Adviesraad voor Bioveiligheid
Conseil consultatif de Biosécurité

Advice of the Belgian Biosafety Advisory Council on the notification B/BE/24/BVW6 of the sponsor AstriVax NV for deliberate release in the environment of genetically modified organisms other than higher plants for research and development

23/01/2025
Ref. SC/1510/BAC/2025_0100

Context

The notification B/BE/24/BVW6 has been submitted by AstriVax NV to the Belgian Competent Authority in October 2024 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 randomised, double-blind, placebo-controlled, multi-centre, phase I study to evaluate the safety, reactogenicity and immunogenicity of AstriVax’ investigational therapeutic hepatitis B virus (HBV) vaccine (AVX70371) in adult patients with chronic HBV (CHB) infection”.

The purpose of this study is to assess safety, reactogenicity and immunogenicity of AVX70371 vaccine in healthy adults aged of 18 to 50 years.

The investigational medicinal product consists of a DNA-based vaccine corresponding to a plasmid-launched live attenuated virus (PLLAV). The PLLAV plasmid contains the full genome of the live attenuated yellow fever virus strain 17D [YF17D] with the coding sequence of the hepatitis B virus core antigen (HBc) inserted within the YF17D genome and is indicated for prophylactic vaccination against hepatitis B virus.

This Phase I first-in-human study will consist of two parts. Part 1 will follow a dose escalation design. In this part of the study, the effect of two vaccinations with two different dose levels of AVX70371 will be evaluated. Part 2 is conducted to further evaluate the effect of two vaccinations with the selected dose level of AVX70371 in different populations of patients with CHB infection. Vaccination will be performed intradermally in the volar aspect of the forearm.

It is estimated that approximately 40 patients will receive AVX70371 in this Phase I study, which is planned to be conducted in one clinical site located in Flanders. The national territory is considered as the potential release area of PLLAV-YF17D/HBc.

The dossier has been officially acknowledged by the Competent Authority on 25 October 2024 and forwarded to the Biosafety Advisory Council (BAC) for advice.

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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. Four experts from the common list of experts drawn up by the BAC and the Service Biosafety and Biotechnology (SBB) of Sciensano answered positively to this request. The experts 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 13 December 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 09 January 2025 and transmitted to the secretariat of the BAC on the same day. This complementary information was reviewed by the coordinator and the experts, 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 three reactions 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 both parts of clinical study is the plasmid DNA vaccine PLLAV-YF17D/HBc. PLLAV-YF17D/HBc contains the full genome of the live attenuated yellow fever virus (YFV) strain 17D (YF17D) with the coding sequence of the hepatitis B virus core antigen (HBc) inserted, and is the precursor DNA that leads to the production of replicating LAV-YF17D/HBc virions in the vaccinated host. The insertion of the HBc sequence within the YF17D genome sequence is associated with a certain level of instability, which results in LAV-YF17D/HBc virions that are more attenuated than the parental YF17D virions, leading to decreased virulence of LAV-YF17D/HBc virions.

The monoclonal DNA vaccine, AVX70731 is based on a live-attenuated yellow fever vaccine strain, YF17D, derived from a clinical isolate Asibi strain and attenuated by serial passaging. According to Kum *et al*. (2019[1]), YF17D has a high degree of genetic stability during *in vivo* replication, which correlates with the fact that only one occurrence of mutational event has been identified up to now corresponding to one fatal case of encephalitis in a 3-year-old child who received commercial YF17D vaccine in 1965 (A.D. Jennings et al. 1994[2]).

The transgene, the hepatitis B virus core antigen (HBc), is a structural component of the HBV viral nucleocapsid. The hepatitis B virus is pathogenic to humans and the HBc protein may impact HBV pathogenesis and viral persistence. However, HBc protein on its own cannot create infectious HBV particles and in the DNA vaccine AVX70731, the HBc antigen is not expressed in its native form. As mentioned by the notifier, in-house studies have been conducted to evaluate the replication capacity of HBc-containing live-attenuated yellow fever virus *in vitro* with regards to viral persistence. Furthermore, several HBc-containing therapeutic vaccines have entered clinical phase, where full-length HBc is expressed as part of a fusion protein or a separate antigen and to date, none of the clinical trials with HBc-containing therapeutic vaccines have reported pathogenic findings related to HBc expression.

Both YF17D and HBc 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[3]). T Antigen protein is an essential replication protein involved in the oncogenic properties of the SV40 virus and has been postulated to be required for DNA integration (Pipas *et al*., 2009[4]). As clarified by the notifier, only the early promoter/enhancer sequence of the SV40 genome, which does not contain the sequence of the T Antigen protein, is included in PLLAV vaccines. SV40 sequence is only present in the plasmid backbone, and is not part of the genetically modified LAV-YF17D/HBc sequence. It is not present in the produced LAV-YF17D/HBc virions and will not be shed if shedding were to occur.

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

Information related to the molecular characteristics of LAV-YF17D/HBc were found to be adequately described in the dossier.

Following BAC's request, in order to improve the reading of the file and to allow access to the information of the DNA vaccine composition, information on the elements that compose the plasmid backbone has been included in section 2 of the CAF document related to the description of the clinical vector, LAV-YF17D/HBc.

3. The conditions of the release

This first in human study is divided in two parts. Patients enrolled in Part I will either be injected intradermally in the forearm with LAV-YF17D (AVX70120) or with a Placebo. Patients enrolled in the

[1] Kum *et al*., 2019. Limited evolution of the yellow fever virus 17d in a mouse infection model. Emerg Microbes Infect. 8(1): 1734-1746
[2] Jennings *et al*., 1994. Analysis of a yellow fever virus isolated from a fatal case of vaccine-associated human encephalitis. J Infect Dis. 169(3): 512-518
[3] Dean *et al*., 1997. Import of plasmid DNA into the nucleus is sequence specific. Experimental Cell Research Volume 230, Issue 2, 1 February 1997, Pages 293-302
[4] Pipas *et al*., 2009. SV40: Cell transformation and tumorigenesis. Virology 384(2): 294-303.

Part II will either be injected with LAV-YF17D/HBc (AVX70371) or with a Placebo. Each subject will be closely observed for at least 60 minutes at the centre.

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 detailed instructions for the patients with respect to good hygiene practices. Patients are not allowed to take part in the trial if they live with close contact such as young children or immunocompromised people. Patients are requested to perform good hand hygiene for the first two months after vaccination. Other restriction measures such as egg / ovum donation, sperm donation, pregnancy, breastfeeding, the use of contraception method will also have to be followed for a period of two months after vaccination. Considering potential differences in the kinetics between commercial YF17D and LAV-YF17D/HBc and taking into account that the viraemia data from study AVX1248-101 (B/BE/23/BVW3) are not yet available to date, in order to avoid any risks, a period of three months after vaccination has been selected in this study AVX37-101 for restriction on blood or organs donation.

4. The risks for the environment or human health

The IMP that will be administrated intradermally is the plasmid DNA vaccine PLLAV-YF17D/HBc, that contains the full genome of the live attenuated yellow fever virus strain 17D (YF17D) with the sequence of the hepatitis B virus core antigen (HBc) inserted. Following administration, PLLAV-YF17D/HBc enters mammalian cells via transfection. PLLAV-YF17D/HBc relies on the human transcription and translation machinery to produce genetically modified replicating LAV-YF17D/HBc virions. LAV-YF17D/HBc 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.

Biodistribution, viraemia and shedding analysis of LAV-YF17D/HBc have not been evaluated non-clinically, because several non-clinical biodistribution, viraemia and shedding evaluations have been performed with LAV-YF17D (clinical vector without transgene) and with LAV-YF17D/RabG (clinical vector that has the glycoprotein of the rabies virus as a transgene) and because it can reasonably be assumed that the presence of HBc coding sequence, which is not expressed as a surface protein on the LAV-YF17D/HBc, is not affecting biodistribution, viraemia nor shedding of LAV-YF17D/HBc compared to LAV-YF17D. Viraemia and shedding of LAV-YF17D/RabG following vaccination with AVX70481 is evaluated in Part II of the Phase I clinical study AVX1248-101 (B/BE/23/BVW3). The notifier commits to provide with the results once available and analysed.

For this first in-human study with LAV-YF17D/HBc, levels of LAV in shedding samples following vaccination will be analysed in serum, urine, faeces and buccal swabs at different time points, in participants in Part II of the study.

No transmission of YF17D through close contact with vaccinated person has been reported up to now and shedding of LAV-YF17D/HBc virions is expected to be limited and similar to that of YF17D. Although recombination of LAV-YF17D/HBc with other Flaviviridae, such as hepatitis C virus, is theoretically possible if a co-infection were to occur in the same cells of the vaccinated host, it can be considered as negligible. Furthermore, as confirmed by the applicant, participants with concomitant HCV infection will not be allowed to take part in this study.

Finally, considering that the sequences coding for HBc protein cannot give rise on its own to infectious hepatitis B virus particles, the BAC concludes that the risk for the environment and human health associated to possible shedding of the LAV-YF17D/HBc 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.
So, for example, in case of accidental self-administration of the precursor DNA vaccine AVX70371 into the body (e.g. needle stick injury), the incident will be reported to the responsible person of the clinical site and AstriVax confirms that the injured caregiver will also receive appropriately follow-up

Given that the assessment of the likelihood of further propagation of PLLAV-YF17D/HBc 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/HBc developed as vaccine against hepatitis B virus, 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 :
    - Latest version of the AVX37-101_ICF_ENG
    - Latest version of the AVX37-101_Protocol
    - LAV-YF17D_HBc_Instruction sheet for study personnel_v1.0 : Please make sure, the word "peronnel" in the title of the document has been corrected into "personnel".
    - LAV-YF17D_HBc_CAF_Public
    - LAV-YF17D_HBc_CAF_Confidential_Version 2.0_clean
    - LAV-YF17D_HBc _SNIF

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

- As committed by the notifier, viraemia and shedding data of the AVX1248-101 trial with LAV-YF17D/RabG (EU CT number 2024-511194-29; notification number B/BE/23/BVW3) will be

provided once available and analysed. Furthermore, the applicant is requested to take the necessary measures to protect health and the environment if new information from this shedding analysis comes to light that may impact human health or 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/HBc.

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/24/BVW6 (ref. SC/1510/BAC/2024_1561 and SC/1510/BAC/2025_0099)
Annex II: Answers to the public reaction to dossier B/BE/24/BVW6 in NL (ref. SC/1510/BAC/2025_0102) and FR (ref. SC/1510/BAC/2025_0101)

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Compilation of comments of experts in charge of evaluating the dossier B/BE/24/BVW6
And comments submitted to the notifier

13 December 2024
Ref. SC/1510/BAC/2024_1561

Mandate for the Group of Experts: Mandate of the Biosafety Advisory Council (BAC) of 18 October 2024
Coordinator: Véronique Fontaine (ULB)
Experts: Nicolas van Larebeke-Arschodt (UGent, VUB), Willy Zorzi (ULiège), Anton Roebroek (KULeuven), Rik Gijsbers (KULeuven)
SBB: Sheela Onnockx

INTRODUCTION

Dossier **B/BE/24/BVW6** 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 25 October 2024 and concerns a clinical trial entitled "*A randomised, double-blind, placebo-controlled, multi-centre, Phase I study to evaluate the safety, reactogenicity and immunogenicity of AstriVax' investigational therapeutic hepatitis B virus (HBV) vaccine (AVX70371) in adult patients with chronic HBV (CHB) infection".* The trial will involve the use of a plasmid-launched live-attenuated vaccines (PLLAV). The genetically modified PLLAV-YF17D/HBc that encodes the full genome of the live-attenuated YF strain YF17D-204 with the coding sequence of hepatitis B virus core antigen (HBc) inserted in the YF17D-204 genome. PLLAV-YF17D/HBc is indicated for vaccination against hepatitis B virus.

♦ 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 13-12-2024 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 (**Willy Zorzi**)*
Has evaluated this item and has no questions/comments.

*Comment 2 (**Nicolas Van Larebeke**)*
P5: do we know with certainty that the plasmid does not integrate into host DNA (chromosomal or mitochondrial DNA)?

SBB's comment:
The question related the plasmid integration within the host DNA genome due to the presence of the SV40 promoter in the PLLAV plasmid has been developed by the applicant during the previous dossier from AstriVax (B/BE/23/BVW3):

Coordinator's comment:
This is not in this reference. https://doi.org/10.1016/j.celrep.2013.03.002 presents the role of SV40 in unwinding the viral DNA from the ori, but other proteins I assume could do the same job. Rommerskirch W., Graeber I., Grassmann M., Grassmann A., Homologous Recombination of SV40 DNA in Cos7 Cells Occurs with High–Frequency in a Gene Dose Independent Fashion, Nucleic Acids Res. 16 (1988) 941–952, presents the high integration rate of SV40. The most important information is that integration rate could be linked to the SV40 enhancer (doi: https://doi.org/10.1101/2024.01.09.574829), the applicant could be asked how this information has been evaluated in their environmental risk assessment.

SBB's comment:
A complete risk assessment on any potential risk of genomic integration in the host genome has already been provided by the applicant during the evaluation of the dossier B/BE/23/BVW3 and has been reviewed and approved by the coordinator in the past. According to the applicant, only the early promoter / enhancer sequence of the SV40 genome is included in PLLAV-based vaccines, which is not involved in the oncogenic properties of the SV40 virus. Both T-ag and the SV40 ori sequences, which have been postulated to mediate SV40 integration, are not included in the PLLAV vaccines. The early promoter / enhancer sequence of the SV40 is not known to promote integration of DNA into the human genome. Senigl *et al.* results, to which the coordinator is referring, show that SV40 enhancer has a strong targeting activity for somatic hypermutation, that certain type of mutation accumulate in the large tumor antigen and suggest that a truncated version of the large tumor could also be harmful. It is not clear however how these findings can lead to the hypothesis that the SV40 enhancer, as expressed from the PLLAV system (and with PLLAV lacking T-ag and the SV40 ori sequences), could have an impact on the integration rate, as suggested by the coordinator.

Coordinator's comment:
A question should be added about the recent publication (pre-print, now published in Tumour Virus Res. 2024 Oct 25:18:200293. doi: 10.1016/j.tvr.2024.200293), whether the applicant assessed the impact on the recent discovery of the role of the SV40 enhancer sequence on strong somatic hypermutation targeting activity in several cell type in the safety of the injected DNA and produced GMO.

P6: YF17D vaccines are heterologous mixtures of multiple virion subpopulations. To which extent are we certain that no viruses with hazardous properties can be present in these mixtures

SBB's comment:
Aspects related to the purification method of YF17D vaccines do not belong to the data requirements for the biosafety dossier. Aspects related to the quality of the IMP will be evaluated by quality assessors within the FAGG.

Coordinator's comment:
Ok

P7: "YF17D can hence most probably not be transmitted under natural environmental conditions." Can a low probability be associated with risks in the case of the intended vaccine treatment?

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.
Under natural environmental conditions, various mosquitoes species are involved in the maintenance and transmission of wild type YFV. However, the parental virus, YF17D, which is the commercial vaccine against yellow fever, does not have a natural host range. The use of YF17D could theoretically lead to the risk of secondary spread by mosquitoes, as vaccine viraemia has been shown in vaccinated adults. The risk of this is however deemed negligible 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 most probably 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).

Coordinator's comment:
Ok

P7: "YF17D superinfection resistance was observed in vertebrate and arthropod cells harbouring a primary infection with wild type YFV Asibi strain."
Do we know how this superinfection resistance comes about? According to Zou et al. (2009) flavivirus superinfection resistance may result from sequestering of critical host factors by the replicative complex of the primary virus, which exists in abundance relative to the secondary virus.

SBB's comment:
Question related to cause of this superinfection resistance goes beyond the scope of the environmental risk assessment or the biosafety assessment of the proposed trial.

Coordinator's comment:
The coordinator doesn't agree, she thinks it will be interesting to have their answers, though in her point of view, the risk is here very low, probably negligible

SBB's comment:
It is indeed important to understand the underlying mechanisms of resistance to superinfection when developing a new vaccine. However, the mechanisms and clinical consequences of YF17D superinfection in patients are related to the patient safety and the efficacy of the vaccine and go beyond the scope of the environmental risk assessment or the biosafety assessment of the proposed trial.

P7 point 2.5 "Therefore, even in the unlikely event of a high level acute co-infection of 2 distinct YFV genomes, the generation of viable recombinants was considered highly unlikely (McGee et al., 2011)." The possibility of recombination is probably the most critical and potentially pathogenic aspect of vaccination with viruses, even flaviviruses. Gee et al.(2011) observed non-homologous intragenic recombination between CHIKV viruses, but did not detect homologous recombination. That they did not find any homologous recombination is not immediately understandable. Also Gee et al used a system requiring recombination to occur within the coding sequence of a single protein, the JEV system employed by Taucher et al., (2009), who did find recombination, allowed for expression of both truncated and full length C and E from a single covalently linked genome. Therefore, it is possible that the efficiency of generating a viable recombinant within the 17D system was less than that using the JEV system. Furthermore, it is possible that the ability for different viruses to undergo recombination and/or for recombinants to be detected may be highly influenced by the specific cell culture conditions employed. Gee et al.(2011) argue that the data suggest that the efficiency of flavivirus recombination may be extremely low and in fact may require long-term sustained or persistent co-infection to allow for sporadic template switching to occur. They however also mention that reports of naturally occurring mosquito-borne flavivirus and alphavirus recombinants suggest that these viruses may undergo precisely homologous recombination in nature, with no aberrant sequence duplications, insertions, or deletions. Twiddy & Holmes (2003), who report not to have found recombination between Mosquito-borne flaviviruses, however also mention that this lack of findings might be due to methodological problems. The experiments of Taucher et al.(2010) are certainly reassuring, but absolute certainties cannot be derived from the available data, as unidentified parameters, such as structural differences between different RNA sequence regions, might permit recombinations not detectable with the systems used by Gee et al.(2011). and Taucher et al.(2010).

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

Coordinator's comment:
I believe the concern is most probably related to the P5 question (DNA integration and recombination) and RNA recombination. In my point of view, I don't see any additional threat. So, no further questions about it as it is already mentioned above.

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

Comment 4 (**Rik Gijsbers**)
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 (**Willy Zorzi**)*
Has evaluated this item and has no questions/comments.

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

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

Comment 4 (**Rik Gijsbers**)
The intended age range for the patients included in the clinical trial is supposed to adults. Considering the reported SAE, vaccination of pregnant or breastfeeding mothers, and immune compromised persons is disadvised? What about possibly pregnant mothers? (this may be a concern more for FAGG instead?)

SBB's comment:
According to the exclusion criterion 27, pregnant or lactating women won't be included in this clinical trial

Coordinator's comment:
OK, I have an additional concern/remark: what about the additional expression of HBc? The information provided on HBc biological activities and impact in pathogenesis and viral persistence is not sufficiently described. Indeed, HBc CTD facilitated annealing and unwinding of DNA and enhanced hammerhead ribozyme cleavage *in vitro*, rendering HBc CTD a bona fide nucleic acid chaperone (Chu et al., 2014). This could have an impact of the frequency of DNA recombination, integration. Furthermore, HBc has been also reported to have different transcriptional regulating activity . What are the applicant evidences to state "HBc on its own ….and is hence not pathogenic " and "The addition of the transgene does hence neither provide an advantage for replication / survival of the clinical vector ".

SBB's comment:
The applicant could indeed be requested to provide further information on HBc biological activities and its potential impact on pathogenesis and viral persistence (in relationship with the interaction with host proteins). HBc CTD facilitated annealing and unwinding of DNA and enhanced hammerhead ribozyme cleavage *in vitro*, rendering HBc CTD a bona fide nucleic acid chaperone (Chu *et al.,* 2014). This could have an impact of the frequency of DNA recombination, integration. Furthermore, HBc has also been reported to have different transcriptional regulating activity (Diab *et al*. 2019). The applicant is requested to clarify the evidence to state that "HBc on its own is not pathogenic" and "The addition of the transgene does hence neither provide an advantage for replication / survival of the clinical vector compared to the parental virus, nor does it alter its transmission route" as mentioned in section 2.15 of the confidential CAF.

A.3. Ability to colonise

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

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

*Comment 2 (**Nicolas Van Larebeke**)*
P10 “They collected 44 urine samples from 44 vaccinated persons (1 sample per person), 16 of which were collected within 1 month after vaccination, 21 of which at 4-7 months after vaccination, and the other 7 between 8-11 months after vaccination. YF17D RNA was detected by reverse transcription polymerase chain reaction (RT-PCR) in 2 out of 44 samples. The time B- vaccination was 21 days for one sample and 198 days for the other (Martinez et al., 2011). While the detection of YF17D RNA in urine at 198 days after vaccination could be indicative of the possibility of persistent infection with YF17D, the Applicant considers this highly unlikely as there are no other (nonclinical or clinical) data supporting the persistence of YF17D in the body of the vaccinee.”
The most likely explanation of this finding is that the virus replicated and persisted for 198 days. That no other signs were detected does not implicate that the virus was not replicating, as it is well known that many viral infections go unnoticed.

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

Coordinator’s comment:
We should suggest to the applicant to be more cautious in his text as indeed the absence of clinical signs cannot support the absence of viral persistence

SBB’s comment:
In the study described by Martinez et al. (2011), YF17D RNA was detected by reverse transcription polymerase chain reaction (RT-PCR) in one sample collected 198 days after vaccination. Although the applicant considered the possibility of persistent infection with YF17D highly unlikely, the notifier could be requested to be more cautious in the wording because many viral infections go unnoticed and the absence of clinical signs cannot rule out viral persistence..

P11 “there are no reports of transmission of YF17D through close contact with a vaccinated person.”
I wonder to what extent this phenomenon has been studied.

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, in this study, the precursor vaccine PLLAV-YF17D/HBc is a DNA plasmid and it does neither persist nor biodistribute through the body of the vaccinee.

Coordinator’s comment:
Ref: WHO, 2021 for DNA persistence and biodistribution. But here, the comment was more on viral transmission. So, the applicants could better develop the evidences, taking also in consideration that we have the additional expression of HBc.

SBB's comment:
Till now, there are no reports indicating that YF17D, the vaccine strain of the yellow fever virus, can be transmitted through close contact with a vaccinated person. The vaccine is generally considered safe, and the risk of transmission from a vaccinated individual to another person is extremely low. A discussion of the potential impact of he HBc insert has been included in the question proposed in section 2.1 here above.

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

Comment 4 (**Rik Gijsbers**)
The applicant provides info on studies indicating that YF17D RNA can be detected in urine, in rare instances 21 and even 198 days post vaccination. Even though this study is rather old and presence of RNA does not imply functionality, techniques have evolved. Would it be interesting to assess this in the current study for example using ddPCR? Or has the urine been used to demonstrate functionality (see also 2.9 – p11/32 B_BE_24-BVW6_Part1_CAF_BE_Confidential Version.pdf where is indicated that the YF17D is fragile). Is data available from the earlier trial for LAV-YF17D/RabG that is in line?

SBB's comment:
According to the protocol synopsis, biological samples will be collected throughout the part I of the study. Blood, urine, faeces and buccal swabs for LAV viraemia and shedding analysis will be collected at different time point until 85 days after vaccination. As mentioned in the SNIF, PCR methods will be used. According to section 2.18 of the confidential CAF, no previous clinical experience with LAV-YF17D/HBc has been performed. Biodistribution, viraemia and shedding of LAV-YF17D/HBc have not been evaluated non-clinically, because non-clinical biodistribution, viraemia and shedding evaluation has been performed for LAV-YF17D (clinical vector without transgene) and for LAV-YF17D/RabG (clinical vector that has the glycoprotein of the rabies virus as a transgene).
In addition, viraemia and shedding of LAV-YF17D/RabG following vaccination with AVX70481 is evaluated in Part 2 of the Phase I clinical study AVX1248-101 (EU CT number 2024-511194-29; Deliberate Release Reference Number B/BE/23/BVW3). At the time of the finalisation of this document, the data were not yet available.

Coordinator's comment:
Almost same comment as Nicolas, it will be interesting to ask for these results.

SBB's comment:
The following question could be sent to the applicant:
Viraemia and shedding of LAV-YF17D/RabG following vaccination with AVX70481 is evaluated in Part 2 of the Phase I clinical study AVX1248-101 (EU CT number 2024-511194-29; Deliberate Release Reference Number B/BE/23/BVW3). At the time of the finalisation of the CAF document, the data were not yet available. As shedding data collected from previous studies will contribute to a proper environment risk evaluation and will help us to judge whether any special measures are necessary when handling the vaccine or once treated with the vaccine, the notifier is requested to provide the results of this study as soon as they are available.

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

*Comment 2 (**Nicolas Van Larebeke**)*
P12 What is the function and the precise action of the 2A self-cleaving peptide?

SBB's comment:
As described in the section 2.14 on page 12, following translation of the clinical vector RNA, the 2A self-cleaving peptide linker ensures that HBc is expressed separately from the YF17D polyprotein.

Coordinator's comment:
Ok

P12 "HBc remains N-terminally covalently linked to the 21 amino acid YF17D C peptide."
Do these 21 amino acids remain as part of the HBc core antigen in the vaccinated persons?

SBB's comment:
As mentioned in a previous STA for a similar plasmid, the PLLAV-YF17D/RabG, the insertion was done into the YF17D polyprotein sequence at the junction between the YF17D E and NS1 protein. This specific insertion allows HBc to be cleaved out of the YF precursor polyprotein.

Coordinator's comment:
Ok

P13 "The clinical vector does not contain unknown sequences or elements of which the origin or functions is unknown". However, the HBc core antigen is modified

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

Coordinator's comment:
Could not find in the files that the HBc protein sequence has been modified? If, I am wrong, they should be an assessment on the known activities of this protein (in relationship with the interaction with host proteins).

SBB's comment:
The transgene consist of the core antigen from the hepatitis B virus.
The coordinator's comment has been integrated in the proposed question to the applicant related to the HBc insert in section A.2 here above.

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

Comment 4 (**Rik Gijsbers**)
In 2.10, p11/32 B_BE_24 BVW6_Part1_CAF_BE_Confidential Version.pdf the clinical vector is referred to as LAV-YF17D/HBc, whereas in the map of the precursor DNA plasmid, PLLAV-YF17D/HBV(HBc) is used. In the intro the applicant mentions: "The clinical vector (live attenuated virus [LAV]-YF17D/HBc) is produced *in vivo* in the vaccinated host (refer to Section 2.0 for more information). There is hence no manufacturer of the clinical vector as such. The information provided below is on the manufacturer of

the precursor DNA vaccine, plasmid-launched live attenuated virus [PLLAV]-YF17D/HBV(HBc) (compound code: AVX70371).”
The nomenclature is complicated here. I’m not sufficiently informed on the specific definitions of “clinical vector” and “drug product”. I would expect here to read info on how the drug product is prepped (the plasmid), and maybe additionally info on the production of the RNA and how this results in self-replication. I did not review the earlier file, was this the same?

SBB’s comment:
The clinical vector LAV-YF17D/HBc corresponds to the full genome of the LAV YF17D, with the gene encoding for HBc protein inserted. This vector is inserted in the plasmid DNA vaccine, the PLLAV-YF17D/HBc (AVX70371). AVX70371 is the precursor DNA that leads to the production of replicating LAV-YF17D/HBc virions in the vaccinated host. These virions are genetically modified organisms (GMOs) as their RNA contains the sequence of HBc inserted into that of YF17D.
According to the World Health Organization (WHO) guideline on the quality, safety and efficacy of plasmid DNA vaccines (WHO, 2021), “plasmid DNA vaccine is not an organism; thus, it is not a GMO per se”. Therefore, as for the previous dossier, the content of this dossier only focuses on the GMO, the LAV-YF17D/HBc and the applicant did not developed on the plasmid production.

Coordinator’s comment:
Totally understand Rik’s remark as it was similar to her remark in the previous AstriVax application. This is why ask per se asked whether vaccine recipients (and AFMPS) will have access to this information. Transparency is very important.

SBB’s comment:
Aspects related to the production and the quality of the IMP will be evaluated by quality assessors within the AFMPH.

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

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

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

Comment 4 (**Rik Gijsbers**)
In 2.15 in p13/32 B_BE_24-BVW6_Part1_CAF_BE_Confidential Version.pdf the title also indicates ‘the DNA inserted’. What is meant here? The plasmid DNA used for vaccination? No information on the plasmid used is provided.

SBB’s comment:
This section is describing the clinical vector which is composed of the clinical vector backbone (YF17D) and in the DNA inserted (HBc).

In section 2.0, page 5/32, few information on the plasmid is provided.

Coordinator's comment:
The coordinator proposes that the applicant improve the reading of the file, allowing easier access to the information of the DNA vaccine composition.

SBB's comment:
In order to improve the reading of the file and to allow access to the information of the DNA vaccine composition, the applicant could indeed be requested to provide more information in section 2.10 on the elements that compose the plasmid backbone.

2.17. Potential for recombination

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

*Comment 2 (**Nicolas Van Larebeke**)*

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

Comment 4 (**Rik Gijsbers**)
P13/32 in p11/32 B_BE_24-BVW6_Part1_CAF_BE_Confidential Version.pdf: one but last paragraph: 'combination' should be 'recombination'

SBB's comment:
In section 2.17, page 13/32, "combination » should indeed be corrected into "recombination" in the following sentence: *Even if a co-infection of LAV-YF17D/HBc and YF17D in a single cell were to occur, the generation of viable recombinants is highly unlikely, in line with what described in Section 2.5 on the combination properties of YF17D*. The request to correct the sentence could be reported as a "Typos and other errors/omissions".

Coordinator's comment:
OK but what about the additional expression of HBc? Indeed, HBc CTD facilitated annealing and unwinding of DNA and enhanced hammerhead ribozyme cleavage *in vitro*, rendering HBc CTD a bona fide nucleic acid chaperone (Chu et al., 2014). This could have an impact of the frequency of DNA recombination

SBB's comment:
Question related to the HBc insert has been included in the question proposed in section 2.1 here above.

2.18. Biodistribution and shedding

*Comment 1 (**Willy Zorzi**)*
Please find my comment in the point 3.6.

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

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

Comment 4 (**Rik Gijsbers**)
Has evaluated this item and has no questions/comments.

3. INFORMATION RELATED TO THE CLINICAL TRIAL

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

*Comment 2 (**Nicolas Van Larebeke**)*
P19 “It is planned to evaluate 2 different dose levels, AVX70371. The proposed dose levels may be lowered subsequent data review during the study. The maximum dose level that will be administered will not be exceed.”
I think there is a contradiction here

SBB’s comment:
According to section 3.5, two different doses will be tested. This maximum dose will not be exceeded. Depending on the intermediated results obtained during the study, these proposed doses may be lowered.

Coordinator’s comment:
Ok

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

Comment 4 (**Rik Gijsbers**)
Has evaluated this item and has no questions/comments.

3.3. Storage of the clinical vector at the clinical site
(e.g. storage location, conditions of storage, …)

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

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

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

Comment 4 (**Rik Gijsbers**)
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 (**Willy Zorzi**)*
Has evaluated this item and has no questions/comments.

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

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

Comment 4 (**Rik Gijsbers**)
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 (**Willy Zorzi**)*
Has evaluated this item and has no questions/comments.

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

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

Comment 4 (**Rik Gijsbers**)
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 (**Willy Zorzi**)*
In the p5 of the « B-BE-24-BVW6_LAV-YF17D-HBc_Information for the Public_Belgium_English_Version 1.0 » document, in the section CONTAINMENT, CONTROL AND MONITORING MEASURES, it is written that:

1)Measures to Limit the Risks for Human Health
-While it is very unlikely that LAV-YF17D/HBc particles will accidently spread to people outside the clinical study, the following measures will be put in place to completely avoid this
Personnel at the hospital taking part in the clinical study will be trained. They will wear a lab coat and gloves when they handle AVX70371, or when they sample bodily fluids (for instance blood) from people who take part in the clinical study.
- All bodily fluids sampled from people in the clinical study will be stored in tubes that have a screw cap. If someone accidently spills a sample, the area will be thoroughly disinfected.
- All waste that may contain LAV-YF17D/HBc will be treated as hazardous medical waste.

2) Measures to Limit the Risks for the Environment
If a sample taken from people who take part in the clinical study is accidentally spilled, the surface will be thoroughly disinfected. No other measures will be put in place because the risk related to release of LAV-YF17D/HBc into the environment is negligible (as LAV-YF17D/HBc cannot survive outside the body).

In view of the above describing measures to limit the risks in hospital, we didn't find any information about the « Package leaflet: Information for the user/patient » concerning the management of the vaccinated patients at home, especially about the risks and the management of certain situations after returning home.

Therefore, the following questions must be raised:
-About the treatment of faecal matter and urine: should they be treated with standard hygiene home practices or specific protocols?
-In case of diarrhea, should it be treated as infectious material or not?
-The « soiled material » as vomit should it be treated as infectious material or not?
-In case of some accidental situations at home requiring for example wound treatment, use of compress which can be soiled by blood potentially contaminated by LAV-YF17D/HBc …, the question is: should it be necessary to treated all these wastes as hazardous medical waste or not? If yes, it would be necessary to describe the procedure of management for these situations and provide biohazard bag to the vaccinated patients with the obligation to return the bag, after the trial, to the hospital for elimination treatment.

Could the notifier clarify these points ?

SBB's comment:
In accordance with the expert's comment, the following requirement could be sent to the applicant:
According to the CAF confidential document p22/32, 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 is 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 on the treatment of faecal matter, urine, diarrhoea and vomit. Should it be treated as infectious material or not? Should they be treated with standard hygiene home practices or specific protocols?
- Instructions and effective solutions to decontaminate possible contaminated areas, tissues, skin, …
- Instruction in case of some accidental situations at home requiring for example wound treatment, use of compress… which can be soiled by blood potentially contaminated by LAV-YF17D/HBc. Should it be necessary to treated all these wastes as hazardous medical waste or not? If yes, it would be necessary to describe the procedure of management for these situations.

Biohazard bag could be provided to the vaccinated patients with the obligation to return the bag, after the trial, to the hospital for elimination of the waste.
- The period during which these instructions must be followed

Coordinator's comment:
Ok

*Comment 2 (**Nicolas Van Larebeke**)*
P20 "The only potential means of dissemination of LAV-YF17D/HBc into the environment during the handling and administration of the DNA vaccine AVX70371, is hence through accidental self-administration (e.g. needle stick injury). "
This is not correct. LAV-YF17D/HBc can also be disseminated through excretion by patients

SBB's comment:
LAV-YF17D/HBc dissemination through excretion by patients has not been studied yet. Shedding analysis has been planned during this clinical trial. Here the applicant is mentioning potential means of dissemination of LAV-YF17D/HBc into the environment during the handling and administration of the DNA vaccine AVX70371 and not after the administration.

Coordinator's comment:
Ok

P22 "Recommendations Given to Clinical Trial Subjects to Prevent Dissemination"
In my clinical experience with hepatitis B patients I observed that these patients have an increased possibility to suffer from drug addiction. Drug addicts are particularly unreliable. It would be advisable to exclude drug addicts from the trial.

SBB's comment:
Question related to the patient's safety goes beyond the scope of the environmental risk assessment or the biosafety assessment of the proposed trial. According to the exclusion criterion 8, "Alcohol, prescription drug, or substance (ab)use that, in the opinion of the Investigator, might interfere with the study conduct and / or participant safety" are excluded.

Coordinator's comment:
Ok

Comment 3 (**Anton Roebroek**)
In several documents (CAFs, SNIF) the word 'disinfectant(s)' is mostly used in combination with the adjective 'organic'. However, in case these disinfectants are exemplified also inorganic disinfectants (e.g. hypochlorite (Javel), 2-3% hydrogen peroxide) are listed. This could be resolved by placing the adjective 'organic' between brackets or just by deleting 'organic'.

SBB's comment:
The request to correct the sentence could be reported as a "Typos and other errors/omissions".

Coordinator's comment:
Ok

Comment 4 (**Rik Gijsbers**)

The measures proposed in 2.6.f&h in p21/32 B_BE_24-BVW6_Part1_CAF_BE_Confidential Version.pdf. How did the applicant defines the time frames? Why are the time windows different for donating blood or organs (3 months) compared to sperm donation or 'close contact' (2 months post vaccination). Also, is there a specific requirement for women to undergo a pregnancy test?

SBB's comment:
According to section 3.6, page 21/32 of the confidential CAF document, several precautionary exclusion criteria are proposed to avoid exposure of LAV-YF17D/HBc to people not included in the clinical study. Some of these restriction measures will have to be followed for a period of two months (close contact, egg / ovum donation, sperm donation, pregnancy, breastfeeding, the use of contraception method), whereas restriction on blood or organs donation should be followed up to three months after the last study vaccination. The applicant is requested to clarify these different time windows and how these time frames were defined.

Coordinator's comment:
Ok

3.7. Sampling and further analyses of samples from study subjects

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

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

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

Comment 4 (**Rik Gijsbers**)
Has evaluated this item and has no questions/comments.

3.8. Emergency responses plans

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

*Comment 2 (**Nicolas Van Larebeke**)*
P23 "In case of accidental self-administration of the precursor DNA vaccine AVX70371 into the body (e.g. needle stick injury), the incident must be reported to the responsible person of the clinical site". This victim of an accident should receive a follow up similar to the patients included in the trial"

SBB's comment:
According to section 3.8 « Emergency Response Plan" of the confidential CAF document, (p23/32), in case of accidental self-administration of the precursor DNA vaccine AVX70371 into the body (e.g. needle stick injury), the incident must be reported to the responsible person of the clinical site. The notifier could be requested to ensure that injured caregiver receives appropriate follow-up after their injuring.

Coordinator's comment:
Ok

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

Comment 4 (**Rik Gijsbers**)
Has evaluated this item and has no questions/comments.

Additional SBB's comment:
According to the applicant, a 2-4 'instructions for study staff personal' document, similar to the one for the clinical trial with AVX1248-101, will be created. This document wasn't ready yet at time of the validation period. In order for us to verify the instructions that will be provided to the healthcare personal, the applicant could be requested to provide us with this instruction sheet :

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

Coordinator's comment:
Ok

5. ENVIRONMENTAL RISK ASSESSMENT

*Comment 1 (**Willy Zorzi**)*
Please find my comment in the point 3.6.

*Comment 2 (**Nicolas Van Larebeke**)*

P26 “Mutational event during *in vivo* replication that increases pathogenicity. As the LAV-YF17D/HBc virions replicate *in vivo*, the occurrence of a mutational event during replication that increases pathogenicity cannot fully be excluded. 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. *In vivo* mutational events that increase pathogenicity of the commercial YF17D vaccines are hence extremely rare.”
In fact I think that this mutation frequency is simply not known. However, the frequency of dangerous mutational events will maximally amount to the frequency of serious adverse effects after vaccination

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

P27” It has been shown that YF17D has a high degree of genetic stability during *in vivo* replication.”
This statement is somewhat at variance with the observation that the YF17D vaccines are heterologous mixtures of multiple virion subpopulations.

SBB’s comment:
The live-attenuated yellow fever vaccine (YF17D) strain YF17D was developed empirically in the 1930s by passaging a highly virulent West African patient isolate (Asibi strain) in minced mouse and chicken tissues. According to section 2.2 of the confidential CAF document (page 6/32), commercial YF17D vaccines corresponds to a heterologous mixtures of multiple virion subpopulations. On page 27/32, when clarifying the likelihood of occurrence of a mutational event during *in vivo* replication, the applicant mentioned that YF17D has a high degree of genetic stability during *in vivo* replication. The applicant could be requested to clarify both statements which seem contradictory.

Coordinator’s comment:
Ok, this will also solve the previous comment

P27 “Likelihood of occurrence of adverse events (pathogenicity). Following the same reasoning as above, the frequency of AEs related to exposure to LAV-YF17D/HBc can be considered similar to that seen after vaccination with commercial YF17D vaccines. The frequency of occurrence of mild non-serious AEs is expected to be low to moderate. Indeed, as described in Section 2.6, there are large differences between studies in the incidence of reported AEs following vaccination with commercial YF17D vaccines, with reported incidences ranging from 0.2% to 71.9% of the vaccinated individuals. The risk of occurrence of a SAE is expected to be low to negligible.”
Correct would be :negligible to high
P28 “…likelihood of recombination … negligible”
Correct would be very low
P28 ”recombination with other flaviviruses”: very low to negligible
P28 “taken together”: low to negligible
P30 “ close contacts”: low to negligible

SBB’s comment:
The overall incidence of SAEs following vaccination with commercial YF17D vaccines between 1990 and 2002 was 1.6 per 100 000, with the rates in the (younger) military population being half of that in the civilian sector (Khromava et al., 2005). A similar analysis of VAERS data performed between 2000 and 2006 including the civilian population only, show an overall incidence of SAEs of 4.7 per 100 000 (Lindsey et al., 2008). Two (2) types of extremely rare cases of SAEs following vaccination with

commercial YF17D vaccines have been reported which may be fatal. Based on these results, the risk of occurrence of a SAE should not be considered as high.
Regarding pages 28 and 30, the expert is referring to the following sentences:

- the likelihood of recombination with other (attenuated) flaviviruses is therefore considered negligible.
- Recombination with other (attenuated) flaviviruses. While the hazard may potentially be severe, the risk of occurrence is considered negligible.
- Taken together, the overall risk to healthcare professionals and / or close contacts of the study participants (including vulnerable groups) is considered very low to negligible.
- The overall risk to healthcare professionals and / or close contacts of the study participants (including vulnerable groups) is considered very low to negligible.

Coordinator's comment:
OK but what about the additional expression of HBc by this GMO? The information provided on HBc biological activities and impact in pathogenesis and viral persistence is not sufficiently described. Indeed, HBc CTD facilitated annealing and unwinding of DNA and enhanced hammerhead ribozyme cleavage *in vitro*, rendering HBc CTD a bona fide nucleic acid chaperone (Chu et al., 2014). This could have an impact of the frequency of DNA recombination, integration. Furthermore, HBc has been also reported to have different transcriptional regulating activity.

SBB's comment:
Question related to the HBc insert has been included in the question proposed in section 2.1 here above.

P28 "where the LAV vaccines against yellow fever, Japanese encephalitis"…
Maybe persons having received these vaccines recently should be excluded from the trial

SBB's comment:
According to the exclusion criterion 13, administration / planned administration of any vaccine not foreseen by the study protocol within 1 month preceding the first study vaccination and up to 1 month after the last study vaccination is not allowed.

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

Comment 4 (**Rik Gijsbers**)
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 (**Willy Zorzi**)*
Has no additional comment

*Comment 2 (**Nicolas Van Larebeke**)*
SNIF P3 "Risk of occurrence of a mutational event during *in vivo* replication that increases pathogenicity. "
The risk of occurrence of a mutational event is not known, but dangerous mutations cannot have a frequency that is superior to the frequency of serious adverse health events.

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

SNIF P3 "Risk of recombination with other (attenuated) flaviviruses."
I wonder whether only attenuated viruses should be considered, although the prevalence of non-attenuated viruses is probably low. Also I think that stating that the risk is negligible is somewhat imprudent.

SBB's comment:
As "attenuated" has been reported into parenthesis, one can suppose that the risk of recombination with both "flavivirus" and "attenuated flavivirus" has been considered.
As developed by the applicant, recombination with other flaviviruses could theoretically occurred if a co-infection were to occur in the same cell of the vaccinated host. However, it has been shown that the generation of viable recombinants in case of recombination between (live attenuated) flaviviruses is highly unlikely. Moreover, this would require a co-infection of LAV-YF17D/HBc with another (attenuated) flavivirus in the same host cell. Considering that clinical study AVX37-101 will take place in Europe, where endemic human flavivirus infections are rare, and where live attenuated vaccines against yellow fever, Japanese encephalitis and dengue are not part of the routine immunization schedule, the likelihood of a co-infection with other (attenuated) flaviviruses is considered low to negligible.

Coordinator's comment:
Do not forget that HCV virus are flaviviridae. So I am not OK with this sentence "flavivirus infections are rare". Maybe we should ask whether they can provide us with a quick assessment of recombination risk with flavivirridae such as the HCV (prevalence is around 0.5% in Europe, see https://doi.org/10.1016/j.lanepe.2023.100792)

SBB's comment:
The following question could be sent to the applicant:
As mentioned by the applicant, this clinical study AVX37-101 will take place in Europe, where endemic human flavivirus infections are rare. However, HCV is also a flaviviridae virus. According to the WHO fact sheet from July 2022, Hepatitis C affects the lives of 12 million people in the WHO European Region – approximately one in every 75 individuals. The notifier is therefore requested to develop the likelihood of recombination with flaviviridae such as HCV.

Comment 3 (**Anton Roebroek**)
Has no additional comment

Comment 4 (**Rik Gijsbers**)
Has no additional comment

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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/24/BVW6

23 January 2025
Ref. SC/1510/BAC/2025_0099

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

INTRODUCTION

Dossier **B/BE/24/BVW6** concerns a notification from AstriVax NV for a clinical trial entitled "A randomised, double-blind, placebo-controlled, multi-centre, Phase I study to evaluate the safety, reactogenicity and immunogenicity of AstriVax' investigational therapeutic hepatitis B virus (HBV) vaccine (AVX70371) in adult patients with chronic HBV (CHB) infection".

On 13 December 2024, based on a list of questions prepared by the BAC (SC/1510/BAC/2024_1558), 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 09 January 2025. This complementary information was reviewed by the coordinator and the experts in charge of the evaluation of this notification.

Evaluation Expert 1

Concerning the evaluation of the notifier answers on the questions of the Biosafety Council for dossier B/BE/24/BVW6 (clinical trial submitted by AstriVax related to the use of plasmid-launched live-attenuated vaccine, the PLLAV-YF17D/HBc that encodes the full genome of the live-attenuated YF strain YF17D-204 with the coding sequence of hepatitis B virus core antigen (HBc) inserted for adult patients with chronic HBV (CHB) infection) :

I would like to inform you that the notifier answered correctly and satisfactorily to all the questions raised in December 2024, in particular the question n°10, through the "4. AVX37-101_Instruction sheet for study personnel_V1.0" document.

Minor correction : in the title : INSTRUCTION SHEET FOR STUDY PERONNEL– HANDLING OF AVX70371 AND BIOLOGICAL SAMPLES IN STUDY AVX37-101

Please change "peronnel" by "personnel"

SBB's comment:
This minor request could be added in the final recommendation of the advice.

Evaluation Expert 2

According to me, the notifier addressed correctly and satisfactorily the comments/questions that have been raised in December.

Evaluation Expert 3

I reviewed the answers of the applicant to the comments and questions raised and indicate some suggestions below.

I have no further comments, concerning Q1, Q3-12.

For Q2: I only wanted to comments on a detail. The statement that “in the paper by Schmidt et al (Schmidt et al., 2024), uses a full-length core that is fused to HBsAg by a P2A cleavable linker” is not correct. P2A peptides are not cleaved, instead there is a peptide bond that is not generated during translation by ribosome-skipping (see also Fig2 https://www.intechopen.com/chapters/1183218 ; some of the confusion may come by the somewhat odd legend at the Wikipedia page https://en.wikipedia.org/wiki/2A_peptides). It is correct that the resulting HBc will be C-terminally tagged by about 20 aminoacid 2A tag (in the answer of the applicant referred to as “a C-terminal overhang, similar to ours”.

SBB’s comment:
The expert’s comments relates to a reply in the applicant’s response document and does not have an impact on the regulatory files from the notification dossier.

Evaluation coordinator

Question 1 : response is satisfactory for question 1
Question 2 :

- “These elongations may impact the folding and could interfere with the functionality and nuclear shuttling of HBc, therefore reducing the likely manifestation of any of the reported activities and associated concerns” : This is not proved and cannot be considered
- Phase I (Schimdt et al. 2021 : OK but phase I, so little amount of participants
- So, cautious should be introduced about HBc pathogenicity (without in vivo experiment results). What about "HBc may also function as a transcriptional repressor of host genes. For example, binding of HBc to E2F1 reduced the DNA-binding ability of E2F1 at the p53 promoter, thereby inhibiting p53 transcription (Kwon and Rho, 2003). In hepatoma cell lines expressing the core protein, HBc blocked the human death receptor 5 (DR5) by repressing its promoter, and inhibition of DR5 desensitized hepatocytes to TRAIL-induced apoptosis (Du et al., 2009). These results suggest a role for HBc in development of chronic infection by preventing hepatocyte death via blocking DR5 expression. The gene regulatory function of HBc may have evolved as a means whereby the virus may evade host immunity. HBc has been shown to downregulate IFN-induced host antiviral responses in hepatoma cell lines, by interacting with the promoter of MxA, an INF-inducible gene widely implicated in antiviral response (Fernandez et al., 2003)" and "Accumulating evidence supports a regulatory role of lncRNAs in cellular gene expression (Hutchinson et al., 2007)." as mentioned in the article of Diab et al., 2019? The answer here is thus in my point of view not completely satisfactory

SBB's comment:

A phase III clinical trial has evaluated the efficacy of a therapeutic vaccine candidate containing both hepatitis B surface antigen (HBsAg) and core antigen (HBcAg) versus pegylated interferon (Peg-IFN) in 160 naïve chronic hepatitis B patients (MA Mahtab et al. 2018. https://doi.org/10.1371/journal.pone.0201236).

A phase II clinical trial has been conducted with 178 patients with chronic HBV infection treated with, a hepatitis B virus-specific therapeutic vaccine GS-4774. GS-4774 consists of heat-inactivated yeast cells that express well-conserved regions of HBV proteins, namely HBsAg, hepatitis B core antigen (HBcAg) and hepatitis B X protein (HBx) expressed as a single fusion protein (A Gaggar et al. 2014. Vaccine Volume 32, Issue 39, 3 September 2014, Pages 4925-4931. https://www.sciencedirect.com/science/article/pii/S0264410X14009530).

Both examples indicate that the phase I clinical study from Schmidt *et al*. (2024), mentioned by the applicant, is not the only clinical trial that has been carried out using HBc. Furthermore, no pathogenic effect have been observed during those clinical trials.

According to the applicant, several HBc-containing therapeutic vaccines have entered clinical phase, where full-length HBc is expressed as part of a fusion protein or a separate antigen. To date, none of these trials have reported pathogenic findings related to HBc expression.

Question 3 : OK, satisfactory response

Question 4 : OK

Question 5 : This is not satisfactory, as SV40 enhancer is a mutation enhancer without the presence of T Ag, however this was indeed shown in cell lines, Ramos or DT40 cell lines

SBB's comment:

As explained by the applicant in the CAF document, once administrated, AVX70371 relies on the human transcription and translation machinery to produce replicating LAV-YF17D/HBc virions, which are genetically modified organisms (GMOs) as their RNA contains the sequence of HBc inserted into that of YF17D.

Although, in the AVX70371 plasmid, the YF17D and HBc sequences are under the control of the early promotor / enhancer sequence of the eukaryotic Simian virus 40 (SV40) genome, the LAV-YF17D/HBc virions do not contain the SV40 early promotor / enhancer which is only present in the plasmid backbone and is not part of the LAV-YF17D/HBc sequence.

Therefore, if LAV-YF17D/HBc virions were to be shed, these will not include any sequences of the plasmid backbone.

Furthermore, there shouldn't be any deliberate release of the vector backbone sequences in the environment, as DNA vaccines do not persist or biodistribute through the body of the vaccinee when delivered parenterally into muscle, subcutaneous tissue or various dermal layers. And the plasmid DNA clears from the injection site over time by degradation (WHO 2021). Therefore, the DNA vaccine, which contains the SV40 sequence, will not be shed in biological fluids.

This question, although justified, should be considered as a question related to the safety for the treated patient only as SV40 sequence will not be shed, and is therefore considered as outside the scope of this evaluation.

Question 6 : This is a minimum... Could we ask to wait for this results? As this is important for spreading assessment.

SBB's comment:
As for previous dossier (B/BE/23/BVW3), the notifier could also be requested to take the necessary measures to protect health and the environment if new information from the shedding analysis of LAV-YF17D/RabG from the clinical study AVX1248-101 comes to light that may impact human health and/or environment.

Questions 7-11 : OK satisfactory answer

Coordinator's comment:
Please drop then all, as SV40 enhancer is more linked to DNA and I believe we cannot assess this. All answers are satisfactory.

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/24/BVW6 de AstriVax NV pour l'introduction volontaire dans l'environnement, à des fins de recherche et développement, d'organismes génétiquement modifiés autres que les plantes supérieures

23/01/2025
Ref. SC/1510/BAC/2025_0101_FR

Contexte

La notification B/BE/24/BVW6 a été soumise en octobre 2024 par AstriVax NV 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 25 octobre 2024.
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_2025_0100). 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 - Apparemment, aucune surveillance de l'excrétion n'est effectuée. Peut-être que ce serait finalement souhaitable ?

Dans le cadre de cet essai clinique AVX37-101, le vecteur clinique évalué est composé du génome complet de la souche vivante atténuée 17D du virus de la fièvre jaune (YF17D) avec la séquence codante de l'antigène de la protéine centrale du virus de l'hépatite B (HBc) insérée dans le génome YF17D (LAV-YF17D/HBc ou AVX70371).

Une évaluation non clinique de la biodistribution, de la virémie et de l'excrétion a déjà été réalisée pour le vecteur clinique sans transgène (LAV-YF17D) ainsi que pour ce même vecteur clinique LAV-YF17D contenant la glycoprotéine du virus de la rage comme transgène (LAV-YF17D/RabG).

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L'analyse de l'excrétion de LAV-YF17D/RabG suite à la vaccination de patients avec AVX70481 est actuellement en cours d'évaluation dans la partie II de l'étude clinique de phase I AVX1248-101 (B/BE/23/ BVW3).
Au moment de la consultation publique, les données de cette analyse n'étaient pas encore disponibles. Une question a été soumise à l'appliquant afin de nous fournir les premiers résultats de l'analyse dès que possible.

Dans cet essai clinique-ci impliquant le vecteur LAV-YF17D/HBc, des échantillons biologiques seront collectés tout au long de la partie I de l'étude. Du sang, de l'urine, des selles et des écouvillons buccaux seront collectés à différents moments jusqu'à 85 jours après la vaccination pour l'analyse de la virémie et de l'excrétion du vecteur par les patients vaccinés.

Question 2 - Pouvez-vous confirmer que le risque de propagation de ce vaccin au-delà du génome humain (et très probablement du moustique) est négligeable, comme l'indique le demandeur ? Cela simplifierait le processus de signature du côté de l'environnement.

La question mentionne l'excrétion du vaccin en dehors du génome humain. Cependant, nous pouvons supposer qu'il s'agit de la dissémination en dehors du corps humain. L'excrétion correspond à la dissémination des particules du vecteur, sous quelque forme que ce soit, dans l'environnement par l'intermédiaire des excréments (matières fécales, sécrétions (urine, sueur, salive, fluides nasopharyngés, fluides lacrymaux, sperme)), de la peau (plaies, pustules, lésions) et du sang du sujet traité.

L'analyse de l'excrétion des virus vivants atténués (LAV) dérivés du LAV-YF17D/HBc (AVX70371) n'a pas encore été évaluée et le sera au cours de cette étude. Le protocole de cette étude d'excrétion proposée par le demandeur est basé sur les résultats d'analyses non cliniques réalisées avec le vecteur clinique sans transgène (LAV-YF17D) et ce même vecteur clinique LAV-YF17D contenant la glycoprotéine du virus de la rage comme transgène (LAV-YF17D/RabG) (dossier B/BE/23/ BVW3).

Une analyse d'excrétion a déjà été réalisée chez des hamsters après une administration intradermique unique de la dose maximale des plasmides d'ADN précurseurs AVX70120 (PLLAV-YF17D) et AVX70481 (PLLAV-YF17D/RabG). Seul un animal ayant reçu une injection d'AVX70120 présentait des niveaux détectables d'ARN YF17D dans les selles 10 jours après la vaccination. En utilisant un vecteur viral similaire, Li *et al*. (2022) ont détecté sporadiquement de l'ARN viral dans des écouvillons buccaux de hamsters vaccinés par voie intrapéritonéale avec une dose élevée (10e4 PFU) du vaccin vivant atténué contre la fièvre jaune YF17D contenant un virus vaccinal SARS-CoV-2 (YF-S0). Li *et al*. ont conclu que le virus vaccinal YF-S0 ne présentait qu'un risque minime, voire nul, d'excrétion ou de problèmes de biosécurité environnementale (Li et al., 2022).

En raison de la similarité de la structure du vecteur viral de ces différents virions et du fait qu'il n'existe aucune donnée montrant qu'un transgène différent ait un impact sur le comportement d'excrétion des virions, on peut s'attendre à ce que l'excrétion de ces différents virions soit identique. Par conséquent, l'excrétion du LAV-YF17D/HBc devrait donc être négligeable.

Au cours de cette nouvelle étude, la présence de virus vivants atténués (LAV) dérivés du LAV-YF17D/HBc (AVX70371) dans le sérum, l'urine, les selles et les écouvillons buccaux sera évaluée à différents moments jusqu'à 85 jours après vaccination chez les participants de la partie I de l'étude.
Dans l'essai clinique précédent avec un LAV similaire, le LAV-YF17D/RabG, l'excrétion a également été évaluée dans le sérum, les écouvillons buccaux, les selles et l'urine des participants à la partie II de l'étude clinique de phase I AVX1248-101 (B/BE/23/BVW3). Au moment de la consultation publique du dossier B/BE/24/BVW6, les données d'excrétion du dossier B/BE/23/BVW3 n'étaient pas encore disponibles. Suite à une question posée à l'appliquant, il nous a certifié que nous serons tenus informés des résultats de cette analyse dès qu'ils seront disponibles.

Le risque de propagation secondaire par les moustiques peut être considéré comme négligeable pour les raisons suivantes : (i) les niveaux de virémie après vaccination avec des vaccins commerciaux contre l'YF17D sont très faibles et inférieurs au seuil d'infection orale du moustique vecteur, et (ii) il a été montré que l'YF17D est peu infectieux pour les moustiques et que le vecteur a très probablement perdu sa capacité à être transmis par les moustiques, probablement en raison de l'incapacité du virus à traverser la barrière de l'intestin moyen (Danet et al., 2019). YF17D ne peut donc très probablement pas être transmis dans des conditions environnementales naturelles.

Question 3 - Deux points à examiner : le risque de mutations après l'administration du vaccin (également évoqué dans le dossier) et la nécessité d'un document à emporter pour informer les participants, qui n'est pas mentionné dans le dossier.

Comme les virions LAV-YF17D/HBc se répliquent *in vivo*, l'apparition d'un événement mutationnel pendant la réplication qui augmenterait la pathogénicité, ne peut pas être totalement exclue. Cependant, selon Kum et al. (2019), comme YF17D présente un degré élevé de stabilité génétique lors de la réplication *in vivo*, la probabilité d'apparition d'un événement mutationnel lors de la réplication *in vivo* peut être considérée comme faible voire négligeable.

Comme le mentionne le demandeur, sur les 800 millions de personnes qui ont été vaccinées avec des vaccins commerciaux YF17D, un seul événement mutationnel a été identifié dans un cas mortel d'encéphalite chez un enfant de 3 ans qui avait reçu un vaccin commercial YF17D en 1965 (AD Jennings et al. 1994).

Les événements mutationnels *in vivo* qui augmentent la pathogénicité des vaccins commerciaux YF17D sont donc extrêmement rares.

La stabilité du vaccin sera évaluée plus en détail par le comité d'évaluation de la qualité du FAGG.

Dans la liste des questions adressées à l'appliquant le 13/12/2024, il lui a été demandé de réaliser un résumé à emporter contenant toutes les informations relatives à une bonne hygiène afin que les patients et leur famille puissent facilement consulter les informations et toutes les instructions dans un format compréhensible chaque fois que cela est nécessaire. Suite à notre demande, le formulaire de consentement éclairé (ICF) a été adapté afin de regrouper ces informations.

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.

Jennings et al. 1994. Analysis of a yellow fever virus isolated from a fatal case of vaccine-associated human encephalitis. J Infect Dis. 1994 Mar;169(3):512-8.

Kum *et al.*, 2019. Limited evolution of the yellow fever virus 17d in a mouse infection model. Emerg Microbes Infect. 8(1): 1734-1746.

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

Adviesraad voor Bioveiligheid
Conseil consultatif de Biosécurité

Antwoorden van de Adviesraad voor Bioveiligheid op opmerkingen gekregen tijdens de publieksraadpleging over de kennisgeving B/BE/24/BVW6 van AstriVax NV voor doelbewuste introductie in het leefmilieu van genetisch gemodificeerde organismen met uitzondering van hogere planten voor onderzoek en ontwikkeling

23/01/2025
Ref. SC/1510/BAC/2025_0102_NL

Context

De kennisgeving B/BE/24/BVW6 werd in oktober 2024 door AstriVax NV 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 pas opgestart worden door de bevoegde overheid (BO) op 25 oktober 2024.
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 meegenomen bij het uitbrengen van het advies van de Adviesraad voor Bioveiligheid (referentie BAC_2025_0100). 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 - Blijkbaar wordt er geen controle op de uitscheiding uitgevoerd. Misschien zou dat uiteindelijk wel wenselijk zijn?

In deze klinische studie met AVX37-101 bestaat de klinische vector die wordt geëvalueerd uit het volledige genoom van de levende verzwakte gele koorts virusstam 17D (YF17D) met coderende sequentie van de hepatitis B virus kerneiwit antigeen (HBc) ingevoegd in het YF17D genoom (LAV-YF17D/HBc of AVX70371).

Niet-klinische evaluatie van biodistributie, viremie en uitscheiding is al uitgevoerd voor de klinische vector zonder transgen (LAV-YF17D) en voor dezelfde klinische LAV-YF17D-vector met het rabiësvirusglycoproteïne als transgen (LAV-YF17D/RabG).
Analyse van de uitscheiding van LAV-YF17D/RabG na vaccinatie van patiënten wordt momenteel beoordeeld in deel II van de fase I klinische studie AVX1248-101 (B/BE/23/ BVW3).

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Ten tijde van de openbare raadpleging waren de gegevens voor deze analyse nog niet beschikbaar. Er werd een vraag gesteld aan de aanvrager om ons zo snel mogelijk de eerste resultaten van de analyse te bezorgen.

In deze klinische studie met de LAV-YF17D/HBc-vector worden in deel I van de studie biologische monsters verzameld. Bloed, urine, ontlasting en buccale swabs zullen worden verzameld op verschillende tijdstippen tot 85 dagen na vaccinatie voor analyse van viremie en vectoruitscheiding door gevaccineerde patiënten.

Vraag 2 – Kunnen jullie bevestigen dat het risico dat dit vaccin zich kan verspreiden buiten het humaan genoom (en heel misschien de mug) negligent is, zoals de aanvrager aangeeft? Dit zou het teken-proces aan de leefmilieu-kant vergemakkelijken.

In de vraag wordt gesproken over uitscheiding van het vaccin buiten het menselijk genoom. We kunnen echter aannemen dat dit verwijst naar verspreiding buiten het menselijk lichaam. Uitscheiding verwijst naar de verspreiding van vectordeeltjes, in welke vorm dan ook, in het milieu via de uitwerpselen (feces, afscheidingen (urine, zweet, speeksel, nasofaryngeale vloeistoffen, traanvloeistoffen, sperma)), huid (wonden, puisten, laesies) en bloed van de behandelde persoon.

De uitscheidingsanalyse van levende verzwakte virussen (LAV) afgeleid van LAV-YF17D/HBc (AVX70371) is nog niet geëvalueerd en zal in de loop van deze studie worden geëvalueerd. Het door de aanvrager voorgestelde protocol van deze uitscheidingsstudie is gebaseerd op de resultaten van niet-klinische analyses die zijn uitgevoerd met de klinische vector zonder transgen (LAV-YF17D) en dezelfde klinische vector LAV-YF17D met rabiësvirusglycoproteïne als transgen (LAV-YF17D/RabG) (dossier B/BE/23/ BVW3).

Uitscheidingsanalyse werd eerder uitgevoerd bij hamsters na een eenmalige intradermale toediening van de hoogste dosis van de precursor DNA-plasmiden AVX70120 (PLLAV-YF17D) en AVX70481 (PLLAV-YF17D/RabG). Slechts één dier geïnjecteerd met AVX70120 had 10 dagen na vaccinatie detecteerbare niveaus van YF17D-RNA in de ontlasting. Met een vergelijkbare virale vector detecteerden Li et al. (2022) sporadisch viraal RNA in buccale swabs van hamsters die intraperitoneaal waren gevaccineerd met een hoge dosis (10e4 PFU) levend verzwakt YF17D-vaccin tegen gele koorts dat SARS-CoV-2-vaccinvirus (YF-S0) bevatte. Li et al. concludeerden dat het YF-S0-vaccinvirus een minimaal tot geen risico op uitscheiding of bezorgdheid over de bioveiligheid in het milieu met zich meebrengt (Li et al., 2022).

Door de gelijkenis van de virale vectorstructuur van deze verschillende virionen en het feit dat er geen gegevens zijn die aantonen dat een verschillend transgen een invloed heeft op het uitscheidingsgedrag van de virionen, kan verwacht worden dat de uitscheiding van deze verschillende virionen identiek is. Daarom wordt verwacht dat de uitscheiding van LAV-YF17D/HBc verwaarloosbaar is.

In deze nieuwe studie wordt de aanwezigheid van levend verzwakt virus (LAV) afgeleid van LAV-YF17D/HBc (AVX70371) in serum, urine, ontlasting en buccale swabs beoordeeld op verschillende tijdstippen tot 85 dagen na vaccinatie bij deelnemers aan deel I van de studie.
In de vorige klinische studie met een vergelijkbaar LAV, LAV-YF17D/RabG, werd de uitscheiding ook beoordeeld in serum, buccale swabs, ontlasting en urine van deelnemers aan deel II van de fase I

klinische studie AVX1248-101 (B/BE/23/BVW3). Op het moment van de openbare consultatie van het dossier B/BE/24/BVW6 waren de excretiegegevens van dossier B/BE/23/BVW3 nog niet beschikbaar. Na een vraag aan de aanvrager verzekerde hij ons dat we op de hoogte zouden worden gehouden van de resultaten van deze analyse zodra ze beschikbaar zouden zijn.

Het risico van secundaire verspreiding door muggen kan om de volgende redenen als verwaarloosbaar worden beschouwd: (i) viremieniveaus na vaccinatie met commerciële YF17D-vaccins zijn zeer laag en onder de drempel voor orale infectie van de muggenvector, en (ii) er is aangetoond dat YF17D minimaal besmettelijk is voor muggen en de vector heeft hoogstwaarschijnlijk zijn vermogen om door muggen te worden overgedragen verloren, waarschijnlijk als gevolg van het onvermogen van het virus om de middendarmbarrière te passeren (Danet et al., 2019). Het is daarom zeer onwaarschijnlijk dat YF17D onder natuurlijke omgevingsomstandigheden wordt overgedragen.

Vraag 3 - twee puntjes om naar te kijken: zijnde de kans op mutaties na toediening van het vaccin (ook besproken in het dossier) en de noodzaak van een take-home brief voor deelnemers, dit zie ik niet vermeld in het dossier

Aangezien LAV-YF17D/HBc virionen in vivo repliceren, kan mutatiegebeurtenis tijdens replicatie die de pathogeniteit zou verhogen niet volledig worden uitgesloten. Echter, volgens Kum et al. (2019), aangezien YF17D hoge genetische stabiliteit vertoont tijdens in vivo replicatie, kan de waarschijnlijkheid van een mutatiegebeurtenis tijdens in vivo replicatie als laag tot verwaarloosbaar worden beschouwd.
Zoals vermeld door de aanvrager, is van de 800 miljoen mensen die zijn gevaccineerd met commerciële YF17D-vaccins, slechts één mutatiegebeurtenis geïdentificeerd in een fataal geval van encefalitis bij een driejarig kind dat in 1965 een commercieel YF17D-vaccin kreeg (AD Jennings et al. 1994). In vivo mutatiegebeurtenissen die de pathogeniciteit van commerciële YF17D-vaccins verhogen, zijn daarom uiterst zeldzaam.
De stabiliteit van het vaccin zal verder worden beoordeeld door het kwaliteitsbeoordelingscomité van het FAGG.

In de lijst met vragen die op 13/12/2024 aan de aanvrager werd geadresseerd, werd hem gevraagd samenvatting voor thuis op te maken met alle informatie met betrekking tot goede hygiëne, zodat patiënten en hun families gemakkelijk toegang hebben tot de informatie en alle instructies in een begrijpelijk formaat wanneer dat nodig is. Op ons verzoek werd het Informed Consent Form (ICF) aangepast om deze informatie samen te brengen.

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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