Adviesraad voor Bioveiligheid
Conseil consultatif de Biosécurité

Advice of the Belgian Biosafety Advisory Council on the notification B/BE/25/BVW2 of the sponsor AstriVax NV for deliberate release in the environment of genetically modified organisms other than higher plants for research and development

Final version : 07/04/2025
Ref. SC/1510/BAC/2025_0515

Context

The notification B/BE/25/BVW2 has been submitted by AstriVax NV to the Belgian Competent Authority in February 2025 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, single centre, Phase I study to evaluate the safety, reactogenicity and immunogenicity of AstriVax’ investigational therapeutic hepatitis B virus (HBV) vaccine (AVX70371) in healthy adults aged 18 to 40 years”.

The purpose of this study is to assess safety, reactogenicity and immunogenicity of AVX70371 vaccine in healthy adults aged of 18 to 40 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 study will consist of a three-day staggered design of vaccine dose. Vaccination will be performed intradermally in the volar aspect of the forearm. Eligible participants will receive three injections over about 2 months, after which they will be followed up for approximately 1 year.

It is estimated that approximately 16 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 24 February 2025 and forwarded to the Biosafety Advisory Council (BAC) for advice.
Within the framework of the evaluation procedure, the BAC, under the supervision of a coordinator and with the assistance of its Secretariat, contacted experts to evaluate the dossier. Three experts from the common list of experts drawn up by the BAC and the Service Biosafety and Biotechnology (SBB) of

Sciensano answered positively to this request. The experts assessed whether the information provided in the notification was sufficient and accurate in order to state that the deliberate release of the genetically modified organism (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 25 March 2025, 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 27 March 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 didn't received any reaction from the public.

Summary of the scientific evaluation

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

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

The genetically modified investigational medicinal product (IMP) that will be administered in this 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.

Both the YF17D and HBc sequences are regulated by the Simian Virus 40 (SV40) promoter. However, PLLAV vaccines include only the early promoter/enhancer region of the SV40 genome. This region does not contain the T Antigen protein sequence, which is associated with the oncogenic properties of the SV40 virus (Pipas *et al*., 2009[3]).

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.

3. The conditions of the release

Patients enrolled will be injected intradermally in the forearm with either 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 or if they are the caretaker of someone with a weakened immune system (for instance someone who has cancer or a baby). 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 be followed for a period of two months after vaccination. A period of three months after vaccination will have to be followed 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

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

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. Therefore, shedding of LAV-YF17D/HBc virions is expected to be limited and similar to that of YF17D. Furthermore, no transmission of YF17D through close contact with vaccinated person has been reported up to now.

During this trial 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, up to approximatively 4 months after vaccination.

Considering that the sequences coding for HBc protein cannot give rise on its own to infectious hepatitis B virus particles and that the HBc coding sequence is not affecting biodistribution, viraemia nor shedding of LAV-YF17D/HBc, 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.

The risk of recombination with other flaviviruses could theoretically occur if a co-infection were to occur in the same cells of the vaccinated host. However, the generation of viable recombinants in case of recombination between (live attenuated) flaviviruses has been shown to be highly unlikely (McGee et al., 2011[4]). Furthermore, potential participants with known or suspected history of any flavivirus infection will be excluded from the study.

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

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.
As confirmed by the notifier, to prevent exposure to biological fluids from study participants, study personnel will wear personal protective equipment (a lab coat and gloves) during collection and handling of biological samples. In case of inadvertent skin contact with biological fluids from study participants, the exposure site will be thoroughly rinsed with water. Any contaminated gloves or lab coat will be removed. Finally, in case of accidental spilling of a biological sample from a vaccinated study participant, the area will be chemically decontaminated with a disinfectant.

[4] McGee et al., 2011. Stability of yellow fever virus under recombinatory pressure as compared with chikungunya virus. PLoS One 6(8): e23247

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 AVX37-102_ICF
    - Latest version of the AVX37-102_Protocol
    - AVX37_102__HBc_Instruction sheet for study personnel_V2.0
    - LAV-YF17D_HBc_CAF_Public_V2.0
    - LAV-YF17D_HBc_CAF_Confidential_V2.0
    - LAV-YF17D_HBc _SNIF_V3.0

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

- Viraemia and shedding analysis will be performed during the AVX1248-101 trial with PLLAV-YF17D/RabG (EU CT number 2024-511194-29; notification number B/BE/23/BVW3) and the AVX37-101 trial with PLLAV-YF17D/HBc (EU CT number 2024-518874-15; notification number B/BE/24/BVW6). The applicant is requested to inform the competent authority, for the attention of the BAC and to take the necessary measures to protect health and the environment if new information from these shedding analyses 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/25/BVW2 (ref. SC/1510/BAC/2025_0466 and SC/1510/BAC/2025_0496)

Adviesraad voor Bioveiligheid
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Compilation of comments of experts in charge of evaluating the dossier B/BE/25/BVW2
And comments submitted to the notifier

25 March 2025
Ref. SC/1510/BAC/2025_0466

Mandate for the Group of Experts: Mandate of the Biosafety Advisory Council (BAC) of 18 February 2025
Coordinator: Véronique Fontaine (ULB)
Experts: Nicolas van Larebeke-Arschodt (UGent, VUB), Willy Zorzi (ULiège), Anton Roebroek (KULeuven)
SBB: Sheela Onnockx

INTRODUCTION

Dossier **B/BE/25/BVW2** 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 24 February 2025 and concerns a clinical trial entitled "*A randomised, double-blind, placebo-controlled, single centre, Phase I study to evaluate the safety, reactogenicity and immunogenicity of AstriVax' investigational therapeutic hepatitis B virus (HBV) vaccine (AVX70371) in healthy adults aged 18 to 40 years".* 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 25-03-2025 to the notifier with a request to provide additional information. The comments or remarks highlighted in grey correspond to the questions addressed to the notifier.

2. INFORMATION RELATED TO THE INVESTIGATIONAL MEDICINAL PRODUCT

A.1. Virus from which the clinical vector was derived (parental virus) (e.g. information on parental virus; phenotypic and genetic markers; host range, zoonotic potential and replication properties of the parental virus ….)

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

Comment 2
I conclude that the LAV-YF17D/HBc clinical vector is derived from only one substrain of YF17D

"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). YF17D can hence most probably not be transmitted under natural environmental conditions."
The Danet paper shows that the YF17D virus certainly replicates much less than the positive control virus YFV-DAK, but does not prove with 100% certainty that the YF17D virus cannot breach the salivary glands YF17D was detected in the legs 2 out of 46 mosquitos, but not in the salivary glands. But in insects with slightly different properties on can fear that the salivary glands would be reached.

SBB's comment:
In the context of vaccination with commercial YF17D vaccines, up to now 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:
I agree with this SBB comment. No need for further information

"The risk of this happening is however considered negligible because different studies assessing the potential for the recombination between (live attenuated) flaviviruses concluded that the generation of viable recombinants is highly unlikely."
As I already mentioned in my report on a previous dossier presented by Astrivax :

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:
The potency of recombination was previously assessed by the BAC and the applicant for the previous LAV-YF17D/HBc vaccine vector. This should not require further assessment. However, I can read in the "...Part2-SNIF…" file, page 3 and 7, that again the applicant omit to mention that HCV, a flavivirus, is highly present in the Belgian and European population and we cannot accept thus that the applicant states in his application " 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. Overall, the likelihood of recombination with other (attenuated) flaviviruses is therefore considered negligible." (extracted from page 3). This sentence should be corrected.

SBB's comment:
Following a previous question to the applicant during the evaluation of the similar dossier B/BE/24/BVW6 involving LAV-YF17D/HBc, the applicant clarified in his Response document (page 6) why he still considered that the likelihood that LAV-YF17D/HBc will recombine with other Flaviviridae such as HCV remains negligible.
If a question should still be sent to the applicant, the following question could be sent:
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. In light of this, the assumption that the likelihood of co-infection with other (attenuated) flaviviruses is low to negligible could be re-evaluated. Likewise, the

conclusion that the likelihood of recombination with other (attenuated) flaviviruses is negligible could be reconsidered. A more cautious wording would be advisable on page 3 of the SNIF.

Coordinator's comment:
In my opinion, there should not be "questions" to be send to the applicant in the matter you proposed, can we just ask them to correct scientifically non sense sentences about flavivirus recombination (as HCV is highly prevalent).

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

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

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

Comment 2
"However, the data obtained so far (consensus sequencing of multiple virus isolates from organs of patients) indicate that, overall, host susceptibility rather than a change in the virus is responsible for such events."
I think this statement has no solid basis. The frequency of severe adverse effects is very low, and it is probably not possible to detect in patients a mutation frequency of that order of magnitude.

SBB's comment:
Expert's comment is related to section 2.7 of the CAF mentioning that over 800 million people who have been vaccinated with commercial YF17D vaccine, only one occurrence of mutational event has been identified in one fatal case of encephalitis in a 3-year-old child who received commercial YF17D vaccine in 1965. As further developed in the "Response to BAC questions" document which was provided by the applicant during the evaluation of the similar dossier B/BE/24/BVW6 involving the same PLLAV-YF17D/HBc, YF17D has a high degree of genetic stability during *in vivo* replication.

Coordinator's comment:
Indeed, it doesn't necessitate an additional request of information

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

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

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

Comment 2

“Moreover, the exploratory study conducted by Martinez et al., has considerable shortcomings, e.g.no medical history or other relevant (e.g. travel history) information was collected from the study participants, which may influence the study findings.”
The fact that the applicant mentions the possibility of shortcomings concerning findings which suggest colonization whereas the applicant almost never discusses the quality of findings suggesting the absence of problems with the vaccine is suspect. It reminds me of reports of chemical firms concerning scientific work finding toxic properties of chemicals.

SBB’s comment:
Martinez *et al* (2011) evaluated the persistence of the yellow fever vaccine RNA in urine and found that urine samples from two vaccine recipients had detectable yellow fever virus RNA up to 21 days and 198 days since vaccination. In the discussion, Martinez *et al*, also mentioned shortcomings in their assessment such as missing confirmation of persistent infection on isolated live virus from the human urine and missing details on underlying or previous health conditions that might influence the likelihood of 17D virus persistence.

Coordinator’s comment:
I partially agree with Nick’s comment and SBB comment. I recommend the applicant to be more careful about his evaluation of this publication results, as nevertheless they observed two patients with YF vaccine RNA in urine samples. Are there other publications with opposite results? In the absence of contradictory publication, the applicant should take this publication into account.

SBB’s comment:
When mentioning Martinez *et al* study, the applicant, also mentioned that there are no other (nonclinical or clinical) data supporting the persistence of YF17D (RNA or virions) in the body of the vaccinee.

Coordinator’s comment:
For the persistence concept, the applicant should only correct also his point of view, as it is not relevant.

SBB’s comment:
As the applicant did include the publication of Martinez *et al.* and did mention that no other (nonclinical or clinical) data supporting the persistence of YF17D (RNA or virions) in the body of the vaccinee exists to date, both comments form the coordinator are fulfilled: 1) absence of other publication confirming or showing opposite results, 2) the applicant has considered results from Martinez *et al*. In agreement with the coordinator, no further action by the applicant is required.

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

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

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

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

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

C. Clinical vector

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

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

Comment 2
I wonder which data demonstrate that LAV-YF17D/HBc might be more attenuated than YF17D

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

2.17. Potential for recombination

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

Comment 2
I am not convinced that natural transmission by mosquitos of AV-YF17D/HBc and YF17D is impossible (see my comment under A1)

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

Additional SBB's comment:
In section 2.17, page 14/31, "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:
Same remark as previous remark on recombination

2.18. Biodistribution and shedding

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

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

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

Comment 2

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

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

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

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

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

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

3.5. Reconstitution, finished medicinal product and administration to the patients (e.g. mode of administration, information on dosing and administration schedule, information on concomitant medication,…)

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

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

Comment 3
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
p13 of the "B_BE_25_BVW2_Part2_SNIF_Belgium_Version 3.0 file", it is reported that:

(c) Methods and procedures to avoid and/or minimise the spread of the GMOs beyond the site of the release

As the LAV-YF17D/HBc virions can most probably not be transmitted under natural environmental conditions, potential routes of spread of the GMO are limited to accidental self-administration of the precursor DNA vaccine AVX70371, or to direct exposure to LAV-YF17D/HBc virions in biological material from a study participant. This will be avoided through the following measures:

- Treating all waste resulting from biological sampling from study participants, as hazardous medical waste.
- Chemical decontamination with an organic disinfectant in case of accidental spilling of a biological sample from a study participant.

Therefore, this concerns advice regarding the treatment of biological samples.
Knowing that, admitting that waste form biological sampling from study participants must be treated as hazardous medical waste, the following information could be included in the instructions for the taking care of vaccinated participants:
- 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 fecal matter, urine, diarrhea, and vomit (inside and outside the sampling field)

In addressing the treatment of these materials, the following questions should be considered:
Outside the sampling field, should these materials be considered and treated as infectious ?
Should they be treated with standard usual hygiene practices or as hazardous medical waste ?

These instructions should make worn on the biosafety of the healthcare personnel handling the samples and taking care of the participants.

Therefore, it could be also necessary to add these instructions in the “B_BE_25_BVW2_AVX37-102_Instruction sheet for study personnel_V1.0” file

SBB’s comment:
The following question could be sent to the applicant:
In the instructions sheet “AVX37-102_Instruction sheet for study personnel_V1.0”, nothing is said about the procedure to prevent and deal with exposure to blood, urine, vomit or other bodily fluids from subjects. Should these materials be considered and treated as infectious ? Should they be treated with standard usual hygiene practices or as hazardous medical waste ?
Admitting that waste form biological sampling from study participants must be treated as hazardous medical waste, the following information could be included in the instructions for study personnel :
- 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 fecal matter, urine, diarrhea, and vomit (inside and outside the sampling field)

Coordinator’s comment:
Good suggestions, comment of the SBB. I agree

Comment 2
Klavinskis et al.,1999 observed a 30 fold change in transfection efficiency between naked DNA and their lipid system. That does not prove that uptake of naked DNA is not possible. Also, all kinds of uncontrollable parameters might render uptake of DNA more efficient in real life conditions.
In view of my clinical experience with drug addicts, I think it is very important to exclude illegal drug users from participating to this phase I study. It should be recalled that phase I studies in which participants receive a financial compensation attract drug users.

SBB’s comment:
Nothing is said about the inclusion and exclusion criteria in the synopsis of the protocol. Question related to drug addicts are related to the patient safety and go beyond the scope of the environmental risk assessment or the biosafety assessment of the proposed trial. However, according to the synopsis of the clinical trial B/BE/23/BVW3, which involve a similar plasmid DNA vaccine, PLLAV-YF17D/RabG that encodes the full genome of the live-attenuated YF strain YF17D-204 with the coding sequence of RabG. PLLAV-YF17D/RabG, the following exclusion criterion is present: “Alcohol, prescription drug, or substance abuse that, in the opinion of the Investigator, might interfere with the study conduct or completion”. The same exclusion criterion can be found in the clinical trial B/BE/24/BVW6 which involve the same plasmid DNA vaccine PLLAV-YF17D/HBc.

Coordinator’s comment:
Ok

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

3.7. Sampling and further analyses of samples from study subjects

Comment 1

Has evaluated this item and has no questions/comments.

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

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

3.8. Emergency responses plans

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

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

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

5. ENVIRONMENTAL RISK ASSESSMENT

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

Comment 2
"As the LAVYF17D/ 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."
That only one observation of a mutation has taken place does by no means prove that such mutations are extremely rare. Measuring the exact rate of such mutations is very difficult.

"Overall, the likelihood of recombination with other (attenuated) flaviviruses is therefore considered negligible." This statemen lacks credibility. Probably the possibility of recombination cannot be excluded with sufficient certainty. It would be wiser to consider it as very low.

To lower the possibility of exposure to biological material from a study participant it is important to exclude illegal drug users from the study

"The overall risk to healthcare professionals and / or close contacts of the study participants (including vulnerable groups) is considered very low to negligible" It seems more reasonable to consider this risk as very low.

SBB's comment:
As mentioned in the SBB's comment in section A.2, the assessment of the genetic stability of the YF17D virus was further developed in the "Response to BAC questions" document which was provided by the applicant during the evaluation of a similar dossier with the reference B/BE/24/BVW6 which also involved the genetically modified PLLAV-YF17D/HBc.

The likelihood of recombination with other flaviviruses has also been discussed in this “Response to BAC questions” document.
The exclusion of drug users has been discussed in section 3.6 here above.

Coordinator’s comment:
I agree with this sentence (It would be wiser to consider it as very low), please consider my previous remark on recombination potency with Flavivirus. Just ask to correct the sentence, not speaking about Flavivirus anymore, but over yellow fever virus.
See, my previous remarks. They should be more cautious when writing. They have to better write this part. This is not requiring any additional information, but they should correct some sentences that are not scientifically correct.

Comment 3
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
Has no additional comment

Comment 2
As so often with advanced genetic engineering there is an ”apprentice sorcer” aspect to the development of these very useful vaccines that should be recognised.

Comment 3
• The documents with information for the public in the four different languages need correction. Apparently these documents are based upon the documents for clinical trial AVX37-101 (B_BE_24_BVW6) and need further adaptation to the novel clinical trial AVX37-102 (B_BE_25_BVW2). On page 6 it is suggested more than once, that the participating people are patients with chronic hepatitis B virus infection.

• In the confidential an public CAFs (page 6) ‘shot’ should be corrected in ‘short’ and regions in region (a shot 5’ non-coding regions -> a short 5’ non-coding region)

SBB’s comments:
Both comments could be reported as as a “Typos and other errors/omissions”.

Coordinator’s comment:
OK. In addition, In the fle «...Part-2-SNIF…» it is mentioned many time that «HBc on its own cannot create infectious particles and is therefore not pathogenic or harmful.». This is a non-sense sentence as pathogenic or harmful aspects are not only linked to the possibility to create infectious particles. This is showing a lack of transparency and this should be corrected.

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.

Klavinskis et al. 1999. Intranasal Immunization with Plasmid DNA-Lipid Complexes Elicits Mucosal Immunity in the Female Genital and Rectal Tracts. J Immunol (1999) 162 (1): 254–262.

Martinez et al., 2011. Persistence of yellow fever vaccine RNA in urine. Vaccine 29(18): 3374-3376.

McGee et al., 2011. Stability of yellow fever virus under recombinatory pressure as compared with chikungunya virus. PLoS One 6(8): e23247.

Taucher et al., 2010. A trans-complementing recombination trap demonstrates a low propensity of flaviviruses for intermolecular recombination. J Virol. 84(1):599-611.

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/25/BVW2

03 April 2025
Ref. SC/1510/BAC/2025_0496

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Experts: Willy Zorzi (ULiège), Anton Roebroek (KULeuven), Nicolas van Larebeke (UGent, VUB)
SBB: Sheela Onnockx

INTRODUCTION

Dossier **B/BE/25/BVW2** concerns a notification from AstriVax NV for a clinical trial entitled "A randomised, double-blind, placebo-controlled, single centre, Phase I study to evaluate the safety, reactogenicity and immunogenicity of AstriVax' investigational therapeutic hepatitis B virus (HBV) vaccine (AVX70371) in healthy adults aged 18 to 40 years".

On 25 March 2025, based on a list of questions prepared by the BAC (SC/1510/BAC/2025_0452), 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 27 March 2025. This complementary information was reviewed by the coordinator and the experts in charge of the evaluation of this notification.

Evaluation Expert 1

By this way, we would like to inform you that the notifier's responses do not yet correctly and satisfactorily address the comments/questions, in particular the following question :

In the response (p 2), the notifier indicated that :

The notifier suggests that the relevant hazardous waste will be resulting from handling, dilution and administration of the DNA vaccine AVX70371, or from biological sampling from participants in clinical study AVX37-102, e.g. syringes, needles, wipes, dressings, gloves.

As reported for example, p16 in the « B_BE_25_BVW2 _LAV-YF17D-HBc_SNIF_Belgium_Version 3.0_clean » document, in the « Information on Post-release and Waste Treatment » section, points :

3. (a) Type and amount of waste generated :

The type of waste generated will be resulting from handling, dilution and administration of the DNA vaccine AVX70371, or from biological sampling from participants in clinical study AVX37-102, e.g. syringes, needles, wipes, dressings, gloves.

The amount of waste generated at the clinical study sites will be within the normal handling capacity that can be managed by the standard operating procedures currently in place.

and

3. (b) Treatment of waste

The waste will be collected and treated as hazardous medical waste, i.e. collected in dedicated and certified waste bins which are hermetically sealed and transported by a certified shipper to a specialized incineration facility.

Advice :

The treatment of fecal matter commonly generated by patients treated with the DNA vaccine AVX70371 and non-collected for biological sampling and analysis is not considered or discussed by the notifier. Should this be considered and treated as biohazardous waste or not?

The notifier is invited to clarify this point.

SBB's comment:
This question was related to the procedure to prevent and deal with unexpected exposure to blood, urine, vomit or other bodily fluids from subjects. Diarrhea, which can be considered as a bodily fluid, could lead to unexpected exposure with fecal matter. As confirmed by the notifier, in case of inadvertent skin contact with biological fluids from study participants, the exposure site will be thoroughly rinsed with water. Any contaminated gloves or lab coat will be removed. To prevent exposure to biological fluids from study participants, study personnel will wear personal protective equipment (a lab coat and gloves) during collection and handling of biological samples. Finally, in case of accidental spilling of a biological sample from a vaccinated study participant, the area will be chemically decontaminated with a disinfectant.

Evaluation Expert 2

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

Evaluation Expert 3

I am not able to criticize this document. I trust coordinator's judgment. I think my task is to critically indicate possible problems.