Adviesraad voor Bioveiligheid
Conseil consultatif de Biosécurité

Advice of the Belgian Biosafety Advisory Council on the notification B/BE/23/BVW3 of the sponsor AstriVax for deliberate release in the environment of genetically modified organisms other than higher plants for research and development

Final version - 28/03/2024
Ref. SC/1510/BAC/2024_0725

Context

The notification B/BE/23/BVW3 has been submitted by AstriVax to the Belgian Competent Authority in December 2023 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, randomized, double-blind, multi-centre, placebo-controlled, dose-escalation study to evaluate the safety, reactogenicity and immunogenicity of AstriVax’ investigational vaccine for the prevention of yellow fever (AVX70120), and of AstriVax’ investigational vaccine for the prevention of rabies (AVX70481), in healthy adults aged 18 to 40 years”.

The purpose of this study is to assess safety, reactogenicity and immunogenicity of both AVX70120 and AVX70481 vaccines in healthy adults aged 18 to 40 years.

The investigational medicinal product consists of two DNA-based vaccines that result in the production of two different plasmid-launched live attenuated viruses (PLLAVs). Both PLLAV plasmids contain the genome of the live attenuated yellow fever virus strain 17D [YF17D]. The AVX70120 vaccine has no additional gene inserted and is indicated for prophylactic vaccination against yellow fever. Whereas, the AVX70481 vaccine has the coding sequence of RabG inserted in the YF17D-204 genome and is indicated for prophylactic vaccination against rabies.

For this first-in-human dose-escalation study, three dose levels will be tested : a lower, a middle and a higher dose of PLLAV-YF17D/RabG..

It is estimated that approximately 48 patients will receive AVX70481 in this Phase I study, which is planned to be conducted in two clinical sites located in Flanders. The national territory is considered as the potential release area of PLLAV-YF17D/RabG.

The dossier has been officially acknowledged by the Competent Authority on 15 December 2023 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 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 19 January 2024, 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 February 2024 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 01 March 2024. This complementary information, received on 12 March 2024, was reviewed by the coordinator and resulted in a third list of questions, which was transmitted to the notifier on 21 March 2024. The answers of the notifier were received on 22 March 2024 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 questions were related to biosafety issues. According to Article 16 §2 of the Royal Decree of 21 February 2005, the 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 genetically modified investigational medicinal product (IMP) that will be administered in Part 2 of clinical study is the plasmid DNA vaccine PLLAV-YF17D/RabG. PLLAV-YF17D/RabG contains the full genome of the live attenuated yellow fever virus (YFV) strain 17D (YF17D) with the sequence of the surface glycoprotein from the rabies virus (RabG) inserted, and is the precursor DNA that leads to the production of replicating LAV-YF17D/RabG virions in the vaccinated host. The insertion of the RabG sequence into that of the YF17D genome is associated with a certain level of instability, which results

in LAV-YF17D/RabG virions that are more attenuated than the parental YF17D virions, leading to decreased virulence of LAV-YF17D/RabG virions.

Although both YF17D and RabG sequences are under the control of the Simian virus 40 promoter, a strong enhancer for transcription that has been shown to promote plasmid entry into the nucleus (Dean DA, 1997), the notifier clarifies that only the early promoter/enhancer sequence of the SV40 genome is included in PLLAV-based vaccines, which does not contain the sequence of the T Antigen protein nor the SV40 ori. T Antigen protein is an essential replication protein involved in the oncogenic properties of the SV40 virus (Pipas *et al*., 2009[1]). Both T Antigen and SV40 ori have been postulated to be required for DNA integration (Strayer *et al*., 2002[2]), suggesting that the SV40 promoter sequence that is present in the DNA plasmid is not expected to promote integration of PLLAV DNA into the human genome.

The plasmid construct also contains a kanamycin resistance gene cassette that functions as a selective marker to ensure stable maintenance of the plasmids in the bacterial cells during manufacturing. Upon BAC's request, and in order to address the likelihood of any potential safety concerns associated to the presence of the antibiotic resistance gene in the plasmid DNA when administered to patients, the notifier further discussed the likelihood of horizontal gene transfer and the possibility of integration of the resistance genes into the patient's genome. Both scenario are considered unlikely given the route of administration. The kanamycin resistance gene is present in the bacterial artificial chromosome (BAC) vector of the PLLAV-YF17D/RabG DNA vaccine, but it is not present in the GMO, LAV-YF17D/RabG. Consequently, if the GMO itself (LAV-YF17D/RabG) were to be shed, it will not include the genetic sequence of the kanamycin resistance gene.

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

The molecular characteristics of LAV-YF17D/RabG were found to be adequately described in the dossier.

3. The conditions of the release

This first in human study is divided in two parts. Patients enrolled in Part I will either be administrated with LAV-YF17D (AVX70120) or with a Placebo. Patients enrolled in the Part II will either be injected with LAV-YF17D/RabG (AVX70481) or with a Placebo. Each subject will be closely observed for at least 60 minutes at the centre.

Upon evaluation of the information provided by the notifier, the BAC revealed a few inconsistenties on the use of personnel protective equipment. Beside the use of a loab coat during the handling, dilution and administration of the PLLAV-YF17D/RabG vaccine, gloves will also have to be worn. Furthermore, open wounds, cuts, scratches and grazes must be covered with waterproof dressings before wearing gloves. The notifier adequately implemented the remarks and requests addressed by the BAC in a revised version of both CAF documents.

[1] Pipas *et al.*, 2009. SV40: Cell transformation and tumorigenesis. Virology 384(2): 294-303.

[2] Strayer *et al.*, 2002. Durability of transgene expression and vector integration: recombinant SV40-derived gene therapy vectors. Mol Ther 6(2): 227-237.

In order to educate patients and patient's family about the potential risk in case of dissemination of the GMO and to help them adhere and practice good hygiene, the Informed Consent Form has been adapted by providing detail instructions for the patients with respect to good hygiene practices. Patients are not allowed to take part in the trial if they live in close contact such as young children or immunocompromised people. Furthermore, patients are requested to perform good hand hygiene for the first 2 months after vaccination.

4. The risks for the environment or human health

The IMP that will be administrated is the plasmid DNA vaccine PLLAV-YF17D/RabG, that contains the full genome of the live attenuated yellow fever virus strain 17D (YF17D) with the sequence of the surface glycoprotein from the rabies virus (RabG) inserted. Following administration, PLLAV-YF17D/RabG enters mammalian cells via transfection. PLLAV-YF17D/RabG relies on the human transcription and translation machinery to produce genetically modified replicating LAV-YF17D/RabG virions. LAV-YF17D/RabG virions actively replicate through infection of host cells and biodistribute in the body of the vaccinee. Replication is self-limiting and stops with the appearance of neutralizing antibodies.

The transgene, RabG, is the surface glycoprotein from the rabies virus. While the rabies virus is pathogenic to humans, the RabG protein on its own cannot create infectious rabies particles and is hence not pathogenic. It is included in the clinical vector to induce an immune response.

Biodistribution, viremia and shedding studies have been conducted on Syrian Golden hamsters with PLLAV-YF17D and PLLAV-YF17D/RabG vaccines. Results have been reported in the Investigator Brochure. There were no apparent differences between both groups in terms of biodistribution, viraemia and shedding. Following BAC's request, the notifier further assessed whether a risk of inadvertent germline transmission could occur. As mentioned by WHO in a recent guideline (2021[3]), data obtained to date have not born out any chromosomal integration of the plasmid DNA in the host cell genome. AV-YF17D/RabG virions contain RNA that replicates in the cytoplasm without DNA intermediate and can therefore not integrate the host cell genome.

Regarding the shedding analysis of the PLLAV-YF17D/RabG-derived LAVs that is currently being assessed in a Good Laboratory Practice (GLP) non-clinical study in hamsters, the notifier highlighted that a more elaborate shedding assessment of PLLAV-YF17D/RabG-derived LAVs has already been performed through the in house non-clinical biodistribution, viraemia and shedding study and results were presented in the Investigator brochure. Nevertheless, the notifier commits that, if any new information that may impact the risks related to the deliberate release of LAV-YF17D/RabG comes to light, they will directly notify the competent authority and if applicable, the notifier will also take the necessary measures to protect health and the environment.

For this first in-human study, levels of LAV in shedding samples following vaccination were first to be analysed in urine, in faeces at different time points, in a sub-cohort of subjects, in both study parts. Following BAC request, the notifier included analysis of buccal swabs in this study as Li et al. (2022[4]) sporadically detected viral RNA in buccal swabs of YF-S0-vaccinated hamsters.

[3] WHO, 2021. Guidelines on the quality, safety and efficacy of plasmid DNA vaccines (WHO TRS 1028, 2021, Annex 2).

[4] Li *et al*. 2022. Biodistribution and environmental safety of a live-attenuated YF17D-vectored SARS-CoV-2 vaccine candidate. Mol Ther Methods Clin Dev. 2022 Jun 9:25:215-224.

No transmission of YF17D through close contact with vaccinated person has been reported up to now and shedding of LAV-YF17D/RabG virions is expected to be limited and similar to that of YF17D. Although recombination of LAV-YF17D/RabG with YF17D is theoretically possible if a co-infection were to occur in the same cells of the vaccinated host, it can be considered as extremely unlikely. Also, considering that the sequences coding for RabG protein cannot give rise on its own to infectious rabies virus particles, the BAC concludes that the risk for the environment and human health associated to possible shedding of the LAV-YF17D/RabG virions, if it were to occur, is low.

Considering all of the above elements, the BAC concludes that the overall risk associated to exposure and transmission to other individuals or animals can be considered low provided that the proposed risk mitigation meaures are adequately implemented.

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

Upon BAC's request, the notifier provided a 2-4 pages technical sheet 'Instructions for study site personnel' giving an overview of all relevant handling instructions, detailed instructions in case of spill or inadvertent exposure of human, waste management and other risk management measures.

Given that the assessment of the likelihood of further propagation of PLLAV-YF17D/RabG 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 PLLAV-YF17D/RabG developed as vaccine against rabbies, will have any adverse effects on human health 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 :
    - AVX1248-101_Model ICF_ENG_v0.3_clean
    - AVX1248-101_Protocol_v1.0
    - AVX1248-101_Instruction sheet for study personnel_final (adapted as requested below)
    - LAV-YF17D_RabG_CAF_Public_Version 2.0_clean
    - LAV-YF17D_RabG_CAF_Confidential_Version 2.0_clean
    - LAV-YF17D_RabG _SNIF

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

- As committed by the notifier, if new information from the current GLP non-clinial study evaluating the shedding of the PLLAV-YF17D/RabG-derived LAVs in hamsters that may impact the risks

related to the deliberate release of LAV-YF17D/RabG comes to light, the notifier will inform the competent authority, for the attention of the BAC, and will take the necessary measures to protect health and the environment.

- As committed by the notifier, if new information from the current GLP repeated dose toxicity study with PLLAV-YF17D and PLLAV-YF17D/RabG that may impact the risks related to the deliberate release of LAV-YF17D/RabG comes to light, the notifier will inform the competent authority, for the attention of the BAC, and will take the necessary measures to protect health and the environment.

- 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 PLLAV-YF17D/RabG.

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

Annex I: Compilations of comments of experts in charge of evaluating the dossier B/BE/23/BVW3 (ref. SC/1510/BAC/2024_0071 and SC/1510/BAC/2024_0317)
Annex II: Answers to the public reaction to dossier B/BE/23/BVW3 in NL (ref. SC/1510/BAC/2024_0466) and FR (ref. SC/1510/BAC/2024_0465)

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Compilation of comments of experts in charge of evaluating the dossier B/BE/23/BVW3
And comments submitted to the notifier

19 January 2024
Ref. SC/1510/BAC/2024_0071

Mandate for the Group of Experts: Mandate of the Biosafety Advisory Council (BAC) of 7 December 2023
Coordinator: Véronique Fontaine (ULB)
Experts: Nicolas van Larebeke-Arschodt (UGent, VUB), Willy Zorzi (ULiège), Anton Roebroek (KULeuven), Aline Baldo (SSB)
SBB: Sheela Onnockx

INTRODUCTION

Dossier **B/BE/23/BVW3** concerns a notification from AstriVax NV 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 15 December 2023 and concerns a clinical trial entitled "*A Phase I, randomized, double-blind, multi-centre, placebo-controlled, dose-escalation study to evaluate the safety, reactogenicity and immunogenicity of AstriVax' investigational vaccine for the prevention of yellow fever (AVX70120), and of AstriVax' investigational vaccine for the prevention of rabies (AVX70481), in healthy adults aged 18 to 40 years".* The trial will involve the use of two plasmid-launched live-attenuated vaccines (PLLAV). One containing the live attenuated yellow fever virus strain 17D (YF17D) and the other the genetically modified PLLAV-YF17D/RabG that encodes the full genome of the live-attenuated YF strain YF17D-204 with the coding sequence of RabG inserted in the YF17D-204 genome. PLLAV-YF17D is indicated for prophylactic vaccination against yellow fever and PLLAV-YF17D/RabG is indicated for prophylactic vaccination against rabies.

♦ 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/questions received from the experts

Remark: The comments below have served as basis for a list of questions that the Competent authority forwarded on 19-01-2024 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.

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

Has evaluated this item and has no questions/comments.

Comment 2

2.3"Spreading through mosquitoes is however not possible for the following reasons: (i) the levels of viraemia following vaccination with commercial YF17D vaccines are very low and below the threshold of oral infection of the mosquito vector, and (ii) it has been shown that YF17D is poorly infectious for mosquitoes and lost its ability to be transmitted by mosquitoes, possibly due to the inability of the virus to cross the midgut barrier (Danet et al., 2019). YF17D can hence not be transmitted under natural environmental conditions." In Danet et al. one reads " Around $5x10^5$ copies of YFV-17D RNA was detected in 2 out of 5 midguts of blood-feed mosquitoes at 3 dpf (Fig 1D). At 12 dpf, around 10^7 copies of YFV-17D RNA was detected in 4 out of 8 mosquitoes, which is 10 time less than in YFV-DAK infected moquitoes." So, while it is certain that the YFV-17D virus multiplies less efficiently than the human pathogen YFV-DAK, the difference is not black-white. It is probably impossible to state with certainty that YFV-17D cannot be transmitted by mosquito's from one human being to another.

SBB's comment:

Further in the article from Danet et al. (2019), it is stated that significantly less midguts were positive for YFV-17D RNA than YFV-DAK RNA at days 7 and 14 post-feeding, suggesting that the midgut infection barrier restricts the replication of the vaccine strain. Based on RT-qPCR, Western blot and immunofluorescence analyses on midgut of mosquitoes, they have observed that the vaccine strain, YFV-17D, replicated poorly in, and disseminated poorly from Ae. aegypti midgut. Furthermore, no dissemination in salivary glands was observed in mosquitoes infected with YFV-17D. Human to human transmission of the virus particles by *Aedes* mosquitoes involves several steps. Firstly, the mosquito feeds on a infected human, the virus needs to be taken up with the blood meal and be able to replicate in the midgut of the mosquito. Secondly, the virus has to overcome the mosquito's midgut barrier, disseminate to and enter the salivary glands of the mosquito, and replicate further. Thirdly, the virus has to be transmitted by transfer of salivary gland fluid when the mosquito feeds on another human.

Coordinator's comment:
I propose to ask investigator to be more cautious and would suggest to replace «however» by « most probably».

2.5"To this means, YF17D constructs were designed to strongly favour recombination, however full length YF17D was never detected under any of the experimental conditions examined." It is however not necessary to incorporate a full YF17D to generate a variant virus with altered pathogenic potency.

SBB's comment:
No question for the applicant has been raised by the expert

Coordinator's comment:
If YF17D constructs were designed to strongly favor recombination, could this have an impact on people receiving the vaccine, knowing that the DNA vaccines harbours the SV40 promoter? Can the applicant confirm the presence of the SV40 promoter in both PLLAV-YF17D and PLLAV-YF17D/RabG vaccines? What would be the non-YFV sequence present in the genome of the produced LAV virion/vaccine? Could there be any homology or complementarity sequence to any human DNA?

SBB's comment:
Agree that we should ask applicant about presence of SV40 promoter and to conduct an assessment on the risk of integration. Also, we need to understand how SV40 promoter could potentially facilitate integration (what is the mechanism ? Should we distinguish between sequences for nuclear import and elements that effectively could play a role in genomic integration?).

2.5"the generation of viable recombinants was considered highly unlikely (McGee et al., 2011)" It is not because full length recombinant YFV17D was not observed in BHK-21 or C710 cells that recombination could not occur in other cell types. That chikungunya virus could show recombination indicates that RNA viruses can show recombination, at least in some circumstances.

SBB's comment:
C.E. McGee *et al* (2011) assessed the potential of YF17D to undergo homologous or non-homologous recombination compared to Chikungunya virus.

2.5 "In line with this, a study of recombination in viruses of the genus Flavivirus did not find any evidence for recombination in YFV (Twiddy and Holmes, 2003)." The Twiddy and Holmes Study mentions, apparently on the contrary: "However, this assumption has now been shown to be invalid, with homologous recombination demonstrated in all three genera of the Flaviviridae".

SBB's comment:
In the introduction, Twiddy and Holmes (2003) state that 'Recent studies have shown this assumption to be invalid, as homologous recombination has now been demonstrated in pestiviruses (bovine viral diarrhoea virus), flaviviruses (all four serotypes of DEN virus, hepaciviruses (GB virus C/hepatitis G virus) and most recently in hepatitis C virus. Twiddy and Holmes further report on their findings using envelope gene sequence data and a combination of graphical and phylogenetic analyses, demonstrating hitherto unreported recombination in Japanese encephalitis virus and St Louis encephalitis virus, as well as further recombinants in DEN virus. However, using this approach, Twiddy and Holmes could not find evidence for recombination for West Nile or YF viruses, or in the tick-borne flavivirus group.

2.5. “The results indicated that while intergenomic recombination can occur between flaviviruses, the frequency appears to be very low and therefore does not represent a major risk in the use of live attenuated “ This might very well be true, but does not prove that there is no risk at all. Use of a vaccine on millions of people provides for the possibility that very rare events occur and give rise to the spread of viruses with altered, possibly dangerous, characteristics.

Coordinator’s comment:
The applicant is already cautious, so I don’t understand why we should highlight this comment

Comment 3
Has evaluated this item and has no questions/comments.

Comment 4
Has evaluated this item and has no questions/comments.

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.

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.

A.3. Ability to colonise

(e.g. transmission routes, survival outside the host….)

Comment 1
Has evaluated this item and has no questions/comments.

Comment 2
2.8 “When determined, mean peak viraemia titres were below 20 pfu and did not exceed 2 log10 pfu/mL, which is far below the infection threshold for mosquito vectors (~3.5 log10/mL). “ Again, this shows that the phenomenon is rare, but rare events can, and probably will, happen when millions of people receive the vaccine.

SBB’s comment:
The use of YF17D could indeed theoretically lead to the risk of secondary spread by mosquitoes, as low vaccine viraemia has been shown in vaccinated adults. However, spreading through mosquitoes is unlikely to occur as the levels of viraemia following vaccination with commercial YF17D vaccines are

very low and below the threshold of oral infection of the mosquito vector, and YF17D is poorly infectious for mosquitoes and lost its ability to be transmitted by mosquitoes.
Furthermore, some measures will be put in place in order to avoid exposure of third the vaccine.

Coordinator's comment:
Nothing to highlight here, except that I found not normal that I had to search myself for reference about the probability of severe AE. This reference should be added in the applicant text when speaking about SAE : https://doi.org/10.1016/j.coi.2009.05.018. So, this comment should be in A2

2.17 "Moreover, as LAV-YF17D/RabG contains the full genome of YF17D, were recombination with the parental virus to occur in vivo, this would have biological effects." I suppose that what was meant here was "NO biological effects"

SBB's comment:
This comment has been combined with the comment of expert 3 in point 2.17.

Coordinator's comment:
Ok

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.

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.

Coordinator's comment:
Here, I have a very important comment, in my opinion. It is in regards to the eventual insertion of the SV40 promoter in both DNA plasmid vaccines (this is mentioned page 10 in the investigator's brochure, but not in any other files, I believe). SV40 is a DNA tumor virus, the SV40 promoter is among others a strong enhancer for transcription. The injection in human of an SV40 positive plasmid in order to favour transfection, could be a real threat for the vaccine receiver. Therefore, it is necessary to verify whether those DNA vaccines are SV40 DNA positive. This would have an impact on human health safety. Furthermore, the DNA plasmid vaccine seems to contain also a kanamycin resistance gene (and additional sequence for high copy maintenance in E. coli). This type of DNA should be assessed for

safety, as recommended in EU directive. Indeed, they could be not only of harm for the vaccine receiver, but also for the environment.

SBB's comment:
As mentioned in section B, a question regarding the presence of the SV40 promoter in the plasmid will be sent to the notifier. If the SV40 promoter is indeed present in both plasmids, a risk assessment on the potential of genomic integration is required.
As the PLLAV plasmid constructs contain a kanamycin resistance gene cassette that functions as a selective marker to ensure stable maintenance of the plasmids in the bacterial cells during manufacturing, the notifier will be requested to address the potential for recombination between the plasmid constructs and genetic material of bacterial cell present in the host and to address the possible fate of the kanamycin selection marker gene, included in the DNA vaccine, and this both for possible genetic exchanges in the environment and with bacteria in the vaccinated host.

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.

Comment 2
Has evaluated this item and has no questions/comments.

Comment 3
Please clarify: is there experimental evidence that the in-frame deletion mutants are still more attenuated than the parental virus, YF17D (bottom page 12/32, top page 13/32 of confidential CAF)? It is not really clear whether this sentence refers to experimental data or an expectation.

SBB's comment:
According to pages 12/13 of the CAF_confidential document, the insertion of the 1.6kb RabG transgene in the LAV-YF17D genome is associated with a certain level of instability caused by the genetic pressure resulting from the insertion of the RabG transgene. This instability results in an emergence of in-frame deletion mutants lacking large parts of the RabG antigen that are still more attenuated than the parental virus, YF17D. The notifier could be requested to clarify whether experimental evidence has demonstrated that the in-frame deletion mutants are still more attenuated than the parental virus, YF17D.

Coordinator's comment:
Has the DNA plasmid sequences the same instability?

Comment 4
Has not evaluated this item

Coordinator's comment:
See my comment above about the molecular characteristics of the DNA vaccines

2.17. Potential for recombination

Comment 1
Has evaluated this item and has no questions/comments.

Comment 2
2.17 “Moreover, as LAV-YF17D/RabG contains the full genome of YF17D, were recombination with the parental virus to occur in vivo, this would have biological effects.” I suppose that what was meant here was “NO biological effects”

SBB’s comment:
This comment has been combined with the comment of expert 3 here below.

Comment 3
Please clarify: What is the significance of the last sentence in 2.17 (page 14/32 in confidential CAF)? Which biological effects are referred to?

SBB’s comment:
This comment together with comment here above of expert 2 could be combined as follow:
According to page 14/32 of the CAF_confidential, as LAV-YF17D/RabG contains the full genome of YF17D, if recombination with the parental virus occurs *in vivo*, this would have biological effects. The notifier could be requested to clarify this sentence. Which biological effects are referred to and are these biological effects expect to occur or not?

Comment 4
Has evaluated this item and has no questions/comments.

2.18. Biodistribution and shedding

Comment 1
Has evaluated this item and has no questions/comments.

Comment 2
Has evaluated this item and has no questions/comments.

Comment 3
See comment at ‘6. Other information’.

Comment 4
In the GLP studies in hamsters, buccal swabs will not be collected. It would have been interesting to collect buccal swabs to evaluate shedding of LAV-YF17D and LAV-YF17D/RabG in saliva even if RNA was not detectable in the previous study in hamsters.
During the first in human clinical trial, all samples should be collected to evaluate the shedding (buccal swabs, feces, urines, serum) because the results in hamsters cannot be extrapolable in humans.

SBB’s comment:
According to the protocol synopsis, page 5/22, to evaluate shedding of LAV following vaccination with AVX70120 and AVX70481, levels of LAV will be analysed in urine and in faeces, at different timepoints, in a sub-cohort of subjects, in both study parts.

Li et al. (2022) detected YF17D viral RNA sporadically in faeces, urine and buccal swabs of vaccinated hamsters. Viral RNA was detectable in vaccinated animals starting at 1 day after vaccination and lasting up to 5 days after vaccination for some of the animals (IB page 15/32). Since, Li et al (2022) also detected viral RNA in buccal swabs, we are wondering why levels of LAV won't be analysed in buccal swabs.
Although viral RNA was not detectable in buccal swabs following administration of PLLAV-YF17D or PLLAV-YF17D/RabG in Syrian Golden hamsters, it should be taken into account that data are not readily extrapolable from animals to human, in particular when different routes of administration are used or when no data have been collected in larger animals, such as non-human primates. As it is important from the perspective of the environmental risk assessment to identify all potential risks for inadvertently infected non-targeted individuals, the notifier could be requested to justify why no buccal swabs will be analysed in both study parts.

Coordinator's comment:
Perfect

3. INFORMATION RELATED TO THE CLINICAL TRIAL

Comment 2
"3.1 A Phase, randomized, double-blind, multi-centre, placebo controlled, dose-escalation study to evaluate the safety, reactiogenicity and immunogenicity of Astrivax investigational vaccine for the prevention of Yellow fever (AVX70120) and of Astrivax' investigational vaccine for the prevention of rabies (AVX70481), in healty adukts aged 18 to 40 years." This seems to me an atypical Phase 1 study

SBB's comment:
Question related to the clinical trial phase goes beyond the scope of the environmental risk assessment or the biosafety assessment of the proposed trial.

Coordinator's comment:
Yes, I don't understand the comment of the expert

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.

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.

3.4. 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.

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.

3.5. 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

In the document « B-BE-23-BVW3_AVX12A_AVX48A-001_Protocol Synopsis » ,
p3 : It is written that :
1)This is the first study in which AstriVax' investigational AVX70120 and AVX70481 vaccines will be evaluated in humans. The purpose of this first in humans (FIH) study is to evaluate the safety, reactogenicity and immunogenicity of different dose levels of the investigational vaccines.
p4 : It is written that :
2)Doses levels
Based on confidential data, the expert has some comments about the clinical dose level that will be administrated.

Coordinator's comment:
I don't know if this will be of any use as we are not hamster

In the document B-BE-23-BVW3_LAV-YF17D-RabG_CAF_Confidential Version,
p26 : It is written that :
3) Adverse events (pathogenicity). For the reasons detailed in Section 2.16, it is possible that LAV-YF17D/RabG may have less pathogenic properties than commercial YF17D vaccines. However, as this has not been shown to date, AEs related to vaccination with commercial YF17D vaccines can be considered as those potentially related to exposure to LAV-YF17D/RabG. These most commonly include erythema and pain at the inoculation site (if applicable, e.g. in case of accidental self-administration through needle stick injury) as well as systemic reactions (headache, asthenia, myalgia, malaise, fever, chills). In addition, there is a small risk of SAEs, including YEL-AND and YEL-AVD.
Considering the point 3) here reported, the notifier extrapolated, (without certainty) that LAV-YF17D/RabG may have less pathogenic properties than commercial YF17D vaccines. However, as this has not been shown to date, AEs related to vaccination with commercial YF17D vaccines can be considered as those potentially related to exposure to LAV-YF17D/RabG.

The present study in humans is elaborated in terms of « doses and trial design » from data obtained from trials in hamsters and in piglets.
Considering the points 1), 2), 3) here reported before and following this « doses and trial design », the notifier is expecting that no adverse consequences in terms of toxicity and immune reactivity will appear in human patients vaccinated both by AVX70120 and AVX70481.
The question is : this Hamster model is it sufficiently closed to Human in terms of physiology and immunology to extrapolate that, considering an average hamster weight of 0.1kg, and an average human weight of 73kg, the dose administrated in hamsters provides sufficient safety margin. Could the notifier provide the scientific data proving the safety of this « doses and trial design », particularly for these vaccines ?
If not, because of all these uncertainties, a trial in Monkey (or other nonhuman primate models, closed to Human) could it/or has it to be proposed to demonstrate and to validate the safety of this protocol in nonhuman primates before trials in humans, and by this way to minimize potential adverse events in vaccinated patients.

→ Would it be possible to request advice from the AFMPS on this concern (particularly in the point of view of health safety for the patients included in this study) ?

SBB's comment:
This question related to the choice of the animal model (hamsters and piglets) to determine the dose to be administrated to human belongs to the patient safety assessment and is out of the scope of the environmental risk assessment. However, we can specify here that the question of the animal model has already been revoked by the Medicines Agency during the STA analysis.

Coordinator's comment:
We have to assess a kind of safety problem: Viruses produced by the DNA vaccines and the DNA plasmid vaccines. Considering the virus GMO, I don't think it is normal to write indeed «it is possible that LAV-YF17D/Rab may have less pathogenic properties than the commercial YF17D vaccines» as it is similar to say we don't know. The file "informations for the public LAV-YF17D/Rab" is not complete. Molecular information on the plasmid DNA is missing. Page 4 of this file said « Le vaccin AND lui-même, PLLAV-YF17D/Rab, ne se propage pas dans le corps. Il est éliminé à l'endroit où le vaccin est administré. » What about the possibility of its integration into the DNA of the host cell, in the nucleus (since that is where it goes to be transcribed), knowing that it could contain an SV40 promoter?»

SBB's comment:
A question regarding the presence of the SV40 promoter in the plasmid will be sent to the notifier. If the SV40 promoter is indeed present in both plasmids, a risk assessment on the potential of genomic integration is required.

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.

3.6. 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

In the document B-BE-23-BVW3_LAV-YF17D-RabG_CAF_Confidential Version, p21 section 3.6. Measures to Prevent Dissemination into the Environment.

It is written that :

b) Personal Protective Equipment
Clinical staff will at a minimum wear a lab coat during the handling, dilution and administration of the PLLAV-YF17D/RabG vaccine. Gloves will be available.
The protection of the eyes seems to be not recommended/required during the handling, dilution and administration of the PLLAV-YF17D/RabG vaccine. Are the goggles not recommended/not required as PPE or are they omitted in the list of PPE ?

SBB's comment:
As confirmed by our colleagues from the Contained Used, protective gown and gloves are sufficient during the handling, dilution and administration of the PLLAV-YF17D/RabG vaccine. Wearing facial protection (protective glasses and mask) is not necessary given the mode of transmission of the parental virus (mosquitoes) and given that the vaccine is a plasmid containing the DNA of the virus.

Coordinator's comment:
For me the main risk is the DNA transfer to commencal bacteria of the clinical staff as the DNA plasmids are kanamycin resistant. This should be taken in consideration and the problem is that assessment is only evaluated at the viral production level mainly, this will be different than reality.

SBB's comment:
As mentioned in section B, the notifier will be requested to address the potential for recombination between the plasmid constructs and genetic material of bacterial cell present in the host and to address the possible fate of the kanamycin selection marker gene, included in the DNA vaccine, and this both for possible genetic exchanges in the environment and with bacteria in the vaccinated host..

Comment 2
Could lipids, present at a site of mucosal contamination, facilitate the entry of PLLAV- YF17D/RabG into a mucosa? Note that Klavinskis et al; (1999) mention only a 30 fold difference between naked DNA and plasmid DNA complexed with DMRIE/DOPE

SBB's comment:
In case of direct contact with mucosa (e.g. eyes, nose, mouth), there is a theoretical risk of DNA uptake, however, the uptake of naked DNA plasmid by mucosal cells has been shown to be very ineffective in comparison with the plasmid DNA-lipid complexes with DMRIE/DOPE that enhances the efficiency of plasmid DNA uptake by nasal epithelium (Klavinskis *et al*. 1999).

Comment 3

With respect to the stated availability of gloves during administration of the product, sampling and further analyses of samples etc., it should be clearly stated that these gloves should be used.

SBB's comment:
This comment has been combined with comment from expert 4 here below.

Comment 4
Clinical staff will at a minimum wear a lab coat during the handling, dilution and administration of the PLLAV-YF17D/RabG vaccine. Gloves will be available.
Open wounds, cuts, scratches and grazes should be covered with waterproof dressings and gloves should be wear.
Could the applicant detail the treatment of accidental spill (contact time of the disinfectant) and provide a written procedure for health care personnel?

SBB's comment:
According to section 3.6.b, clinical staff will at a minimum wear a lab coat during the handling, dilution and administration of the PLLAV-YF17D/RabG vaccine. Gloves will be available. However, gloves should not only be available, they should also be worn. Therefore, the notifier could be requested to adapt this section by clearly indicate that the personal protective equipment should include both a lab coat and gloves that should be used. Furthermore, open wounds, cuts, scratches and grazes should be covered with waterproof dressings before wearing gloves.
The remark regarding the procedure in case of accidental spill has been combined with the remark of point 3.8 of expert 4.

Coordinator's comment:
Here I will had an information for the clinical staff saying that the plasmid DNA is carrying a kanamycin resistance gene

SBB's comment:
This point will be included in the question to the notifier.

Additional SBB's comment:
According to the CAF confidential document p23/32, study participants will be educated about the potential risk in case of dissemination to immunocompromised persons or young infants and how this can be avoided.
In order for patients and patient's family to adhere to and practice good hygiene, it is important to explain why measures are taken and what are the likely sources of contaminated material. Therefore, the notifier could be requested to provide a small take home summary (preferably one-page, plasticized document) to ensure that patients and patient's family easily can consult the information and all the instructions in an understandable format whenever needed.
The following information could be reported in this instruction sheet for the patient:

- The bodily fluids which are anticipated to contain viral vector genome
- Instructions aimed at limiting contact with materials or surfaces frequently contaminated with bodily fluids

- Instructions and effective solutions to decontaminate possible contaminated areas, tissues, skin, …
- The period during which these instructions must be followed

Coordinator's comment:
Should the assessment of such a complex dossier not be in different steps? One step for the DNA plasmids and one step for the virus produced after vaccination? I found the dossier quite «uncomplete» because of : the uncertainty that the DNA vaccine and maybe also RNA vial genome might contain SV40 sequences

SBB's comment:
A question regarding the presence of the SV40 promoter in the plasmid will be sent to the notifier. If the SV40 promoter is indeed present in both plasmids, a risk assessment on the potential of genomic integration is required.

3.7. Sampling and further analyses of samples from study subjects

Comment 1
Has evaluated this item and has no questions/comments.

Comment 2
Has evaluated this item and has no questions/comments.

Comment 3
See 3.6

Comment 4
Has evaluated this item and has no questions/comments.

3.8. Emergency responses plans

Comment 1
Has evaluated this item and has no questions/comments.

Comment 2
Has evaluated this item and has no questions/comments.

Comment 3
Has evaluated this item and has no questions/comments.

Comment 4
A spill kit should be available for the personnel administrating the vaccine.
Could the applicant provide a detailed procedure in the event of accidental occupational exposure through a splash to the eyes or mucous membrane and in case of a needle stick accident?

SBB's comment:
According to the CAF document section 3.6.c, in case of accidental spilling of a biological sample from a vaccinated study participant (which potentially contain the clinical vector, LAV-YF17D/RabG), the area

will be chemically decontaminated with an organic disinfectant. However, detail the treatment of accidental spill (contact time of the disinfectant). The decontamination and cleaning measures after in the case of accidental spilling could have been improved by clarifying the procedure to drain the spillage and the contact time of the disinfectant. It should also be mentioned that a spill kit should be available for the personnel handling and administrating the vaccine. This spill kit should contain appropriate disinfectant, personal protective equipment (PPE, i.e. gloves, safety glasses, laboratory coat, shoe covers, mask), tongs or forceps in order to take broken vials, absorbent paper towels, biohazard waste bags.

Furthermore, in order to help health care personnel when handling the vaccine, the notifier could be asked to provide a 2-4 page 'instructions for study staff personal' provided as a plasticized document with the essentials for preparing and administering the IMP by personnel. This sheet should include all relevant handling instructions, detailed procedures to handling a spill including appropriate disinfectants, waste management and other risk management measures:

- Personal Protective Equipment (PPE)
  - o For the IMP preparation
  - o For the administration to the patients
  - o For the samples collection from the patient
- Management of inadvertent exposure of human to the vaccine
  - o Eye exposure from splash or aerosol
  - o Mouth exposure from splash or aerosol
  - o Needlestick, sharps exposure or non-intact skin exposure
  - o Contact with skin and clothing
- Management of inadvertent exposure to blood, urine, vomit or other bodily fluids from patients in the initial period at the hospital
- Clean-up procedure
  - o After IMP preparation (specify decontamination solution and minimum contact time)
  - o In case of accidental spill or breakage (specify decontamination solution and minimum contact time)
- Waste Management
  - o During IMP preparation
  - o During IMP administration

5. ENVIRONMENTAL RISK ASSESSMENT

Comment 1

Has evaluated this item and has no questions/comments.

Comment 2

5.3. "LAV- YF17D/RabG virions cannot be transmitted under natural environmental conditions" This might well be very rare, but *."* In Danet et al. (2019) one reads *" Around 5x10^5 copies of YFV-17D RNA was detected in 2 out of 5 midguts of blood-feed mosquitoes at 3 dpf (Fig 1D). At 12 dpf, around 10^7 copies of YFV-17D RNA was detected in 4 out of 8 mosquitoes, which is 10 time less than in YFV-DAK infected moquitoes*." So, while it is certain that the LAV- YF17D/RabG virus will not easily be transmitted by mosquito's from one human to another, it is not possible to exclude that possibility with certainty. Indeed, YFV-17D virus multiplies less efficiently than the human pathogen YFV-DAK, the difference is not black-white. It is probably impossible to state with certainty that YFV-17D cannot be transmitted by mosquito's from one human being to another.

SBB's comment:

See SBB's comment here below

A.3 Overall Risk Evaluation and Conclusions
5.3 In the penultimate paragraph of 5.3. one reads: "*As a consequence, the likelihood of occurrence of a mutational event during in vivo replication that increases pathogenicity is considered low to negligible.*"
I estimate that this statement is far from true and unacceptable in scientific terms. Indeed, in Kum et al. (2019) one reads "*Forty-two percent (42%) (36/86) of virus clones that were plaque-purified from the brain of mice that had been inoculated with PLLAV-YFV-17D had no mutations (Figure 2B) (in contrast to 12% observed in YFV-17D inoculated mice, Figure 2A) whereas the remaining 58% (50/86) had either only 1 or 2 mutations (median = 1) per genome. Hence the incidence of nucleotide variants in the PLLAV-YFV-17D derived viruses was markedly lower compared to the frequency of variant clones that were obtained from mice that had been injected with YFV-17D (Stamaril®); where 0–11 mutations per genome (median = 5) were observed (Figure 2B).*"
I am not capable of speaking out on the question to which extent this point influences the risk associated with vaccination with or environmental exposure to PLLAV-YF17D/RabG, but is certainly not negligible.

**"5.4*. Risk Characterisation. Mutational event during in vivo replication that increases pathogenicity.* While the hazard may potentially be severe, the risk of occurrence is considered low to negligible*." Considering this risk as negligible seems inappropriate to me.

"***5.9. Risk Characterisation***
As there are no safety concerns associated with potential LAV-YF17D/RabG shedding into the environment, the risk is considered negligible."
It would be more reasonable to assess this risk as very low.

"A.3 '*The overall risk to the environment is considered negligible"* It would be more reasonable to assess this risk as very low.

Comment 3
See comment at '6. Other information'.

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

In the document B-BE-23-BVW3_LAV-YF17D-RabG_SNIF,

p3 : It is written that :
Risk of adverse effects. As the GMO has similar biological properties as its parental organism, YF17D, it can be assumed that adverse effects related to vaccination with YF17D may be similar to those related to exposure to LAV-YF17D/RabG. The majority of adverse effects related to vaccination with YF17D are mild in intensity, however, there is a small risk of serious adverse events that are of severe intensity: the incidence of serious adverse events following vaccination with commercial YF17D vaccines has been estimated at 1.6 – 4.7 per 100 000 vaccinees. The risk of occurrence of serious adverse events is considered low to negligible.
Considering this point and in the whole dossier, the notifier extrapolated, (without certainty) that LAV-YF17D/RabG may have less pathogenic properties than commercial YF17D vaccines. The notifier proposed an risk analysis for the patients in an evasive approach, especially about serious adverse events and superficially about the others ones. It would be necessary to develop more the risk assessment aspect.

→ Would it be possible to request advice from the AFMPS on risk assessment quality of this dossier, in the point of view of health safety for the patients included in this study?

SBB's comment:
The question merely belongs to the patient safety assessment of LAV-YF17D/RabG.

Comment 2
First a very general remark. As so often in reports by scientific experts working in the field of genetically modified organisms, perhaps a little critical sense is lacking here and there. Indeed, as we learned from the covid 19 pandemic and from some other outbreaks of viral epidemics, small genetic changes and recombinations between viral genomes, or between a viral genome and the cellular genome of a mammal, can lead to dangerous pathogenic viruses. These are events that in themselves are extremely infrequent and highly unlikely, but can have important consequences for human health, and sometimes lead to terrible epidemics. In addition, insertions of viral sequences and recombinations with chromosomal genes can contribute to the risk of malignant tumoral transformation and the development of cancer. If so, however, these are rare phenomena that affect only individuals and may not really have a public health impact. However, these rare phenomena can have a huge impact on an individual, are almost impossible to detect, but their existence should be recognized and taken into account.

This vaccine is an example of a medicinal product that could contribute importantly to the protection of human health and the prevention of human suffering. But there is also a limited probability that it carries a risk. Specifically, in relation to his vaccine it seems that the risk of spreading from one human to another cannot be excluded with certainty. And as with probably many viruses and procedures introducing new genetic information into the cells of a human being, mutational or recombinational events that may contribute to the risk of cancer cannot be excluded with certainty, and their possible occurrence should be acknowledged.

SBB's comment
Risk assessment is based on the principle: Risk = hazard x exposure.
This means that if there is no hazard, there is no risk despite a real chance of exposure. Conversely if there is no exposure, there will be no risk even if a hazard were identified.
In the context of vaccination with commercial YF17D vaccines, there are no data to suggest hazard or adverse effects to the non-vaccinee.
Even if exposure of close contacts to shedding by patients cannot completely be ruled out (although unlikely in this case because of the transmission properties), there are no data to suggest a real risk to the close contacts. Furthermore, the precursor vaccine PLLAV-YF17D/RabG is a DNA plasmid and hence not a GMO, and it does neither persist nor biodistribute through the body of the vaccinee.

Concerning the document SUMMARY NOTIFICATION INFORMATION FORMAT FOR THE RELEASE OF GENETICALLY MODIFIED ORGANISMS OTHER THAN HIGHER PLANTS IN ACCORDANCE WITH ARTICLE 11 OF DIRECTIVE 2001/18/EC"

"*The human and environmental risk assessment show that there is very low to negligible risk to public health and environment*."It seems to me more correct to state that this risk is very low

"Public Health Risk Assessment
"The likelihood of infection with LAV-YF17D/RabG virions of people not included in the clinical study is low to negligible considering:
- The virions cannot be transmitted under natural environmental conditions.
- Any potential means of spread of the GMO (through accidental self-administration of the precursor DNA vaccine PLLAV-YF17D/RabG, or through direct exposure to LAV-YF17D/RabG virions in biological material from a study participant) would involve the exposure to very low amounts of (PL)LAV-YF17D/RabG (if any), by consequence, it would be unlikely that the person would actually get infected with LAV-YF17D/RabG."
I think that infection with LAV-YF17D/RabG virions of people not included in the clinical study cannot be excluded with certainty. *Indeed,* while it is certain that the YFV-17D virus multiplies less efficiently than

the human pathogen YFV-DAK, the difference is not black-white. It is probably impossible to state with certainty that YFV-17D cannot be transmitted by mosquito's from one human being to another.

SBB's comment:
More than 800 million people have been vaccinated since the vaccine became available in 1938. The reactogenicity and safety of YF17D vaccine was monitored in 21 clinical studies conducted between 1953 and 2008. The vaccine YF17D is safe and effective.
As for the wild-type YFV, YF17D does not spread through close contact with a vaccinated person. No case of transmission of the commercial vaccine against yellow fever YF17D have been reported. A mosquito host is required for transmission.
However, efficient transmission of the virions through a mosquito implies several steps:

- The virions have to be taken up by the mosquito during an infectious bloodmeal
- The virus first infects the midgut where it can replicate
- The virus also has to infect the salivary glands from where it can be released into the saliva
- The infected mosquito then transmits the virus through its saliva when it bites another uninfected host

Even if transmission by mosquitoes from one human being to another cannot completely be ruled out, as the levels of viraemia following vaccination with commercial YF17D vaccines are very low and below the threshold of oral infection of the mosquito vector, and as YF17D is poorly infectious for mosquitoes and lost its ability to be transmitted by mosquitoes, possibly due to the inability of the virus to cross the midgut barrier, spreading of YF17D through mosquitoes is can be considered as negligible.

""***Risk of occurrence of a mutational event during in vivo replication that increases pathogenicity.*** *As the LAV YF17D/RabG virions replicate in vivo, the occurrence of a mutational event during replication that increases pathogenicity cannot fully be excluded. If this were to occur, the intensity of the hazard may potentially be severe. The same risk exists for commercial YF17D vaccines, and over the 800 million people who have been vaccinated with commercial YF17D vaccines, one occurrence of this has been identified. The likelihood of occurrence of this type of event is hence considered low to negligible*.
'It is clearly not because only one case of severe adverse effects due to mutation of the vaccine-virus was detected that this occurred only once. Systematic surveillance and detection of this phenomenon is totally impossible. Also from the observations of Kum et al.(2019) it can be deduced that the mutation rate after infection with *PLLAV-YF17D/RabG will probably be somewhat less than after infection by YFV-17D, but still of the same order of magnitude. Also, it was mentioned by the applicant that* "*The insertion of the 1.6kb RabG transgene into the YF17D genome is associated with a certain level of instability caused by the genetic pressure resulting from the insertion of the RabG transgene."*
"*Taken together, the overall risk to public health is considered low to negligible."* I would rather conclude that the overall risk to public health is considered very low

SBB's comment:
In this concluding sentence, the notifier is mentioning the overall risk to public health and not to the patient. Although the overall risk to patient could rather be considered as very low, since spreading through mosquitoes is very unlikely, overall risk can be considered as negligible for the public health in general.

"(c) Is it frequently used in the country where the notification is made?
Yes (x) No (.)
(d) Is it frequently kept in the country where the notification is made?
Yes (x) No (.)" I wonder whether the organism is frequently used and kept in Belgium.

SBB's comment:
This editorial remark could be reported under "Typos/editorial remarks on the SNIF as follow:
On the SNIF page 5/16, YES has been reported for both questions, "Is it frequently used in the country where the notification is made?" and "Is it frequently kept in the country where the notification is made? However, to our knowledge, the parental organism, the Yellow Fever Virus is not frequently used and kept in Belgium. Please either correct the answer to both questions or clarify.

"*10 (b) Factors affecting dissemination*

*Not applicable. YF17D cannot be disseminated under natural environmental conditions."*I think that dissemination is likely to be very rare, but that it cannot be excluded completely.

SBB's comment:
See SBB's comment here above

"2. Intended outcome of the genetic modification
The purpose of the genetic modification is for LAV-YF17D/RabG virions to express the RabG protein in all cells infected by the LAV, in order induce an immune response against rabies virus in the vaccinated host, for the prevention of rabies" I wonder whether the expression of RabG protein can have adverse effects. It is well known that vaccination against rabies frequently has quite severe side effects.

SBB's comment:
RabG mediates entry of the rabies virus into host cells and is as such involved in the pathogenic or harmful properties of the rabies virus. However, the GMO (LAV-YF17D/RabG) only includes the genetic sequence of the RabG glycoprotein from the rabies virus. The RabG protein as such is not enough to create infectious particles.

The following question could be submitted to the notifier:
According to the IB p25/32, a repeated dose toxicity GLP study with PLLAV-YF17D and PLLAV-YF17D/RabG is currently ongoing. This repeated dose toxicity study with Syrian Golden hamsters should be completed and should clearly demonstrate absence of toxicity before the Phase 1 clinical trial can be started. As it is also important from the perspective of the environmental risk assessment to identify all potential risks for inadvertently infected non-targeted individuals (e.g. accidental exposure of heath care professionals at clinical trial site; exposure of close contacts because of shedding), the notifier could be requested to provide, if available, an update of this toxicity study.

"2. Is the site of the release different from the natural habitat or from the ecosystem in which the recipient or parental organism is regularly used, kept or found?" I think that the answer to this question should be YES rather than No

SBB's comment:
This editorial remark could be reported under "Typos/editorial remarks on the SNIF.

"*(i.e. they cannot be transmitted under natural environmental conditions)"*It would be more correct to state that transmission is probably very exceptional

"*(i.e. they cannot be transmitted under natural environmental conditions)"* This cannot be stated with absolute certainty

Comment 3

1. The dossier contains a provisional Investigator's Brochure. In this IB reference is made to several ongoing nonclinical studies investigating shedding, toxicology etc. It is assumed, that these study results not only will be incorporated into an updated IB, but also in updated versions of the CAFs in case this would be relevant. For the moment these does not seem to be a problem with respect to shedding and toxicology (both environmental risk related aspects).

SBB's comment:
See here above question on missing toxicity study.
The following question could also be submitted to the notifier regarding the shedding study:
Shedding data collected from previous studies will contribute to a proper environment risk evaluation. These data will need to be evaluated in light of the observed quantity of shed viral material and the period of time during which shedding is observed. According to the CAF_confidential document, page 14/32, shedding of the PLLAV-YF17D/RabG-derived LAVs is being further assessed in a Good Laboratory Practice (GLP) non-clinical study in hamsters. In order to be able to judge whether no special measures are necessary when handling the vaccine or once treated with the vaccine, the notifier could be requested to provide the results of this study as soon as it is available.

2. RabG Information for the public. Only the version in English and Dutch were evaluated by the expert. It is assumed, that the French and German version have the same content. With respect to these documents with information to the public it should be noted, that the AstriVax' investigational vaccine for the prevention of rabies (AVX70481) is in fact at the same time also an investigational vaccine for the prevention of yellow fever. In Part 2 of the clinical study this aspect of LAV-YF17D/RabG is also investigated as explained in the Protocol Synopsis. The documents with information to the public does hardly address this aspect.

SBB's comment:
Based on the IB, page 7/32, it can be assume that during this phase I study two different investigational vaccines will be tested :

1- The vaccine AVX70120 (PLLAV-YF17D) for the prevention of yellow fever
2- The vaccine AVX70481 (PLLAV-YF17D/RabG) for the prevention of rabies

3. It is assumed that Informed Consent Forms will part of the complete dossier.

SBB's comment:
According to the Belgian Royal Decree from 2005, the Informed Consent Form is not a mandatory document to provide together with the notification to the competent authority to obtain permission. It is recommended to accompany the biosafety dossier with, among other, the ICFs as it would greatly facilitate the evaluation of the application under the "deliberate release" procedure.

Comment 4
Has no additional comment

References

Danet et al., 2019. Midgut barriers prevent the replication and dissemination of the yellow fever vaccine in Aedes aegypti. PLoS Negl Trop Dis. 13(8):e0007299.

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Adviesraad voor Bioveiligheid
Conseil consultatif de Biosécurité

Compilation of the expert's evaluations of the answers of AstriVax NV on the list of questions for dossier B/BE/23/BVW3

08 march 2024
Ref. SC/1510/BAC/2024_0317

Coordinator: Véronique Fontaine (ULB),
Experts: Willy Zorzi (ULiège), Anton Roebroek (KULeuven), Aline Baldo (SBB)
SBB: Sheela Onnockx

INTRODUCTION

Dossier **B/BE/23/BVW2** concerns a notification from AstriVax NV for a clinical trial entitled "A Phase I, randomized, double-blind, multi-centre, placebo-controlled, dose-escalation study to evaluate the safety, reactogenicity and immunogenicity of AstriVax' investigational vaccine for the prevention of yellow fever (AVX70120), and of AstriVax' investigational vaccine for the prevention of rabies (AVX70481), in healthy adults aged 18 to 40 years".

On 19 January 2024, based on a list of questions prepared by the BAC (SC/1510/BAC/2024_0070), 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 February 2024. This complementary information was reviewed by the coordinator and the experts in charge of the evaluation of this notification.

Evaluation Expert 1

In my point of vue, the notifier addressed correctly and satisfactorily the comments/questions that have been raised for the B/BE/23/BVW3 dossier, in January 2024 .

Evaluation Expert 2

In my opinion, the notifier addressed correctly and satisfactorily the comments/questions that have been raised in January.

Evaluation Expert 3

Je suis satisfaite de leurs réponses et des mesures supplémentaires mises en place.

Evaluation Coordinator

I generally find that the answers from the questions were well addressed. Please find here my comment:

Q7: For ethical consideration: “ Lastly, the vector mosquito species that can transmit yellow fever virus are not endemic to Belgium, where the clinical study will be conducted”, is an additional proof that we should not conduct clinical study in non-endemic area…as we cannot investigate all potential adverse effects of vaccine or the release of an OGM (secondary to a pre-established immune response or to the presence of the vector, respectively).

SBB’s comment:
Any ethical consideration related to the current trial application will be analyzed by the ethical committee in charge of this dossier. A clinical trial can only start after having received a favorable opinion from Ethics Committee.

Coordinator’s comment:
Ethical committees never consider this aspect. They are considering the risk/ benefice balance for the patients, never at a global point of view. That is why this aspect will be now part of the cooperation agreement. The BAC previously emphasized the need for this kind of ethical consideration.

SBB’s comment:
Whether ethical consideration will be integrated within the cooperation agreement or not is currently still under discussion.

Adviesraad voor Bioveiligheid
Conseil consultatif de Biosécurité

Antwoorden van de Adviesraad voor Bioveiligheid op opmerkingen gekregen tijdens de publieksraadpleging over de kennisgeving B/BE/23/BVW3 van AstriVax voor doelbewuste introductie in het leefmilieu van genetisch gemodificeerde organismen met uitzondering van hogere planten voor onderzoek en ontwikkeling

Versie – 28/03/2024
Ref. SC/1510/BAC/2024_0466

Contexte

De kennisgeving B/BE/23/BVW3 werd in december 2023 door AstriVax 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 15 december 2023.
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_2024_0468). 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: We merkten dat één van de onderzoeksgroepen van Sciensano betrokken partij is in dit dossier. Welke mechanismen bestaan er binnen Sciensano om de onpartijdigheid van de SBB bij de beoordeling van deze studie te garanderen? (We twijfelen hier voor de duidelijkheid niet aan, maar nemen dit graag op voor de volledigheid)

SBB's Comment:
Er liep een onderzoekscontract tussen de KU Leuven en de Dienst Virale Ziekten van Sciensano over PLLAV-constructies, tot december 2023. Er werden een aantal serologische tests uitgevoerd voor Astrivax waarvoor een vergoeding werd vastgelegd. Er is echter geen intellectueel of financieel belang bij de welvaart van Astrivax.
De SBB is functioneel en hiërarchisch gescheiden van de Dienst Virale Ziekten, zoals weergegeven in het organigram van de Wetenschappelijke directies van Sciensano (https://www.sciensano.be/fr/a-propos-de-sciensano/organigramme-de-sciensano). De SBB is niet betrokken bij de onderzoeks- en ontwikkelingsactiviteiten van Sciensano met betrekking tot PLLAV-constructies.
Sciensano werkt met aparte budgetlijnen. Het budget van de SBB is niet gekoppeld aan bovenvermelde overeenkomsten tussen Sciensano en KU Leuven.

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De SBB is één van de twee pijlers (samen met de Adviesraad voor Bioveiligheid) van het wetenschappelijke evaluatiesysteem dat in België is opgezet om de federale en regionale bioveiligheidsautoriteiten te adviseren over activiteiten waarbij genetisch gemodificeerde organismen (GGO's) en/of pathogenen betrokken zijn. De officiële taken van de SBB zijn vastgelegd in het samenwerkingsakkoord inzake Bioveiligheid van 25 april 1997, wat betekent dat de rol van de SBB als wetenschappelijk adviseur van de autoriteiten gebaseerd is op strikte wettelijke vereisten, in het bijzonder met betrekking tot de onafhankelijkheid en de wetenschappelijke objectiviteit.

Verschillende maatregelen zorgen ervoor dat de wetenschappelijke ondersteuning van de SBB volledig onafhankelijk is:
- Alle uitwisselingen tussen de SBB en het FAGG (Bevoegde Autoriteit) gebeuren via een generiek e-mailadres, waardoor alle wetenschappers die betrokken zijn bij de SBB op de hoogte kunnen worden gebracht. Dit garandeert ook de volledige traceerbaarheid van de procedure.
- De beoordeling van dossiers wordt uitgevoerd volgens duidelijk gedefinieerde procedures en criteria, die gebaseerd zijn op de wettelijke vereisten van de Richtlijnen 2001/18/EG en 2009/41/EG.
- De adviezen van de SBB worden getoetst door minstens twee SBB-experts en goedgekeurd door het diensthoofd.

Ten slotte werd het FAGG op de hoogte gebracht van de situatie, in het kader van een wetenschappelijk en technisch advies (STA) over de GGO-status en de regelgevingsprocedure van de vaccinplatforms PLLAV-YF17D en PLLAV-YF17D/RabG voor de firma Astrivax, dat uitgebracht werd in juni 2023.

Vraag 2: Het publieke dossier spreekt over een bijkomede dierenproef naar toxiciteit, maar de mogelijke toxiciteit komt niet direct terug in het technische dossier. a) in welke sectie van het technisch dossier wordt deze bijkomende proef beschreven en b) zal deze info nog in rekening gebracht kunnen worden bij deze beslissing of bij de opmaak van de vergunning?

SBB's Comment:
Volgens de Investigator Brochure (IB) p25/32 is er momenteel een GLP-toxiciteitsstudie aan de gang bij Syrische goudhamsters met herhaalde PLLAV-YF17D en PLLAV-YF17D/RabG doses. Voordat de klinische studie van fase 1 kan beginnen, moet deze studie voltooid worden en moet de afwezigheid van toxiciteit aangetoond worden. Omdat het vanuit het perspectief van de milieurisicobeoordeling ook belangrijk is om potentiële risico's te identificeren voor niet tot de doelgroep behorende mensen die onbedoeld besmet zouden kunnen raken (bijvoorbeeld door accidentele blootstelling van gezondheidswerkers of door blootstelling van naaste contacten via uitscheiding), werd de kennisgever gevraagd om een update van dit toxiciteitsonderzoek. De kennisgever bevestigde dat de definitieve resultaten van dit onderzoek in het tweede kwartaal van 2024 worden verwacht en dat ze in het IB zullen worden geïntegreerd voordat de klinische proefaanvraag (CTA) wordt ingediend. Bovendien belooft de kennisgever de bevoegde autoriteit op de hoogte te stellen van alle nieuwe informatie die een impact kan hebben op de risico's die gepaard gaan met de doelbewuste introductie van LAV-YF17D/RabG, zodra deze beschikbaar komt. In dit geval zal de kennisgever, indien nodig, ook de nodige maatregelen nemen om de gezondheid en het milieu te beschermen.

Vraag 3: Germline transmission wordt niet genoemd in dit dossier. Kunnen jullie kort motiveren om welke reden, kwestie van dit consequent op te nemen in onze briefing van het politieke niveau (Ik vermoed omwille van de slechts tijdelijke aanwezigheid van het vreemd materiaal in de cel, maar hoor dit graag bevestigd).

SBB's Comment:

Bij het gebruik van genetisch gemodificeerde organismen is het van belang om goed in te schatten of er een risico op accidentele kiembaanoverdracht zou kunnen ontstaan. De kennisgever werd daarom gevraagd te beoordelen of er sprake zou kunnen zijn van een risico op accidentele kiembaanoverdracht. Het geneesmiddel voor onderzoek (Investigational Medicinal Product, IMP) dat zal worden toegediend is het plasmide DNA-vaccin PLLAV-YF17D/RabG, dat het volledige genoom van de levende verzwakte stam van het gele koortsvirus 17D (YF17D), met de sequentie van het oppervlakteglycoproteïne van het rabiësvirus (RabG) ingevoegd, bevat onder controle van de krachtige SV40-promotor. Na toediening komt het PLLAV-YF17D/RabG-plasmide de cellen binnen door transfectie. Het plasmide maakt gebruik van menselijke transcriptie- en translatiemachines om genetisch gemanipuleerde replicatieve LAV-YF17D/RabG-virionen te produceren. LAV-YF17D/RabG-virionen zijn RNA-virussen die zich in het cytoplasma repliceren zonder een DNA-tussenproduct en kunnen daarom het genoom van de gastheercel niet integreren. LAV-YF17D/RabG-virionen repliceren door gastheercellen te infecteren. De replicatie is zelflimiterend en stopt met het verschijnen van neutraliserende antilichamen. Zoals vermeld door de WHO in een recente richtlijn (WHO, 2021), hebben tot nu toe geen gegevens chromosomale integratie van plasmide-DNA in het genoom van de gastheercel aangetoond.

References:

WHO, 2021. Guidelines on the quality, safety and efficacy of plasmid DNA vaccines (WHO TRS 1028, 2021, Annex 2).

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/23/BVW3 de AstriVax pour l'introduction volontaire dans l'environnement, à des fins de recherche et développement, d'organismes génétiquement modifiés autres que les plantes supérieures

Version - 28/03/2024
Ref. SC/1510/BAC/2024_0465

Contexte

La notification B/BE/23/BVW3 a été soumise en décembre 2023 par AstriVax 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 15 décembre 2023.
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_2024_0468). 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: Nous avons remarqué qu'un des groupes de recherche de Sciensano est impliqué dans ce dossier. Quels mécanismes existent au sein de Sciensano pour garantir l'impartialité du SBB dans l'évaluation de cette étude ? (Sachez que nous n'en doutons pas, mais nous souhaitons l'inclure par souci d'exhaustivité).

Commentaire du SBB :
Un contrat de recherche était en cours entre la KU Leuven et le Service Maladies virales de Sciensano sur les constructions PLLAV, jusqu'en décembre 2023. Un certain nombre de tests sérologiques ont été réalisés pour Astrivax pour lesquels une compensation a été définie. Cependant, il n'y a aucun intérêt intellectuel ou financier à la prospérité d'Astrivax.
Le SBB est fonctionnellement et hiérarchiquement distinct du Service Maladies virales comme le montre l'organigramme des Directions Scientifiques de Sciensano (https://www.sciensano.be/fr/a-propos-de-sciensano/organigramme-de-sciensano). Le SBB n'est pas impliqué dans les activités de recherche et de développement de Sciensano liées aux constructions PLLAV.

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Sciensano travaille avec des lignes budgétaires distinctes. Le budget du SBB n'est pas lié aux accords susmentionnés entre Sciensano et la KU Leuven.
Le SBB est l'un des deux piliers (avec le Conseil consultatif de biosécurité) du système d'évaluation scientifique mis en place en Belgique pour conseiller les autorités fédérales et régionales compétentes en matière de biosécurité sur les activités impliquant des organismes génétiquement modifiés (OGM) et/ou pathogènes. Les tâches officielles du SBB sont définies dans l'accord de coopération sur la biosécurité du 25 avril 1997, ce qui signifie que le rôle du SBB en tant que conseiller scientifique des autorités repose sur des exigences juridiques strictes, notamment en matière d'indépendance et d'objectivité scientifiques.

Plusieurs mesures garantissent que le soutien scientifique apporté par le SBB s'effectue en toute indépendance:
- Tous les échanges entre le SBB et l'AFMPS (Autorité compétente) s'effectuent à l'aide d'une adresse email générique, permettant d'informer tous les scientifiques impliqués au SBB. Cela garantit également une traçabilité complète de la procédure.
- L'évaluation des dossiers est effectuée selon des procédures et des critères bien définis basés sur les exigences légales des directives 2001/18/CE et 2009/41/CE.
- Les avis du SBB sont contre-vérifiés par au moins deux experts du SBB et approuvés par le chef de service.

Finalement, dans le contexte d'un avis scientifique et technique concernant le statut et la procédure régulatoire OGM des plateformes vaccinales PLLAV-YF17D & PLLAV-YF17D/RabG pour la firme Astrivax, émis en juin 2023, l'Agence AFMPS a été informée de la situation.

Question 2: Le dossier public mentionne un test supplémentaire de toxicité sur les animaux, mais la toxicité éventuelle n'est pas directement reflétée dans le dossier technique. a) dans quelle section du dossier technique cet essai complémentaire est-il décrit et b) cette information peut-elle encore être prise en compte dans cette décision ou lors de l'établissement du permis ?

Commentaire du SBB:
Selon l'Investigator Brochure (IB) p25/32, une étude GLP de toxicité à doses répétées avec PLLAV-YF17D et PLLAV-YF17D/RabG est actuellement en cours. Cette étude de toxicité à doses répétées sur des hamsters dorés syriens devra être achevée et devra démontrer l'absence de toxicité avant que l'essai clinique de phase 1 ne puisse démarrer. Comme il est également important, du point de vue de l'évaluation des risques environnementaux, d'identifier tous les risques potentiels pour les personnes non ciblées qui pourraient être infectées par inadvertance (par exemple, par exposition accidentelle de professionnels de la santé ou par exposition de contacts étroits via l'excrétion), il a été demandé au notifiant de fournir une mise à jour de cette étude de toxicité. Le notifiant a confirmé que les résultats définitifs de cette étude sont attendus au deuxième trimestre 2024 et qu'ils seront intégrés dans l'IB avant la soumission de la demande d'essai clinique (CTA). De plus, le notifiant s'engage à notifier à l'autorité compétente toute nouvelle information susceptible d'avoir une incidence sur les risques liés à la dissémination volontaire du LAV-YF17D/RabG dès qu'elle sera disponible. Dans ce cas, s'il y a lieu, le notifiant prendra également les mesures nécessaires pour protéger la santé et l'environnement.

Question 3: La transmission germinale n'est pas mentionnée dans ce dossier. Pouvez-vous expliquer brièvement pourquoi, afin d'inclure systématiquement cela dans notre briefing au niveau politique (je suppose qu'en raison de la seule présence temporaire de corps étrangers dans la cellule, mais j'aimerais que cela soit confirmé).

Commentaire du SBB:

Lors de l'utilisation d'organismes génétiquement modifiés, il est important d'évaluer correctement si un risque de transmission germinale accidentelle pourrait survenir. Il a dès lors été demandé au notifiant d'évaluer si un risque de transmission germinale accidentelle pouvait survenir. Le médicament expérimental (Investigational Medicinal Product, IMP) qui sera administré est le vaccin à ADN plasmidique PLLAV-YF17D/RabG, qui contient, sous le contrôle du puissant promoteur SV40, le génome complet de la souche vivante atténuée du virus de la fièvre jaune 17D (YF17D) avec la séquence de la glycoprotéine de surface du virus de la rage (RabG) insérée. Après administration, le plasmide PLLAV-YF17D/RabG pénètre dans les cellules par transfection. Le plasmide s'appuie sur la machinerie humaine de transcription dans le noyau et de traduction pour produire des virions LAV-YF17D/RabG réplicatifs génétiquement modifiés. Les virions AV-YF17D/RabG sont des virus à ARN qui se répliquent dans le cytoplasme sans intermédiaire ADN et ne peuvent donc pas intégrer le génome de la cellule hôte. Les virions LAV-YF17D/RabG se répliquent par infection des cellules hôtes. La réplication est autolimitée et s'arrête avec l'apparition d'anticorps neutralisants. Comme mentionné par l'OMS dans une ligne directrice récente, aucune donnée obtenue à ce jour n'ont montré d'intégration chromosomique de l'ADN plasmidique dans le génome de la cellule hôte (WHO, 2021).

References:

WHO, 2021. Guidelines on the quality, safety and efficacy of plasmid DNA vaccines (WHO TRS 1028, 2021, Annex 2).