Adviesraad voor Bioveiligheid
Conseil consultatif de Biosécurité

Advice of the Belgian Biosafety Advisory Council on the notification B/BE/21/BVW2 of Wageningen Bioveterinary Research (Larissa Consortium), for deliberate release in the environment of genetically modified organisms other than higher plants for research and development

06/08/2021
Ref. SC/1510/BAC/2021_0776

Context

The notification B/BE/21/BVW2 has been submitted by Wageningen Bioveterinary Research (Larissa Consortium) to the Belgian Competent Authority in April 2021 for a request of deliberate release in the environment of genetically modified organisms (GMOs) 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 and the title of the notification is: ***"Phase I, single-centre, randomized, double blind, placebo-controlled study to assess safety, tolerability and immunogenicity of the hRVFV-4s vaccine in healthy subjects".***

The purpose of this study is to generate safety, tolerability and immunogenicity data for the development of a candidate Rift Valley fever (RVF) vaccine that is expected to protect against infections with RVF virus (RVFV) and as a consequence thereof prevent the occurrence of severe and sometimes life-threatening disease that can be caused by this infection.

RVFV is a phlebovirus (order Bunyavirales, family Phenuiviridae) transmitted by mosquitoes that causes abortions and neonatal mortality in ruminants. RVFV also infects humans via contact with animal products or mosquito bites. No direct human-to-human transmission nor human-to-animal transmission has been documented with RVFV. Infected humans generally develop a febrile, self-limiting influenza-like illness. However, 0.5% to 2% progress to severe and often life-threatening disease, including ocular disease with loss of vision, meningoencephalitis with severe neurological sequelae, and haemorrhagic icterus with a high case-fatality rate. Although RVFV is confined to Africa and the Arabian Peninsula, the global distribution of Aedes and Culex mosquito vectors may allow outbreaks in currently unaffected areas.

The investigational medicinal product is a four segments human Rift Valley fever virus (hRVFV-4s) vaccine, which derives from a natural clone (Clone 13) that lacks 69% of the gene NSs, a major virulence determinant of the virus. Clone 13 has been used as a veterinary attenuated vaccine virus. The hRVFV-4s vaccine is further attenuated by splitting the M genome segment into two M-type segments.

The 75 healthy study subjects will be divided over three cohorts each consisting of 25 subjects of whom 20 subjects will receive the active component by intramuscular injection while 5 will be given placebo. The cohorts will be enrolled sequentially such that cohort 1 receives the lowest dose of hRVFV-4s ($3x10^4$ +/- 0.5 log $TCID_{50}$), cohort 2 the middle dose ($3x10^5$ +/- 0.5 log $TCID_{50}$) and cohort 3 the highest dose ($3x10^6$ +/- 0.5 log $TCID_{50}$).

It is planned to conduct the trial in one clinical site located in the Flemish Region.

The dossier has been officially acknowledged by the Competent Authority on 26 April 2021 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. 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. One expert from the SBB took part in the evaluation of the dossier.
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 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 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 01 June 2021, 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 15 July 2021 and transmitted to the secretariat of the BAC on 16 July 2021. This complementary information was reviewed by the coordinator and the expert, and was considered satisfactory.

In parallel to the scientific evaluation of the notification, the Competent Authority also made the dossier available on its website for the one-month public consultation foreseen in the abovementioned Royal Decree. The Competent Authority did not receive reactions from the public.

Summary of the scientific evaluation

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

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

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

During the production process, reconstruction of the M genome segment is theoretically possible through a heterologous recombination event upon transfection of the 4 plasmids in BSR-T7 cells. Considering there are no overlapping M genome segments between the segments of the hRVFV-4s vaccine and taking into account that absence of reconstruction of M genome segment was verified by PCR, the BAC agrees that sufficient evidence has been provided to support the *in vitro* genetic stability of hRVFV-4s.

All of the remaining information related to the molecular characteristics of 4 segments human Rift Valley fever virus (hRVFV-4s) were found to be adequately described in the dossier.

3. The conditions of the release

This study consists of three cohorts each consisting of 25 subjects of whom 20 subjects will receive the active component at low ($3x10^4$ +/- 0.5 log $TCID_{50}$), middle ($3x10^5$ +/- 0.5 log $TCID_{50}$) or high dose ($3x10^6$ +/- 0.5 log $TCID_{50}$) while 5 of each cohort will be given placebo. One single dose of the hRVFV-4s/placebo vaccine will be administrated in the deltoid muscle of the arm. All patients will stay for 60 minutes at the hospital and then leave the reference hospital without quarantine measures.

Upon request of the BAC, the notifier provided further information on the use of personnel protective equipment and on the instructions with respect to the use and disposal of a wound dressing in order to minimize exposure to the GMO following injection.
The notifier further informed that no wound dressings will be collected for assessing the presence of hRVFV-4s virus (e.g. in the absorbent material of the wound dressings), as it is considered very unlikely that vaccine substance will be absorbed from the injection site due to leakage 1 hour after vaccine administration.

The notifier provided adequate answers to some remarks and requests addressed by the BAC. The BAC strongly encourages the notifier to properly improve both documents (SNIF and ERA) in accordance with their answers provided to the BAC. The notifier may also consider to clarify in the adequate document the appropriate disinfectant that will be used to clean the site of injection after the vaccination.

4. The risks for the environment or human health

The h-RVFV-4s virus lacks 69% of the gene NSs, a major virulence determinant of the virus. Due to the further segmentation of the genome and splitting of the M genome segment into two M-type segments, the replication cycle h-RVFV-4s was shown to be slowed down *in vitro* as compared to Clone 13 . The hRVFV-4s vaccine was also shown to be completely avirulent and to be highly immunogenic in (nude) mice, rats, marmosets, lambs, and pregnant ewes. This virus does not harbour any antibiotic or other resistance genes. Furthermore, the notifier intends to complete a toxicity study on rats before the start of the proposed clinical trial, which the BAC deemed both necessary and relevant from an environmental risk point of view since human-to-human transmission of the vaccine cannot be fully excluded yet.

Shedding properties of hRVFV-4s in humans are currently lacking and one of the aims of the current "First-in-Human" clinical trial with the hRVFV-4s vaccine is to investigate viral vector shedding in urine, saliva, semen and blood samples at several time points after administration of the vaccine using the reverse-transcriptase quantitative PCR.

No direct human-to-human transmission nor human-to-animal transmission has been documented with RVFV. On the other hand, even though replication of hRVFV-4s was slowed down as compared to Clone 13 *in vitro*, residual replication in humans still cannot be excluded. The BAC advised to further add an exclusion criteria for participants that may come into contact with immunocompromised human or children whose immune system is not mature and to consider restriction on blood donation. The notifier agreed with these suggestions as part of the proposed first in human study and added both exclusion criteria (no contact with immunocompromised people or children for 4 weeks after hRVFV-4s administration; blood donation restriction for three months after administration) in an update version of the protocol. Considering that wild-type RVFV is not contagious and is not spread via the respiratory route, the BAC can agree with the notifier that wearing of face masks by participants will not contribute to safety.

For safety reasons, an interval of at least 24 hours will be respected between the administration of the first dose of any dosage level to the first subject and the second subject The notifier clarified that no monitoring of potential shedding of hRVFV-4s will be performed within the 24 h following administration. The BAC reflected on the possibility to implement more time between the first patients so as to allow for a better understanding of the shedding data soon after administration of hRVFV-4s in the first trial participants, yet the BAC understands that it is likely that both the limited number of participants (limited data) as well as the double blinded concept of the proposed trial could hamper any proper conclusion on shedding during the course of the clinical trial should. As a conclusion, the BAC encourages the collection of shedding and multiplication data-points for next phases for the vaccine development.

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

Upon BAC's request, the notifier provided a 2-4 pages technical sheet 'Instructions for study staff personal' including all relevant handling instructions, such as those to minimize aerosol formation, detailed instructions in case of accidental spill or breakage of a vial containing the GMO and waste management.
Upon request of the BAC the notifier provided more details on the procedures in case of spill and emergency plan by clarifying the management of accidental exposure of clinical personnel through a splash to the eyes of mucous membrane or in the event of exposure to broken skin or needle stick.

Given that the likelihood of further propagation of hRVFV-4s can be considered highly unlikely in the context of the proposed trial, 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 expert, the Biosafety Advisory Council concludes that it is unlikely that hRVFV-4s developed as a vaccine candidate for protection against infections with Rift Valley fever virus (RVFV), will have any adverse effects on human health or on the environment in the context of the intended clinical trial provided that all the foreseen safety measures are followed.

Therefore, the Biosafety Advisory Council issues a **positive advice with the following conditions**:

- The notifier and the investigators must strictly apply the clinical trial protocol (amended as per July 2021), and all the safety instructions as described in the documents BIOSAFETY-Behandelen van morsen en lekken, POL90027_GMO_HANDLING_V3 as well the biosafety procedures for study staff for all contained use areas at the UZ Ghent campus. It should be specified that whenever hypochlorite solution is used (e.g. for the decontamination of work areas), special attention should be given to the use of freshly prepared hypochlorite solution. Procedures for disinfection of the injection site and/or spill of product on the skin should also be clarified (e.g. use of soft Pad MLS 70% isopropyl alcohol).

- Section 6.4.4 of the protocol, Visit 1 (D0), both undersections "Vaccination procedures" and "Post-vaccination procedures" are improved by adding information related to the use of wound dressing on the vaccination site: *The injection site is covered with a wound dressing to prevent dissemination from the injection site. A new dressing will be placed on the injection site at the time of the first visit of the participant to the physician, which is 60 minutes after the vaccine administration*.

- The final analysis of the results of the repeated dose toxicity study on rats are in line with the primary analysis as provided in the general preliminary summary study, which reveals good tolerability of the IMP.

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

- The notifier is responsible to verify that the study centre has qualified personnel experienced in handling infectious material and that the investigator has the required authorizations 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 Contained use of genetically modified micro-organisms.

- The Biosafety Advisory Council 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 to the competent authority at the attention of the Biosafety Advisory Council a report with details concerning the biosafety aspects of the project. This report shall at least contain:
    - The total number of patients included in the trial 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 hRVFV-4s.

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

Annex I: Compilation of comments of experts in charge of evaluating the dossier B/BE/21/BVW2 (ref. SC/1510/BAC/2021_0517, SC/1510/BAC/2021_0770)

Adviesraad voor Bioveiligheid
Conseil consultatif de Biosécurité

Compilation of comments of experts in charge of evaluating the dossier B/BE/21/BVW2 And comments submitted to the notifier

01June 2021
Ref. SC/1510/BAC/2021_0517

Mandate for the Group of Experts: Mandate of the Biosafety Advisory Council (BAC) of 19 April 2021.
Coordinator: Jozef Anné (KULeuven)
Experts: Rik Gijsbers (KULeuven), Anton Roebroek (KULeuven), Nicolas van Larebeke-Arschodt (UGent, VUB), Willy Zorzi (ULiège), Aline Baldo (SBB)
SBB: Sheela Onnockx and Katia Pauwels

INTRODUCTION

Dossier **B/BE/21/BVW2** concerns a notification of the company Larissa consortium and Wageningen Bioveterinary Research for 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 26/04/2021 and concerns a clinical trial entitled "A Phase I, single-centre, randomized, double blind, placebo-controlled study to assess safety, tolerability and immunogenicity of hRVFV-4s vaccine in healthy subjects". The investigational medicinal product is a genetically modified four segmented human Rift Valley fever virus (hRVFV-4s) derived from a naturally attenuated strain.

♦ 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 01-06-2021 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.

1. INFORMATION RELATED TO THE CHARACTERISTICS OF THE DONOR, THE RECIPIENT OR PARENTAL ORGANISM

(e.g. possibility of natural transfer of genetic material to other organisms, pathological, ecological and physiological characteristics, indigenous vectors ...)

Comment 1

Can we be absolutely certain that the NSs gene is totally absent from hRVFV-4s? I wonder how we can be certain that hRVFV-4s cannot contaminate the environment or animals or other humans as the hRVFV-4s virus is still replication competent? In particular the fact that RVFV virus can be transmitted through many different species of mosquitos, and that the mosquitos in question can be present everywhere, is problematic.
I do not believe in RNA or DNA sequences that cannot show recombination with other sequences . It is precisely such rare events that are the source of new dangerous viruses. I do not understand the statement "that the 69% (549 nucleotides) of the NSs gene, given its size, cannot restore upon replication of the virus." That clone 13 would be completely uncapable of producing any level of viremia is rather unlikely.

SBB comment

The wild-type RFV virus is endemic in African countries and the Arabian Peninsula. The probability that this wild-type virus is present in Belgium where the clinical trial will be performed is very low. Therefore, the probability that the wild-type virus enters in contact with the investigational medicinal product is very low. Furthermore, according to p14/TECH, participants are not allowed to travel to countries where RVFV is endemic . Therefore, we can assume that:
* the probability of recombination with related viruses is very low, if not negligible. Further analyses regarding the probability of recombination has been detailed in point 1 here below.
* the low prevalence of mosquitoes combined with the observed lack of viremia in preclinical studies with hRVFV-4s is considered to alleviate the concern of its dissemination in the population at large (see point 2 here below) upon its use in the context of the proposed first in human (FIH) study.
Finally, with this FIH study, monitoring of shedding is foreseen, the study will contribute to a better understanding of some of the remaining uncertainties in this regard.

1- In the Technical dossier, section 2c (p18), it is stated : "Reconstruction of the M genome segment is theoretically possible through a recombination event. Considering that there are no overlapping sequences on the two M-type genome segments of the hRVFV-4s candidate vaccine, homologous recombination cannot occur. Although heterologous recombination can never be excluded (for any live-attenuated vaccine), it has never been observed in any bunyavirus. Furthermore, repeated passage (>20 times) of hRVFV-4s in vitro in Vero cells did not result in the restoration of the M segment, as observed by next-generation sequencing of the complete genome. According to the introduction in the Technical dossier (p18 section 2c), the attenuating mutations (split M genome segment and deletion NSs) were shown to be stably maintained upon repeated passage in vitro.

2- As mentioned in the ERA document section C, “in vitro studies have demonstrated that packaging of four segments slows down the viral replication cycle, allowing the host’s innate immune response to control dissemination effectively (Wichgers Schreur, 2014).”
Indeed, we cannot exclude that the wild-type virus can be transmitted through many different species of mosquitoes everywhere. But according to ERA page 7, Clone 13 does not induce viremia in natural target species (sheep, goat, cattle), and it cannot be transmitted via mosquitoes. Non-clinical studies with natural target species showed that hRVFV-4s does not cause viremia (ERA, section B1 p15).

Comment coordinator

See also: PLOSNeglectedTropicalDiseases | https://doi.org/10.1371/journal.pntd.0006576March21,201: We found no evidence for intragenic recombination among any of the three segments. This is probably due to the low genetic diversity among the sequences.

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

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

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

Comment 5
Has not evaluated this item.

2. INFORMATION RELATED TO THE VECTOR
(e.g. description, sequence, mobilisation ...)

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
As indicated by the notifier (p18/37_2c in B_BE_21_BVW2_Part 1 A Technical Dossier), there is a possibility that the M genome segment reconstitutes through a recombination event. Even though the chance is low that this occurs at the RNA level (and thus in the Vero cells), this may occur during the transfection of the 4 plasmids in the BSR-T7 cells. Has this been tested for? A simple PCR would be an option to assess M Gn and Gc fusion/restoration.
Even though this would result in the attenuated Clone13, the latter still results in fetal demise in sheep.

SBB comment

Indeed, since there is a possibility that the M genome segment reconstitutes through a recombination event, it is relevant to assess whether other constructs than hRVFV-4s could be generated upon transfection of the 4 plasmids in the BSR-T7 cells that possibly could not by revealed by next-generation sequencing of rescued virus.

Comment 5

Has not evaluated this item.

3. INFORMATION RELATED TO THE CHARACTERISTICS OF THE GMO

3.1. Information related to the genetic modification

(e.g. methods used for the modification, description of the insert/vector construction ...)

Comment 1

Have pregnant ewes been vaccinated and their young studied for any problems after birth?

SBB comment

According to the Technical dossier page 21 section "Safety of hRVFV-4s in pregnant ewes", the safety of the hRVFV-4s vaccine has been studied in pregnant ewes that were inoculated with high dose of hRVFV-4s. No hRVFV-4s viremia was detected by PCR and no clinical signs or other untoward effects were observed. Four weeks after inoculation, all ewes were euthanized and foetuses inspected for abnormalities. No pathology was noted in either ewes or foetuses. Moreover, no hRVFV-4s RNA was detected in organ samples from ewes or foetuses at necropsy. Whereas the vaccine was completely safe for pregnant ewes and their foetuses, all ewes developed neutralizing antibodies within one week after vaccination (Wichgers Schreur, 2017).

Comment 2

Has evaluated this item and has no questions/comments.

Comment 3

Has evaluated this item and has no questions/comments.

Comment 4

Has evaluated this item and has no questions/comments.

Comment 5

Has not evaluated this item.

3.2. Information on the molecular characteristics of the final GMO

(e.g. number of copies of the transgenes, phenotypic and genetic stability of the transgenes, expression of the new genetic material, re-arrangements in the genome, inclusion or suppression of genetic material ...)

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

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

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

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

Comment 5
Has not evaluated this item.

3.3. Considerations for human, animal or plant health

(e.g. invasiveness and virulence, toxic or allergenic effects, possibility of survival outside of receiving host, other product hazards ...)

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

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

Comment 3

According to the ERA, there is no indication that the GMO is toxic or allergenic. However, to demonstrate absence of toxicity, a repeated dose toxicity study with rats will be performed before start of the Phase 1 clinical trial. This repeated dose toxicity study with rats should be completed and should clearly demonstrate absence of toxicity before the Phase 1 clinical trial can be started.

SBB comment

The notifier could be asked to give an update of the toxicity study that is planned to be performed before the start of the phase I clinical trial. Though toxic or allergenic properties are predominantly a patient-related safety issue, it is considered to further complete the environmental risk assessment since human-to-human transmission has never been studied for this vaccine candidate.

According to Annex 1, studies in nude mice show that the hRVFV-4s vaccine is safe in immunocompromised mice and suggest that the vaccine will also be safe for humans with a compromised immune system. Upon completion and positive evaluation of the Phase 1 clinical trial, will there be a follow-up trial including immunocompromised humans to investigate this safety aspect for immunocompromised humans further? This seems to be necessary because of the future application of the vaccine in populations where both RVF and AIDS are endemic

SBB comment

A related question regarding immunocompromised subject was also raised by another expert (see comment 4). Both questions have been combined under comment 4.

Comment 4

p11/21.F in B_BE_21_BVW2_Part 3 ERA: The vaccine only multiplies in cells with a defective type I IFN pathway. What is the consequence of patients suffering from type I IFN deficiency or low IFN I responses receiving the vaccine? Has this been tested, because these patients may theoretically shed material more efficiently, and are themselves at risk.

SBB comment

Although the study in immunocompromised mice shows that the hRVFV-4s vaccine is safe (Annex 1), the notifier could be asked to further discuss

i) any potential adverse effects for immunocompromised humans (like humans suffering from type I IFN deficiency or low IFN I response or HIV patients) being exposed to hRVFV-4s, either upon administration of a dose for vaccination (which seems unlikely considering exclusion criteria 6 – draft protocol) or when being exposed to shed hRVFV-4s (for example close contacts)

ii) how the notifier intends to collect relevant data in this regard in the course of the further clinical development.

Comment Coordinator

Although the comment of the experts (comment 3,comment 4, SBB comment) is correct and useful, this is in my opinion beyond the evaluation of this dossier "The study is a "First-in-Human" clinical trial with the hRVFV-4s vaccine. The study will be conducted with a total of 75 healthy subjects aged 18 to 45 years old.

Comment 5

Has evaluated this item and has no questions/comments.

4. INFORMATION RELATING TO THE CONDITION OF RELEASE

(e.g. description of the activity, quantities of GMO to be released, workers protection measures, elimination of any contaminating material in the preparation of the GMO stock, elimination of the GMO at the end of the experiment …)

Comment 1

How the low, middle and high doses will be determined?(concerns the test on humans)

SBB comment

The method of determination of the doses for human tests is not relevant for the purpose of the biosafety analysis of the clinical trial.

Comment Coordinator

See part 2 SNIF, p13. The GMO will be administered at a maximum dose of 10^7 $TCID_{50}$, present in a volume of 500µl. Quantities of GMOs to be released (injected in volumes of 500µl): 20 human subjects will be injected with a dose of $3x10^4$ +/- 0.5 log $TCID_{50}$ of hRVFV-4s, 20 human subjects will be injected with a dose of $3x10^5$ +/- 0.5 log $TCID_{50}$ of hRVFV-4s, 20 human subjects will be injected with a dose of

$3x10^6$ +/- 0.5 log $TCID_{50}$ of hRVFV-4s. The optimal dose level will be identified based on the available safety and immunogenicity data and data on virus shedding and spreading.

Comment 2

In the ERA document, page 10 of 21: Handling the containers and bottles of candidate vaccine
- Question: What are the titers in candidate vaccine and the volume of these containers/bottles? Please describe the types of the involved containers (glass or plastic).

Comment coordinator :
See technical dossier. The candidate vaccine is provided to the clinical trial centre in 2R (2 ml) vials filled in 0.7 ml aliquots, sufficient for 1 dose (0.5 mL) per vial, and presented as solution for IM injection. The 2 ml injection vial (2R) is manufactured out of clear tubular borosilicate glass of the 1st hydrolytic class. See technical dossier.

- Please describe the kind of handling of these containers and bottles and the aim of such handling: fridge storage, stock bottles for preparation of inoculum doses…? Are they single- or multiple-use bottles? In the last case, please describe the decontamination protocol and the disinfectant (is it generic or specific?).

In the ERA document, page 18 of 21: It is reported that:" Disposable wipes will be used when handling samples". Please describe the type of wipes that are used.

In the ERA document, page 18 of 21: "Hospital workers may accidentally come in contact with the hRVFV-4s vaccine preparation (e.g. shedding from the injection site after administering the vaccine), but based on the characteristics of hRVFV-4s, no untoward effects are foreseen."

Please discuss this sentence in light of the 3 following paragraphs:

1) In the scenario case mentioned p18: "Hospital workers may accidentally come in contact with the hRVFV-4s vaccine preparation (e.g. shedding from the injection site after administering the vaccine), but based on the characteristics of hRVFV-4s, no untoward effects are foreseen." Please describe which profiles of hospital workers are concerned (surgeon, nurse, physician, surface cleaner technician… or only the medical staff in charge of the vaccination).

Comment coordinator :
Prior to study start, CR2O will have completed a site-readiness procedure ensuring all involved site staff are trained on respective legislation and guidelines, protocol specific procedures (including but not limited to subject recruitment and retention strategies). ERA p18

2) "The clinical staff will use good clinical practices and will therefore be protected against inadvertent exposure, should it occur, staff is protected by a lab coat, disposable gloves, safety glasses and a face mask (FFP2). Disposable wipes will be used when handling samples. All waste material will be handled as hazardous medical waste."

3) For this scenario case, which kind of medical follow-up is proposed to people (medical staff or others) accidentally exposed to the hRVFV-4s vaccine preparation. Are skin disinfection, treatment or a medical exam proposed, or nothing of the sort?

In the SNIF document, page 13 of 16, point c): "Exposure during contact with participants in follow-up visits or when handling of samples collected from the participant and preparing for shipment is not expected…"

The fact that the notifier claims that: "exposure during contact with participants in follow-up visits or when handling of samples collected from the participant and preparing for shipment is not expected… "does not signify that this scenario case is not possible nor realistic. In this case, there is no follow-up or remediation clearly proposed by the notifier.

SBB comment

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

Comment 3

Has evaluated this item and has no questions/comments.

Comment 4

Has evaluated this item and has no questions/comments.

Comment 5

Has evaluated this item and has no questions/comments.

5. INFORMATION RELATED TO THE RISKS FOR THE ENVIRONMENT AND HUMAN HEALTH

5.1. Information on spread ("shedding") of the GMO from the treated patient/animal to other persons/animals or to the environment (including indirect/delayed effects due to vertical transmission to offspring).

(e.g. genetic transfer capability, routes of biological dispersal, target organisms ...)

Comment 1

I do not believe that a functioning type I interferon pathway will block ALL multiplication of the virus. Biological systems do not function in a black-white way. Contrary to the statement in point B1 of the Technical Dossier it is evident that a natural habitat is present: humans and mosquitos.

SBB comment

The notifier acknowledges the remaining level of uncertainty with respect to replication capacity of hRVFV-4s in humans (p14, SNIF : ' very little, if any, replication is expected to occur in vaccinated humans') albeit, several lines of evidence points towards a strong attenuation and reduction of replication capacity of the virus. From an environmental risk point of view, considerations should also go beyond replication capacity of the virus. For example, should replication occur, the possibility that mosquitoes could get infected and transmit the virus upon a blood meal of a clinical trial participant seems unlikely in the context of this clinical trial. Also, it remains to be investigated whether potential shedding by clinical participants, if any, lead to a risk for close contacts given the attenuated profile of hRVFV-4s .

Coordinator comment

Technical dossier p27 and SNIF p13 : Exposure during contact with participant in follow-up visits or when handling of samples collected from the participant and preparing for shipment is not expected, as no multiplication or shedding is anticipated. Irrespective, standard clinical practice will be implemented as a general workers protection measure.

Comment 2

Has evaluated this item and has no questions/comments.

Comment 3

Has evaluated this item and has no questions/comments.

Comment 4

The shedding in men is not studied and shedding has not been reported/detected in the animal models tested. Still, all experiments in animals using Clone13 were required to be executed in BSL2 settings, and only allowed D-I conditions from 24hrs after vaccination (p13/37 in B_BE_21_BVW2_Part 1 A Technical Dossier). Should it be considered to keep the patients overnight in the hospital instead of having them travel home (to limit possible shedding)?

SBB comment

Since all experiments in animals using Clone13 were required to be executed in BSL2 settings, and only allowed D-I conditions from 24hrs after vaccination (p13/37 in B_BE_21_BVW2_Part 1 A Technical Dossier), it is suggested in comment 4 that the same conditions should be applied to the patients, meaning that they should stay overnight in the hospital.

Since data demonstrated that RVFV-4s candidate vaccine for veterinary use (vRVFV-4s) does not disseminate in vaccinated animals, is not shed or spread to the environment and does not revert to virulence (Wichgers Schreur, 2020) and since the clinical trial will be hold in Belgium where the wild-type virus should not be present, the measures taken to limit the release of the virus in the environment should be proportionate and adequate to the actual risk. As a (more feasible ?) alternative to overnight stay in the clinical setting, the trial participant could be required to avoid close contact with household contact during 24h or to wear mouth-mask at home for 24h (as proposed in section 5.2-comment 4). An exclusion criterion could also be added for participants that are in close contact with immunocompromised human or with children whose immune system is not mature.

Coordinator comment

With respect to SBB's comment on adding an exclusion criterion for participants that are in close contact with immunocompromised human or children, see annex 1: **Safety of hRVFV-4s in nude mice** The results of this hRVFV-4s dose-escalation (4.4 log10, 5.4 log10, 6.4 log10 TCID50) study with 10 juvenile nude mice demonstrate that the hRVFV-4s vaccine is highly safe, even at high dose, in immunocompromised animals.

Comment 5

Are there information on shedding of hRVFV-4s in semen of NHPs? Could the notifier provide the report on shedding data collected from the study on NHPs (WBVR-LARISSA 2020-06 report)? The notifier says that the vaccine does not disseminate and is not shed in the environment and consider therefore the risk of transmission as negligible. However, it remains uncertainties concerning the shedding of hRVFV-4s vaccine in semen. Could the notifier comment on this?

SBB comment

Indeed, in Annex 3 (report on marmoset study) no detailed information is given on virus detection from oral and rectal swabs because it is referred to another report WVBR LARISSA 2020-06, which has not been included in documentation provided by notifier.
In addition to the question on presence of hRVFV-4s in semen, the notifier could also be asked to clarify whether urine samples have been analysed in the marmoset study.

Comment coordinator

See Tech Dossier p32. Although hRVFV-4s is not expected to cause viremia and not expected to disseminate in vaccinated study subjects based on non-clinical data (Annexes), after vaccination, the presence of the vaccine virus in blood samples, collected on days 0, 1, 3, 7, 14, 28 and 180, will be monitored by RT-qPCR. On the same days, urine and saliva samples will be collected, and evaluated for the presence of hRVFV-4s RNA and live virus. Semen will be investigated by analyses of samples collected on days -28, day 3 and day 14.

5.2. Information on possible effects on human health resulting from interactions of the GMO and persons working with, coming into contact with or in the vicinity of the GMO release (carekeepers, patient relatives, immunocompromised people ...).

Comment 1

It seems impossible to me to exclude with absolute certainty that the test persons could become sick, neither is it possible to exclude with absolute certainty that test persons could transmit the virus to other people.

SBB comment

While the transmission by mosquitoes of hRVFV-4s in the context of the proposed clinical trial is considered negligible, it is agreed that the conduct of the proposed first in human study is inherently associated to a level of uncertainty as regards the potential of RVFV-4s being shed by human beings. However, an evaluation of the environmental risk should also look beyond the potential event of transmission and try to estimate whether, should shedding occur, it would constitute a risk for human population and the environment. Transmission should not be regarded as an environmental risk per se unless it is shown that the RVFV-4s causes adverse effects.

Coordinator comment

From CDC https://www.cdc.gov/vhf/rvf/transmission/index.html
People can also get RVF through bites from infected mosquitoes and, rarely, from other biting insects. Infection with the RVF virus (RVFV) has occurred in laboratories when someone has inhaled virus that was in the air (known as aerosol transmission). Spread from person to person has not been documented, and no transmission of RVF to health care workers has been reported when standard infection control precautions have been put in place.

Comment 2
Please see the third comment section in the point 4 of the present report

Comment 3

Has evaluated this item and has no questions/comments.

Comment 4

The notifier indicates (p27/37 point6 in B_BE_21_BVW2_Part 1 A Technical Dossier) that "Following administration, the hRVFV-4s candidate vaccine is not expected to multiply in the host (based on absence of viremia in non-clinical studies with nude mice, rats, lambs, pregnant ewes, and marmosets)." In the frame of identifying whether the product may result in multiplication, or shedding may occur, would it be advisable to assess shedding and replication in the first patient (or patients) more closely, and fall back to the more 'relaxed' protocol later?

Currently the protocol (p9/21 in B_BE_21_BVW2_Part 3 ERA) indicates that a patient will be injected and at the earliest, the next patient will be administered 24hrs later for safety reasons. Between the second and the subsequent subjects another 24hrs interval will be respected (these time points are very short considering laboratory safety parameters are being examined within that time frame). The notifier indicates "CEVAC-LAB will prepare the serum, plasma, blood, saliva, urine, and semen samples and peripheral blood mononuclear cells (PBMC), cryopreserve and ship these samples to the laboratories that will execute the specific testing." This means that (assuming 24hrs will be in between two patients) safety is actually not considered.

Additionally, since this is a double blinded study, I reckon one should consider the fact that the first two patients may be injected with placebo, which would result in a profound underestimation of the risk of side effects, and also of the shedding of the material.

Last paragraph p11/21 E in B_BE_21_BVW2_Part 3 ERA: in my opinion it would be advisable that (at least the first few patients) are requested to refrain from blood/sperm donation, intercourse and would be advised to wear mouth-masks, until data show that shedding is in line with the results shown in laboratory animals (for example p14/21 and Annex3 B_BE_21_BVW2_Part 3 ERA).

SBB comment

As data on shedding of the vaccine after administration in human is currently lacking , it could be interesting to assess shedding and replication of the human virus in the first patient (or patients) more closely. The notifier could be asked to reconsider the protocol design so as to allow for more time for analysis and a better understanding of the shedding. However, it should be considered that both the limited number of participants (limited data) as well as the double blinded concept of the trial could hamper any proper conclusion on shedding during the course of the clinical trial.

Anyway the notifier should clarify which data will be reviewed by the site during these 24 first hours after patient randomisation. According to the protocol (as mentioned on page 9 of the ERA), they will check only for safety data. Which data are included in this safety analysis? Only the Adverse Event observed during these 24 first hours or will they also check for shedding in urine, saliva, semen and blood samples that will be collected at day 0. This could be clarified. Furthermore, should shedding be observed in combination with sign of virulence, the notifier could be asked to describe their plan for further action.

According to the exclusion criterion 7, men are refrain from sperm donation (see also comment by SBB in section 5.7 comment 4). Restriction on blood donation is not mentioned in any of the exclusions criteria and could be considered.

Comment 5

Has evaluated this item and has no questions/comments.

5.3. Information on possible effects on animal health or on the environment.

Comment 1

As it cannot be excluded that test persons would release any virus, it remains possible(but unlikely) that the virus would be transmitted to an animal.

SBB comment

Indeed, we cannot exclude the possibility that the virus could be transmitted to an animal. Therefore, during the clinical trial, after vaccination, urine, saliva, semen and blood samples will be collected at regular intervals and will be tested for the presence of vaccine virus RNA using sensitive reverse-transcriptase quantitative PCR. Furthermore, as mentioned in section 6.3, comment 4, subjects could be advised to avoid contact with other animals, and other human beings.

Coordinator comment

No human-to-human transmission has been documented; no human to animal has been described

Comment 2

Has evaluated this item and has no questions/comments.

Comment 3

Has evaluated this item and has no questions/comments.

Comment 4

Has evaluated this item and has no questions/comments.

Comment 5

Has evaluated this item and has no questions/comments.

5.4. Information on selective advantages or disadvantages conferred to the GMO compared to the parental organism.

Comment 1

Has evaluated this item and has no questions/comments.

Comment 2

Has evaluated this item and has no questions/comments.

Comment 3

Has evaluated this item and has no questions/comments.

Comment 4

Has evaluated this item and has no questions/comments.

Comment 5

Has evaluated this item and has no questions/comments.

5.5. Information on the possibility of the GMO to reconvert to his wild type form and possible consequences for human health or the environment.

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

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

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

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

Comment 5
Has evaluated this item and has no questions/comments.

5.6. Information on the possibility of the GMO to exchange genetic material with other micro-organisms and possible consequences for human health or the environment.

Comment 1
Has not evaluated this item.

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

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

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

Comment 5
Has evaluated this item and has no questions/comments.

5.7. Information on the possibility of gene transfer to other organisms and about the selective advantages or disadvantages conferred to those resulting organisms (possible consequences for human health or the environment).

Comment 1
Has not evaluated this item.

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

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

Comment 4
P16/21 G. in B_BE_21_BVW2_Part 3 ERA: In my opinion more caution should be considered (at least at the start of this trial), and the results cannot just be extrapolated from laboratory animals. The very set-up of this study is to determine the risk of the GMO.
Men should abstain from intercourse with any person (instead of only WOCBP) or use a condom. In addition, also blood donation should be prohibited, which is not indicated as far as I know.

SBB comment
As mentioned in the protocol, the inclusion criteria 7 refers to men who has to refrain from donating sperm, abstain from intercourse with a WOCBP or use a male condom and advise partner to use a highly effective contraceptive method until 6 months after hRVFV-4s administration. Since, transmission from human to human seems to be very low but cannot be excluded, it could indeed be interesting to prohibit blood donation during the course of the study .

Comment 5
Has evaluated this item and has no questions/comments.

6. INFORMATION RELATED TO THE MONITORING, SURVEILLANCE AND CONTROL, WASTE TREATMENT AND EMERGENCY PLANS PROPOSED BY THE NOTIFIER

6.1. Monitoring plan proposed by the notifier and possibility to identify the occurrence of non-anticipated adverse effects.

(adequation between the monitoring plan and risks identified during the risk assessment, when appropriate measures to minimize the potential risks to offspring …)

Comment 1

Concerning the statement" In the unexpected event that hRVFV-4s vaccination results in viremia, the risk of further dissemination is considered negligible," I think that it is impossible to be certain that further dissemination is impossible. So it is advisable to isolate the test person if viremia is detected.

SBB comment
As there is some uncertainty about shedding of the virus by human and as transmission cannot be fully excluded yet, the notifier could further describe which action they will perform in case viremia is detected in the patient.

Coordinator comment
Since no human-to-human transmission and human-animal transmission has been documented with the WT RVFV I am questioning myself if the remark on detection of viremia in test person makes sense.

See also below. Overview of the expert's comments in regards risk management measures and instructions (Emergency plan).

Comment 2

Informations on the emergency plan(s) are very light in description and situation scenarios presented in the ERA dossier

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

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

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

Comment 5
Has evaluated this item and has no questions/comments.

6.2. Surveillance and control of the release

(adequation between the procedures to avoid and/or minimise the spread of the GMO and risks identified during the risk assessment...)

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
At p28/37 point10 in B_BE_21_BVW2_Part 1 A Technical Dossier: 'Techniques foreseen for elimination or inactivation of the GMOs at the end of the experiment', only bleach is proposed to inactivate the product. Even though this can be used on surfaces, this will not be possible as a method to sterilize the injection site of the vaccine, or a possible spill on the skin of a caretaker or patient. Still, to prevent shedding in the environment, a way of containment should be clearly stipulated.
In addition, will patients be advised to avoid contact with other animals, and other human beings?

At p31/37 point6 in B_BE_21_BVW2_Part 1 A Technical Dossier aerosols are indicated as possibly allowing spreading of the vaccine. Aerosol formation should specifically be prevented and this should be clarified in the operational procedures to minimize spreading to the environment.

SBB comment

As the possibility that the virus could be transmitted to an animal cannot be excluded, subjects could be advised to avoid contact with immunocompromised humans (like humans suffering from type I IFN deficiency or low IFN I response or HIV patients) or animals during two week after the injection. See below. Overview of the expert's comments in regards risk management measures and instructions.

Comment 5
Are there any restriction for blood donation after the inoculation of hRVFV-4s?

SBB comment
Already mentioned in section 5.7 comment 4.

6.3. Information on the waste generated by the activity and its treatment.
(e.g. type of waste, amount ...)

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

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

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

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

Comment 5
Has evaluated this item and has no questions/comments.

6.4. If applicable, information on the emergency plan(s) proposed by the notifier.

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

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

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

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

Comment 5
Could the notifier provide the spill procedure in case of accidental spill or breakage of a vial containing the GMO ?

Could the notifier provide procedures in the event of an accidental occupational exposure through a splash to the eyes of mucous membrane or in the event of exposure to broken skin or needle stick? In case of incident, the medical staff should report the incident to the responsible of the site.

SBB comment

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

6.5 Information related to the identification of the GMO and the detection techniques

(e.g. identification methods and detection techniques, sensitivity, reliability and specificity of the proposed tests ..)

Comment 1

Has evaluated this item and has no questions/comments.

Comment 2

Has evaluated this item and has no questions/comments.

Comment 3

Has evaluated this item and has no questions/comments.

Comment 4

Has evaluated this item and has no questions/comments.

SBB comment

On p4 of the SNIF it is stated that The parental virus (Clone 13) can be detected by conventional reverse-transcriptase quantitative PCR (RT-qPCR) using RVFV-specific primers and probe and by Sanger- or next-generation sequencing. The notifier could be asked to clarify whether a technique is available to distinguish hRVSV-4s from the parental clone 13.

Furthermore, we propose to send a reminder to the notifier that he is expected to provide, along with the delivery of the control sample (due at the latest 15 days after the start of the trial), a detailed protocol for the method of conservation and analysis of the control sample.

Coordinator comment

See Technical dossier RVFV (Clone 13, hRVFV-4s) can be identified by conventional reverse-transcriptase quantitative PCR (RT-qPCR) using RVFV-specific primers and probe and by Sanger- or next-generation sequencing. The virus can also be detected using antibodies against the N, Gn or Gc protein in Western blots, immunoperoxidase monolayer assays (IPMA) or virus neutralization test (VNT).

Comment 5

Has not evaluated this item.

7. OTHER INFORMATION

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

Comment 1

I consider this type of vaccine as carrying a potentially considerable risk. All vaccines carry a risk, mainly concerning the induction of auto-immune diseases, but these risks are very small and negligible in comparison with the risk of the disease against which they confer at least some immunity. Here however we are confronted with a vaccine that should be considered a live attenuated vaccine that does not present the same safety margins as some recent vaccines based on RNA or replication incompetent viruses that have to pass many hurdles before being able to cause illness. The fact that transmission between humans by mosquitos has never been demonstrated is not a strong argument, because proving that such a transmission by mosquitos occurred is not a simple and easy thing to do. So I think that as well the phase I study of the vaccine as the later massive use of the vaccine carry a significant risk. However, the severity of the Rift Valley fever disease might well justify both the development and the later massive use of the vaccine.

SBB comment

One should be cautious with the risk qualification. If risks are deemed significant or considerable, it would mean that an actual risk has been identified.
In an environmental risk assessment context, the term 'risk' refers to a threat to the environment or human health. A 'possibility' or 'likelihood of occurrence', or un identified uncertainty is only one element leading to the determination of the risk. According to the EMA guideline on environmental risk assessment for medicinal products consisting of, or containing, genetically modified organisms (EMEA/CHMP/BWP/473191/2006 – Corr), the risk associated to the use of an investigational medicinal product is determined by the combination (or product) of the magnitude of the adverse effect and the likelihood of occurrence of such an adverse effect.
The epidemiology and biology of the wild-type RVFV, an analysis of the preclinical data provided in the dossier and the proposed intended use of hRVFV-4s leads to an appropriate estimation of the level of uncertainty. Inherent to the proposed FIH study, it is agreed that a level of uncertainty remains on the safety and possibility of replication and shedding upon IM administration of hRVFV-4s in human beings. In this regard, the proposed study allows for collecting data on all of this three aspects. These data will inform whether the (environmental) safe profile of hRVFV-4s, as demonstrated through the preclinical studies conducted in mice, rats, young lambs, pregnant owes and marmosets, will be confirmed.
Precautionary measures can be taken so as to limit exposure of non-vaccinees and the environment, taking into account that measures should be proportionate and adequate to the actual risk posed. As a precautionary measure, the notifier could be asked to detail/improve measures taken to further minimize potential exposure of study staff and potentially vulnerable people to hRVFV-4s.

Comment 2

None .

Comment 3

None .

Comment 4
None

Comment 5
None

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

In addressing all of the questions below, the notifier could also be recommended to provide a 2-4 page technical sheet 'instructions for study staff personal' including all relevant instructions for risk management when handling hRVFV-4s in the context of the proposed clinical trial.

Handling of the vaccine (internal transport):
(see section 4 – comment 2)
- Describe the kind of handling of these containers and bottles and the aim of such handling: fridge storage
- Describe the type of wipes that are used when handling samples (ERA p18/21)

PPE:
SBB comment: Information regarding the personal protection equipment should be revised throughout the documents. SNIF (section F.4.c page 13/16) mentioned that "Healthcare personnel administrating the vaccine will wear protective gloves." whereas, in ERA (section E, page 11/21), staff will wear a lab coat, disposable gloves, safety glasses and a face mask (FFP2). It should be clarified what kind of activity would require a lab coat , disposable gloves, safety glasses and face mask (FFP2) (p11/ERA) and what type of manipulation would only require protective gloves (p13/SNIF). The notifier could be asked to provide 'study staff' instructions with all handling instructions for health care workers and staff, including detailed instructions in case of spill and on waste management.

Procedures in case of spill and emergency plan:
(see section 4 – comment 2; section 6.1 – comment 2; section 6.4 – comment 5 ; section 6.3 – comment 4 ; section 6.1 – comment 1)

Contact with the vaccine preparation should be restricted to a few hospital workers. The notifier should clarify all potential profiles of hospital workers that could be concerned with accidental contact with the hRVFV-4s vaccine preparation (e.g. shedding from the injection site after administering the vaccine) as mentioned in the ERA p18/21. An emergency plan should be more detailed.
In case of accidental contact with vaccine preparation, which kind of medical follow-up is proposed to people (medical staff or others) : Are skin disinfection, treatment or a medical exam proposed?
Could the notifier provide the spill procedure in case of accidental spill or breakage of a vial containing the GMO ?
Could the notifier provide procedures in the event of an accidental occupational exposure through a splash to the eyes of mucous membrane or in the event of exposure to broken skin or needle stick?
In case of incident, the medical staff should report the incident to the responsible of the site.

At p31/37 point6 in B_BE_21_BVW2_Part 1 A Technical Dossier aerosols are indicated as possibly allowing spreading of the vaccine. Aerosol formation should specifically be prevented and this should be clarified in the operational procedures to minimize spreading to the environment.

Injection site leakage and wound dressing:
(see section 6.2 - comment 4)

At p28/37 point10 in B_BE_21_BVW2_Part 1 A Technical Dossier, only bleach is proposed to inactivate the product. Procedure of disinfection of the injection site and appropriate disinfectant to be used to sterilize possible skin of a caretaker or a patient should be clarified.

SBB comment: In the SNIF, section F.4.c (page 13/16), it is stated that after injection of the vaccine, the injection site is covered with a wound dressing to prevent dissemination from the injection site. However, it remains unclear whether the patient will leave the hospital with the same wound dressing or whether the wound dressing will be changed and replaced with another one before the patient leaves the hospital. This could be done before the study physician examination that will be done 60 minutes after vaccine administration (ERA section D page 9).
Furthermore, the notifier could be asked to indicate whether wound dressings will be collected for assessing the presence of hRVFV-4s virus (eg in the absorbent material of the wound dressings), as it could inform on the possibility of injection site leakage ?

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Adviesraad voor Bioveiligheid
Conseil consultatif de Biosécurité

Compilation of the expert's evaluations of the answers of Wageningen Bioveterinary research on the list of questions for dossier B/BE/21/BVW2

04 August 2021
Ref. SC/1510/BAC/2021_770

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SBB: Sheela Onnockx and Katia Pauwels

INTRODUCTION

Dossier **B/BE/21/BVW2** concerns a notification from Wageningen Bioveterinary research (Larissa consortium) for a clinical trial entitled "A Phase I, single-centre, randomized, double blind, placebo-controlled study to assess safety, tolerability and immunogenicity of hRVFV-4s vaccine in healthy subjects".

On 01 June 2021, based on a list of questions prepared by the BAC (SC/1510/BAC/2020_1170), 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 15 July 2021. This complementary information was reviewed by the coordinator and the experts in charge of the evaluation of this notification.

Evaluation expert 1

I've read through the replies of the applicant. Even though several questions have been answered, some were thoroughly answered in my opinion.
Edited files have been provided for the SNIF and the ERA. The ERA file only had two numbers added, is that correct? In other edited files, references to tables and figures are indicated to be changed (as track changes), but are replaced by identical versions. Again, is this correct?

SBB comment

Most of the changes were brought in the edited version of the SNIF and are in line with the notifier's answer on question 8. It is correct that reference to Figure 1 on p 8/12 of the ERA document has been omitted for a reason that is not fully clear.

Q1-2.
The applicant has provided sufficient additional info in response to the question asked.

Q3-4.
The reply on the shedding is a bit strange to me. *'Although data on shedding of the vaccine from humans is currently lacking, we would like to stress that even the wildtype virus is not shed from infected persons,*

nor from even the most susceptible livestock species.' Shedding data are available for the virus and have been shown to be negative, both in men and animals, is that the correct interpretation? As far as I could deduce from the provided documents, this was not clear to me in the first reading (or I have read over it). If so, I reckon this would be a good addition to the document.

SBB comment

While no evidence of human-to-human transmission has been reported with RVFV (Monath *et al*. 2020), there are indeed no data supporting the absence of shedding of wild-type virus in human.
On the other hand, even though no direct human-to-human transmission nor human-to-animal transmission has been documented with RVFV and replication of hRVFV-4s was slowed down as compared to Clone 13 *in vitro,* residual replication in humans still cannot be excluded for hRVFV-4s .
The question here is whether asking participants to wear a face-mask when in contact with other people during the first 24 hrs back home is a measure that is proportionate to the risk posed by hRVFV-4s.
Because the notifier agrees to add an exclusion criterion for participants that are in close contact with immune-compromised human or children, it could be considered that the concern related to protection of vulnerable people is met and that the proposal of wearing a face-mask can be dropped.

Coordinator comment
From CDC https://www.cdc.gov/vhf/rvf/transmission/index.html
People can also get RVF through bites from infected mosquitoes and, rarely, from other biting insects. Infection with the RVF virus (RVFV) has occurred in laboratories when someone has inhaled virus that was in the air (known as aerosol transmission). Spread from person to person has not been documented, and no transmission of RVF to health care workers has been reported when standard infection control precautions have been put in place.

Q5.
I understand that the low numbers of participants do not allow detailed shedding studies. If shedding studies have been performed for patients undergoing a 'live' infection, this would be a good addition to the dossier. Still, with this being a new vaccine, collection of shedding and multiplication data-points would be interesting for next phases for the vaccine development.

Q6-7-8.
The applicant has provided sufficient additional info in response to the question asked.

Q9.
Again, here the reasoning is not clear to me. The txt on p31 reads: '***6. Routes of biological dispersal, known or potential modes of interaction with the disseminating agent, including inhalation, ingestion, surface contact, burrowing, etc.*** *Considering that wild-type RVFV is likely to be infectious via aerosol exposure (although not experimentally demonstrated), the hRVFV-4s vaccine virus is also likely to be infectious via this route. Nevertheless, the very limited or even absent replication in even the most susceptible target species suggests that the risk of dissemination is negligible (the virus is not contagious)*.' It would be easier to stipulate that the virus is not spreading through aerosol, and thus the vaccine neither.

SBB comment

No direct human-to-human transmission nor human-to-animal transmission has been documented with RVFV. However, certain type of activities conducted in laboratories or close occupational contact with infected livestock (sample collection, slaughtering, meat processing) have been reported as source for infection through inhalation of infectious aerosols (https://www.cdc.gov/vhf/rvf/transmission/index.html).

Q10.

The applicant does not reply to the question put forward. The topic considers injection site leakage and spill of product to the skin of the patient or the caretaker. Skin disinfection using bleach is not an option at that moment. Procedure of disinfection of the injection site and appropriate disinfectant to be used to sterilize possible skin of a caretaker or a patient should be clarified.

SBB comment

According to the applicant's answer to question 9, wipes that are used to clean the skin of the participant are alcohol swabs (soft Pad MLS 70% isopropyl alcohol) and cotton swabs (Lohmann & Rauscher).

Coordinator comment

Procedures considering injection site leakage and spill of product to the skin of the patient should have been mentioned in Procedure to be followed in case of spills and leakage :
"BIOSAFETY-Behandelen van morsen en lekken"

Evaluation expert 2

La procédure en cas de spill a été ajoutée et est bien détaillée et complète. Ils disent bien qu'ils vont renseigner un éventuel incident aux personnes responsables sur le site.
Les critères d'exclusion demandés ont été ajoutés au protocole (éviter le contact avec les individus immunodéprimés, ne pas donner de sang après l'injection de l'IMP).
Les mesures de protection lors de la préparation de l'IMP au laboratoire sont bien détaillées aussi (L2, utilization d'une ESM, gants, tablier de laboratoire).
L'injection est réalisée par voie intramusculaire, ce qui limite le risque de création d'aérosols durant l'injection.
Les réponses aux questions sont satisfaisantes, je n'ai pas de remarques.

Evaluation expert 3

The expert has no further remarks nor advice.

Evaluation expert 4

Ik heb het geüpdatete dossier en de antwoorden intussen bekeken. Ik ben van mening, dat op adequate manier geantwoord en gereageerd werd op de vragen en opmerkingen van de experten. De voorlopige resultaten van de toxiciteit studie suggereren, dat de finale resultaten geen onoverkomelijke problemen wat betreft toxiciteit te zien zullen geven. Op voorwaarde, dat dit in het finale toxiciteit rapport bevestigd wordt, zie ik geen bezwaren met betrekking tot dit dossier.