactivate it. For ChAdV IPA 70% should be used, for MVA EtOH could be used to break the membrane.

SBB comment

A proposal to disinfect the injection site after the procedure was also raised by another expert, see section 6.2. of this compilation document.

Coordinator comment:

The applicant should be asked to add to the procedure that the injection site will be disinfected after it has been allowed to dry.

PartA1-3.6g – p37; first paragraph

The applicant indicates no 'viral' shedding (should be 'viral vector' shedding) is anticipated. Still to prevent potential exposure during pregnancy they inform participants to use contraception 4 weeks before and 8 weeks after the last dose. Yet, no advice is stipulated for men.

See also risk management strategies for section 5.5 for both viral vector types. Also male participants (if any) should be recommended to use contraception during the study period and for several months after the viral vectors have been applied.

Coordinator comment:

According to the title of the trial (page 31) only women will be included in the study.

Comment 2 **(van Larebeke)**

Has evaluated this item and has no questions/comments.

Comment 3 **(Zorzi)**

Has evaluated this item and has 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 **(Gijsbers)**

PartA1-3.3 – p34

At one site the vaccine will be prepared in a BSL2 lab instead of the hospital pharmacy. When considering ERA, how will the product be transported once prepared? Are procedures the same at all test sites?

SBB comment:

The applicant provided the following information (PartA1-3.4) :
Before the vaccine is transported for administration to the participant, a Self-Righting Luer Lock Tip Cap is secured tightly on the end of the syringe to avoid spillage; this is placed in a sterile plastic bag which is sealed and placed inside a second sealed bag. This is then placed in a transport pack which is labelled as “GMO transport box” and remains in this until at the site of administration. A commercially available spill kit, will accompany all vaccines that are transported to participants for administration. Any spillages will be cleaned up according to the study site SOPs.

Coordinator comment:

The coordinator agrees with the SBB comment.

Comment 2 **(van Larebeke)**

Has evaluated this item and has no questions/comments.

Comment 3 **(Zorzi)**

Please describe the storage conditions of the vials and the number of tubes stored in one fridge or freezer.

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 **(Gijsbers)**

PartA1-2.11 – p18; last paragraph

The applicant indicates that MVA-HPV has a host range restriction to avian cells and consequently does not require transcomplementation by the cells for growth. This makes the vector safe for human use (since it does not replicate). However, does this mean that MVA-HPV replicates on all avian cells?
What is the chance of shedding to birds in the environment? This is not discussed in the application.

SBB comment :

Same remark as under 3.3, see also comment of SBB.

Coordinator comment:

The coordinator agrees with the SBB comment.

PartA1-3.6a – p35; last paragraph

The applicant indicates that a dressing is applied for 10 minutes to absorb any ‘virus’ (should be ‘viral vector’) that may leak. Next, the dressing is removed and disposed of as GMO waste, the injection site is left to dry for 20 more minutes. After that the patient can go.
In my opinion it would be best that after this procedure, the injection site is again decontaminated using the appropriate disinfectant. If vector is leaking, the vector will be in the dressing, but also on the skin of the patient. In order to limit the spread to the environment, an additional disinfection will wipe the residual

vector and inactivate it. For ChAdV IPA 70% should be used, for MVA EtOH could be used to break the membrane

SBB comment
A proposal to disinfect the injection site after the procedure was also raised by another expert, see section 6.2. of this compilation document.

Coordinator comment:
The applicant should be asked to add to the procedure that the injection site will be disinfected after it has been allowed to dry.

Comment 2 **(van Larebeke)**
Has evaluated this item and has no questions/comments.

Comment 3 **(Zorzi)**
Has evaluated this item and has no questions/comments.

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 **(Gijsbers)**

PartA1-3.8 Emergency response plans
Upon needle stick injury. It is indicated that the area should be cleaned with 'a suitable disinfectant'. What would be adviced? It is important to clearly provide advice on how to inactivate the viral vectors on different surfaces (skin, surfaces, eyes, ..). Here I would expect the applicant to provide a SOP for each of the viral vectors.

Coordinator comment:
In section 3.6, page 36, item c) it is mentioned in detail which desinfectant can be used for the two types of vectors. For both vectors, ethanol (e.g. skin) and 70% IPA (e.g. other surfaces) could be used. However, section 3.8 could state more clearly what should be used in case of the different events: needle stick, skin and eyes. Especially for the latter two, two different procedures should be worked out. In the present description, there is not sufficient difference in action upon these two different events, which could lead to inappropriate action.

Comment 2 **(van Larebeke)**
Has evaluated this item and has no questions/comments.

Comment 3 **(Zorzi)**
Has evaluated this item and has no questions/comments.

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

Comment 1 **(Gijsbers)**

PartA1-3.8 – p39; 3rd paragraph

Here is referred to section 2.9. Shouldn't this be 2.8?

SBB comment:
Agrees with expert's comment

Coordinator comment:
A remark should be added to a list of 'typos and other errors/omissions'

Comment 2 **(van Larebeke)**
Has evaluated this item and has no questions/comments.

Comment 3 **(Zorzi)**
Has evaluated this item and has no questions/comments.

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

Comment 1 **(Gijsbers)**
Has evaluated this item and has no questions/comments.

Comment 2 **(van Larebeke)**
Has evaluated this item and has no questions/comments.

Comment 3 **(Zorzi)**
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 **(Gijsbers)**
Has evaluated this item and has no questions/comments.

Comment 2 **(van Larebeke)**
I consider that the likelihood of a recombination event leading to a wild type virus is very low, but cannot be excluded with 100% certainty. However, even if the unlikely event happens I expect that the consequences would not be important.

SBB comment:
See also SBB comment (additional information) under 3.2. with respect to the review Wold & Toth *et al*., 2013.

Coordinator comment:
The coordinator agrees with the SBB comment.

Comment 3 **(Zorzi)**
Has evaluated this item and has 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 **(Gijsbers)**
Has evaluated this item and has no questions/comments.

Comment 2 **(van Larebeke)**
This event is very unlikely and I see no reason to expect important consequences

Comment 3 **(Zorzi)**
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 **(Gijsbers)**

PartA1-2.11 – p18; last paragraph
The applicant indicates that MVA-HPV has a host range restriction to avian cells and consequently does not require trans-complementation for growth. This makes the vector safe for human use (since it does not replicate). However, does this mean that MVA-HPV replicates on all avian cells?
What is the chance of shedding to birds in the environment? This is not discussed in the application.

SBB comment :
Same question as the one under 3.3. of this compilation. See SBB comment under 3.3.

Coordinator comment:
The coordinator agrees with the SBB comment.

Comment 2 **(van Larebeke)**
Has not evaluated this item and has no questions/comments.

Comment 3 **(Zorzi)**
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 **(Gijsbers)**
Has not evaluated this item

Comment 2 **(van Larebeke)**
Has evaluated this item and has no questions/comments.

Comment 3 **(Zorzi)**
In the Document B-BE-20-BVW2 Part1A Common Application form ChAdOx1-HPV :
Part of the risk assessment should be revised.
Indeed, as mentioned in SECTION 5 – ENVIRONMENTAL RISK ASSESSMENT (p41), the common risks categories are to be classified as “high”, “moderate”, “low” or “negligible”.
However, the following risk assessment present on p39: ‘ *The vaccines present effectively zero risk to human health or the environment* » does not seem to follow the require classification and is also in contradiction with the A. 3 Overall risk evaluation and conclusions, line 5, p72: « The overall risk to the environment and human health is also considered low. »

Coordinator comment:
The coordinator agrees with the remark of the expert. The applicant should use the correct classifications and be consequent in its evaluation of risks. E.g. ‘Biological activity of the transgenes…..’ similar arguments for both vector types are resulting in different Hazard Characterizations: Low (ChAdOx1-HPV) (page 44) and Moderate (MVA-HPV) (page 60).

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 **(Gijsbers)**

PartA1-3.4
It is not clear what is meant with ‘study site SOPs’. Are SOPs different at different study sites? Would the installation of a single SOP procedure not be better (both for the study and for ERA). It is mentioned in point 3.5 later in the application that training will be provided by the sponsor. Are the SOP for vector transport included?

SBB comment:
According to legislation implementing GMO Directives in Belgium, each study site involved in the clinical trial should also submit a ‘contained use’ dossier which are subject to regional legislative procedures. It is anticipated that the relevant SOPs, such as those describing vector transport, will be included in each of the contained use dossiers. The SBB acts here as a scientific and technical expert for the regional competent authorities. Hence, this comment can be taken on board by the SBB when evaluating the corresponding ‘contained use’ dossiers.

Coordinator comment:
The coordinator agrees with the SBB comment.

Comment 2 **(van Larebeke)**
I wonder why the injection site is not disinfected after it has been allowed to dry.

SBB comment
A similar comment was raised by another expert in section 3.3. of this compilation document.

Coordinator comment:
The applicant should be asked to add to the procedure that the injection site will be disinfected after it has been allowed to dry.

Comment 3 **(Zorzi)**
Has evaluated this item and has no questions/comments.

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

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

Comment 1 **(Gijsbers)**
Has evaluated this item and has no questions/comments.

Comment 2 **(van Larebeke)**
Has evaluated this item and has no questions/comments.

Comment 3 **(Zorzi)**
Has evaluated this item and has no questions/comments.

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

Comment 1 **(Gijsbers)**

PartA1-3.8 Emergency response plans
It is important to clearly provide advice on how to inactivate the viral vectors on different surfaces (skin, surfaces, eyes, ..). Emergency plans should be more detailed.
For example, now it is adviced ‘that upon needle injury the contaminated area should be cleaned with ‘a suitable disinfectant’’. It should be clearly stated and detailed what would be the preferred procedure for each adverse situation.

Coordinator comment:
Already discussed in 5.2.

Comment 2 **(van Larebeke)**
Has not evaluated this item.

Comment 3 **(Zorzi)**
Has evaluated this item and has no questions/comments.

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 **(Gijsbers)**
Has not evaluated this item.

Comment 2 **(van Larebeke)**
Has not evaluated this item.

Comment 3 **(Zorzi)**
Has evaluated this item and has 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 **(Gijsbers)**
None

Comment 2 **(van Larebeke)**
I consider that the proposed first in human study does not entail significant risks and that performing the study is not in contradiction with general interest.

Comment 3 **(Zorzi)**
See the comment in the 6.1. section about the overall risk assessment

References

The references indicated below were also referenced by the applicant.

Altenburg, A.F., van de Sandt, C.E., Li B.W.S., MacLoughlin, R.J., Fouchier R.A.M., van Amerongen, G., Volz A., Hendriks, R.W., de Swart, R.L., Sutter, G., Rimmelzwaan, G.F., de Vries, R.D. 2017. Modified Vaccinia Virus Ankara Preferentially Targets Antigen Presenting Cells *In Vitro, Ex Vivo* and *In Vivo*. Sci Rep 7, 8580 doi:10.1038/s41598-017-08719-y

Dicks, M.D., Spencer, A.J., Edwards, N.J., Wadell, G., Bojang, K., Gilbert, S.C., Hill, A.V. and Cottingham, M.G. 2012. A novel chimpanzee adenovirus vector with low human seroprevalence: improved systems for vector derivation and comparative immunogenicity. *PloS one*, *7*(7).

Goossens, M., Pauwels, K., Willemarck, N. and Breyer, D. 2013. Environmental risk assessment of clinical trials involving modified vaccinia virus Ankara (MVA)-based vectors. *Current gene therapy*, *13*(6), pp.413-420.

Verheust, C., Goossens, M., Pauwels, K. and Breyer, D. 2012. Biosafety aspects of modified vaccinia virus Ankara (MVA)-based vectors used for gene therapy or vaccination. *Vaccine*, *30*(16), pp.2623-2632.

Wold, WSM. and Toth, K. 2013. Adenovirus vectors for gene therapy, vaccination and cancer gene therapy. *Current gene therapy*, *13*(6), pp.421-433.

Typos and other errors/omissions

PartA1-2.6 – p10; last paragraph
The study for the 120.000 people in Germany should be referenced.

PartA1-2.7 – ChAdOx1 paragraph
Throughout the text the word 'non-integrative' is used. This should be replaced with 'non-integrating'.

PartA1-2.16 – p25; third paragraph
Throughout the text the words 'virus' and 'infect/infection' are used when 'viral vector' and 'transduction/transduce' should be used. For example in third paragraph. But also further on in the application.

PartA1-3.8 – p39; 3rd paragraph
Here is referred to section 2.9. Shouldn't this be 2.8?