Adviesraad voor Bioveiligheid
Conseil consultatif de Biosécurité

Advice of the Belgian Biosafety Advisory Council on the notification B/BE/19/BVW4 of the company MSD Belgium for deliberate release in the environment of genetically modified organisms other than higher plants for any other purpose than for placing on the market

17/09/2019
Ref. SC/1510/BAC/2019_0730

Context

The notification B/BE/19/BVW4 has been submitted by MSD Belgium BVBA/SPRL to the Belgian Competent Authority in May 2019 for a request of deliberate release in the environment of genetically modified organisms (GMO) other than higher plants for any other purpose than for placing on the market according to Chapter II of the Royal Decree of 21 February 2005.

The planned activity concerns a deliberate release in the environment entitled "*Importation of doses of V920 for Emergency Use*". It relates to the import in Belgium of doses of a vaccine candidate for protection against Ebola Virus Disease (EVD), for use in vaccination of health care workers traveling to Africa under Emergency Use conditions to support outbreak situations in Africa.

The Ebola virus, a filovirus, is responsible for a severe and often fatal haemorrhagic fever in humans and other mammals, known as Ebola Virus Disease. There are five species of Ebola viruses, all from the African continent and named from the place where they were identified: Z (Zaire) or ZEBOV appeared for the first time in 1976 in the Democratic Republic of the Congo (DRC, formerly Zaire). Other epidemics emerged in 1994-1995 and the last is still ongoing in the DRC in 2018-2019.

The GMO subject to this notification is V920 (also known as rVSVΔG-ZEBOV-GP), a vaccine candidate for protection against EVD caused by Zaire Ebola virus (ZEBOV). V920 is a genetically modified (GM) vesicular stomatitis virus (rVSV) in which the gene encoding the VSV glycoprotein G has been deleted and replaced with the gene encoding the ZEBOV glycoprotein (GP). The vaccine is a replication-competent, attenuated live virus that induces immune responses after a single dose.

There are currently no specific medical interventions licensed to treat Ebola haemorrhagic fever globally and there are no licensed vaccines in the European Union (EU). Two Ebola virus vaccines were recently approved in Russia and China, but without any efficacy data in humans. The Ebola virus is classified as a Category A priority pathogen, the highest level of risk to national security and public health. Current treatment of Ebola haemorrhagic fever is mainly supportive, involving fluid and electrolyte replenishment and pain reduction. A preventive vaccine could be used to protect individuals at high risk in advance of exposure and could be used for outbreak control at a population level to interrupt transmission. Since V920 elicits rapid immunity after a single dose, it has important potential for use in this context.

The V920 candidate vaccine is currently being deployed under ring vaccination using the Expanded Access/Compassionate Use protocol as recommended by WHO Strategic Advisory Group of Experts. V920 is also currently under regulatory review by the European Medicines Agency (EMA) for active

Biosafety Advisory Council - Secretariat • Service Biosafety and Biotechnology (SBB)
Sciensano • Rue Juliette Wytsmanstraat 14 • B-1050 Brussels • Belgium
T + 32 2 642 52 93 • bac@sciensano.be • www.bio-council.be

immunization of at-risk individuals 18 years of age and older to protect against Zaire Ebola virus disease (Marketing Authorisation Application according to Regulation (EC) 726/2004 of the European Parliament and of the Council of 31 March 2004 laying down Community procedures for the authorisation and supervision of medicinal products for human and veterinary use and establishing a European Medicines Agency).

Awaiting authorization from EMA, Médecins Sans Frontières (MSF) requested to receive doses of V920 in Belgium to support vaccination of local health care workers to be deployed in the DRC, where an outbreak of Ebola virus disease is currently ongoing. MSD plans to ship V920 doses from the United States to a reference hospital in Brussels where the vaccination will take place.
About five health care workers should be vaccinated per week, each receiving a 1 mL dose containing ≥ 72 million plaque-forming units (pfu) of V920. After the vaccination, the patient should stay for 30 minutes under surveillance and then leave the reference hospital without quarantine measures. The health care workers will then be deployed to the field in an epidemic zone, sometimes already within 48 hours of vaccination.
The national territory is considered as the potential release area of V920 in the context of the current request of deliberate release in the environment of a GMO.

The dossier has been officially acknowledged by the Competent Authority on 20 June 2019 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. Two experts from the common list of experts drawn up by the BAC and the Service Biosafety and Biotechnology (SBB) of Sciensano answered positively to this request. The SBB also took part in the evaluation of the dossier. The experts and the SBB assessed whether the information provided in the notification was sufficient and accurate in order to state that the deliberate release of the 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.

According to the above-mentioned Royal Decree of 21 February 2005, the notifier should deliver to the SBB (Sciensano) control samples and relevant scientific information to allow detection of the GMO in case of inspection or accidental release to the environment. Manufacturing of V920 vaccine drug substance and underpinning know-how are considered Dual-Use technology and are subject to Export Administration Regulations by the United States Bureau of Industry and Security (BIS), which requires licenses to export samples and technology to countries outside of the United States. Due to these special restrictions, shipment of reference standards and sharing sequence information to support method transfer is under the remit of the BIS License, and an updated BIS License listing Sciensano as an end-user would have been needed with a formal written acknowledgment of the conditions for export of V920 technology. In view of these restrictions the Belgian Competent Authority agreed on the principle of an alternative approach, where an independent laboratory would support testing of biological samples according to a quantitative Real-time Reverse Transcription Polymerase Chain Reaction Assay (qRT-PCR).

The scientific evaluation of the dossier 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 vaccine candidate 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 22 July 2019, 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 August 2019 and transmitted to the secretariat of the BAC on the same day. This complementary information was reviewed by the coordinator and the experts, and was considered for the preparation of this advice.

In parallel to the scientific evaluation of the notification, the Competent Authority also made the dossier available on its website for the one-month public consultation foreseen in the above-mentioned Royal Decree. The Competent Authority received eight comments from the public, none of them related to biosafety issues.

Summary of the scientific evaluation

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

The recipient organism is a vesicular stomatitis virus (VSV), a single-stranded negative sense RNA virus, belonging to the family *Rhabdoviridae*, genus *Vesiculovirus*. Wild-type VSV (wt-VSV) causes significant disease in pigs, cattle, and horses. Additionally, wt-VSV also circulates in bats. The VSV-related disease is limited to the Americas; Europe is not an endemic area for VSV. Occupational exposure to wt-VSV or lab-adapted VSV strains (veterinarians, lab workers, agricultural workers) is known.

The donor organism is the species *Zaire ebolavirus* (ZEBOV), a filamentous virus containing a single-strand of non-segmented, negative-sense viral genomic RNA. The EBOV glycoprotein (GP) is the only virally expressed protein on the virion surface and is critical for attachment to host cells and catalysis of membrane fusion. EBOV GP is not cytotoxic when expressed constitutively at a moderate level.

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

V920 comprises a single rVSV isolate (11481 nt, strain Indiana) in which the gene of the VSV envelope glycoprotein (VSV-G) has been deleted and replaced with the ZEBOV (Kikwit 1995 strain) envelope glycoprotein (GP) gene. V920 was constructed using a reverse genetics system based on five plasmids. Besides replacement of the VSV-G by the ZEBOV-GP no additional sequences were added to the V920 genome.

Upon request of the BAC the notifier provided the sequence of the full rVSVΔG-ZEBOV-GP genome as well as the predicted location and amino acid sequence of the Ebolavirus glycoprotein. It also provided clarification about the criteria on which the clone that was selected for premaster virus production was chosen, based on its nucleotide sequence.

RNA-viruses are known to have a high error rate during replication. The notifier provided additional information on the type of single nucleotide substitutions variants, including four type of variants located in the ZEBOV-GP, observed across three successive viral passages and in a viral passage with varying multiplicity of infection (MOI). Data indicate that the frequency of single nucleotide substitutions is not exceeding 12% in the viral passaging and not exceeding 15,2% in the MOI study. The notifier argued that the frequency of variants in vaccine production is low and that there are no specific modifications to either GP nor the VSV backbone that are responsible for attenuation where a single mutation would have caused a reversion to wild-type VSV or impacted tropism or viral replication.
The BAC agrees with these conclusions but would like to point out that the possible impact of the minor variants on tropism or function of the vaccine cannot be fully reliably predicted on the basis of the provided data.

The BAC also raised some questions with respect to the genetic stability of V920 *in vivo*. Because RNA-viruses are much less stable compared to DNA-viruses, and shedding-based transmission of V920 cannot be ruled out (see below), the genetic (in)stability of shed viral particles is an important aspect in the context of the environmental risk assessment. The whole set of clinical data seems to support the notifier's position that low-level genetic variants observed *in vitro* have no apparent impact on patient safety (V920 seems generally well tolerated though systemic and local adverse effects have been reported). However, the BAC is of the opinion that uncertainties remain concerning the genetic robustness of V920 during *in vivo* replication in vaccine recipients in terms of the safety of subjects exposed to shed vaccine during secondary transmission, if it would occur.

It is suggested that soluble (free) Ebola GP (shed GP, sGP) could result in some of the pathogenic effects of Ebola infection. When examining the vaccine sequence, it was determined that the coding region is predicted to preferentially translate the full GP and not the sGP construct. Furthermore, the downstream purification will significantly reduce any free/soluble protein such as the free-GP in the final product. The notifier further indicated that no evidence of detectable sGP was found in Western blot studies with sGP-reactive monoclonal antibody. While no *in vivo* studies were conducted, the notifier further pointed out that the attenuated phenotype demonstrated by rVSVΔG ZEBOV-GP in human subjects would suggest that significant amounts of sGP are not being produced.

3. The conditions of the release

MSD will import V920 doses in Belgium for use in vaccination of health care workers traveling to Africa under Emergency Use conditions to support outbreak situations. Vaccination will occur in a controlled clinical setting. Emergency use vaccination is expected to be for a limited number of health care workers (about five per week), each receiving a 1 mL dose containing ≥ 72 million plaque-forming units (pfu) of V920. The vaccine should be administered by intramuscular (IM) injection. After the vaccination, the patient should stay for 30 minutes under surveillance and then leave the reference hospital without quarantine measures. The health care workers will then be deployed to the field in an epidemic zone, sometimes already within 48 hours of vaccination.

The BAC is of the opinion that clear instructions must be provided to all involved personnel at the clinical center. Personnel will be trained in best biosafety practices to be applied during transport of the vaccine candidate to the administration room, during administration and at disposal of any biological waste. Such training will involve, among others, handling of live viral vaccines, applying safe handling procedures for needles and syringes, and using disinfectants. In the laboratory Good Microbiology Practices should be applied as a minimum for handling the GMO: the use of gloves, safety glasses and lab coat or scrub suit during the manipulation, washing one's hands after manipulation, disposing of the material used as biological waste, disinfecting the area after the manipulation, applying sharp objects handling policies, having collection and disinfection of waste procedures and materials and an action plan in case of accidental spillage. Detailed concentrations, chemical compositions and precise treatment times for disinfectants must be provided.
The notifier indicated that relevant instructions will be provided in a Package Leaflet. It also mentioned that detailed instructions for spill clean-up and disinfection have been developed and provided with each shipment of V920 in a Vaccine Agent Summary Sheet (VASS).

Vesicular lesions of the skin appearing in the first two weeks after vaccination are rare, but are a potential source of virus infection. Care should be taken to avoid contact spread from such lesions to others, including animals, by covering the vesicles until healing occurs.
The notifier did not clearly indicate the specificities of the bandage and the modalities of use to prevent fluid from being exposed to others. The BAC is of the opinion that the bandage should seal on all four sides, be properly applied without folds against the skin and be watertight. It should be applied on the injection site directly after injection and should be worn, if necessary, until lesions have completely disappeared. It should be changed immediately if for any reason it no longer properly sealed and at

least every 48 hours. This information should be communicated to vaccine recipients since they are likely to be the ones changing the bandage at places outside healthcare institutions.

4. The risks for the environment or human health

V920 is a GM, replication-competent, attenuated live virus. Attenuation of V920 is based principally on the reduction of viral replication and virulence linked to deletion of the VSV glycoprotein G gene (the viral determinant for neurotropism and pathogenicity), and replacement with the Ebolavirus glycoprotein gene. V920 has no selective advantage for replication, virulence or pathogenicity compared to wt-VSV or wt-ZEBOV.

VSV replicates within the cytoplasm of infected cells without intermediate DNA, and does not undergo genetic recombination or integration into the cellular genome. This precludes the possibility of genetic recombination of host cell sequences.

Reversion back to a wt-VSV is not expected since the attenuation is driven by the deletion of the full VSV-G gene sequence. Reversion back to a wild type EBOV is not considered a possibility as the only EBOV sequence in V920 is the ZEBOV GP.
The notifier provided an evaluation of the probability of recombination between the vaccine vector and wt-VSV based on the likelihood of co-infection of two non-segmented negative strand RNA viruses and the occurrence of RNA polymerase switching templates during replication. Although the frequency is low, the notifier provided some examples demonstrating that recombination in negative strand RNA viruses cannot be excluded. However, the notifier further pointed out that there is no documented evidence for recombination between viral vaccine vectors and wild-type virus strains outside of the laboratory.
The BAC agrees with the notifier that based on the limited use of V920 to protect people at risk of ZEBOV exposure in the context of the current deliberate release, the limited replication of rVSVΔG-ZEBOV-GP compared to wild-type strains including limited timeframe, the absence of wt-VSV or wt-ZEBOV in the Belgian environment, the highly unlikely coinfection of a susceptible cell by two viruses, and infrequency of recombination events even in the face of co-infection, the risk of homologous recombination of V920 with wt-VSV or wt-ZEBOV can be considered negligible.
In addition, homologous recombination of VSV strains or non-homologous recombination with other non-related RNA viruses is not believed to occur to any significant extent and as V920 does not cause long-lasting viremia in humans or animals, the probability of coinfection is further minimized. Thus, the generation of new chimeric viruses affecting new animal species is only a low probability theoretical possibility.

Gene transfer from V920 to other species is not expected. V920 is an RNA virus and does not contain homologous sequences with bacteria which would allow for such a transfer, even if reverse transcriptase would convert RNA in DNA.
There is no documented evidence for human to human transmission or human to animal transmission of VSV.

Specific stability in the environment of V920 is unknown. However, rVSVΔG-ZEBOV-GP is an enveloped virus which by its nature tends to be somewhat labile. The V920 vaccine has been shown to lose potency when held at 37°C or 25°C and thus is expected to lose potency under ambient conditions in case of an inadvertent environmental release.

According to the notifier, individuals vaccinated with V920 typically had low levels of virus in their blood for up to 1 week after vaccination, and all subjects assessed to date have cleared the virus from their blood by Day 28 post vaccination. Transfer of active rVSVΔG-ZEBOV-GP from urine, saliva, or vesicular lesions of the skin of vaccinated individuals may also occur during the first few weeks after vaccination. Therefore transmission of V920 from vaccinated persons to other individuals or through close personal contact with susceptible farm animals is a theoretical possibility. Should transmission of V920 occur,

the vaccine would retain its attenuated phenotype, and the individual could develop immunity against EVD.

The BAC is of the opinion that this potential transmission represents a negligible risk, due to the minimum levels of virus shedding by human vaccinees and the management strategies recommended to prevent contact of vaccinated individuals with the environment and animals. Vaccinated individuals should be informed about the potential for shedding and the need to avoid close association with and exposure of immuno-compromised individuals to blood and bodily fluids, and close contact with livestock animals for at least 6 weeks following vaccination.
Vaccinated individuals should also be required not to donate blood for 6 weeks following vaccination.

The BAC concludes that, based on the above-mentioned considerations, uncertainties remain concerning genetic robustness of V920 during *in vivo* replication in vaccine recipients. However, taking into account the whole set of data available, the BAC considers that the overall risk for individuals exposed to shed vaccine during secondary transmission (if it would occur) can be considered negligible in the context of the deliberate release subject to this notification (vaccination of a limited number of health care workers traveling to Africa under Emergency Use conditions).

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

Broadly speaking the BAC is of the opinion that the information provided is sufficient and does not raise safety concerns. In terms of risk for the environment or human health, the measures proposed by the notifier are proportionate and adequate in the context of the intended deliberate release.

The notifier outlined that none of the variants observed in the vaccine material have mutations present in the PCR amplicon region for the primer/probe set and that the primer/probe set was designed in a highly conserved region, thereby indicating that the specificity of the qRT-PCR is unlikely to be impacted by the of occurrence of mutation variants.

The notifier provided general scientific information with respect to the detection of the GMM.
The BAC can agree with the principle that an independent laboratory would support testing of biological sample, thereby deviating from the standard applied conditions foreseen in the Royal Decree of 21 February 2005, where it is required that the applicant provides the SBB (Sciensano) with control samples and relevant scientific information. However this is possible only if the notifier can demonstrate that the independent laboratory is applying the internationally accepted standard conditions required for monitoring viruses such as V920. The laboratory needs to be accredited according to ISO 17025, guaranteeing that it is able to report analytical data that reflect correctly the presence/absence/quantity of the virus used as vaccine. It should also be guaranteed that the laboratory will have access to the relevant reference materials and that the laboratory will be authorized to use the validated detection method (as referred to in the dossier) to analyze samples that could be sent by the Belgian competent authority for inspection or monitoring purposes. These services should be performed according to market conform prices applied in European laboratories.

Conclusion

Based on the scientific assessment of the notification made by the Belgian experts, the Biosafety Advisory Council concludes that it is unlikely that V920 developed as a vaccine candidate for protection against EVD caused by Zaire Ebola virus, will have any adverse effects on human health or on the environment in the context of the intended deliberate release and 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, including the specificities of the bandage applied on vesicular lesions of the skin, as described in section 3 of this advice.
- Vaccinated individuals should be informed about the potential for shedding and the need to avoid close association with and exposure of high-risk individuals to blood and bodily fluids, and close contact with livestock animals for at least 6 weeks following vaccination. Vaccinated individuals should also be required not to donate blood for 6 weeks following vaccination.
- The BAC can agree with the principle that an independent laboratory would support testing of biological sample, thereby deviating from the standard applied conditions foreseen in the Royal Decree of 21 February 2005, where it is required that the applicant provides the SBB (Sciensano) with control samples and relevant scientific information. However this is possible only if the notifier can demonstrate that the independent laboratory is applying the internationally accepted standard conditions required for monitoring viruses such as V920. The laboratory needs to be accredited according to ISO 17025, guaranteeing that it is able to report analytical data that reflect correctly the presence/absence/quantity of the virus used as vaccine. It should also be guaranteed that the laboratory will have access to the relevant reference materials and that the laboratory will be authorized to use the validated detection method (as referred to in the dossier) to analyze samples that could be sent by the Belgian competent authority for inspection or monitoring purposes. These services should be performed according to market conform prices applied in European laboratories.
- Any protocol amendment has to be previously approved by the Competent Authority.
- The notifier is responsible to verify that each clinical centre where the vaccination is taking place has qualified personnel experienced in handling the GMO and that the investigator has the required authorisations to perform the 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 health care worker receives the vaccination.
- At the latest six months after the vaccination of the last patient included in the deliberate release, the notifier must send the competent authority for the attention of the BAC a report with details concerning the biosafety aspects of the release. This report shall contain at least:
  - The total number of individuals included in the vaccination in Belgium;
  - A summary of all adverse events marked by the investigators as probably or definitely related to the vaccination;
  - A report on the accidental releases, if any, of V920.

Dr. Corinne Vander Wauven
President of the Belgian Biosafety Advisory Council

Annex I: *Compilation of comments of experts in charge of evaluating the dossier B/BE/19/BVW4 (ref. SC/1510/BAC/2019_0619)*

Compilation of comments of experts in charge of evaluating the dossier B/BE/19/BVW4

19 July 2019
Ref. SC/1510/BAC/2019_0619

Mandate for the Group of Experts: Mandate of the Biosafety Advisory Council (BAC) of 13 March 2019.
Coordinator: Jozef Anné (KUL)
Experts: SBB (Sciensano), Viggo Van Tendeloo (UZA), Willy Zorzi (ULiège)
Secretariat: Didier Breyer

INTRODUCTION

Dossier **B/BE/19/BVW4** concerns a notification from Merck Sharp & Dohme B.V. for the deliberate release in the environment of genetically modified organisms (GMOs) other than higher plants according to Chapter II of the Royal Decree of 21 February 2005.
The notification has been officially acknowledged on 20 June 2019 and concerns a deliberate release entitled "*Importation of doses of V920 for Emergency Use"*.

♦ 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.

The comments highlighted in yellow have been sent to the notifier as request for additional information.

Comments/questions received from the experts

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

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.

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.

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

- Contrary to what is mentioned in the technical dossier, the sequence of the full rVSVΔG-ZEBOV-GP genome as well as the predicted location and amino acid sequence of the Ebolavirus glycoprotein are not provided. The notifier is requested to provide this information.
- Information on the outcome of the sequencing results of clone P6PP5C4 that was selected for premaster virus production is missing. As it concerns an RNA-virus of which the replication occurs by

an RNA-dependent RNA polymerase lacking proofreading capability, alterations in the sequence are expected to occur. On which criteria clone P6PP5C4 was selected? The notifier is requested to provide a summary of the results of this study or to provide the corresponding literature reference.
- Given the higher error rate during replication for RNA-viruses, the notifier is asked to indicate of which material (e.g. virus master seed, virus working seed or other) sequencing results were obtained and to describe the outcome of these sequencing results with respect to possible or anticipated impact on i) the tropism of rVSV ZEBOV-GP ii) the replication properties of V920 in the host iii) the specificity of the real-time qRT-PCR, which is proposed as method to specifically detect and quantify rVSVΔG-ZEBOV-GP.
- The notifier is requested to clarify on which lots non-clinical studies have been performed and to detail whether these lots contained sequence variants compared to the V920 consensus reference.

Comment 2

Has evaluated this item and has no questions/comments.

Comment 3

Has evaluated this item and has no questions/comments.

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

- The notifier is requested to indicate whether there are any data available that confirm the absence of soluble GP in vaccine recipients of V920.
- The notifier does not provide any reference to substantiate the verification of the genetic stability *in vivo*. Because RNA-viruses are much less stable compared to DNA-viruses, the notifier is requested to provide more information that could lead to the statement that the genome sequence of V920 is stable. Because shedding-based transmission of this replicating recombinant RNA-virus cannot be ruled out, the genetic (in)stability of shed viral particles is an aspect that should not be neglected in the context of the environmental risk assessment. In addressing this request the notifier is asked not only to focus on the extent to which intentional modifications in the viral genome are kept during viral replication or on the presence of truncated genomes (known to be associated with defective interfering VSV particles), but also to describe how the presence of potential sequence variants was analysed and assessed with respect to their possible involvement in potential altered attenuation or tropism of V920.

Comment 2

Has evaluated this item and has no questions/comments.

Comment 3

Has evaluated this item and has no questions/comments.

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

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

Technical dossier, section 2.3.1. (and in other relevant documents where these aspects are mentioned)
(v) The last sentence should be changed as follows: *Medical personnel should be trained in the handling of live viral vaccines and apply safe handling procedures for needles and syringes*.
(vi) Detailed concentrations, chemical compositions and precise treatment times for disinfectants must be provided.
(vii) Details for transportation must be provided. It is insufficient to say "*Follow local requirements for administration sites. Any unused vaccine or waste material should be disposed of in accordance with local requirements in order to achieve inactivation of V920*". It must be made clear that this is biohazardous waste that should be disposed of in a rigid biohazard container that is then destroyed by incineration.

Technical dossier, section 2.3.2.
The notifier mentions that individuals who develop vesicular rash after receiving the vaccine should cover the vesicles until they heal. The notifier is requested to clearly indicate with what kind of material (e.g. bandage, should it be watertight?) vesicular lesions should be covered to prevent fluid from being exposed to others.

- The notifier is requested to clarify why exposure of high-risk individuals to blood and bodily fluids is recommended to be avoided for up to 6 weeks post vaccination while it is asked not to donate blood or plasma or to avoid close contact of livestock to blood or bodily fluids for 1 month (and not 6 weeks) post vaccination.
- The proposed packaging could easily lead to release of V920 in case the packing container (made of carton) is crushed during an accident and glass vials are thawed and broken. A sealed, plastic bag

surrounding the carton (for example each 10 vial cartons) should minimize these risks. The notifier is recommended to adapt the proposed packaging accordingly.

Comment 2

1) Technical dossier, point: 1.5.9 Measures to take in case of loss, unintended release or misuse of V920

It is stated that: "*Unintended loss or release as well as potential misuse are prevented by the need for storage in a controlled environment. Commercial carriers with manifest systems are used to track individual shipments. If breakage/spillage were to occur during administration of the product, disinfectants such as aldehydes, alcohols, and detergents are proven to reduce viral infection potential after only a few minutes.*"

The applicant refers to a paper from Zimmer et al. (2013) "Stability and inactivation of vesicular stomatitis virus, a prototype Rhabdovirus" (reference 20 in the list). This paper showed that VSV viruses may remain infectious outside the host cell for considerable time and represent a source of accidental infection if not properly inactivated. VSV can ''survive'' outside the host for considerable time, not only in suspension but also in dried form on surfaces. This relatively high tenacity may favour the vector-independent dissemination of the virus. The high stability of VSV on surfaces and in suspension may facilitate dissemination of the virus in livestock by contaminated feeding and water troughs, hands… This study showed also that the virus was highly sensitive to inactivation by commonly used disinfectants such as aldehydes, alcohols, and detergents. Among the compounds tested, 1-propanol, glutaraldehyde, and Triton X-100 turned out to be particularly effective at low concentrations.

Despite this reference article, there is, in this dossier, a lack of a clear procedure for disinfection and decontamination after the vaccination or in case of accidental spillage. Are there different protocols or procedures? Or is it the same universal procedure to decontaminate all the potentially contaminated surfaces (floors, walls of care rooms, tables, chairs, stools, computers…). The applicant is requested to clearly describe the procedure for disinfection of the vaccination equipment (e.g. detailing the type of disinfectant, time of contact) and of the vaccination room after the vaccination process.

Is there any responsibility or obligation for the applicant or for the vaccine care user to inform the commercial carriers supplying the vaccine vials about the decontamination procedure in case of accidental spillage (broken containers)?

Comment Secretariat:
In answer to these comments the expert was informed about the measures implemented at the clinical center where the vaccination is going to take place, regarding the use of disinfectants and the decontamination procedure in case of accidental spillage.
The expert was satisfied with these measures.

2) Technical dossier, point: 2.3.1 (vi) Disinfection and decontamination

It is stated that: "*Instruments, benches, surfaces, etc. should be decontaminated using disinfectants after the vaccination of individuals. If breakage/spillage were to occur, disinfectants such as aldehydes, alcohols, and detergents are proven to reduce viral infection potential after only a few minutes (less than 5) [20]*".

Same comment as for point 1.5.9 above.

Comment Secretariat:
See comment Secretariat above.

3) Technical dossier, point 2.4.1.3 (iv) Known or predicted environmental conditions which may affect survival, multiplication and dissemination (wind, water, soil, temperature, pH, etc.)

It is stated that: "*Specific stability in the environment of V920 is unknown. However as noted previously rVSVΔG-ZEBOV-GP is an enveloped virus which by its nature tends to be somewhat labile.*

The V920 vaccine has been shown to lose potency when held at 37 °C (1.137 log10 pfu/ml potency loss per day) or 25 °C (0.0790 log10 pfu/ml potency loss per day) [90] and thus is expected to lose potency under ambient conditions in case of an inadvertent environmental release. Importantly, the ambient temperatures for regions where Ebola is endemic/epidemic and where V920 is most likely to be used are closer to 37 °C than 25°C suggesting that survival in that environment is likely to be short-lived."

For this point, it will be considered that people outside of endemic/epidemic tropical regions (e.g. the foreign healthcare workers or simple travellers coming from Europe, US…) will be very probably vaccinated in their own country before going to these regions (maybe at a temperature closer to 25 °C than 37 °C).

Examples of optimal temperatures commonly used in hospital:

- Emergency service: 20 °C
- Medical Centre: from 20 °C (for an external temperature of -12 °C) to 26 °C (for an external temperature of 32 °C)

Please integrate this aspect (temperature in °C) in the comments at the end of point 1.5.9 (about the procedures of decontamination/disinfection…) and at point 2.4.2 (i) of this report.

4) Technical dossier, point: 2.4.2 Exposure scenarios and quantities of the product possibly released by accident during administration - (i) Spread following dispersal of drug product during normal handling or use

It is stated that: "*V920 is not intended for dispersal in the environment at large, but for direct administration by intramuscular injection into people to be vaccinated against Zaire Ebola virus.*

In case of accidental needle stick injury by medical personnel during intended administration of the vaccine to individuals, a small percentage of the vaccine dose might be injected with no untoward effects expected based on existing safety data.

Any other exposure of medical personnel should be prevented through personal protective equipment and safe vaccination techniques (section 2.3.1). In the case of an accidental spill of a vial of V920 to surfaces or tools that could lead to splashes on the skin or droplets exposed to the airway/mucous membranes of healthcare professionals, the exposure would once again be expected to represent a small percentage of the full vaccine dose with no untoward effects."

Same comments as for point 1.5.9 and 2.4.1.3 (iv) above + describe precisely the worker's protection measures during the decontamination of the vaccination areas in case of accident, especially in case of large spillage due to accidental release (broken containers).

Comment Secretariat:

See comment Secretariat above.

5) Document "Protocol Utilization", Section 6. Mesures organisationnelles et d'hygiène pour l'administration du vaccin.

Concerning the point 4 of the « Précaution de sécurité » part , it is written that : "*S'il y a des gouttes de vaccin qui se versent, essuyer avec un papier absorbant imbibé de désinfectant Anios Surfasave et le mettre dans le container jaune rigide. Tout le matériel non désinfectable et potentiellement contaminé par le vaccin lors d'un incident devra aussi être éliminé.*"

Please propose a universal treatment without trademark (generic treatment as possible).

6) SNIF - Point J. Information on emergency response plans - 1. Methods and procedures for controlling the dissemination of the GMO(s) in case of unexpected spread

It is stated that: "*If breakage/spillage were to occur, disinfectants such as aldehydes, alcohols, and detergents are proven to reduce viral infection potential after only a few minutes (less than 5).*"

In this dossier, there are several proposed disinfection/decontamination procedures and it is difficult, for the final user, to choose one of them. It will be perhaps necessary to select only one clear procedure.

Comment Secretariat:

See comment Secretariat above.

Comment 3

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

- RT-PCR assays have been used to detect viremia or shedding in saliva and urine. From the information provided in the dossier it is not clear what is the fraction of shed virus that is infectious and susceptible to contribute to effective transmission of V920. The notifier is requested to clarify whether any results have been obtained to distinguish the fraction of infectious particles among the shed virus material.
- The notifier is requested to indicate the limit of detection of the RT-PCR used in the studies references in Table 5.
- No information has been made available in the dossier with regards the lower limit of quantification of the RT-PCR assay in study V920-007 (table 9 and 10). The notifier is requested to clarify what is the limit of detection (LOD) by RT-PCR of viremia studies, the LOD by RT-PCR of saliva samples and the LOD by RT-PCR of urine samples and to indicate whether each of these detection methods have been qualified.
- The notifier concludes that shedding of virus in adults is infrequent, at low levels, and appears to pose minimal, if any, risk of transmission to other persons. We would like to point out that the dissemination of V920 into the environment through shedding is not an adverse event *per se* but rather a mechanism by which an adverse effect may occur. Therefore, any conclusions on the risk for human population or the environment should take into account several aspects such as the capacity for functional viral particles to survive in the environment, the route of transmission, the capacity of V920 to infect cells of other persons or animals and the potentially adverse effects observed in humans and/or animals. It is noted that shedding-based transmission to third parties among the human population has not been documented by experimental data. Therefore, the potential risk for third parties due to shedding-based transmission can only be assessed on the basis of a weight of evidence of aspects involved in successful transmission.

- With regards the study conducted with VSVΔG-ZEBOV-GP in pigs (de Wit et al. , 2015), we would like to point out that the number of pigs included in the study is very limited. Hence, it is difficult to conclude whether the obtained results can substantiate the probability of spread beyond the exposed animal.

Comment 2

Has evaluated this item and has no questions/comments.

Comment 3

Has evaluated this item and has no questions/comments.

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

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.

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

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.

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.

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

The notifier gives an evaluation of the probability of recombination between the vaccine vector and wt-VSV based on the likelihood of co-infection of two non-segmented negative strand RNA viruses and the occurrence of RNA polymerase switching templates during replication. Although the frequency is low, the notifier provides some examples demonstrating recombination in negative strand RNA viruses cannot be excluded. However, the applicant further points out that there is no documented evidence for recombination between viral vaccine vectors and wild-type virus strains outside of the laboratory.
We can agree with the notifier that based on the limited use of V920 to protect people at risk of ZEBOV exposure in the context of the current deliberate release, the limited replication of rVSVΔG-ZEBOV-GP compared to wild-type strains including limited timeframe, the absence of wt-VSV or wt-ZEBOV in the Belgian environment, the highly unlikely coinfection of a susceptible cell by two viruses, and infrequency

of recombination events even in the face of co-infection, the risk of genetic recombination of V920 with wt-VSV or wt-ZEBOV can be considered negligible.

Comment 2

Has evaluated this item and has no questions/comments.

Comment 3

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 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.

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

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

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.

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.

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.

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

Please see the comment of the point 4 of this document.

Comment 3

Has evaluated this item and has no questions/comments.

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.

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

None

Comment 2

None

Comment 3

None

References

-