Adviesraad voor Bioveiligheid
Conseil consultatif de Biosécurité

# Advice of the Belgian Biosafety Advisory Council on the notification B/BE/22/BVW4 of the sponsor Nouscom srl for deliberate release in the environment of genetically modified organisms other than higher plants for research and development

19/01/2023
*Ref. SC/1510/BAC/2023_0051*

## Context

The notification B/BE/22/BVW4 has been submitted by Nouscom srl to the Belgian Competent Authority in October 2022 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.

The planned activity concerns a clinical trial entitled “A Phase I/II, Multicenter, Open-Label Study of Nous-209 Genetic Vaccine for the Treatment of Microsatellite Unstable Solid Tumors”.

The purpose of this study is to assess safety, tolerability and immunogenicity, and to detect any preliminary evidence of anti-tumor activity of Nous-209 genetic polyvalent vaccine in patient ≥18 years of age with microsatellite unstable solid tumors.

The investigational medicinal product consists of two viral vaccines composed of a recombinant replication-defective Great Ape Adenovirus GAd20-209-FSP used for priming and a highly attenuated orthopoxvirus Modified Vaccinia Ankara virus MVA-209-FSP used for boosting. GAd20-209-FSP is composed of four recombinant replication-incompetent non-human Great Ape Adenoviruses (gorilla-derived GAd20). MVA-209-FSP is composed of four recombinant attenuated, replication-defective orthopoxvirus MVA.
Each of the four GAd20 vectors and each of the four MVA vectors present in the vaccine encode a synthetic transgene, named FSP-A1, FSP-A2, FSP-A3 and FSP-A4. Each transgene encodes a string of approximately 50 selected FSPs (Frame-Shifted Peptides) neoantigens selected among the most common found in colorectal, gastric and endometrial MSI (Microsatellite Instable) cancer patients. Both GAd20-209-FSP and MVA-209-FSP vaccines encode in total for an identical set of 209 different FSPs.

The prime component of the investigational Nous-209 vaccine, GAd20-209-FSP, will first be administered by intramuscular injection at a dose of 1.88 $x10^{10}$ viral particles (vp) (low dose) or 1.88 $x10^{11}$ vvp (high dose), and after three weeks the boost component, MVA-209-FSP, will be administered three times with a 3-week interval by intramuscular injection at the dose of 1.65 $x10^{7}$ infectious unit (ifu) (low dose) or 1.65 $x10^{8}$ ifu (high dose).

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It is estimated that approximately 15 patients will be included in this Phase I/II study, which is planned to be conducted in one clinical site located in Brussels. The national territory is considered as the potential release area of Nous-209.

The use of recombinant replication-defective Great Ape Adenovirus for priming and a highly attenuated orthopoxvirus Modified Vaccinia Ankara virus for boosting for the treatment of cancer patients, has already been assessed by the BAC in the framework of notification B/BE/20/BVW5[1], submitted by Nouscom srl.

The dossier has been officially acknowledged by the Competent Authority on 10 October 2022 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. Two 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 SBB also took part in the evaluation of the dossier. 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 (GMO) 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 the following legislation:
- 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, economic or ethical considerations, are outside the scope of this evaluation.

On 17 November 2022, 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 05 December 2022 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 list of questions, which was transmitted to the notifier on 16 December 2022. The answers of the notifier were received on 10 January 2023 and transmitted to the BAC, after which the BAC was able to come to a conclusion with respect to the environmental aspects associated to the proposed clinical trial.

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 above-mentioned Royal Decree. The Competent Authority received one reaction from the public of which some questions were related to biosafety issues. According to Article 16 §2 of the Royal Decree of 21 February 2005, the

[1] Advice of the Belgian Biosafety Advisory Council on the notification B/BE/20/BVW5 - *Ref. SC/1510/BAC/2021_0127*

comments that are relevant for biosafety received in the framework of the public consultation, have been taken into account in the preparation of the advice below.

## Summary of the scientific evaluation

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

The donor, recipient and parental organisms were found to be adequately described in the dossier.

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

The human tumor FSP neoantigens encoded by both viral vectors are not expected to have any function in the cells and do not promote spreading or alters viral vector tropism as determined by the biodistribution studies.
Information related to the molecular characteristics of GAd20-209-FSP and MVA-209-FSP were found to be adequately described in the dossier.

### 3. The conditions of the release

Upon evaluation of the information provided by the notifier, the BAC revealed a few inconsistenties throughout the documents on the use of personnel protective equipment and on the procedure to be followed in case of accidental spill. The notifier adequately implemented the remarks and requests addressed by the BAC in a revised version of the SNIF, the Technical sheet, the Pharmacy Manual and the Information to the public documents that were submitted in the context of this notification. The personal protective equipment includes eye protection, masks, gloves and a lab coat for exposed personnel. The procedure to be followed in case of accidental spill has been implemented according to the Laboratory biosafety manual (Fourth edition) of the World Health Organization. The disinfectant solution used to clean accidental spillage will stay on the area where the spillage occurred for 30 minutes.

Given that no specific data are currently available on GAd20-FSP-209 and MVA-209-FSP shedding from secretions and/or excreta of animals and that shedding analysis has not been tested in patients, the notifier, following BAC's request, prepared a short, readable format document that will be provided to each patient detailing all required instructions for the patients with respect to good hygiene practices. Among other things, patient working in a zoo will be advised to avoid contacts with the GAd20 natural host in order to eliminate the risk of recombination between the wild type GAd20 and the GMO for a period of 8 days, which corresponds to the time required to allow the vector clearance from most of the tissues. Since biodistribution studies with similar viral vectors in rats, rabbits (Stokes et al, 2022; Sheets et al 2008; Volkmann et al, 2021)[2]

[2] Sheets RL *et al*. J Immunotoxicol 2008; 5(3) 315-335 - Biodistribution and toxicological safety of adenovirus Type 5 and Type 35 vectored vaccines against human immunodeficiency virus-1 (HIV-1) Ebola or Marburg are similar despite differing adenovirus serotype vector, manufacturer's construct or gene inserts.

Stokes AH *et al*. Int J of Toxicol 2022; 41(4): 263-275 - Repeated-dose toxicity, biodistribution and shredding assessments with ChAd155 Respiratory Synctial Virus vaccine candidate evaluated in rabbits and rats.

Volkmann A *et al*. Vaccine 2021; 39(22): 3050-3052 - The Brighton Collaboration standardised template for collection of key information for risk/benefit assessment of a Modified Vaccinia Ankara (MVA) vaccine platform.

and mice (Hanke et al, 2005; Hanke et al, 2002)[3] have shown that both viral vectors mainly remain at the injection site (muscle) up to several days, the first bandage will be replaced by a new one instead of leaving the injected area unprotected when leaving the hospital. Instruction on bandage management at home have also been provided in the instructions sheet to the patient.

**4. The risks for the environment or human health**

GAd20-209-FSP is unable to replicate due to the deletions of viral E1, E3 and E4 coding regions and is consequently unable to persist and to multiply. MVA-209-FSP is a non-integrative vector unable to produce vector particles in human cells.

In light of the biodistribution studies conducted on mice with MVA vectored vaccines injected intramuscularly, the notifier further clarified that neither infectious virus nor viral DNA was found in any other of the organs tested (liver, spleen and gonads) at Day 1 and at Day 8 after injection.

The proposed clinical trial is a first-in-human study, hence no shedding and biodistribution data with both viral vectors are available. As further developed by the notifier, except for injection site leakage, several clinical studies performed with very similar replication-deficient adenoviral vectors as well as non-clinical results show absence of shedding when the vector is administered intramuscularly. One clinical trial with a similar MVA viral vector DNA in cancer patients confirm very limited spread over biological fluids for MVA-based vectors upon local injection.

Given the replication-defective properties of the GAd20-209-FSP vector, its low probability of shedding especially when administered by the intramuscular injection route, 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 FSP transgenes could influence the shedding behavior of recombinant GAd vectors, the BAC concludes that the risk for the environment and human health associated to possible shedding of the GAd20-209-FSP vector, if it were to occur, is low.

Taking into account that i) wild type vaccinia virus and the parental MVA are not naturally found in the environment ii) the MVA vector has lost about 15% of its parental genome, precluding the ability of poxviruses to complement MVA iii) MVA is a non-integrative vector unable to produce vector particles in human cells iv) the lack of viral shedding observed from subjects vaccinated with MVA vectors, the BAC concludes that it is unlikely that the proposed intended use of MVA-209 would confer risks to the human health or the environment.

Considering all of the above elements, the BAC concludes that, based on the non-pathogenic and non replicative nature of Nous-209 (and the assumed lower amounts of shed Nous-209), the overall risk

[3] Hanke T et al. Vaccine 2002; 21(1-2): 108-114 - Lack of toxicity and persistence in the mouse associated with administration of candidate DNA- and Modified Vaccinia Virus Ankara (MVA)-based HIV vaccines for Kenya.

Hanke T et al. Vaccine 2005; 23(12): 1507-1514 - Biodistribution and persistence of an MVA-vectored candidate HIV vaccine in SIV-infected rhesus macaques and SCID mice.

associated to exposure and transmission to other individuals or animals can be considered negligible provided that the proposed risk mitigation meaures are adequately implemented.

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

The BAC raised a few comments on the assessment of procedures with respect to the handling of accidental spills and the procedure to be followed in case of mouth exposure.
The notifier adequately implemented the remarks addressed by the BAC regarding the procedure to be followed in case of mouth exposure in a revised version of Pharmacy manual v3.0 dated 18 January 2021, the three information documents for the public and the Technical sheet 26Sep2022.

Given that the assessment of the likelihood of further propagation of Nous-209 can be considered highly unlikely, the BAC supports the view that, in terms of risk for the environment or human health, the proposed measures are proportionate and adequate in the context of the intended clinical trial.

## Conclusion

Based on the scientific assessment of the notification made by the Belgian experts, the Biosafety Advisory Council concludes that it is unlikely that Nous-209 developed as a anti-tumor therapy for melanoma and non-small cell long carcinoma, 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**:

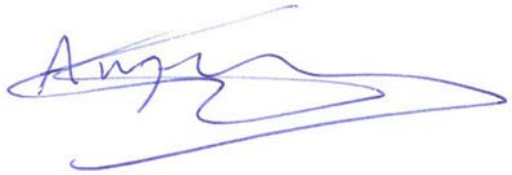
- The notifier and the investigators must strictly apply the clinical trial protocol and the safety instructions as described in the following documents :
    - EU_Pharmacy manual v3.1_BEL_22Dec2022
    - Common application form for GAd20-209-FSP and MVA-209-FSP v2.0 27sep2022
    - Summary Notification Information Format Gad-209-FSP and MVA-209-FSP BEL_SNIF_v3.1_22Dec22
    - IB, v4.1, dated 4 Nov 2021
    - BEL_Technical sheet_v2.1_22Dec22
    - BEL_Patient Instructions_v1_03Jan2023

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

- The notifier is responsible to verify that the 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 the contained use of genetically modified micro-organisms.

- The BAC 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 the competent authority for the attention of the BAC a report with details concerning the biosafety aspects of the project. This report shall contain at least:
  - 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 Nous-209.



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/22/BVW4 (ref. SC/1510/BAC/2022_1456)
Annex II: Answers to the public reaction to dossier B/BE/22/BVW4 in NL (ref. SC/1510/BAC/2023_0053) and FR (ref. SC/1510/BAC/2023_0052)

# Compilation of the expert's evaluations of the answers of Nouscom srl on the list of questions for dossier B/BE/22/BVW4

19 December 2022
*Ref. SC/1510/BAC/2022_1456*

**Coordinator:** Jozef Anné (KUL)
**Experts:** Rik Gijsbers (KULeuven), Willy Zorzi (ULiège), Aline Baldo (SBB)
**SBB:** Sheela Onnockx

## INTRODUCTION

Dossier **B/BE/22/BVW4** concerns a notification from Merck Sharp & Dohme Corp. for a clinical trial entitled "A Phase I/II, Multicenter, Open-Label Study of Nous-209 Genetic Vaccine for the Treatment of Microsatellite Unstable Solid Tumors".

On 17 November 2022, based on a list of questions prepared by the BAC (SC/1510/BAC/2022_1285), 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 05 December 2022. This complementary information was reviewed by the coordinator and the experts in charge of the evaluation of this notification.

## *Evaluation Expert 1*

We do not agree with the response to the section "Q6" provided by the notifier.

The applicant seems to mistake the assessment and the management of the biorisk of a normal work situation during the preparation or administration of the IMP with a situation in case of bioemergency.

In case of normal preparation and administration situations, it seems clear that the risk of aerosol generation could be evaluated as very low (the vial is kept in vertical position, gentle and not vigorous swirling is applied, and foam production is avoided).

However, in case of accidental spill, aerosols will be spreading even if in a case of small volume (such as 1 ml) of GMO preparation.

1) In case of such a spill, it is better to wait for a certain length of time (generally 30 min) to allow aerosols to be carried away and heavier particles to settle. Despite of application of this awaiting time, if the notifier would like immediately decontaminate the spill area surface, it could be underlined that during an early step of decontamination as suggested previously, part of the aerosols are continuing to float suspended in the room air while others are settling on the room surfaces. Therefore, this leads to a significant drop in the effectiveness of decontamination if applied without delay.

2) In case of non-compliance with this awaiting time, if the work is continuing in the spilled room without worrying about aerosols, it could lead to continuous exposure of all the material and the people present in the room. Moreover, if the notifier consider that spread of GMO during a spill in the area as negligible or irrelevant matter, it could result in the absence of sufficient care and remediation measures. It could also lead the notifier to neglect or abandon the culture of precaution as well as the assessment and the management of exposure to direct and indirect risks during bioemergency situations.

To conclude, concerning the point “Q6”, the notifier is invited to follow the previous advice provided by the Biosafety Council.

***SBB’s and coordinator’s Comment:***

It should be noted that even with small volumes (such as 1 ml), aerosol could be produced in case of accidental spill.

The NOUS-209 products IMP for Protocol NOUS-209-01 are supplied to the site/pharmacy in 3 ml vials each vial containing 1,2 ml of IMP. Therefore, the maximum volume of spillage cannot exceed the 1,2 ml volume extractable from the vial. The injection volume is 1 ml for each of the vaccines (GAd20-209-FSP and MVA-209-FSP).

All procedures associated with a high risk of aerosolization, such as centrifugation or sonication, are expected to be performed in a biosafety cabinet (see answer to question 6), and all personnel handling GAd20-FSP-209 and MVA-209-FSP will wear protective gowns, masks, gloves and eye protection (see answer to question 10).

The instruction to wait a certain length of time (generally 30 min) to allow aerosols to be carried away and heavier particles to settle was not requested in the previous and similar dossier B/BE/20/BVW5 from Nouscom srl with a recombinant GAd20 Gorilla adenovirus and a recombinant vaccinia virus Ankara (MVA). Whereas for dossier B/BE/21/BVW6 from GlaxoSmithKline Biologicals SA using a recombinant simian adenoviral vector and a modified Vaccinia Ankara viral vector, this instruction was requested and added in the final advice.

***Evaluation Expert 2***

Ils ne fournissent pas de nouvelles informations concernant le shedding. Ils disent que le risque est négligeable car ce sont des vecteurs déficients pour la réplication et donnent des exemples d’absence de shedding chez l’homme avec d’autres vecteurs similaires (pour le MVA même vecteur mais pas le même transgène et le site d’insertion ; pour l’Ad, il s’agit de vecteurs différents et il ne donne pas le % d’identité/homologie avec ces vecteurs). Le notifiant ne juge pas nécessaire de donner un message à la maison aux participants de l’étude concernant les mesures à prendre de nouveau car il estime que le risque est négligeable (réponse à la Q7). Je ne sais pas si nous devons aller plus loin et leur imposer de récolter des données de shedding chez l’homme et de fournir un message aux participants avec des mesures à prendre (celles proposées) ? Je pense que pour les dossiers précédents, nous n’avons pas demandé de telles mesures.
Concernant les mesures à prendre en cas de spill, je suis d’accord avec leur argumentation, si ils ont des volumes de 1 ml, ils ne vont pas quitter la pièce.

***SBB's and coordinator's Comment:***

GAd20-209-FSP is a replication-incompetent GMO based on a group C gorilla adenovirus, engineered to express FSP antigens, with the deletion of the E1 gene that is essential for virus replication. The applicant provided the results of a few studies with replication-defective recombinant adenoviruses that failed to detect viral release in biological samples such as nasal and throat swabs, urine or feces. GAd20-209-FSP will be administered by intramuscular injection which corresponds to a route that was shown to limit its potential shedding and spread to other tissues, since the virus vector remains localized to the site of injection.
MVA-209-FSP is a highly attenuated strain of vaccinia virus derived from the Chorioallantois Vaccine Ankara (CVA) strain which is defective for replication in human cells and unable to cause infection in mammals. One phase I study with a similar MVA that expressed human MUC1 failed to observe the presence of vector sequences in urine samples up to 8 days after injection. As mentioned by the applicant, Imvanex®, a live non-replicating Modified Vaccinia Ankara (MVA) vaccine has already been registered for smallpox protection in Europe since 2013 and in the US since 2019.

For a previous and similar dossier B/BE/20/BVW5 from Nouscom srl with a recombinant GAd20 Gorilla adenovirus and a recombinant vaccinia virus Ankara (MVA), no patient instruction sheet has been requested. As well as for the dossier B/BE/21/BVW6 from GlaxoSmithKline Biologicals SA using a recombinant simian adenoviral vector and a modified Vaccinia Ankara viral vector.

***Evaluation Expert 3***

Having read through the provided replies of the notifier, I largely agree with the provided answers. Still, there are some points that in my opinion require further clarification or correction.

I indicate these below.

**Q1** answered sufficiently

**Q2**: Even though the applicant clearly adopted the txt (see Q1) in the file to replace "virus" by "viral vector", still the words "infection" and "virus" are used in the answers (for example Q2 – 'vaccine infected cells' this should be 'vaccine transduced cells' ; "virus tropism" should be "viral vector tropism"). This is particularly sloppy.
Similarly, in the SNIF file Attachment 1_NOUS-209-01_BE_SNIF_v3_01Dec22_track.pdf the words virus is still used when viral vector is meant (eg p12/30, p14/30, 'small quantities of the virus could be detected by PCR at the injection site' p28/30).

**Q3** answered sufficiently

**Q4**: I do not agree that shedding analysis in animal models is sufficient and allows to extrapolate shedding predictions in humans. Therefore, the analysis of shedding and biodistribution in mouse and rat models should at least be supported by shedding analysis in human when possible. Even though it is interesting to have supportive data from other trials in human, the vectors used, and the doses and injection sites applied are different, and therefore not readily extrapolable. In addition, technologies evolve, and current ddPCR platforms are more sensitive than the former qPCR approaches to pick up shed particles. Thus, I would still strongly suggest including a shedding analysis when possible.

**Q5** answered sufficiently

**Q6**: I agree that volumes are low and therefore follow the reasoning of the applicant. Still, it seems to me that the current way of handling whether one or the other procedure should be applied is subjective.

**Q7**: The answer to Q7 is not sufficient. In my opinion, we cannot conclude that there will be no shedding if we do not assess the shedding for the current vector and injection dose/route. (see also point2 higher up)

**Q8** is answered sufficiently.
**Q9** was answered sufficiently

**Q10** was answered sufficiently. “Masks” are added as PPE. Is this a universal reference, or should it be indicated as “surgical mask”?

**Q11** was answered sufficiently

**Q12** was answered sufficiently

**Typos and suggestion** to clarification:
the ones indicated in the document have been corrected well.

In addition, I provide some small additions below:

- In Attachment 1_NOUS-209-01_BE_SNIF_v3_01Dec22_track.pdf at p10/30: ‘parenteral’ should be “parental”. Also, in my opinion it would be best to stipulate the specific hosts that should be avoided.

- At p29/30 point6: the total number indicated for the monitoring is not correct (should be 29 and 21?) – or the number of visits should be adapted.

***SBB’s and coordinator’s Comment:***

Q2 : Should a second list of questions be sent to the notifier, this point could be added to the list.

Q4 : Interaction of the viral vector with the host and the clinical trial conditions (administration route, doses…) may have an effect on the shedding pattern. Therefore, one should stay cautious with extrapolating pre-clinical data to human beings. The applicant provided some results of shedding analysis with similar viral vectors in pre-clinical and clinical studies. Although no shedding analysis with GAd20-FSP-209 and MVA-209-FSP seems to have been done yet in animals. When assessing the overall risk associated with conducting the trial and taking into account the non-pathogenic and replication-deficient nature of both IMP, the remaining uncertainty on the shedding pattern of GAd20-FSP-209 and MVA-209-FSP is likely to be alleviated by the proposed risk management measures, provided that instructions and precautionary measures mentioned in the Pharmacy Manual and the technical sheet are applied by the personnel handling both viral vectors, that the patients are clearly instructed on the precautionary measures to be applied in order to minimizing exposure of thirds outside. Furthermore, as recommended by EMA for a potential Marketing authorisation application, the notifier is expected to present shedding analysis data in human should the notifier plan to carry out subsequent steps in the development of the IMP in Belgium.

Q6 : See SBB’s comment to expert 1

Q7 : As long as the applicant is not fully aware of the shedding properties of the IMP, clear instructions should be provided to the patients in order for them to adhere to and practice good hygiene. These risk management measures should focus on minimizing exposure of thirds outside the clinical setting, thereby giving particular attention to immunosuppressed or any vulnerable people (e.g. infants, elderly people).

It is very important to explain which measures must be followed once the patient has left the hospital :

- bandage management at home : Since biodistribution studies in rats, mice and rabbits have shown that both viral vectors mainly remain at the injection site (muscle) up to several days, it could be advice to replace the bandage by a new one instead of leaving the injected area unprotected. In this case, detailed instructions for the patient should be provided regarding the appropriate time for removal of the second bandage, the disposal of the second bandage and injection site waste management.
- washing hands procedure
- close contact management with immunosuppressed or any vulnerable people (e.g. infants, elderly people)

Although GAd20-209-FSP is replication-incompetent is, and MVA non-infectious virus produces in men, and handled as Biosafety level 1 (BSL-1), it might be advisable to inform patients about the procedure to be followed the first week, when leaving the hospital

Q10: The biosafety level required for manipulating both viral vectors corresponds to a BSL-1 for all operations including transportation, storage and handling for administration to patients. Personal protective equipment required for biosafety level 1 (BSL-1) laboratories includes lab coats, gloves, and eye protection.

Typos 1 : Should a second list of questions be sent to the notifier, this point could be added to the list.

Typos 2 : According to the protocol, table 6 on page 88/150, and the ICF, patients enrolled in cohort C will undergo in total 30 visits and patients enrolled in cohort D will only undergo 22 visits in total.
The administration of GAd20-209-FSP corresponds to visit 1, week 1. The administration of MVA-209-FSP corresponds to visit 2, week 2. Therefore, after the first administration of the GMO MVA-209-FSP (visit 2, week 2), each patient will undergo an additional 28 visits, which corresponds to a total of 30 visits in Cohort C and an additional 20 visits (total 22) in Cohort D.

Adviesraad voor Bioveiligheid
Conseil consultatif de Biosécurité

# Réponse du Conseil consultatif de Biosécurité aux observations formulées pendant la consultation du public concernant la notification B/BE/22/BVW4 de Nouscom srl pour l'introduction volontaire dans l'environnement, à des fins de recherche et développement, d'organismes génétiquement modifiés autres que les plantes supérieures

Adopté le 19/01/2023
*Ref. SC/1510/BAC/2023_0052_FR*

## Contexte

La notification B/BE/22/BVW4 a été soumise en août 2022 par Nouscom srl à l'autorité compétente belge pour une demande de dissémination volontaire dans l'environnement, à des fins de recherche et développement, d'organismes génétiquement modifiés autres que les plantes supérieures, conformément au chapitre II de l'arrêté royal du 21 février 2005. La notification a été lancée par l'autorité compétente (AP) le 23 juillet 2021, après que le notifiant aie suffisamment répondu aux questions de validation.
Conformément à l'article 17 de l'arrêté royal, l'AC a organisé une consultation du public pendant une période de 30 jours. À la suite de cette consultation, l'AC a transmis les observations du public au Conseil consultatif de biosécurité, parmi lesquelles un certain nombre d'observations pertinentes en matière de biosécurité.
Conformément à l'article 16§2 de l'arrêté royal, ces observations ont été prises en compte lors de la préparation de l'avis du Conseil consultatif de Biosécurité (référence BAC_2023_0051). La réponse à ces observations est donnée ci-dessous.
Les questions/observations du public qui ne sont pas pertinentes en matière de biosécurité (telles que les questions liées au patient, les questions économiques ou éthiques) ne sont pas prises en compte par le Conseil de Biosécurité.

**Question 1**: En quoi ce processus diffère-t-il d'une réaction allergique, où (d'après ce que nous savons) le système immunitaire est également (trop) déclenché ? Il s'agit d'avoir une bonne compréhension du résultat attendu de cette étude.

### Commentaire du SBB

Le mécanisme d'activation du système immunitaire après l'administration de vaccins à base d'adénovirus est un aspect lié à la sécurité du patient. Cet aspect est traité dans le cadre de l'évaluation clinique de l'étude et dépasse le cadre de l'évaluation des risques environnementaux ou de l'évaluation de la biosécurité de l'essai proposé. Comme l'ont observé Kloor *et al* (2020), la vaccination par le néoantigène FSP est bien tolérée et induit des réponses immunitaires humorales et cellulaires chez tous les patients vaccinés, représentant ainsi une nouvelle approche prometteuse pour le traitement et même la prévention du cancer déficient en MMR.

**Question 2:** Il est indiqué dans le dossier public que l'infection et la réplication ne sont explicitement pas le but de cette étude, mais l'introduction d'une séquence d'ADN codant pour les néoantigènes. Est-il exact de dire que le résultat final d'un essai réussi est un fragment d'ADN flottant dans le noyau cellulaire qui est transcrit comme le reste du génome, ce qui donne les néoantigènes ? Dans quelle mesure cette situation est-elle stable à long terme, ou la libération des antigènes n'est-elle nécessaire que pendant une courte période ?

**Commentaire du SBB:**
Les vecteurs adénovirus sont des vecteurs viraux très intéressants pour le développement de vaccins en raison de leur immunogénicité puissante et de l'absence d'expression transgénique prolongée. Les délétions des régions codantes virales E1, E3 et E4 inactivent la réplication de l'ADN viral et la formation de nouveau particules. Il est donc incapable de répliquer son ADN pour amplifier le transgène et par conséquent incapable de se propager, contrairement à un adénovirus avec les régions E1, E3 et E4 intactes et toujours compétent pour la réplication de son génome.
Le principe de cette vaccination est d'induire une réponse immunitaire puissante contre les FSP codées par le vaccin : Le GAd20-209-FSP amorce les cellules T produisant de l'INF-γ contre les antigènes FSP des 4 vecteurs du mélange. Les injections de MVA stimulent les réponses immunitaires amorcées par GAd20.

**Question 3:** Le dossier indique que rien n'est encore connu sur l'excrétion et la biodistribution de ces vecteurs spécifiques. Le notifiant a-t-il l'intention de collecter également des données à ce sujet ?

**Commentaire du SBB:**
Une requête a été envoyée au notifiant lui demandant de justifier pourquoi aucune analyse de l'excrétion des vecteurs viraux ne sera effectuée pour GAd20-209-FSP et MVA-209-FSP et d'énumérer et détailler les résultats (échantillons où l'excrétion a été observée, la période de temps pendant laquelle une excrétion a été observée) des études cliniques et non cliniques sur l'excrétion qui ont été réalisées avec des vecteurs similaires pour nous permettre de juger correctement si l'excrétion est pertinente pour l'évaluation des risques environnementaux . Selon les lignes directives de l'EMA, une recommandation concernant la nécessité d'effectuer des analyses d'excrétion si le notifiant envisage une éventuelle demande d'autorisation de mise sur le marché, sera également donnée.

**Question 4 :** Y a-t-il une chance que le transgène puisse être transmis par les gamètes à la génération suivante ? Nous posons cette question en pensant en particulier aux ovocytes, qui sont en grande partie dormants pendant toute la durée de vie de la femme et où le transgène peut donc être encore activement présent après 180 jours. Ou bien le fragment d'ADN n'est-il plus actif après 180 jours (pour des raisons de stabilité, par exemple) ?

**Commentaire du SBB:**
Comme le virus parental dont le vecteur GAd20-209-FSP est dérivé, le génome devrait rester épichromosomique, évitant ainsi le risque de mutagenèse par insertion et excluant la transmission germinale. Les études de biodistribution avec le vecteur GAd20-209-FSP chez les rats Sprague-Dawley n'ont pas détecté de quantités quantifiables d'ADN dans les gonades à 24 heures, 7 jours et 30 jours après l'injection (IB p28/102).

En ce qui concerne la dispersion possible du MVA recombinant dans les cellules germinales et donc la transmission à la descendance, il convient de noter que l'intégration du matériel génétique du MVA recombinant dans le chromosome de l'hôte est considérée comme négligeable puisque le MVA ne se réplique que dans le cytoplasme des cellules permissives non humaines [Goossens *et al*. 2013], par conséquent, la transmission du MVA recombinant dans les cellules germinales et donc la transmission

à la descendance peut être considérée comme négligeable. Une étude de biodistribution avec un vaccin similaire à base de MVA recombinant, le MVA MSP1 administré par voie intramusculaire, n'a pas observé de virus infectieux, ni d'ADN viral dans les gonades au jour 1 et au jour 8 après l'injection (IB p12/102).

Selon les critères d'inclusion des patientes inclues dans les cohortes C et D, une femme en âge de procréer (WOCBP) doit accepter d'utiliser des méthodes contraceptives hautement efficaces pendant la période de traitement et pendant au moins 180 jours après la dernière dose du traitement de l'étude ainsi que de s'abstenir de donner des ovules pendant cette période. Les patients masculins doivent accepter d'utiliser un contraceptif pendant la période de traitement et pendant au moins 180 jours après la dernière dose du traitement à l'étude ainsi que de s'abstenir de donner du sperme pendant cette période. Ce délai est conforme à la recommandation de Goossens *et al* (2013) qui suggéraient que les patientes, injectées avec des vecteurs à base de MVA, devaient utiliser une contraception efficace pendant la période d'étude et pendant plusieurs mois après la dernière administration d'IMP afin de prévenir toute transmission éventuelle.

**Question 5**: Sur base de quelles informations la période de 30 minutes a-t-elle été choisie pour couvrir le site d'injection ? Cela semble très limité par rapport à d'autres dossiers, surtout compte tenu de la persistance d'études similaires pour l'AVM, ou est-ce lié au caractère non réplicatif et au mode d'administration ?

**Commentaire du SBB:**
Étant donné que des études de biodistribution réalisées avec des vecteurs viraux similaires chez le rat, le lapin (Stokes et al, 2022 ; Sheets et al 2008 ; Volkmann et al, 2021) et la souris (Hanke et al, 2005 ; Hanke et al, 2002) ont montré que les deux vecteurs viraux restent principalement au site d'injection (muscle) jusqu'à plusieurs jours, une demande a été envoyée au notifiant pour remplacer le bandage par un nouveau plutôt que de laisser la zone injectée sans protection lorsque le patient quitte l'hôpital. Il a été demandé au notifiant de préparer pour le patient un petit résumé à emporter chez soi avec des instructions détaillées concernant le moment approprié pour le retrait du second pansement, l'élimination du second pansement et la gestion du nettoyage du site d'injection.

**References:**

Goossens M *et al*. Curr Gene Ther. 2013 Dec; 13(6): 413–420 - Environmental Risk Assessment of Clinical Trials Involving Modified Vaccinia Virus Ankara (MVA)-Based Vectors

Hanke T *et al*. Vaccine 2002; 21(1-2): 108-114 - Lack of toxicity and persistence in the mouse associated with administration of candidate DNA- and Modified Vaccinia Virus Ankara (MVA)-based HIV vaccines for Kenya.

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Stokes AH *et al*. Int J of Toxicol 2022; 41(4): 263-275 - Repeated-dose toxicity, biodistribution and shredding assessments with ChAd155 Respiratory Synctial Virus vaccine candidate evaluated in rabbits and rats.

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Adviesraad voor Bioveiligheid
Conseil consultatif de Biosécurité

# Antwoorden van de Adviesraad voor Bioveiligheid op opmerkingen gekregen tijdens de publieksraadpleging over de kennisgeving B/BE/22/BVW4 van Nouscom srl voor doelbewuste introductie in het leefmilieu van genetisch gemodificeerde organismen met uitzondering van hogere planten voor onderzoek en ontwikkeling

Goedgekeurd op 19/01/2023
*Ref. SC/1510/BAC/2023_0053_NL*

## Contexte

De kennisgeving B/BE/22/BVW4 werd in augustus 2022 door Nouscom srl bij de Belgische bevoegde overheid ingediend voor een verzoek om doelbewuste introductie in het leefmilieu van genetisch gemodificeerde organismen, met uitzondering van hogere planten voor onderzoek en ontwikkeling, overeenkomstig hoofdstuk II van het koninklijk besluit van 21 februari 2005. De kennisgeving kon opgestart worden door de bevoegde overheid (BO) op 10 oktober 2022 nadat de kennisgever de validatievragen voldoende beantwoord had.
Volgens artikel 17 van het koninklijk besluit organiseerde de BO een openbare raadpleging van het publiek voor een periode van 30 dagen. Als resultaat van deze raadpleging heeft de BO de opmerkingen van het publiek doorgestuurd naar de Adviesraad voor Bioveiligheid, waarvan een aantal opmerkingen betreffende bioveiligheid.
Overeenkomstig artikel 16§2 van het koninklijk besluit zijn deze opmerkingen in beschouwing genomen bij het uitbrengen van het advies van de Adviesraad voor Bioveiligheid (referentie BAC_2023_0051). Het antwoord op deze opmerkingen wordt hieronder gegeven.
Vragen/opmerkingen van het publiek die niet relevant zijn inzake bioveiligheid (zoals patiënt gerelateerde vragen, economische of ethische kwesties) worden door de Bioveiligheidsraad niet in aanmerking genomen.

**Vraag 1**: Op welke manier verschilt dit proces van een allergische reactie, waarbij (naar ons begrip) het immuunsysteem ook (te veel) getriggered wordt? Kwestie van een goed begrip te hebben van het beoogde resultaat van deze proef.

**SBB's Comment:**
Het activeringsmechanisme van het immuunsysteem na een adenovirus gebaseerde vaccinatie is een aspect dat verband houdt met de veiligheid van de patiënt. Het wordt beoordeeld in het kader van de klinische beoordeling van de voorgestelde studie en valt buiten de scope van de milieurisicobeoordeling en de bioveiligheidsbeoordeling van de studie. Zoals waargenomen door Kloor *et al* (2020), wordt FSP-neoantigeenvaccinatie goed verdragen en induceert het humorale en cellulaire immuunresponsen bij alle gevaccineerde patiënten, waardoor het een veelbelovende nieuwe aanpak is voor de behandeling en zelfs de preventie van MMR-deficiënte kanker.

**Vraag 2:** Er wordt in het publiek dossier gesteld dat infectie en replicatie expliciet niet het doel is van deze studie, maar het invoeren van een DNA-sequentie die codeert voor de neoantigenen. Is het correct

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om te stellen dat het eindresultaat van een succesvolle proef een vrij zwevend DNA-fragment in de celkern is dat wordt getranscripteerd zoals de rest van het genoom, met de neoantigenen als resultaat? Hoe stabiel is deze situatie op langere termijn, of is de vrijgave van de antigenen slechts voor een korte periode noodzakelijk?

**SBB's Comment:**
Adenovirale vectoren zijn aantrekkelijke virale vectoren voor de ontwikkeling van vaccins vanwege hun krachtige immunogeniciteit en het ontbreken van langdurige transgene expressie. De deleties van de virale E1-, E3- en E4-coderingsgebieden inactiveren de virale DNA-replicatie en de vorming van nieuwe partikels. De virale vector kan zijn DNA niet repliceren om het transgen te amplificeren en kan zich niet verspreiden, in tegenstelling tot een E1-, E3- en E4-intact replicatiecompetent adenovirus.
Het principe van deze vaccinatie is het opwekken van een krachtige immuunrespons tegen gevaccineerde FSP's: GAd20-209-FSP induceert T-cellen die INF-γ produceren tegen FSP-antigenen van de 4 vectoren in het mengsel. De MVA-injecties versterken de GAd20 immuunresponsen.

**Vraag 3:** Er wordt in het dossier gesteld dat er nog niets geweten is over shedding en biodistributie voor deze specifieke vectoren. Is het de intentie van de aanvrager om hier dan ook gegevens over te verzamelen?

**SBB's Comment:**
Bij een aanvraag voor bijkomende informatie werd de kennisgever gevraagd om uit te leggen waarom er geen analyse van de uitscheiding van virale vectoren zal worden uitgevoerd voor GAd20-209-FSP en MVA-209-FSP en om de resultaten op te sommen en in detail te beschrijven (monsters waar uitscheiding werd geobserveerd, de tijdsperiode waarin uitscheiding werd geobserveerd) van klinische en niet-klinische onderzoeken naar uitscheiding die uitgevoerd werden met vergelijkbare vectoren om goed te kunnen beoordelen of de mogelijke uitscheiding relevant is voor de milieurisicobeoordeling. Zoals ook wordt geadviseerd door EMA-richtlijnen, zullen we ook een advies geven over de noodzaak om uitscheidingsanalyses uit te voeren indien de kennisgever een mogelijke marktoelatingsaanvraag overweegt.

**Vraag 4 :** Is er een kans dat het transgen kan doorgegeven worden via de geslachtscellen naar de volgende generatie? We stellen deze vraag met in het bijzonder eicellen in gedachte, die grotendeels dormant aanwezig zijn gezien de hele levensfase van de vrouw en waar het transgen dus na 180 dagen nog steeds actief aanwezig kan zijn. Of is het DNA fragment na 180 dagen niet langer actief (vanwege bv. stabiliteit)?

**SBB's Comment:**
Net als het ouderlijke virus waarvan de GAd20-209-FSP-vector is afgeleid, wordt verwacht dat het genoom epichromosomaal blijft, waardoor het gevaar van insertionele mutagenese wordt vermeden en kiembaanoverdracht wordt uitgesloten. Biodistributiestudies met GAd20-209-FSP-vector bij Sprague-Dawley-ratten hebben geen kwantificeerbare hoeveelheden DNA in de gonaden gedetecteerd na 24 uur, 7 dagen en 30 dagen na injectie (IB p28/102).

Aangezien MVA alleen repliceert in het cytoplasma van permissieve niet-menselijke cellen, kan het optreden van integratie van genetisch materiaal van het recombinant MVA in het gastheerchromosoom als verwaarloosbaar worden beschouwd [Goossens *et al*. 2013]. Daarom kan de mogelijke verspreiding van recombinant MVA in de kiemcellen en dus de kans op overdracht naar het nageslacht als verwaarloosbaar worden beschouwd. In een biodistributiestudie met een vergelijkbare recombinante op MVA gebaseerde vaccin, MVA MSP1 die intramusculair werd toegediend, werd op dag 1 en op dag 8 na injectie geen infectieus virus of viraal DNA in de gonaden waargenomen (IB p12/102).

Volgens de inclusiecriteria voor patiënten in cohorten C en D moet een vrouw in de vruchtbare leeftijd (WOCBP) ermee instemmen om tijdens de behandelingsperiode en gedurende ten minste 180 dagen na de laatste dosis studiebehandeling zeer effectieve anticonceptiemiddelen te gebruiken en zich tijdens deze periode te onthouden van eiceldonatie. Mannelijke patiënten moeten ermee instemmen tijdens de behandelingsperiode en gedurende ten minste 180 dagen na de laatste dosis studiebehandeling een anticonceptiemiddel te gebruiken en mogen gedurende deze periode geen sperma doneren. Deze periode komt overeen met de aanbeveling van Goossens *et al*. (2013), die stelt dat patiënten die met MVA-gebaseerde vectoren worden geïnjecteerd, tijdens de studieperiode en gedurende enkele maanden na de laatste IMP-toediening een effectief anticonceptiemiddel moeten gebruiken om mogelijke overdracht te voorkomen.

**Vraag 5**: Op basis van welke informatie werd de periode van 30 minuten geselecteerd voor de bedekking van de injectiesite? Dit lijkt zeer beperkt vergeleken met andere dossiers, zeker gezien de persistentie in gelijkaardige studies voor MVA, of heeft dit te maken met de niet-replicatieve aard en de manier van toediening?

**SBB's Comment:**
Aangezien biodistributiestudies met vergelijkbare virale vectoren bij ratten, konijnen (Stokes et al, 2022; Sheets et al, 2008; Volkmann et al, 2021) en muizen (Hanke et al, 2005; Hanke et al, 2002) hebben aangetoond dat beide virale vectoren voornamelijk tot enkele dagen op de injectieplaats (spier) aanwezig blijven, is er een verzoek naar de kennisgever gestuurd waarin we ten zeerste aanbevelen om het verband te vervangen door een nieuw verband in plaats van het geïnjecteerde gebied onbeschermd te laten bij het verlaten van het ziekenhuis. De melder is verzocht een kleine samenvatting op te stellen voor thuis met gedetailleerde instructies voor de patiënt met betrekking tot het geschikte tijdstip voor het verwijderen van het tweede verband, het weggooien van het tweede verband en het schoonmaken van de injectieplaats.

**References:**

Goossens M *et al*. Curr Gene Ther. 2013 Dec; 13(6): 413–420 - Environmental Risk Assessment of Clinical Trials Involving Modified Vaccinia Virus Ankara (MVA)-Based Vectors

Hanke T *et al*. Vaccine 2002; 21(1-2): 108-114 - Lack of toxicity and persistence in the mouse associated with administration of candidate DNA- and Modified Vaccinia Virus Ankara (MVA)-based HIV vaccines for Kenya.

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