Adviesraad voor Bioveiligheid
Conseil consultatif de Biosécurité

Advice of the Belgian Biosafety Advisory Council on the notification B/BE/20/BVW5 of the sponsor Nouscom srl for deliberate release in the environment of genetically modified organisms other than higher plants for research and development

11/02/2021
Ref. SC/1510/BAC/2021_0127

Context

The notification B/BE/20/BVW5 has been submitted by Nouscom srl to the Belgian Competent Authority in November 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.

The planned activity concerns a clinical trial entitled “An Open-Label, Multicenter, Non-Randomized, Dose-Confirmation and Cohort-Expansion Phase 1b Study to Evaluate the Safety, Tolerability, and Anti-Tumor Activity of Nous-PEV, with pembrolizumab, in Patients with Unresectable Stage III / IV Cutaneous Melanoma and with Stage IV NSCLC (PDL1≥ 50%) ”.

The proposed prime/boost Personalized vaccination (PEV) regimen encompasses the use of two viral vaccines encoding tumor-specific neoantigens that are selected for each subject individually and that are based on specific mutations identifed and collected from individual tumor biopsy. The investigational PEV vaccines consist of a recombinant replication-defective Gorilla Adenovirus vector (GAd) encoding PEV neoantigen (GAd-PEV) and a highly attenuated orthopoxvirus Modified Vaccinia Virus Ankara (MVA), replication-deficient in humans and other mammals, with the same set of selected tumor-specific neoantigens as GAd-PEV, but in different order to avoid boosting of potential immunogenic epitopes generated at the junction of the different neoantigens as present in the prime vaccine GAd-PEV. The synthetic gene generated by the head-to-tail fusion of neoantigens encoding sequences present in GAd-PEV has another sequence compared to the synthetic gene harboured by MVA-PEV but will encode the same set of antigens.
The prime component of the investigational Nous-PEV vaccine will be administered by the intramuscular route (IM) at a dose of 1 x 10^{11} viral particles (vp) whereas the boost component (MVA-PEV) will be administered three times at a dose of 1 x 10^{8} to 3 x 10^{8} IFU.

This study is planned to be conducted in two clinical sites located in Flanders and Wallonia. The national territory is considered as the potential release area of Nous-PEV.

The dossier has been officially acknowledged by the Competent Authority on 05 November 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 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 IMP 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 11 December 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 22 January 2021 and transmitted to the secretariat of the BAC on the same day. This complementary information was reviewed by the coordinator and the experts, and was considered satisfactory.

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 didn't receive any reactions from the public.

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

Information related to the molecular characteristics of GAd-PEV and MVA-PEV were found to be adequately described in the dossier.

3. The conditions of the release

Upon the evaluation of the information provided by the notifier, the BAC revealed a number of inconsistenties throughout the documents on the use of personnel protective equipment. The BAC also recommended to improve the description of measures with respect to the internal transport of the IMP inside the clinical setting and to detail the instructions with respect to use and disposal of a bandage in order to minimize exposure to the GMO following injection site leakage.

The notifier adequately implemented the remarks and requests addressed by the BAC in a revised version of documents that were submitted in the context of this notification, including the Pharmacy manual v3.0 dated 18 January 2021, the common application form for GAd-PEV and MVA-PEV and the Summary Notification Information Format (SNIF) GAd-PEV and MVA-PEV.

As a last remark, the notifier may consider to adapt the instructions with respect to the recapping of needle of the syringes that are prepared for administration and that are transported from pharmacy to administration site (see Pharmacy and Administration Instructions). As a general principle, recapping of needles should be avoided so as to minimize the risk of needle stick injury.

4. The risks for the environment or human health

E1, E3 and E4 genes have been deleted in the viral vector GAd20 as compared to its parental GAd virus from which it is derived. On the other hand, the GAd20 viral vector carries the gene sequence of E4Orf6 of the human Adenovirus 5. As both GAd and Ad5 belong to group C adenoviruses, the notifier was requested to elaborate on the degree of similarity with Ad5 and/or the presence of other sequences (besides E4Orf6) with increased homology to further substantiate the notifier's statement that the chance of recombination is very low.
As part of the environmental risk assessment, the notifier was also asked to elaborate on any potential adverse effects that could arise as a result of co-infection of GAd20-PEV and a wt virus like HuAd5 upon recombination in the shared E4Orf6 sequences (only about 900bp). Such recombination could result in a putatively replication-competent chimeric HuAd5 viral genome with GAd20-PEV sequences at its 3-'end, harbouring the polyepitope transgene from the cassette in the G2 region and the GAd20 inverted terminal repeat ITR-R. Consideration was given as to whether HuAd5 pre-terminal proteins could interact with GAd20 ITR and could give rise to a replication-competent chimeric virus resulting from replication of such a chimeric viral genome with dissimilar ITRs of two different serotypes. The notifier stated that the presence of dissimilar ITRs indeed could interfere with the mechanisms of adenoviral replication. Upon recombination in the shared E4Orf6 sequences, however, also a larger chimeric viral genome (about 107 % compared to wt HuAd5) would be generated having a presumed negative impact on genomic stability and efficient packaging. With respect to the generation of such a hypothetical chimeric virus the BAC considered that, besides HuAd5 pathogenicity, the expression of non-functional artificial polypeptide encoding neoepitopes against human proteins could potentially elicit an immune response resulting in 'autoimmune type' of disease upon dissemination of such a hypothetical chimeric virus in the human population. The severeness of the adverse effect associated to this worst case scenario was weighted against the very low probability of co-localization of HuAd5 and GAd20 upon intramuscular injection of Nous-PEV, the low probability of recombination between the shared E4Orf6 sequences, the presumed negative impact of dissimilar ITRs, the increased genomic seize of such a hypothetical chimeric virus on replication, genomic stability and efficient packaging, and the implementation of management measures (see below) in the context of the proposed use of Nous-PEV.

In light of the biodistribution studies conducted on rats, the notifier further clarified that no further data were obtained with GAd20 that could allow to conclude on transduction capacity of shed particles. The proposed clinical trial is a first-in-human study, hence no shedding and biodistribution data are available. Except for injection site leakage, several clinical studies performed with very similar vectors as well as non-clinical results show absence of shedding when the vector is administered intramuscularly.

The environmental risk assessment associated to the intended use of MVA-PEV was found to be adequately described in the dossier. 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-PEV 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-PEV (and the assumed lower amounts of shed Nous-PEV), the overall risk 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, the management of accidental exposure of clinical personnel or any other person other than the patient raised.
The notifier adequately implemented the remarks addressed by the BAC in a revised version of Pharmacy manual v3.0 dated 18 January 2021, the common application form for GAd-PEV and MVA-PEV and the Summary Notification Information Format (SNIF) GAd-PEV and MVA-PEV.
Given that the assessment of the likelihood of further propagation of Nous-PEV 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-PEV 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**:

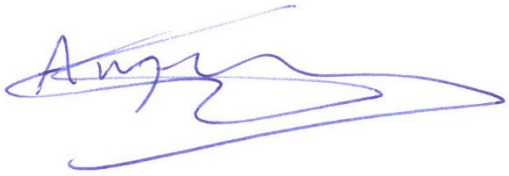
- The notifier and the investigators must strictly apply the clinical trial protocol and the safety instructions as described in the amended documents
    - Pharmacy manual v3.0 dated 18 January 2021 - *the notifier may consider to adapt the instructions so as to avoid recapping of needle of the syringes that are prepared in the pharmacy prior administration of Nous-PEV*
    - Common application form for GAd-PEV and MVA-PEV
    - Summary Notification Information Format (SNIF) Gad-PEV and MVA-PEV
    - IB, version3.0, dated 21 Jan 2021
    - Proposal for public information dd18 Jan 2021

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

- The notifier is responsible to verify that each study centre has qualified personnel experienced in handling infectious material and that the investigator has the required authorisations to perform the clinical trial activities inside the hospital (laboratory, pharmacy, hospital room, consultation room...) according to the Regional Decrees transposing Directive 2009/41/EC on 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-PEV.



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/BVW5 (ref. SC/1510/BAC/2020_1169)*

Adviesraad voor Bioveiligheid
Conseil consultatif de Biosécurité

Compilation of comments of experts in charge of evaluating the dossier B/BE/20/BVW5
And comments submitted to the notifier

11 December 2020
Ref. SC/1510/BAC/2020_1169

Mandate for the Group of Experts: Mandate of the Biosafety Advisory Council (BAC) of 18 September 2020.
Coordinator: Anton Roebroek (Jules Bordet Institute, ULB)
Experts: Jozef Anné (KUL), Rik Gijsbers (KUL), Willy Zorzi (ULiège), Amaya Leunda (Sciensano, SBB)
SBB: Katia Pauwels

INTRODUCTION

Dossier **B/BE/20/BVW5** concerns a notification from Nouscom Srl for the deliberate release in the environment of genetically modified organisms other than higher plants according to Chapter II of the Royal Decree of 21 February 2005.
The notification has been officially acknowledged on 18 September 2020 and concerns a clinical trial entitled "*An Open-Label, Multicenter, Non-Randomized, Dose-Confirmation and Cohort-Expansion Phase 1b Study to Evaluate the Safety, Tolerability, and Anti-Tumor Activity of Nous-PEV, with pembrolizumab, in Patients with Unresectable Stage III / IV Cutaneous Melanoma and with Stage IV NSCLC (PDL1≥ 50%)".* The trial will involve the use of several recombinant Gorilla adenovirus-based investigational medicinal products which will express different set of tumor neoepitopes specific for each patient (GAd20-PEV). The first vaccination with GAd20-PEV will be followed by three boost vaccination with recombinant modified vaccinia Ankara (MVA-PEV).

♦ 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 11-12-2020 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

2. INFORMATION RELATED TO THE INVESTIGATIONAL MEDICINAL PRODUCT

A.1. Virus from which the clinical vector was derived (parental virus)

(e.g. information on parental virus; phenotypic and genetic markers; host range, zoonotic potential and replication properties of the parental virus ….)

Comment 1

The expert has evaluated this item and has no questions/comments.

-MVA is derived from the chorioallantois vaccinia virus strain Ankara by serial passaging in chicken embryo fibroblasts (CEF) over 500 times.
-GAd20 Gorilla adenovirus (similar to human subgroup C adenoviruses)

Comment 2

P9/77 in 2019-004759-35_1211_Nous-PEV_IB_v1-0_20200709.pdf, it is not clear whether the applicant uses virus or viral vector. It is stated "*The presence of infectious virus was assessed at the injection site and draining lymph nodes, spleen, liver and gonads at day 1 and at day 8 after the injection. As expected, virus was detected at the injection site immediately after injection. One week later the virus was still detected at the injection site but at a markedly reduced amount."*. I think viral vectors is meant here. For clarity it would be better to stick to viral vector when replication deficient single round particles are meant, and to use virus only for replication competent version of the product. Especially in the IB it is key to be clear on the status of the vectors used.

General question: MVA is regularly used in vaccine development. No doubt people that will use the DP may have previously been treated with an MVA based vaccine. Will the treatment be as effective under those conditions? Moreover, Gad will here be used for the first time. However, is there info available on cross-reactivity with Human AdV (and thus potential neutralizing Abs)? Further, are GAd specific Abs assessed in the patients?

SBB comment

Proposed wording for first remark (*if retained for a list of question*) :
In the IB it is stated on p9/77 "'*The presence of infectious virus was assessed at the injection site and draining lymph nodes, spleen, liver and gonads at day 1 and at day 8 after the injection. As expected, virus was detected at the injection site immediately after injection. One week later the virus was still detected at the injection site but at a markedly reduced amount."* In the assumption that viral vector is meant, the applicant is asked to change the wording so as to stick to viral vector whenever replication deficient single round particles are meant.

While relevant from a clinical point of view, the second remark on the efficacy of the proposed therapeutic approach in light of potential neutralizing antibodies goes beyond the scope of the environmental risk assessment.

Coordinator comment
Agreed with SBB comment on neutralizing antibodies

Comment 3

Has evaluated this item and has no questions/comments.

Comment 4

In CAF p2: "The unique host is Gorilla gorilla gorilla" should be : "the unique natural host…"

SBB comment
To be consistent with the expert's remark, the CAF should also be adapted in section 2.17 at p7 of Part 1A_1211_BEL_CAF_GAD-PEV (p7): '*not expected to be found into humans being Gorilla its unique host*' should read '*not expected to be found into humans being Gorilla its unique* ***natural*** *host*'.

A.2. Pathogenicity
(e.g. pathogenic properties, available treatment methods, attenuation and biological restrictions of the parental virus ….)

Comment 1

Has evaluated this item and has no questions/comments.

- MVA is not pathogenic. It has already been successfully tested as a vector vaccine for various infectious diseases in several clinical trials.
- No previous experience or information about zoonosis of GAd20 is known.

Comment 2

In how far is the pathogenic GAd compatible with human Ad? May a human Ad infection complement the genes deleted in GAd?
At p9/15 in Part 2_1211_BEL_SNIF app form_GAd-PEV_Non-confidential_20201007.pdf: it is stated "However, a deliberate release cannot be excluded referring to potential shedding of the GMO through body fluids of the treated patients inside and outside the clinical centers." Would it be advisable to make sure that as long as the applicant is not fully aware of the shedding properties of the DP, that the patients are advised not to come into contact with original natural host of the GAd?
I agree chances are low, but nevertheless this additional measure will not jeopardize the study either.

SBB comment :
Agreed that the potential of gaining replication competence by trans-complementation should be discussed by the applicant as part of the identification of potential hazards. Furthermore, the applicant is also supposed to do a risk assessment and to address the risk associated to trans-complementation,

which implies an assessment of the likelihood of occurrence that such an event would happen. This requires a consideration of the likelihood that both the GAd and a trans-complementing wild type adenovirus would co-infect the same cell.

Comment 3

Has evaluated this item and has no questions/comments.

Comment 4

Has evaluated this item and has no questions/comments.

A.3. Ability to colonise
(e.g. transmission routes, survival outside the host….)

Comment 1

Has evaluated this item and has no questions/comments.

- Modified Vaccinia Ankara (MVA) is a highly attenuated vaccinia virus strain with an exceptional safety profile due to its inability to productively replicate in human and most mammalian cells. MVA is not known to be able to infect and replicate in wild organisms; it only replicates in avian cell cultures.
- Gorilla adenoviruses could possibly be transmitted among host animals via the fecal–oral route and inhalation of aerosols, but there are no available specific data for GAd20.

Comment 2

Has evaluated this item and has no questions/comments.

Comment 3

Has evaluated this item and has no questions/comments.

Comment 4

Has evaluated this item and has no questions/comments.

B. Genetic modification and manufacturing of the clinical vector
(e.g. manufacturing process of the vector; characteristics of the cell lines used for production, information on replicating –competent virus…)

Comment 1

Has evaluated this item and has no questions/comments.

-MVA is modified by insertion of antigen sequences corresponding to patients-specific cancer neoantigens. This modification is finalized at the expression of the specific neoantigens upon IMP administration to the patient and enhancing the natural immunological response against the tumour.

- GAd20: The insertion fragments are cloned in a vector derived from GAd20 in which the E1, E3 and E4 sequences involved in replication have been deleted.

Comment 2

Has evaluated this item and has no questions/comments.

Comment 3

Has evaluated this item and has no questions/comments.

Comment 4

Has evaluated this item and has no questions/comments.

C. Clinical vector

2.13. - 2.16 . Map of the clinical vector and molecular characteristics, coding genes and regulatory sequences, biologic profile of the clinical vector versus parental virus

Comment 1

Has evaluated this item and has no questions/comments.

Each GAd20-based IMP and MVA-based IMP will express a different set of epitopes specific for each patient but the overall manufacturing scheme and the resulting IMP genomic array will be the same as pilot lots. The resulting final GMP product, both pilot lots and each individual GAd- and MVA based on individual patients' sequences, are unable to replicate (outside permissive cells in culture), due to the deletions made in the GAd20 original vector sequence, hence they are not able to productively infect animals and humans and to propagate in the environment.

Comment 2

Has evaluated this item and has no questions/comments.

Comment 3

Has evaluated this item and has no questions/comments.

Comment 4

Has not evaluated this item.

2.17. Potential for recombination

Comment 1

Has evaluated this item and has no questions/comments.

Both he GMO and the vector do not replicate outside lab cultures, so no recombination can take place in the cell.

Comment 2

P79/123 of 2019-004759-35_1211_NOUS-PEV-01_IMPD GAd NSCLC Pilot 1_v1.0_20200724.pdf and P7/24 (2.17) in Part 1A_1211_BEL_CAF_GAd-PEV.pdf: RCA analysis of the DP is performed by qPCR using primers for GAd. Earlier it is indicated in the text that the chance of recombination with human Ad5 is very low (which implies not completely absent). Is Ad5 the closest (genetically) to GAd? What about other adenoviruses ?
Same goes for p83/128 in 2019-004759-35_1211_NOUS-PEV-01_IMPD GAd NSCLC Pilot 2_ v1.0_20200724.pdf and P83/126 in 2019-004759-35_1211_NOUS-PEV-01_IMPD GAd MM Pilot 3_v1.0_20200724.pdf.

SBB comment
Referring to the comment 4 here below, it is remarked that Gad20-PEV actually carries the gene sequence of E4Orf6 of the human Adenovirus 5.

As both Gad and Ad5 belong to group C adenoviruses, the applicant could be asked to discuss the degree of similarity with Ad5 and/or the presence of other sequences (besides E4Orf6) with increased homology to further substantiate the applicant's statement that the chance of recombination is very low.

Comment 3

Has evaluated this item and has no questions/comments.

Comment 4

Recombination of the Gad20-PEV with wild-type human Adenoviruses are described as unlikely. However, in this viral vector, the gene sequence of E4Orf6 of the human Adenovirus 5 has been inserted. Does this increase the possible recombination events between the IMP and Ad5 in case of *in vivo* co-infection?

SBB comment
For information, the following remark as regards the effect of the presence of Ad5 derived E4 orf4 in a ChAd vectored IMP has also been addressed for dossier B/BE/20/BVW2 (ref BAC_2020_0467) :
'*Assuming that the E4 gene with the E4Orf4,6/7 is close to the 3'-ITR in both ChAdOx1-HPV and AdHu5 and all other genes including the hexon, pentonbase and fiber genes are located 5' to E4Orf4,6/7 in the viral map, the applicant is asked to clarify whether a replication competent ChAdOx1/AdHu5 chimeric virus harbouring the E1 gene, generated by homologous recombination, would be more or less an AdHu5 virus carrying at its 3'-end a chimeric ChAdOx1/AdHu5 E4 gene and the ITR of ChAdOx1. If correct, what is the consequence of two presumably different ITRs for replication of such chimeric virus*?'

Comment coordinator

The answer of the applicant (B/BE/20/BVW2) on this remark was considered as sufficiently. However, a similar remark I have made with respect to dossier EMEA/H/C/005737. My progressive, but still limited insight in the role of the ITR in adenoviral assembly and replication suggests that a better substantiation is needed to support the answer on the remark by the applicant (B/BE//BVW2) that the chimeric AdHu5 virus would likely not be viable due to mismatched ITRs. Furthermore, if it were viable, its growth characteristics would at best resemble those of AdHu5 (no enhanced virulence, in case of B/BE//BVW2).

In case of the current application, the situation seems similar, but there is a clear difference, because in this case, in theory, a non-wildtype type, chimeric replication-competent virus could emerge. In conclusion I want to suggest to add the following remark/question:

In case of co-infection of Gad20-PEV and a wt virus like HuAd5 a single homologous recombination event between both viruses in the E4Orf6 gene regions could result on one hand in a chimeric Gad20-PEV virus with HuAd5 sequences at its 3'-end, no longer encoding the polyep.G2 region and the Gad20 ITR-R, but instead HuAd5 sequences including a HuAd5 ITR-R. Due to the lack of E1, this chimera would be, anyhow, replication-defective. The other chimeric virus which could emerge is a chimeric HuAd5 virus with Gad20-PEV sequences at its 3-'end, harbouring the polyep.G2 region and the Gad20 ITR-R. Of the E4 gene, only the E4Orf6 gene region would be present. In theory, such virus could be replication-competent, because all necessary adenoviral genes, including E1 and E3 seem to be present. With respect to the potential generation of such a chimeric virus the next questions are relevant:

1. Are HuAd5 pTP and PT proteins, which interact with the huAd5 ITR sequences in viral assembly and replication processes functional in combination with a Gad20 ITR in this presumed chimeric virus? If yes, then this chimeric virus could be replication-competent. Or do other arguments exist to exclude such chimeric virus from being replication-competent?
2. If such chimeric virus would be replication-competent, what could be the consequence? What about its virulence compared to wt HuAd5?

2.18. Biodistribution and shedding

Comment 1

Has evaluated this item and has no questions/comments.

GAd-PEV and MVA-PEV will be experimented for the first time in the proposed FIH clinical trial, hence no shedding and biodistribution data are available. Several clinical studies performed with very similar vectors as well as non-clinical results show absence of shedding when the vector is administered intramuscularly.

Comment 2

P47/77 2019-004759-35_1211_Nous-PEV_IB_v1-0_20200709.pdf : biodistribution in rats showed qPCR positive samples till a month after administration of GAd20-209-FSP. Has this been tested whether this positive PCR also coincided with a transduction positive particles? Also, it is indicated that the DNA decreases. What is meant here? 'A trend of decreased DNA' is used: does this imply no statistical difference was detected?

P13/24 in Part 1A_1211_BEL_CAF_GAd-PEV.pdf: following injection and disposal of the bandage after 30 minutes, it is advised to rinse the area, apply 70% EtOH, and replace the bandage by a new one instead of leaving the injected area unprotected. In the unlikely event of additional bleeding the patient may get in contact with the injected vector.
See also p11/15 in Part 2_1211_BEL_SNIF app form_GAd-PEV_Non-confidential_20201007.pdf and p12/15 in Part 2_1211_BEL_SNIF app form_MVA-PEV_Non-confidential_20201007.pdf. This is also indicated in the patient information documents: e.g. p5/9 Part 3_1211_BEL_Information to the public_20201004_EN.pdf. It would be advisable to use the same procedure as suggested on p8/9 of Part 3_1211_BEL_Information to the public_20201004_EN.pdf: "Wipe the spill area with disinfectant and then remove and dispose of gloves properly and wash hands with soap or suitable alternative."

P14/24 in Part 1A_1211_BEL_CAF_GAd-PEV.pdf: donation of eggs is not allowed. In addition, blood/cell donation and organ donation should also be restricted (only for the clinical trial purposes).

SBB comment

From the information provided it is not clear whether treated patients may return home shortly after the treatment. If the applicant is recommended to give instructions for replacing the first bandage with a second one, then instructions for the patients should also be sufficiently clear in case the patient has left the clinical site and still carries the second bandage. In this case, detailed instructions for the patient should be considered as regards the appropriate time for removal of the second bandage, the disposal of the second bandage and waste management.

As regards risk minimization measures following possible injection site leakage, see also comment raised in section 3.6 (comment 4)

As regards blood/cell donation and organ donation, it is possible that the condition of the patients treated, de facto, imply the non-eligibility of the patient as a future donor[1].

Comment coordinator

See below. Overview of the expert's comments in regards risk management measures and instructions.

Comment 3

Has evaluated this item and has no questions/comments.

[1] Eligibility criteria for donors of whole blood and blood components are laid down in Annex III to *Commission Directive 2004/33/EC of 22 March 2004 implementing Directive 2002/98/EC of the European Parliament and of the Council as regards certain technical requirements for blood and blood components* (OJ L91, 30.3.2004, p. 25), as amended.
Selection criteria for donors of tissues and cells are laid down in Annex I to *Commission Directive 2006/17/EC of 8 February 2006 implementing Directive 2004/23/EC of the European Parliament and of the Council as regards certain technical requirements for the donation, procurement and testing of human tissues and cells* (OJ L38, 9.2.2006, p. 40), as amended.
Organ and donor characterisation criteria for organs are laid down in the Annex to *Directive 2010/45/EU of the European Parliament and of the Council of 7 July 2010 on standards of quality and safety of human organs intended for transplantation* (OJ L207, 6.8.2010, p. 14).

Comment 4

Has evaluated this item and has no questions/comments.

3. INFORMATION RELATED TO THE CLINICAL TRIAL

3.3. Storage of the clinical vector at the clinical site

(e.g. storage location, conditions of storage, …)

Comment 1

Has evaluated this item and has no questions/comments.

IMP will be stored according to study requirements at or below -60°C. Access to the area is limited to qualified personnel of the facility.
Each individual personalised vaccine injection with the GAd vector vaccine will be received around week 9 of the respective patient's trial participation and used on week 10 (day 64 +/- 2 days), as stipulated by the study protocol. After injection, used vials will be destroyed immediately as per local biohazard destruction procedures. The anticipated maximum storage duration on site is therefore less than 4 weeks for each individual personalised vaccine injection with the GAd vector.
Each individual personalised vaccine injection with the MVA vector will be received around week 9 of the respective patient's trial participation and used on weeks 13, 16 and 19 (day 127 +/- 2 days), as stipulated by the study protocol. After injection, used vials will be destroyed immediately as per local biohazard destruction procedures. The anticipated maximum storage duration on site is therefore less than 12 weeks for each individual personalised vaccine injection with the MVA vector

Comment 2

P34/77 2019-004759-35_1211_Nous-PEV_IB_v1-0_20200709.pdf : The Drug Product GAd and MVA are assigned an expiry date of 24 months starting from the date of manufacturing was assigned to the Investigational Product. It is not clear why. For the Nous-209 stability was only shown for 1 year.

SBB comment

This question goes beyond the scope of the environmental risk assessment

Coordinator comment

Agreed with SBB comment

Comment 3

Has evaluated this item and has no questions/comments.

Comment 4

Has evaluated this item and has no questions/comments.

3.4. Logistics for on-site transportation of the clinical vector

(information on logistics of in-house transportation, characteristics of the container, disinfection procedures, labelling of the containers, ...)

Comment 1

Has evaluated this item and has no questions/comments.

GAd-PEV and MVA-PEV viral vaccine is supplied within a 3 mL borosilicate glass vial closed with a rubber stopper and sealed with an aluminum tear-off cap. The vial is labeled with a technical label and a primary label. The internal transportation of the vial(s) from the GMP facility to the administration site must be done, as for the thawing, putting it in an upright vertical position in a rack. Once administered by I.M. injection, the empty/used IMP packaging must be discarded per local biohazard materials disposal standards.

Comment 2

Has evaluated this item and has no questions/comments.

Comment 3

Has evaluated this item and has no questions/comments.

Comment 4

IMP internal transport (inside the clinical setting) for example, from pharmacy to the hospital room, needs a double packaging. It is recommended to use a double packaging with a watertight, shockproof, easily decontaminated container as outer packaging. This container is handy, designed so that the vials are not overturned or damaged during handling. (Part 1A_1211_BEL_CAF_GAd-PEV p11 and 12).

Comment coordinator

See below. Overview of the expert's comments in regards risk management measures and instructions.

3.5. Reconstitution, finished medicinal product and administration to the patients

(e.g. mode of administration, information on dosing and administration schedule, information on concomitant medication,…)

Comment 1

Has evaluated this item and has no questions/comments.

GAd-PEV (Dose will be $1x10^{11}$ vp) will be administered IM only once as prime component of the candidate vaccine Nous-PEV. After the unique GAd-PEV administration, 3 administrations of MVA-PEV will be performed IM as a boosting injection. Dose range will be $1\text{-}3x10^{8}$ ifu. Protocol of the NOUS-PEV-01 clinical study included in the CTA)..

Comment 2

P39&41/77 2019-004759-35_1211_Nous-PEV_IB_v1-0_20200709.pdf : in the table the dose is indicated as vp or ifu. Is the dose always the same notwithstanding the bodyweight?

SBB comment

While the question is relevant from clinical point of view it may be out of the scope of the environmental risk assessment given that preclinical studies indicate a low probability of shedding following intramuscular injection. At the other hand, leakage at the injection site can be seen as the main source of release of the GMO into the environment.

Comment coordinator

The common application documents for both viral vectors mention clearly a dose or dose range. The dose of GAd-PEV that will be administered to melanoma or NSCLC patients in the NOUS-PEV-01 clinical trial is 1x10^11 vp (page 7 of 24). The dose range of MVA-PEV that will be administered to melanoma or NSCLC patients in the NOUS-PEV-01 clinical trial is 1-3x10^8 ifu (page 6 of 23).

Comment 3

Has evaluated this item and has no questions/comments.

Comment 4

Has evaluated this item and has no questions/comments.

3.6. Measures to prevent dissemination into the environment

(e.g. control measures, PPE, decontamination/cleaning measures after administration or in the case of accidental spilling, waste treatment, recommendation given to clinical trial subjects, …)

Comment 1

Procedures according to study Pharmacy manual. In summary, drain the area with paper tissues, treat the surface with 1:10 dilution of household bleach (the solution must not be older than one day) for at least 30 minutes, wipe the area with disinfectant ethanol 70% or Virkon S. Rinse and dry. Dispose residual contaminated material of as per local biohazard destruction procedures.

However in SNIF GAd p11 “Spilled vector can be decontaminated as per Biosafety Protocols using a 1:10 dilution of household bleach and wiping the area with disinfectant 70% Ethanol or Virkon S.” .The following specification should be added : ‘ using a freshly prepared 1:10 dilution of household bleach”

In **PHARMACY MANUAL and ADMINISTRATION INSTRUCTION** p23**:** The healthcare professional performing the procedure must wear gloves and appropriate eye protection. Here “a lab coat or white coat or gown” should be added.

SBB comment

The last remark is in line with comment 4 (section 3.6) here below

Comment coordinator

See below. Overview of the expert's comments in regards risk management measures and instructions.

Comment 2

Has evaluated this item and has no questions/comments.

Comment 3

Has evaluated this item and has no questions/comments.

Comment 4

There are discrepancies in instructions given to personnel for risk management in clinical setting. They should be consistent throughout the documents.
For example, concerning personal protection equipment:
In documents "Part 1A_1211_BEL_CAF_GAd-PEV" p19 (and SNIF p11): PPE needed for personnel handling GAd-PEV consists in protective gowns, gloves, eye protection and mask. In p13 and 23 of the same document and in document Part 6_1211_NOUS-PEV_Pharmacy Manual and Admin_v1-0_20200721, p24 mask is not required.
It is very likely that GAd20-PEV could be transmitted through aerosols generated in case of accidental splash and during syringe preparation, it is thus recommended to wear an adequate respiratory protection.
Concerning spill management:
Part 1A_1211_BEL_CAF_GAd-PEV p13: surface that has been decontaminated after an accidental spill should not be rinsed and dried.
Part 6_1211_NOUS-PEV_Pharmacy Manual and Admin_v1-0_20200721 describes the correct and detailed management of spill (without rinsing and drying).

Management of accidental exposure of clinical personnel (or any other person than the patient):
Part 6_1211_NOUS-PEV_Pharmacy Manual and Admin_v1-0_20200721 p25
A procedure in case of eye exposure should be included
Part 1A_1211_BEL_CAF_GAd-PEV p19 there is no instruction for management of an accidental needle stick injury (could refer to the safety data sheet?)

An adapted spill kit (with personnel protection equipment and adequate disinfectant) available at proximity of IMPs could be foreseen (in the storage room and hospital room)

The injection site will be covered by a bandage during 30 minutes. When removed, presence of IMP on the skin cannot be discarded. An additional disinfection of the site could be useful to prevent dissemination of IMP.

SBB comment

As regards risk minimization measures following possible injection site leakage, see also comment raised in section 2.18 (comment 2).

The lack of consistency between the instructions for PPE throughout several documents has also been raised in comment 1 , section 3.6. here above.

Comment coordinator

See below. Overview of the expert's comments in regards risk management measures and instructions.

3.7. Sampling and further analyses of samples from study subjects

Comment 1

Has evaluated this item and has no questions/comments.

These biological samples will be handled and stored at site according to appropriate biohazard procedures and instructions provided by Nouscom in the Lab Manual which will be sent to each site. The biological samples will then be transported and analyzed for the immediate objectives of the trial at the corresponding Central lab. Nouscom will manage the transport from the site to the corresponding central lab according to GCPs. Currently proposed procedures for the various patients' samples storage are the following and will be included in lab manuals.

Comment 2

Has evaluated this item and has no questions/comments.

Comment 3

Has evaluated this item and has no questions/comments.

Comment 4

Has evaluated this item and has no questions/comments.

3.8. Emergency responses plans

Comment 1

Procedures are well described.
One question: .. p25/30 in Pharmacy manual: "and notify the Principal Investigator and the Medical Monitor". Nothing is mentioned what the PI or Med Mon has to do with it.

SBB comment:

For clinical trials conducted in Belgium the applicant could be directed to the website https://www.biosafety.be/content/laboratory-acquired-infections-and-bio-incidents where a direct link is provided to a dynamic online tool or online occupational bio-incident notification platform

Comment coordinator

See below. Overview of the expert's comments in regards risk management measures and instructions.

Comment 2

P25/30 in Part 6_1211_NOUS-PEV_Pharmacy Manual and Admin_v1-0_20200721.pdf: the paragraph states
'*Procedures in case of unexpected contamination of clinical site personnel by contact with IMP or injection of the IMP by needle injury*.' In my opinion it would be better to treat a possible splash on the skin by first covering the splash/spill with paper towels (to absorb) and next to pour disinfectant (70% EtOH or betadine) on the towels. Finally, the surface area can be washed with soap and water.
Moreover, the measures provided do not address a possible needle injury. This should be explicitly discussed instead.

SBB comment
The need to provide measures that specifically addresses possible needle injury has also been raised in comment 4 here below.

Comment coordinator
See below. Overview of the expert's comments in regards risk management measures and instructions.

Comment 3

Has evaluated this item and has no questions/comments.

Comment 4

Management of accidental exposure of clinical personnel (or any other person than the patient):
Part 6_1211_NOUS-PEV_Pharmacy Manual and Admin_v1-0_20200721 p25: a procedure in case of eye exposure should be included (an eye washer for example).
Part 1A_1211_BEL_CAF_GAd-PEV p19 there is no instruction for management of an accidental needle prick (could refer to the safety data sheet?)

An adapted spill kit (with personnel protection equipment and adequate disinfectant) available at proximity of IMPs could be foreseen (in the storage room and hospital room)

SBB comment
The need to provide measures that specifically addresses possible needle injury has also been raised in comment 2 here above.

Comment coordinator
See below. Overview of the expert's comments in regards risk management measures and instructions.

5. ENVIRONMENTAL RISK ASSESSMENT

Comment 1

Has evaluated this item and has no questions/comments.

The chances to detect even traces of the GMOs in the environment are considered extremely low.

Comment 2

Has evaluated this item and has no questions/comments.

Comment 3

In the file "Part 3_1211_BEL_Information to the public_20201004_FR" (p7), in the following paragraph: "*Évaluation des risques potentiels pour la santé humaine et l'environnement liés à la dissémination volontaire :*
On ne connaît aucune dissémination volontaire provenant des patients après l'administration des ME (dans l'étude NOUS-PEV-01, les produits seront utilisés pour la première fois chez l'Homme), mais elle ne peut être exclue. Outre le fait qu'il ne s'agit que d'une possibilité non démontrée, en répondant principalement à l'hypothèse d'une excrétion virale par les fluides corporels, il doit également être considéré que les risques inhérents à une dissémination hypothétique des ME dans l'environnement sont nuls ou négligeables."

We do not agree on the risk assessment level, characterised as "nul". We invite the notifier to change this term, considering section H "Information relating to monitoring", in the file "Part 2_1211_BEL_SNIF app form_GAd-PEV_Non-confidential_20201007" (p13):
"1.Methods for monitoring the GMOs
In summary, the chances to detect even traces of the GMOs in the environment are considered extremely low, let alone to establish a rudimentary distribution pattern."

SBB comment :
The same comment applies to the Dutch and English version of the information to the public.

Comment coordinator:

Indeed, the mentioning of "nul (or zero)" should be removed from the information documents.

Comment 4

Has evaluated this item and has no questions/comments.

6. OTHER INFORMATION

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

Comment 1

Investigator's brochure p37/77 neoaare ??

Comment 2

The text contains several typos, or words that are not included together with verbs that are wrongly conjugated. Some examples are described below. A spell check would be advisable. Especially in the IB this is disturbing, since this is supposed to be the document to inform about the DP.

2019-004759-35_1211_Nous-PEV_IB_v1-0_20200709.pdf : PEV is not mentioned in the list of abbreviations. It is present in the txt further on, but may be added when possible.

P28/77 2019-004759-35_1211_Nous-PEV_IB_v1-0_20200709.pdf : adventituos should be adventitious; clincal should be clinical

P28/77 2019-004759-35_1211_Nous-PEV_IB_v1-0_20200709.pdf : AVA is used as abbreviation, but this is not explained. Adventitious agents??

P34/77 2019-004759-35_1211_Nous-PEV_IB_v1-0_20200709.pdf : “The Drug Product GAd NSCLC Pilot-2 vaccine Lot No. RL20-0004.PEV, manufactured on 08 April 2020 has been place on stability under long-term storage conditions at or below -60°C.” Sentence does not make sense. Also lower the same construction is used for the MVA product.

Comment coordinator

These remarks should be communicated to the applicant in a kind of annex with typos and suggestions to improve the text of the documents.

Comment 3

None

Comment 4

None

Overview of the expert's comments in regards risk management measures and instructions

Comment coordinator :

The remarks and questions below should be communicated to the applicant. I understand that this a compilation of all issues raised regarding risk management measures and instructions.

Injection site leakage

(See Section 2.18 – comment 2 and section 3.6 comment 4)

P13/24 in Part 1A_1211_BEL_CAF_GAd-PEV.pdf: following injection and disposal of the bandage after 30 minutes, it is advised to rinse the area, apply 70% EtOH, and replace the bandage by a new one instead of leaving the injected area unprotected.

SBB comment

From the information provided it is not clear whether treated patients may return home shortly after the treatment. If the applicant is recommended to give instructions for replacing the first bandage with a second one, then instructions for the patients should also be sufficiently clear in case the patient has left the clinical site and still carries the second bandage. In this case, detailed instructions for the patient should be considered as regards the appropriate time for removal of the second bandage, the disposal of the second bandage and waste management.

Alternatively, an additional disinfection of the site could be useful to prevent dissemination of IMP.

Internal transport

IMP internal transport (inside the clinical setting) for example, from pharmacy to the hospital room, needs a double packaging. It is recommended to use a double packaging with a watertight, shockproof, easily decontaminated container as outer packaging. The container should be designed so as to prevent that vials can be overturned or damaged during handling.
The applicant is requested to adapt the following documents accordingly : Part 1A_1211_BEL_CAF_GAd-PEV p11 and 12.

Procedure in case of spill

(Section 3.6 Comment 1 and comment 4)

The measures to handle spills presented in the Part 6_1211_NOUS-PEV_Pharmacy Manual and Admin_v1-0_20200721 and on p11 of Part 2_1211_BEL_SNIF app form_GAd-PEV_Non-confidential_20201007.pdf should be aligned with particular attention to use freshly prepared 1:10 dilution of household bleach :
Also, surface that has been decontaminated after an accidental spill should not be rinsed and dried unlike to what is stated in Part 1A_1211_BEL_CAF_GAd-PEV p13.

PPE

(Section 3.6 Comment 1 and comment 4)

According to p19 of “Part 1A_1211_BEL_CAF_GAd-PEV” p19 and p11 of Part 2_1211_BEL_SNIF app form_GAd-PEV_Non-confidential_20201007.pdf, the handling of GAd-PEV necessitates protective gowns, gloves, eye protection and mask while on p13 and 23 of the same document and in document Part 6_1211_NOUS-PEV_Pharmacy Manual and Admin_v1-0_20200721, p 23 and p24, a protective gown, gloves and a mask are not required.

Management of accidental exposure of clinical personnel (or any other person than the patient)

(Section 3.6 - comment 4 and section 3.8 comment 2 and comment 4)

Part 6_1211_NOUS-PEV_Pharmacy Manual and Admin_v1-0_20200721 (p25) should include a procedure in case of eye exposure.

Part 1A_1211_BEL_CAF_GAd-PEV - p19 should also include instructions for management of an accidental needle stick injury or should at least refer to a safety sheet. p25/30 in Part 6_1211_NOUS-PEV_Pharmacy Manual and Admin_v1-0_20200721.pdf should also include specific measures to address a possible needle injury.

An adapted spill kit (with personnel protection equipment and adequate disinfectant) available at proximity of IMPs should be foreseen (in the storage room and hospital room)

P25/30 in Part 6_1211_NOUS-PEV_Pharmacy Manual and Admin_v1-0_20200721.pdf: the paragraph states
‘*Procedures in case of unexpected contamination of clinical site personnel by contact with IMP or injection of the IMP by needle injury*.’ It would be better to treat a possible splash on the skin by first covering the splash/spill with paper towels (to absorb) and next to pour disinfectant (70% EtOH or betadine) on the towels. Finally, the surface area can be washed with soap and water.

Emergency response plan

(*Section 3.8- comment 1)*

According to Part 6_1211_NOUS-PEV_Pharmacy Manual and Admin_v1-0_20200721, p25/30, it could be further clarified which steps the principal Investigator and the medical monitor could undertake.
For clinical trials conducted in Belgium the principal Investigator and the medical monitor could be directed to the website https://www.biosafety.be/content/laboratory-acquired-infections-and-bio-incidents where a direct link is provided to a dynamic online tool or online occupational bio-incident notification platform.