Adviesraad voor Bioveiligheid
Conseil consultatif de Biosécurité

Advice of the Belgian Biosafety Advisory Council on the notification B/BE/21/BVW4 of the company AMAL Therapeutics, for deliberate release in the environment of genetically modified organisms other than higher plants for research and development

17/02/2022
Ref. SC/1510/BAC/2022_0207

Context

The notification B/BE/21/BVW4 has been submitted by AMAL Therapeutics to the Belgian Competent Authority in October 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: ***"An Open-Label, Multicenter, Non-Randomized, Dose-Confirmation and Cohort-Expansion Phase 1b Study to Evaluate the Safety, Tolerability, and Anti-Tumor Activity of ATP128, VSV-GP128 and BI 754091, in Patients with Stage IV Colorectal Cancer ".***

The purpose of this study is to assess the safety, tolerability and the anti-tumor effect of the study treatment in patients with stage IV Colorectal cancer (CRC).

CRC regroups two closely related diseases: colon and rectal adenocarcinomas. It is a common and lethal disease, ranking third as the most commonly diagnosed cancer and second in terms of incidence and mortality in developed countries. Microsatelite stable (MSS) CRC patients represent a large majority (> 95%) of the Stage IV CRC population but are facing a serious lack of treatment efficacy. Surgery and chemotherapy have long been the first choices for cancer patients. However, the prognosis of CRC has never been satisfying. Therapeutic cancer vaccines able to induce tumour specific immune responses are becoming a promising therapeutic approach in oncology. Tumour cell infection with oncolytic viruses leads to cancer cell killing and to host anti-tumour immune system responses stimulation.

VSV-GP is a recombinant chimeric vesicular stomatitis virus (VSV, Indiana strain Rhabdoviridae) which carries the envelope glycoprotein (GP) of the visceral non neurotropic WE-HPI strain of the Lymphocytic choriomeningitis virus (LCMV) instead of the native VSV glycoprotein (G). The GP of the LCMV abrogates neurotoxicity in mice even after direct injection of high doses directly into the brain (Muik *et al.*, 2014). VSV-GP128 expresses the multi-antigenic domain (Mad) which comprises the Carcinoma embryonic antigen (CEA), survivin and ASCL2 antigens and which induce an immune response against the included tumour antigens.

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This first-in-human clinical trial is composed of 4 parts. The genetically modified viral vaccine VSV-GP128 will only be administrated to patients included in Parts 3 and 4 of the study. Recombinant VSV-GP128 will be administrated in a triple combination treatment : the recombinant protein cancer vaccine ATP128 + the PD-1 inhibitor BI 754091 + the recombinant VSV-GP128. These patients will be given a single intravenous (iv) injection of VSV-GP128 on Day 15. A minimum of 45 (6 + 39) patients will be included in Part 3 and 4 of the study. In Belgium, an estimated number of 10 patients will be enrolled at two clinical sites located in the Flemish Region.

Viral shedding will be closely monitored in Part 3. Buccal swabs, nasal swabs and urine samples will be collected to assess viral shedding at indicated time points. Patients will also be instructed to follow biosafety measures at home to avoid transmission to close contacts and the environment.

The dossier has been officially acknowledged by the Competent Authority on 27 October 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. Three experts from the common list of experts drawn up by the BAC and the Service Biosafety and Biotechnology (SBB) of Sciensano answered positively to this request. 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 expert.

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 14 December 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 03 February 2022 and transmitted to the secretariat of the BAC on the same day. 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 received a few reactions from the public of which some were related to biosafety issues. According to Article 16 §2 of the Royal Decree of 21 February 2005, the comments

that are relevant for biosafety received in the framework of the public consultation, have been taken into account in the preparation of the advice below.

Summary of the scientific evaluation

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

VSV-GP128, which expresses the multi-antigenic domain (Mad), is derived from a highly similar virus, the VSV-GP recombinant virus. The VSV-GP recombinant virus is derived from the wt VSV where the native VSV glycoprotein has been replaced by the LCMV glycoprotein in order to abrogate neurotoxicity. While the notifier considered the VSV-GP recombinant virus as parental organism to conduct the environmental risk assessment, he repeatedly refers to the wt VSV to characterize the parental organism. Upon request from the BAC, the notifier further justified the approach taken since VSV-GP128 was generated using the genome of VSV-GP as template without the involvement of wt-VSV. Because both recombinant viruses are not naturally found and no epidemiological information is available, basic virology data from the wt-VSV was used to inform the environmental risk assessment.

Following the notifier's responses, the BAC is of the opinion that, the donor, recipient and parental organisms are adequately described in the dossier.

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

Upon request from the BAC regarding the characteristics of the viral vector, the notifier clearly stated in the documents that *VSV-GP128* corresponds to a replication competent virus. VSV-GP and its variant VSV-GP128 are sensitive to type I IFN responses. H.R. Thacore, 1978[1], demonstrated in non-clinical studies that the virus replicates well in interferon system deficient cancer cells leading to cell lysis while virus propagation in normal tissues is suppressed by an antiviral IFN response, resulting in an abortive infection.

3. The conditions of the release

Patients included in Parts 3 and 4 of the study, will be treated with the triple combination treatment : the related cancer vaccine ATP128 + the PD-1 inhibitor BI 754091 + the recombinant VSV-GP128. After injection, patients will be observed in hospital for 8 hours, where after they will be discharged . As requested by the BAC, during their 8 hours hospital stay, the patients will be asked to stay in their room with a private bathroom. Any movement within the hospital will be strictly limited to those required for study exams. When outside the room, the patient must wear a surgical grade mask and ensure that the injection site is covered with a dressing. Several instructions (such as bringing back to hospital any potentially contaminated material (e.g. plasters), reduction of close contact with other people, avoiding contact with livestock…) will also be given to patients to help prevent dissemination of the viral vector once they are at home. The notifier agreed to implement these instructions by adding the prohibition of sexual intercourse, the collection of gloves that were used to change the dressing and single-use tissue used when coughing or sneezing and by adding rodents in the list of animals that should be avoided

1. H.R. Thacore, Effect of Interferon on Transcription and Translation of Vesicular Stomatitis Virus in Human and Simian Cell Cultures J. gen. Virol 1978, 41, 421-426

since rodents also correspond to the wt virus host according to the Pathogen Safety Data Sheets: Infectious Substances – Vesicular stomatitis virus (VSV). Since data from non-GLP study in cynomolgus monkeys has shown that in blood RNA levels decreased over time and were no longer detectable 7 days after VSV-GP128 boost and that no infectious virus was detected at any time point, in blood and in any shedding samples, the notifier decided to keep the 7-day time period for the patient to respect these instructions. The BAC concludes that, based on the above-mentioned arguments, the 7-day time period for the patient to respect these instructions is acceptable.

The notifier was asked to further specify the properties of the bandage and the modalities of use to prevent fluid from being exposed to others. Upon BAC's request, the notifier adapted the recommendations to be given to the patient. The injection site (puncture) will be immediately covered with an air-and- watertight dressing. The patient is asked to keep the bandage for 48 hours. After 2 days the patient can remove the bandage (dressing). If the injection site is still visible then the patient should add another airtight and watertight dressing. The person changing the dressing is advised to wear a surgical grade mask and single use plastic gloves.

All these instructions for the patients with respect to good hygiene practices have been detailed in a short, readable format document that will be provided to each patient.

Muik *et al*. 2014[2] showed that replacing the glycoprotein G of the VSV by the GP of the LCMV abrogates neurotoxicity. As confirmed by the notifier, the safety profile of the VSV-GP vector has currently only been studied in mice and not yet in humans. Oncoselectivity of VSV is generally based on the lower type I IFN-associated antiviral potential of cancer cells compared to normal cells. Since immunocompromised persons could present deficiencies in a type I IFN response pathway and therefore not eliminate the virus as quickly as a healthy person, the notifier confirmed that any patients with a known immunodeficiency (beyond that acquired from their cancer treatment) will be excluded from this VSV-GP128 study.

In non-clinical data on animals, shedding samples (nasal, buccal and injection site swabs, urine and feces), as well as blood (for viremia assessment) were collected. Upon BAC's request, the notifier confirmed that no shedding of infectious VSV-GP was observed in tumor-bearing mice, healthy rabbits, healthy dogs and pigs. The absence of infectious material was confirmed either by using a plaque forming unit assay or by using the $TCID_{50}$ when shedding levels were high enough to allow the analysis. Non-clinical data in cynomolgus monkeys showed that viral clearance is observed after 7 days post-injection. The BAC addressed the fact that surgery of trial participants will be performed approximately 22 days after virus injection and concluded, also on the basis of the notifier's commitment to inform the surgery team about the patient status and data on viral clearance, that the proposed measures to contain/ limit exposure to VSV-GP128 are acceptable.

4. The risks for the environment or human health

VSV-GP128 is a GM, replication-competent attenuated live virus. Attenuation of VSV-GP128 is based on the replacement of the wt VSV glycoprotein G gene (the viral determinant for neurotropism and pathogenicity) with the LCMV glycoprotein gene.

2. A Muik et al., Re-engineering vesicular stomatitis virus to abrogate neurotoxicity, circumvent humoral immunity, and enhance oncolytic potency, Cancer Res. 2014 Jul 1;74(13):3567-78

VSV is a negative-sense single-stranded RNA viruses (ssRNA(-)) which replicates within the cytoplasm of infected cells without intermediate DNA, and does not undergo re-assortment or integration into the cellular genome. This precludes the possibility of genetic recombination of host cell sequences. Although, the frequency of recombination among negative sense RNA viruses seems to be relatively low, Chare *et al*., 2003[3] reported patterns of sequence variation compatible with, but with no direct evidence for, recombination for 10 viruses including Vesicular Stomatitis virus, which suggests that it is not possible to rule out recombination.
Reversion might be possible in the presence of the wild type VSV virus, generating a wild type virus with no additional survival or pathogenicity benefit.
Gene transfer from VSV-GP128 to other species is not expected. VSV-GP128 is a RNA virus with no DNA intermediates and does not contain homologous sequences with bacteria which would allow for such transfer, even if reverse transcriptase would convert RNA in DNA.
Genetic stability of VSV-GP128 has been demonstrated by Sanger sequence analysis at the end of production.

As infected animals with wt VSV salivate excessively and release between 4 and 6 logs of virus per milliliter of saliva (P. Rozo-Lopez *et al*, 2018[4]), the notifier further developed the results of viral shedding in the saliva obtained from the non-clinical studies. The notifier also provided further results regarding shedding in the faeces that have been obtained from the non-clinical studies on cynomolgus monkey upon request from the BAC.

For this first in-human study with VSV-GP128, after administration of the VSV-GP128 vector, patients included in cohort 3 will be closely monitored for shedding and viremia. To evaluate the possible transmission routes of the vector, virus shedding analysis (viral RNA detection by PCR) will be performed on buccal swabs, nasal swabs and urine samples at different time points after injection: Day 15 (pre-dosing), Day 15 (1h post-dosing), Day 15 (8h post-dosing), Days 19-22-29-36, and additional samples will be collected at the next scheduled visits (D43, D57, D64, D85, etc.) until three negative consecutive PCR results are obtained.

Upon BAC's request, instructions for the in-house transportation of the vector on site have been clarified and developed both in the CAF document and in the 2-4 pages technical sheet 'Instructions for study site personal'.

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

The BAC was of the opinion that instruction given to the pharmacy personnel on departure from the room could be improved by clarifying that the staff must wash their hands after removing protective equipment. The "Instructions for study site personal" document has correctly been implemented with this additional instruction.

Following BAC's request, the notifier made sure that at both clinical sites in Belgium, any spill incident will be reported to the internal prevention service of the hospital. The notifier also implemented the information related to elimination or inactivation of left-over finished product at the end of the clinical trial for UZ Leuven.

3. E Chare et al., Phylogenetic analysis reveals a low rate of homologous recombination in negative-sense RNA viruses, Journal of General Virology (2003), 84, 2691–2703
4. P. Rozo-Lopez et al., Vesicular Stomatitis Virus Transmission: A Comparison of Incriminated Vectors, Insects 2018,9, 190

Concentration and percentage of disinfectants proposed in the CAF for the inactivation of the viral vector VSV-GP128 are coming from publicly available documents. The notifier included in the decontamination/cleaning measures section of the CAF, the specific disinfectants used by both Belgian clinical sites.

Upon BAC's request, the notifier provided a 2-4 pages technical sheet 'VSV-GP128 Instructions for study site personal' including all relevant handling instructions, detailed PPE, detailed instructions in case of accidental spill or breakage of a vial containing the GMO, clean-up procedure, waste management.

The notifier adequately implemented the remarks and requests addressed by the BAC in a revised version of the CAF and in the "Instructions for study site personal" sheet and provided missing documents such as the UZ Leuven internal guidelines on Internal transport and the emergency response plans for accidental self-contamination during handling or administering the vector and/or accidental release of the vector into the environment of the UZ Leuven.

Given the assessment of the likelihood of further propagation of VSV-GP128, the BAC supports the view that, in terms of risk for the environment or human health, the proposed measures as described in the revised documents are proportionate and adequate in the context of the intended 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 VSV-GP128 developed as a gene therapy approach for the treatment of Stage IV Colorectal Cancer will have 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 as described in the following new or updated documents:

- 1.3a KISIMA-01_CAF_VSV_GP128_Clean_24Jan2022
- 1.4 KISIMA-01_VSV-GP128 Instructions for study site personal
- 1.5 KISIMA-01_Intructions for participants

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

- Referring to the vector-borne properties of the wt-VSV, and as a precautionary measure, the patient should be recommended to use mosquito repellent during the day and the night (alternatively a mosquito net could be used when sleeping) for 7 days, unless the notifier can provide supporting evidence on the low likelihood of transmission through arthropod vectors. In this regard, the notifier is asked to clarify whether any replication data of VSV-GP128 in arthropods are available (e.g. replication data in relevant arthropod cell cultures or live mosquitoes).

- The notifier and the investigators must strictly apply the clinical trial protocol version 10, and all the safety instructions as described in the dossier and the updated and new documents listed here above.

- 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.

- At the latest 15 days after the start of the trial, the notifier should provide, along with the delivery of the control sample, a detailed protocol for the method of conservation and analysis of the control sample.

- 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 and the number of patients included in Belgium;
    - A report of the shedding data obtained from the clinical trial (monitoring of viral vector excretion/secretion in buccal swabs, nasal swabs and urine samples after injection at Day 15 (pre-dosing), Day 15 (1h post-dosing), Day 15 (8h post-dosing), Days 19-22-29-36 compared to baseline)
    - 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 VSV-GP128.

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/BVW4 (ref. SC/1510/BAC/2021_1224)
Annex II: Answers to the public reaction to dossier B/BE/BVW4 in NL (ref. SC/1510/BAC/2022_0205) and FR (ref. SC/1510/BAC/2022_0206)

Adviesraad voor Bioveiligheid
Conseil consultatif de Biosécurité

Compilation of comments of experts in charge of evaluating the dossier B/BE/21/BVW4 And comments submitted to the notifier

14 December 2021
Ref. SC/1510/BAC/2021_1224

Mandate for the Group of Experts: Mandate of the Biosafety Advisory Council (BAC) of 08 october 2021.
Coordinator: Karen Willard-Gallo (Jules Bordet Institute, ULB)
Experts: Rik Gijsbers (KULeuven), Anton Roebroek (KULeuven), Willy Zorzi (ULiège), Amaya Leunda Casi (SBB)
SBB: Sheela Onnockx

INTRODUCTION

Dossier **B/BE/21/BVW4** concerns a notification from AMAL Therapeutics for the deliberate release in the environment of genetically modified organisms other than higher plants according to Chapter II of the Royal Decree of 21 February 2005.
The notification has been officially acknowledged on 27 October 2021 and concerns a clinical trial entitled "*An Open-Label, Multicenter, Non-Randomized, Dose-Confirmation and Cohort-Expansion Phase 1b Study to Evaluate the Safety, Tolerability, and Anti-Tumor Activity of ATP128, VSV-GP128 and BI 754091, in Patients with Stage IV Colorectal Cancer*".
The trial will involve the use of a genetically modified viral vaccine, VSV-GP128, which is a recombinant vesicular stomatitis virus carrying the glycoprotein (GP) of the visceral non-neutropic WE-HPI strain of the LCMV virus and a gene coding for the multi-antigenic domain (Mad). Patients enrolled in Cohorts 3, 4a and 4b, will receive one single injection of VSV-GP128 on Day 15 in between the first and second dose of ATP128 (a chimeric recombinant protein developed for the treatment of colorectal cancer (CRC)).

♦ 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 14-12-2021 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.

2. INFORMATION RELATED TO THE INVESTIGATIONAL MEDICINAL PRODUCT

A.1. Virus from which the clinical vector was derived (parental virus)

(e.g. information on parental virus; phenotypic and genetic markers; host range, zoonotic potential and replication properties of the parental virus ….)

Comment 1

In the CAF (B_BE_21_BVW4_Part 1A_CAF_VSV-GP128_revised_18Oct2021.pdf) it is not clear whether the drug substance consists of a replicating virus (this in only indicated in 2.9 p15/43 in CAF), a conditionally replicating virus (only in cancer cells) or a viral vector (replication deficient & single-round). It would be advisable to state this clearly at the beginning of the CAF (and to adapt the nomenclature accordingly when necessary).

SBB Comment:
VSV-GP128 is derived from a highly similar virus, VSV-GP. The recombinant VSV-GP virus is carrying the LCMV glycoprotein instead of the native VSV glycoprotein. The oncolytic virus VSV-GP is designed to be replication competent, and its intent is to infect, replicate in and kill interferon deficient cancer cells (section 2.9, p15/43 in CAF_VSV-GP128).
Since the wt VSV is sensitive to type I IFN responses, it preferentially replicates in cancer cells. The chimeric VSV-GP virus has been engineered so that it lacks its natural neurotoxicity while retaining potent oncolytic activity (Muik *et al*, Cancer, Res, 2014). The transgene, Mad, expressed by VSV-GP128 is a fusion of three tumour specific antigens frequently found in colorectal cancer cells.

Coordinator Comment:
The coordinator thinks that expert 1 is right, it needs to be more clearly stated. The SBB's comment above in blue clarifies better.

P8/43 in CAF B_BE_21_BVW4_Part 1A_CAF_VSV-GP128_revised_18Oct2021.pdf indicates the titers and doses applied. For clarity, 106 and 107 should be 10e6 and 10e7.

SBB and Coordinator Comment:
This comment could be added together with other typo errors.

For VSV a functional type I IFN response pathway is a key determinant of VSV oncoselectivity (see also p12/43 in CAF). Patient with immunodeficiencies in this pathway should be excluded from the study

design. In addition, considering the info provided in 2.6 p14/43 in CAF, pregnant women and small children should be excluded.

SBB Comment:
Exclusion criterion 12 excludes pregnant and nursing women. Inclusion criterion 2 allows only patient older than or having 18 years.

Oncoselectivity of VSV is generally based on the lower type I IFN-associated antiviral potential of cancer cells compared to normal cells. Most tumours have defective or inhibited type I IFN signalling, likely because many IFN responses are anti-proliferative, anti-angiogenic and pro-apoptotic. As WT VSV is sensitive to type I IFN responses, it preferentially replicates in cancer cells *(*S.A.Felt *et al*., J Gen Virol. 2017*)*.
Based on available data, the notifier could be asked to elaborate on the impact of deficiencies in a type I IFN response pathway on the biodistribution and shedding of VSV-GP128. For example, is it conceivable that patients with deficiencies in a type I IFN response pathway could present unanticipated shedding pattern following administration of VSV-GP128.

Coordinator Comment:
"Most tumours" : More likely SOME tumours – it can be hit or miss and varies for each solid tumour type.
Proposition from SBB would be difficult to do as a routine analysis. The point here is that patient's with a known immunodeficiency (beyond that acquired from their cancer treatments) should be excluded.

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

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

Comment 4
Is the choice of VSV-GP as the parental organism for risk assessment adequate? Furthermore, the applicant refers repeatedly to the wt-VSV to characterise the parental organism (CAF) which seems logic from the point of view of basic virus biology (genome, replication, …). As the modification of the glycoprotein in wt-VSV determines VSV-GP128 properties relevant for the environmental risk assessment, the choice of VSV-GP as parental organism is a bit confusing. This choice should be explained.

SBB Comment:
VSV-GP128 is derived from a highly similar virus, VSV-GP. The recombinant VSV-GP128 virus is carrying the glycoprotein (GP) of the visceral non-neutropic WE-HPI strain of the lymphocytic choriomeningitis virus (LCMV; Arenaviridae) instead of the native VSV-G glycoprotein and in addition a gene coding for the multi-antigenic domain (Mad) (CAF, p7/43).
The oncolytic virus VSV-GP has been obtained by removing and replacing the envelope VSV glycoprotein G of the wt VSV, the key neurovirulence determinant with the glycoprotein of the lymphocytic choriomeningitis virus (LCMV-GP), thereby generating the oncolytic virus VSV-GP lacking the Mad antigenic cargo.

The notifier considered the VSV-GP as the "Parent virus" as the Mad antigens were inserted into the VSV-GP vector sequence.

Coordinator Comment:
Again this goes back to Rik's comment – they need to clarify their text – it reads like a patchwork of different authors writing different parts.

A.2. Pathogenicity

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

Comment 1

VSV is not considered a human pathogen, but living in enzootic areas have a high seroprevalence rate. Is this the case for Belgium? If so, people working with animals should be guided and take extra care.

SBB and Coordinator Comment:
Vesicular stomatitis (VS) is a viral disease of veterinary importance, enzootic in tropical and subtropical regions of the Americas. VSV outbreaks occur most frequently in the central and south-western United States, Canada, and Mexico. Once the disease is introduced into a herd, it may move from animal to animal by contact or exposure to saliva or fluid from ruptured vesicles. Humans can contract vesicular stomatitis by coming into contact with lesions, saliva, or nasal secretions from infected animals. There are no reports of humans transmitting the infection to other humans or to animals. Furthermore, the following instruction will be given to the patients and will have to be followed for 7 days following VSV-GP128 administration: Avoid close contact with young children, pregnant women, immunocompromised people and livestock (e.g. pigs, cows, horses, etc.). When unavoidable, a surgical grade mask should be worn when within touching distance.
Finally, it could be noted that due to the mild, self-limiting nature of the disease and unlikely international spread through trade of animals, VSV has been de-listed by the World Organization for Animal Health (OIE) as a reportable animal disease (http://www.oie.int/en/animal-health-in-the-world/oie-listed-diseases-2019/).

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

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

Comment 4
wtVSV is classified in risk class 2 for human and 3 for animal (Belgian classification list). Attenuation is obtained in VSV-GP by removing neurotoxicity (VSV glycoprotein removal) which has been shown in animal models. It is however not shown in humans until now. Applicant cannot claim that VSV-GP is not pathogenic for humans.

SBB and Coordinator Comment:
As mentioned in the SNIF, p5/16, as VSV-GP is a genetically modified virus there is no natural host. No clinical data is currently available. Neither VSV-GP128 nor the highly similar virus VSV-GP, which does not contain the cancer antigens, have been previously used as a treatment in humans, meaning this will

be a First-In-Human clinical trial. According to section 2 of the CAF (page 7/43), replacing the glycoprotein G of the VSV by the GP of the LCMV abrogates neurotoxicity, even after direct injection of high VSV-GP doses directly into the brain (Muik *et al*, 2014, Cancer Res). These results have been obtained in mice and even if VSV-GP vector seems to be safe in mice, this hasn't been showed in human yet. The notifier could be requested to clearly indicate in the text of sections 2 (p13/43) and 2.16 (p21/43) of the CAF that the safety profile of the VSV-GP vector has till now only been obtained in mice.

A.3. Ability to colonise

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

Comment 1

P14/43 in CAF: "Non-clinical data from VSV-GP presented in Section 2.18 show that shedding and transmission is considered low." I do not agree with this statement. For clarity, it should be amended with "considered low in the x, y and z animal studies". Considering the species-specific infection routes and distribution, and studies that are not conducted in human are informative, but we should envision that shedding and transmission features my differ, are thus should be stated as 'to be determined'.

SBB and Coordinator Comment:

Since no previous shedding analysis of the VSV-GP128 has been performed in humans yet, in section 2.8 of the Part 1A_CAF, the sentence "Non-clinical data from VSV-GP presented in Section 2.18 show that shedding and transmission is considered low." could indeed be improved by clarifying in which studies these shedding results have been observed and that these results have only been obtained till now on animals and not on humans where such analysis still need to be performed.

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.

B. Genetic modification and manufacturing of the clinical vector

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

Comment 1

Has evaluated this item and has no questions/comments.

Comment 2

Has evaluated this item and has no questions/comments.

Comment 3

Has evaluated this item and has no questions/comments.

Comment 4

Has not evaluated this item.

C. Clinical vector

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

Comment 1

The applicant indicates that an artificial transgene was constructed that assembles different antigens expressed in colorectal cancer cells. Even though I realize the latter falls beyond the scope of the ERA, it should be assessed whether this RNA sequence may encode protein sequences that may have adverse effects in the human cell (transcription factor function, or whether the RNA may exert siRNA/asRNA like effects).
Has the multi-antigenic domain (Mad) been expressed and assessed for its properties in human laboratory cell lines?

SBB and Coordinator Comment:
The VSV-GP-128 is derived from a highly similar virus, VSV-GP. In addition to the glycoprotein LCMV-GP, the recombinant VSV-GP128 virus is carrying a gene coding for the multi-antigenic domain (Mad). As mentioned in section 5.1 of the CAF (p33/43), VSV-GP128 expresses the same three tumour antigens (Mad) as ATP128, the therapeutic protein vaccine already undergoing clinical evaluation in the ongoing KISIMA-01 trial. Available safety data show that the construct is safe and induces antigen-specific immune responses in stage IV colorectal cancer patients.

Considering that the transmission routes of either of the parental viruses for the drug product (DP) are unclear, one should proceed with caution, since we do not know what the effect may be when the DP would enter the environment. If shedding is excluded from human patients, the latter can be considered nil. The applicant indicates (p20/43 in CAF) that the drug product will be assessed under contained use, but this is not detailed.

SBB Comment:
As mentioned on page 20/43 in the CAF_VSV_GP128, VSV-GP128 or VSV-GP will only be used in a clinical setting under contained use. Administration of VSV_GP128 will only be performed in the clinic under contained use in order to control the spread and unintended release. In order to evaluate the possible transmission routes of the viral vector, viral shedding analysis (viral RNA detection by PCR) will be performed at different time points after injection of the viral vector VSV-GP128: Day 15 (pre-dosing), Day 15 (1h post-dosing), Day 15 (8h post-dosing), Days 19-22-29-36, on buccal swabs, nasal swabs and urine.

Coordinator Comment:
Yes, they need to justify why it is contained use. I am not sure how contained the use is. The virus will be shed for a minimum of 7 days and likely longer. In addition, surgery is planned between the 3rd and 4th injection (or earlier if medically necessary). Surgeries are messy and it is possible that the medical team is exposed during the procedure – cuts happen during surgery. So for me this is deliberate release. I do not see why they have declared it contained use.

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 not evaluated this item.

2.17. Potential for recombination

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 not evaluated this item.

2.18. Biodistribution and shedding

Comment 1

P8/43 of B_BE_21_BVW4_Part 1A_CAF_VSV-GP128_revised_18Oct2021.pdf indicates 'no shedding of infectious VSV-GP was observed': this is not clear, does this imply there is clearly shedding detected, but the virus was infectious?

> **SBB Comment:**
> The notifier could be requested to clarify the following sentence reported on p8/43 of the CAF_VSV-GP128 : "No shedding of infectious VSV-GP was observed in tumor-bearing mice (n00279794), healthy rabbits (n00284577), healthy dogs (n00279792) and pigs (n00282666; n00282980)". Has shedding of non-infectious particles been observed? Which analyses have been performed to determine the infectivity of the shedded particles? From the information provided in the dossier it is not clear what would be the fraction of shed virus that is infectious and susceptible to contribute to effective transmission of VSV-GP. Furthermore, the notifier could be requested to clarify if any results VSV-GP128 have been obtained to distinguish the fraction of infectious particles among the shed virus material.
>
> **Coordinator Comment:**
> This is important

Considering the large amounts of viral load reported in saliva of host animals for VSV (Rozo-lopez et al. 2018), why has this not been sampled in the studies presented? (p22/43 in CAF)

SBB and Coordinator Comment:

The notifier could be asked to clarify whether shedding in the saliva has been analysed or not in the non-clinical studies. If shedding in saliva has been tested results should briefly be developed in the CAF section 2.18. However, if shedding in the saliva has not been performed, notifier could be asked to clarify why this has not been tested since it has been showed that infected animals salivate excessively and release between 4 and 6 logs of virus per milliliter of saliva (P. Rozo-Lopez *et al*, Insects, 2018).

As indicated by the applicant (p10/43 in B_BE_21_BVW4_Part 1A_CAF_VSV-GP128_revised_18Oct2021.pdf) the host range depends on the envelope protein. Thus, shedding studies in animals other than human do not add substantial info on the shedding and biodistribution of the drug product. The fact that no shedding is observed in other species does not imply that in patients shedding will be similar. In addition, in section 2.18, it is not clear how the in vitro replication kinetics were determined (which cells?).

If no data are available on VSV shedding in human subjects, extra caution should be considered and this should be clearly stated to health care personnel, patients. In the opinion of the expert, the results presented in 2.18 (p21/43 CAF) are promising but cannot be used to consider spreading negligible (conclusion p23/43).

SBB Comment:

This study corresponds to a first-in-human study with the recombinant VSV-GP128. There are no reports of humans transmitting the infection to other humans or to animals. Furthermore, no transmission to sentinel mice co-housed with VSV-GP-treated tumor-bearing mice (n00279795) was observed (CAF, section 2, p8/43). As mentioned in section 3.6 of the CAF_VSV-GP128, the pharmacy staff will need to follow specific instructions during the preparation and the administration of the VSV-GP128 and for patients' management.

In order to help the health care personnel, a 2-4 page 'instructions for study staff personal' that can be provided as a plasticized document to personnel preparing and administering the MP detailing could be prepared. The detail of the information to be provided on this sheet has been developed in comment 1 of point 3.4 below.

Coordinator Comment:

Totally agree and both the health care workers, particularly surgeons, and the family and friends of the patient are a significant risk of exposure. Administration of the VSV-GP128 and for patients' management is done by the study nurses and doctors. A 2-4 page 'instructions for study staff personal' is clearly necessary.

In the opinion of the expert, the arguments included on VSV based vaccines to be safe (p23-24/43 in CAF) do not apply, since VSV-GP contains a different envelope, and may have a different host range and shedding profile. Thus, the first patients treated should be closely followed to underscore the hypothesis that spreading is indeed negligible (in the best case).

When assessing the sampling for viral shedding/viremia, only urine, blood and swabs will be used (p100/154 in B_BE_21_BVW4_Protocol_V10.0_Clean_09Aug2021.pdf). Considering the recommendations (point 3.6 in CAF) given to the patient, the expert would assume that saliva should be included.

SBB Comment:

Shedding-based transmission to third parties among the human population has not been documented by experimental data for VSV-GP128 viral vector. Therefore, the potential risk for third parties due to shedding-based transmission should indeed carefully be assessed during this first in human study involving the VSV-GP128.

As mentioned in the protocol section 1.2.6.4 and under table 13, buccal swabs, nasal swabs and urine samples will be collected until 3 negative, consecutive results are obtained. The viral load in the blood (viremia) will also be tested with the objective to confirm viral clearance. Shedding samples will be collected at Day 15 (VSV-GP-128 injection), Days 19, 22, 29 and 36. Buccal swabs, nasal swabs, urine and blood samples will be collected 3 times on Day 15 (day of VSV-GP128 administration): pre-dose / 1h ±10mn / 8h ±60mn post-dose.

Coordinator Comment:

Yes this is an unknown, and the fact that this will be done in academic centres permits this kind of follow-up.

Additional SBB Comments:

As mentioned here above, buccal swabs, nasal swabs and urine samples will be collected until 3 negative, consecutive results are obtained. Shedding samples will be collected at Day 15 (VSV-GP-128 injection), Days 19, 22, 29 and 36. As mentioned in the protocol, additional samples will be collected beyond Day 36 if three negative, consecutive results are not obtained. The notifier could be requested to clarify at which time point beyond D36, these samples collection for shedding analysis will be performed.

In the non-clinical study with cynomolgus monkey, shedding samples (urine, faeces, nasal and oral swabs) from animals treated with at least 10^7 $TCID_{50}$ of VSV-GP128 have been collected (CAF, section 2.18, p22/43). No data has been reported regarding shedding results from faeces and oral swabs samples. The notifier could be requested to clarify in the CAF document whether viral shedding has been observed in faeces and oral swabs samples. Furthermore, since shedding properties of VSV-GP128 in humans are currently lacking, the notifier could be requested to clarify why no faeces samples will be collected in human for shedding analysis in this first in-human study with VSV-GP128.

Data from the VSV-GP128 cynomolgus monkey GLP toxicity study shows that RNA levels were the highest at Day 17 and Day 18, then decreased over time and were no longer detectable by Day 22 (7 days post-VSV-GP128 injection) (CAF, section 2.18, p22/43). The notifier could be requested to clarify in this section the study design that has been applied for this toxicity study in cynomolgus monkey. Has VSV-GP128 injection been performed at D15 post ATP128 injection?

On p 24 of the CAF, the notifier mentions that 'This VSV oncolytic virus was demonstrated recently to be safe for caregivers, with no viral shedding, even with increased infusion duration (*Merchan et al.*, 2020).' However, the document 'Merchan_2020' provided as reference in the dossier only discloses the abstract and a table, which are not very informative on the shedding data that were collected. More detailed information such as the nature of clinical samples taken, the time point at which samples were taken or the limit of detection are deemed necessary to assess the information in the table. Could the notifier provide further information that would allow a proper assessment of the results reported in the table ?

Coordinator Comment:
Regarding this last additional point suggested by SBB, since the doses are given every three weeks and surgery is planned between 2 doses, this exposure needs to be addressed.

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

Comment 3
See 3.7

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

3. INFORMATION RELATED TO THE CLINICAL TRIAL

3.3. Storage of the clinical vector at the clinical site

(e.g. storage location, conditions of storage, …)

Comment 1

It surprises the expert that the containment level is different at the UZ Leuven and Antwerp (p26/43 in CAF), especially the fact that BSL1 would be sufficient, considering this is a replicating virus. At p10/43 BSL2 is indicated as biosafety classification of the DP.

In the remark in 1.3e KISIMA-01_CAF_Appendix 2 UZL_27Sep2021 the applicant indicates that containment level will be adapted during the periods and times the activities take place. First, the current level is the lowest (HR1/L1) and thus does not need to be adapted. Still, the expert is not sure whether the current product can be considered safe to use under BSL1 since no shedding info is available.
Additionally, the document provided now indicates also for UZA BSL1 containment, whereas the CAF indicated BSL2.

SBB Comment:
As reported in section 3.3 (Safety instruction) of the Pharmacy manual, pg 13/34, VSV-GP128 is classified as Risk Group 2 (BSL class 2) animal pathogen. All procedures that may produce aerosols, or involve high concentrations or large volumes should be conducted in a laminar flow cabinet (class II biological safety cabinet).

In Appendix 1 (UZA, p1) and Appendix 2 (UZL, p1), the following information has been reported:
UZA: Preparation IMP dosage will be performed in a biosafety cabinet (class 2)
UZL: Thawing and reconstitution of the IMP will be performed in a clean room with a BSC class II.

The viral vector VSV-GP128 will be administered using the IV route of injection using a syringe pump. As mentioned in the Appendix 1 (UZA, p1), administration of the viral vector will be performed in the hospitalization unit which present a containment level L1. Administration of the viral vector at UZL will be done in a HR1 level room. Following an internal discussion with our colleagues from the Contained use team, it turns out that the administration of the viral vector to patients could be done in rooms presenting a L1 containment level since the preparation of the viral vector will be performed under a class II Biosafety Cabinet. The use of a syringe pump should reduce the risk of exposure and the risk

of aerosols formation since the flowrate of the VSV-GP128 injection volume will be very low with the pump (10 mL/hour on the pump).

Coordinator Comment:
The coordinator agrees that the PREPARATION needs to be done at BSL2 but that the patient in a BSL1 hospital room (not a shared room) will receive the IMP in a contained system.

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

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

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

3.4. Logistics for on-site transportation of the clinical vector

(information on logistics of in-house transportation, characteristics of the container, disinfection procedures, labelling of the containers, ...)

Comment 1

At UZL there is no description on the logistics for on-site transportation provided. The CAF (p27/43) indicates the SOP is provided in Appendix2, but Appendix2 has no additional information, and the SOP is not accessible (see B_BE_21_BVW4_Part 1C_CAF_Appendix 2 UZL_27Sep2021.pdf).

> **SBB Comment:**
> A related question regarding in-house transportation was also raised in comment 3. Both questions have been combined under comment 3.

In the remark in 1.3e KISIMA-01_CAF_Appendix 2 UZL_27Sep2021 the applicant indicates that containment level will be adapted during the periods and times the activities take place. First, the current level is the lowest (HR1/L1) and thus there is no adaptation (is this considered sufficient?), and second, there is no report/flowchart on how it will be ensured that non-authorized persons for example are excluded to enter the room or facilities. How will the facility (and BSC) be cleaned after preparation of the drug product to prevent contamination of other preparations that will take place in the same room later?

In conclusion, in both UZA and UZL, there is only a general procedure provided on how to handle a product and how to act specifically in case of spills or contamination. In the opinion of the expert, it would be better to have a specific procedure for the use and clean-up (and handling of a possible spill) of the specific MP. There is no clear description on how personnel will be informed on how to handle the specific product, and how to manage a spill.

SBB Comment:
The notifier could be asked to provide a 2-4 page 'instructions for study staff personal' that can be provided as a plasticized document to personnel preparing and administering the MP detailing. This

sheet should include all relevant handling instructions, detailed instructions in case of spill, waste management and other risk management measures:

- Containment Level
    - o For IMP preparation
    - o For IMP administration
    - o Samples collection from the patient
    - o Samples storage
- Personal Protective Equipment (PPE)
    - o For the IMP preparation
    - o For the administration to the patients
    - o For the samples collection from the patient
- Management of inadvertent exposure of human to VSV-GP128 product
    - o Eye exposure from splash or aerosol
    - o Needlestick, sharps exposure or non-intact skin exposure
    - o Contact with skin and clothing
- Management of inadvertent exposure to blood, urine, vomit or other bodily fluids from patients in the initial period at the hospital
- Clean-up procedure
    - o After IMP preparation (specify decontamination solution and minimum contact time)
    - o In case of accidental spill or breakage (specify decontamination solution and minimum contact time)
- Waste Management
    - o During IMP preparation
    - o During IMP administration
    - o During the 8h hospitalisation of the patient
    - o During samples collection from the patient

Coordinator Comment:
Agreed – a detailed protocol sheet as mentioned above is absolutely necessary

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

Comment 3
The information with respect to transport of the GMO-IMP after reconstitution in the pharmacy (UZL) or CCRG, cleanroom (UZA) to a room for GMO therapy administration is very limited. The common application form (page 27) does not especially mention details on this transport step (UZA). For UZL reference is made to a SOP of the pharmacy (B_BE_21_BVW4_UZL_SOP_GMO procedure for UZ Leuven pharmacy) which states on page 18 (section 5.8 Transport of GMO-IMP) that the sponsor is responsible for providing a suitable transport container since there is none available at the UZ Leuven hospital pharmacy. The study protocol should specify such transport container.

The study protocol (B_BE_21_BVW4_Protocol_V10.0_Clean_09Aug2021) and the referred pharmacy manual (B_BE_21_BVW4_VSV-GP128_Pharmacy Manual_v3.2_14Oct2021) mention that syringes prepared should be transported in a disposable impermeable plastic container.

Both hospital sites should specify in greater detail how this transport step will be executed: type of suitable transport container and who is responsible for the availability of such a suitable transport container.

SBB comment:

On the basis of comments 1, 3 and 4 , the following wording is proposed :
In-house transportation of the clinical vector at the UZA has been described in section 3.4.1 of the CAF document. As described in this section, the cryopreserved investigational medical product shall be placed inside a container that carries enough absorbent material inside it to soak up any spill that may occur, and securely closed. The notifier could be asked to clarify what will happen with the absorbent material that will be placed inside the container. Will this absorbent material be treated as potential contaminated waste material and be eliminated as such?
For UZL, although in section 3.4.2 (p27/43) of the CAF, the notifier refers to the SOP "GMO procedure for UZ Leuven pharmacy" (Appendix 2) for the description of the in-house transportation, this description has not been developed in this SOP "GMO procedure for UZ Leuven pharmacy" (Appendix 2). The notifier could be requested to develop, in section 3.4.2 of the CAF, the instructions regarding to the in-house transportation of the clinical vector at the UZL.

According to the protocol, p149/154 (appendix 8), the pharmacy manual p14/34 (section 3.3.2) and the public CAF p28/43 (section 3.6) : Syringes prepared should be transported in a disposable impermeable plastic container. The notifier could also be requested to describe in more details in the 2-4 page 'instructions for study staff personal' the instructions regarding the transportation of the prepared clinical vector from the pharmacy to the hospital room at both sites: type of suitable transport container, labelling of the container… If needle are used, how will the syringe and the needle be protected for the transportation of the reconstitute viral vector in order to ensure protection of staff and environment against an accidental exposure (prick or spill) during this transportation.

Coordinator Comment:

The absorbent material should be treated as potential contaminated waste material and be eliminated as such

Comment 4

Pharmacy manual 3.3.2 Precaution for Study Staff: It's not clear if UZA and UZL will use the CSTD (Closed System Transfer Device) or needles to prepare the IMP (reconstitution of VSV-GP128). If needles are used, how is the syringe and needle protected for the transportation of the reconstitute VSV-GP128 from the pharmacy to the hospital room in order to ensure protection of staff and environment against an accidental exposure (prick or spill) during this transportation? These measures should be precisely described.

SBB Comment:

Regarding the transportation of the reconstitute VSV-GP128 from the pharmacy to the hospital room, a related question was also raised in comment 3. Both questions have been combined under comment 3. According to the pharmacy manual, section 3.4.1, The Sponsor will supply the study drug VSV-GP128. If the clinical site is using a CSTD (Closed System Transfer Device), the 18G needle is not needed. It is the responsibility of the clinical site to check in advance the compatibility of the in-house CSTD with the syringe pump and the requested flow speed.

According to the SOP_GMO procedure for UZ Leuven pharmacy (p6/19), the hospital pharmacy, together with the biosafety coordinator will assess whether CSTD will be used or not for the reconstitution of the vector. No further information have been found for UZA.

3.5. Reconstitution, finished medicinal product and administration to the patients

(e.g. mode of administration, information on dosing and administration schedule, information on concomitant medication,…)

Comment 1

No information is provided on the use of concomitant medication. The statement that 'Necessary supportive measures for optimal medical care will be given throughout the study but do not affect shedding' does not suffice to cover this topic in my opinion.

SBB Comment:
In section 3.5 of the CAF_VSV-GP128, the notifier could be requested to develop which concomitant medications is expected to influence the shedding of the clinical vector and what measures are taken in this respect.

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

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

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

3.6. Measures to prevent dissemination into the environment

(e.g. control measures, PPE, decontamination/cleaning measures after administration or in the case of accidental spilling, waste treatment, recommendation given to clinical trial subjects, …)

Comment 1

The applicant indicates the patient's movement should be limited to a minimum (point b) and g)), but there is no time-window indicated (how long is this to be done?). Can the patient be asked to stay inside the room for a specific time? Could/should the room be equipped with a personal bathroom for the patient to limit (possible) shedding to the environment?

SBB Comment:
As mentioned in section 3.6 point B of the CAF, following their treatment with VSV-GP128, patient's movements within the hospital should be limited to the minimum necessary. When outside the room, the patient must wear a surgical grade mask and ensure that the injection site is covered with a dressing.
In order to further control the spread and unintended release of the viral vector, the notifier could be asked to strongly encourage the patient to stay in his room during his 8 hour stay in hospital. His movement into the hospital during his 8 hour stay should be strictly limited to necessary.
According to the plan of the hospitalization unit at UZA, rooms planned for this study have a

private bathroom. The notifier could be asked to check with UZL if rooms with private bathroom have been foreseen for patients included in this study. The notifier could be suggested to strongly encourage the site to provide rooms with a private bathroom to patients enrolled in this study.

Coordinator Comment:
Totally agree with comment 1, I thought the same thing. How long will the patient be in the hospital room and will they be quarantined in the room for the 8h stay?
Patient's movements within the hospital should be limited. Just for medical exams – should not be allowed to go into the public areas of the hospital.

In point 3.6 c) it is indicated that spill incidents should be reported to the intern prevention service of UZA. Assuming that spills could also occur in UZL (and assuming reporting at UZA would be questionable), this procedure should be included as well for UZL.

SBB Comment:
As mentioned in section 3.6 point C of the CAF, spill incident must be reported to the intern prevention service of UZA. The reporting of any spill incident that occurred at the UZL should also be reported. Therefore, the notifier could be requested to make sure a similar procedure has been put in place at UZL and to adapt the CAF accordingly.

Again, in point d) there is a reference to Appendix2 which is not informative.

SBB Comment:
According to section 3.6 point D of the CAF (p29/43), information related to Elimination or inactivation of left-overs of the finished product at the end of the clinical trial for UZL can be found in Appendix 2 of the CAF document. However, elimination or inactivation of left-overs of the finished product at the end of the clinical trial is not mentioned in the SOP GMO procedure for UZ Leuven pharmacy. Therefore, the notifier could be requested to improve section 3.6.D of the CAF by adding a brief explanation on the management of medical wastes applied at UZL.

In point g) a period of 7 days is suggested. It is not clear on what this period is based. Is this a first period, and may this be adapted depending on the shedding results of the first patients? It is not clear whether the patients expected to remain in the hospital during that period, or would they be allowed to go home?

SBB Comment:
After injection of VSV-GP128 on Day 15, patients will be observed in hospital for 8 hours where after they will be released home.
As reported in section 3.6 point G, several instructions will be given to the patients to prevent dissemination of the viral vector. These instructions are to be implemented for 7 days following VSV-GP128 administration. The notifier is requested to clarify why these instructions should be implemented for 7 days following injection. Since this study corresponds to a First-in-human study, no previous data relating to VSV-GP128 viral vector shedding is available. Although non-clinical data indicate that shedding of infectious VSV-GP128 particles is expected to be negligible, we cannot exclude that VSV-based viral vaccine may be present in biological fluids shed by the human subjects. Therefore, we could suggest the notifier to adapt this time period during which

particular attention should be taken by the patient from 7 days to "until 3 negative, consecutive results are obtained from the RT-PCR analysis of viral shedding samples".

As a general advice, the expert would advise to avoid close contact with any person or living being for at least a minimum time. Would it make sense to advice in addition to avoid close contact with livestock, to include close contact with pets as well?

SBB Comment:
After injection of the viral vector, patients will be recommended to avoid close contact with young children, pregnant women, immunocompromised people and livestock (e.g. pigs, cows, horses, etc.) for 7 days. When unavoidable, a surgical grade mask should be worn when within touching distance.

VSV infection occurs primarily in domesticated cattle, horses, swine, and rarely in llamas and humans. Infection of horses is particularly significant in the US (Rozo-Lopez *et al*., 2018, Insects). According to the Pathogen Safety Data Sheets: Infectious Substances – Vesicular stomatitis virus (VSV), rodents also corresponds to host range of the wt virus. Furthermore, serological surveys have shown that small grass-eating rodents, such as cotton rats and deer mice, might play a role in viral maintenance (Rozo-Lopez *et al*., 2018, Insects).
Small pets, such as cats or dogs, have not been reported as host ranges for the wt VSV. Therefore, the notifier could be requested to adapt the recommendations given to the patient to prevent dissemination of the viral vector by adding rodents in the list of animals to avoid.

Coordinator Comment:
I hope these patients are not living with rodents but you never know – some people do have them as pets!

The combination of instructions is not logic in my opinion. How can one advice to avoid common use of cutlery and drinking vessel, store clothing separately, add bleach to the toilet after use, for safety reasons, but still allow 'protected' intercourse? The expert would assume the treated patients are very sick and would remain in the hospital during the procedure.

SBB Comment:
As mentioned here above, after injection of VSV-GP128 on Day 15, patients will be observed in hospital for 8 hours where after they will be released home.
The notifier could be requested to clarify why patients are still allowed to have protected intercourse when, on the other hand, for safety reasons, they are recommended to avoid common usage of unwashed cutlery, crockery, and drinking vessels, to store any soiled clothing separately from any other people living in the same accommodation and to use a separate toilet when possible and to add bleach or equivalent products to the toilet after each use. In order to be consistent with the recommendations provided to the patient, the notifier could be requested to strongly encourage the patient to abstain from sexual intercourse until 3 negative, consecutive results are obtained from the RT-PCR analysis of viral shedding samples.

Coordinator Comment:
I thought the exact same thing as expert's comment

The expert does not agree with point h). One should consider that in the worst case, when the patient would not respond well to the treatment, or the treatment would not work, that organs could be used for scientific research as well. These organs could be considered as the 'best human model to study these types of cancer and their treatment' (see https://www.nature.com/articles/nbt.4157.pdf). As such donation may occur, and should be considered.
In addition, these patients should be monitored for cancer development, so the expert assumes biopsies may be collected, which also falls under this topic.

SBB and Coordinator Comment:
Section 3.6 point G "Recommendations on donation of blood/cells/tissues/organs by the clinical trial subject" is related to donation of organs to living persons and not to donation of tissue/organs for *in vitro* use for scientific research on biological material. This comment falls therefore beyond the scoop of the ERA.

Additional SBB Comment:
Section 3.6 point B should be improved by developing the personal protective equipment that will be used by the medical staff during the different phases when the viral vector is manipulated: preparation of the medication and administration of the medication to the patient.

Comment 2
Regarding the data reported in the B_BE_21_BVW4_Part 2_SNIF_revised_13Oct2021.pdf document, page 12/16, on the instructions to be given to the patients to prevent dissemination, the expert has the following comment: The list of instructions given to the patients to prevent dissemination is very exhaustive but we have the following question:
1-Should treated patients be abstained from sexual intercourse?
2-Viral shedding (feces, urine, oral and nasal swabs) risk has been assessed as negligible. Is it necessary to disinfect the familial toilets after each use by the treated patient?
3-For the transport of the collected trial waste (e.g. bandages, plasters), stored separately, by the treated patient to the clinical site, at his next visit, what kind of bag or biohazard container is provided to the patient?

SBB Comment:
1- A related question regarding sexual intercourse was also raised in comment 1. Both questions have been combined under comment 1 here above.
2- Though RT-qPCR data from 2 studies of VSV-GP128 in cynomolgus monkeys has not revealed shedding, shedding properties of VSV-GP128 in humans are currently lacking. Therefore, as a precautionary measure, patient could still be asked to disinfect the familial toilets after each use until 3 negative, consecutive results are obtained from the RT-PCR analysis of viral shedding samples
3- For the transport of the collected trial waste (e.g. bandages, plasters), that have to be stored separately, and brought back to the hospital by the treated patient, the notifier could clarify in the documents what kind of bag or biohazard container should be used? Will these bags be provided to the patient?

Additional SBB comment:
Regarding the data reported in section F.4 of the SNIF (p12/16), section 3.6.g of the CAF (p30/43) and section 4.4.1 of the protocol (p56/154) on the instructions to be given to the patients to prevent dissemination, the notifier could be requested to implement the following instruction: Ensure gloves are

worn when changing dressing to ensure patient and close contacts do not come in contact directly with any of the dressings or with the injection site. It could be suggested to collect gloves that were used to change the dressing together with trial waste, to store them separately and to bring them back to the clinical site until 3 negative, consecutive results are obtained from the RT-PCR analysis of viral shedding samples.

If sexual intercourse are still allowed, the notifier could be suggested to instruct the patient to also collect condoms, store them separately and bring them back to the clinical site until 3 negative, consecutive results are obtained from the RT-PCR analysis of viral shedding samples.

Coordinator Comment:
Yes, gloves that were used to change the dressing are part of the waste that should be collected.
Collect condoms this will be difficult to control – these are advanced cancer patients and generally rather ill, I think it is just better to say that they should abstain.

Comment 3
The common application form, the protocol, the investigator's brochure, and the Pharmacy Manual describe sufficiently the necessary measures to prevent dissemination into the environment. Three remarks, however:
1. For inactivation, several different disinfectants are mentioned, but for some of them the exact compound (phenolics) or the precise percentage or concentration is not stated (phenolics, chlorinated phenol, sodium hypochlorite).
2. Measures for decontamination/cleaning/waste treatment after administration and patient care or accidental spill should be summarized in a preferably one-page, plasticized document, which must be made available to the involved health care professionals in the room where the administration of the GMO-IMP and the patient care will take place.
3. Also, the recommendations given to the clinical trial subjects to prevent dissemination should be, next to clear instruction and explanation to them, made available in a preferably one-page, plasticized document to consult at home.

SBB Comment:
1- In the CAF, section 3.6.c (p29/43), the applicant proposed that all disinfectants for enveloped viruses can be used for inactivation of the viral vector VSV-GP128 : 1% cresylic acid, phenolics, chlorinated phenol, 2.5% phenol, 0.4% HCl, 2% sodium orthophenylphenate 14, and sodium hypochlorite.
The notifier is requested to mention in the text the exact compound (phenolics) and/or the precise percentage or concentration in the final solution (phenolics, chlorinated phenol, sodium hypochlorite).
2- A related question regarding technical sheet was also raised in comment 1 at point 3.4. Both questions have been combined under comment 1 at point 3.4 here above.
3- In order for the patients to adhere and practice good hygiene, it is important to explain why measures are taken and what are the likely sources of contaminated material. The applicant could be requested to create a small take home summary (preferably one-page, plasticized document) in order to make sure that the information for the patients can be consulted in a readable format whenever they want.

Coordinator Comment:
Important to provide the details of the different disinfectants.

Regarding point 3, I think this is important and that it is lacking also struck me as an oversight.

Additional SBB Comment:
According to section 3.6.g (Recommendations given to clinical trial subjects to prevent dissemination), the surface injection site will be covered with an airtight and watertight dressing for 2 days following VSV-GP128 treatment. Based on our advice for V920 (dossier B/BE/19/BVW4), the notifier could be recommended to clearly indicate the specificities of the bandage and the modalities of use to prevent fluid from being exposed to others. 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 through the small take home summary.

Comment 4
Pharmacy manual – 3.3.2 Precaution for study staff, the last sentence should also precise that on departure from the room, staff must wash its hands after removing protective equipment.
Pharmacy manual – 3.3.3 Precaution for patients. The single-use tissue used when coughing or sneezing, should be disposed of as biohazard waste material, stored separately and returned to the clinical centre for proper management and final incineration. A specific waste bag or container could be foreseen to collect trial and patient waste at home.
Pharmacy manual – Appendix 3 PCI IMP returns for destruction. Remind the adequate labelling for transport of GMO (UN 3245) where required.

(CAF 3.6 Measures to prevent dissemination into the environment)

SBB Comment
- As mentioned in CAF section 3.6.a (p28/43) and in the pharmacy manual, section 3.3.2 (p14/34), on departure from the room, staff must remove all protective equipment and dispose of appropriately within the patient's room or within pharmacy accordingly. The notifier could be requested to improve the text by clarifying that, on departure from the room, staff must also wash their hands after removing protective equipment.
- Furthermore, in section 3.6.g of the CAF (p30/43) and section 3.3.3 of the pharmacy manual (p15/34), as an additional precaution for the patient, the patient could be informed that single-use tissue used when coughing or sneezing should also be disposed of as biohazard waste material, stored separately and returned to the clinical centre for proper management and final incineration until 3 negative, consecutive results are obtained from the RT-PCR analysis of viral shedding samples.
- A related question regarding the plastic bag/container was also raised in comment 2. Both questions have been combined under comment 2.
- Finally, in the appendix III of the pharmacy manual (p32/34), the notifier could be requested to mention the adequate labelling for transport of GMO (UN 3245) where required.

Coordinator Comment:
Regarding the first point, it's good to state it but this is an SOP

3.7. Sampling and further analyses of samples from study subjects

Comment 1

In B_BE_21_BVW4_Protocol_V10.0_Clean_09Aug2021.pdf info is provided on the sampling (timing) and analyses of the samples, but not on the procedure themselves.

SBB Comment:
In section 7.6.3 of the protocol, the method that will be used to detect the viral RNA (PCR) and the virus functionality ($TCID_{50}$) have been mentioned. Instructions regarding the buccal swabs, nasal swabs and urine collection in Cohort 3 have been developed in the Laboratory Manual.

Coordinator Comment:
This is enough.

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

Comment 3
VSV-GP128 will be administered to humans in a first time in human study. Based on available non-clinical data, viral shedding from VSV-GP128 is expected to be negligible. Shedding and viremia patients, however, will be closely monitored after administration for the patients included in Cohort 3 (first cohort involving administration of VSV-GP128). As shedding is most likely to occur in the period immediately following product administration (fda.gov, europa.eu), buccal swabs, nasal swabs and urine samples will be collected until 3 negative, consecutive results are obtained. The viral load in the blood (viremia) will also be tested with the objective to confirm viral clearance.
What will be the impact of detection of undesirable high levels of shedding or prolonged shedding periods on the execution of the protocol parts for Cohorts 4a and 4b? The protocol mentions that precautions shall be reconsidered based on patients' shedding results. Do any criteria exist concerning the aspect of shedding to determine when and which extra precautions are necessary and could it result in shutdown of the trial for Cohorts 4a and 4b?

SBB Comment:
For this first in-human study with VSV-GP128, after admission of the viral vector VSV-GP128, patients included in cohort 3 will be closely monitored for shedding and viremia analysis. In order to evaluate the possible transmission routes of the viral vector, viral shedding analysis (viral RNA detection by PCR) will be performed at different time points after injection of the viral vector VSV-GP128: Day 15 (pre-dosing), Day 15 (1h post-dosing), Day 15 (8h post-dosing), Days 19-22-29-36, on buccal swabs, nasal swabs and urine samples. These shedding analysis will be performed until 3 negative consecutive PCR results are obtained. As mentioned in section 7.6.3.2.1 of the protocol (p97/154), depending on results at the different time points, the patient may be requested to continue or re-apply patients' precautions. Will these changes also be applied for cohorts 4a and 4b? The notifier could be requested to clarify what will be the impact of detection of undesirable high levels of shedding or prolonged shedding periods on the execution of the protocol part 4 for Cohorts 4a and 4b? In such case, will the protocol be amended accordingly?

The start of Part 4 of the clinical trial will depend on the analysis of the Safety monitoring board (SMB). Upon completion of the 35-day safety evaluation period of the last enrolled patient in Cohort 3, the SMB will, based on available cumulative safety data, decide whether the study may safely proceed with Part 4 (Cohorts 4a, 4b).

Coordinator Comment:
These are cancer patients that may be immunocompromised and thus not eliminate the virus as quickly as a healthy person would.

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

3.8. Emergency responses plans

Comment 1

These procedures are not provided for UZL. Why are these considered confidential and only available via intranet? This information should be available for the HCP but are also an intrinsic part of the ERA application in my opinion.

SBB Comment:
As mentioned in section 3.8 (p31/43) of the CAF, emergency response plans for accidental self-administration during handling or administering the clinical vector and for accidental release into the environment of the clinical vector can be found on the intranet of UZ Leuven. As we don't have access to the intranet of the UZ Leuven, we could ask the notifier to provide us the adequate document in order to evaluate the emergency response plan that have been put in place at the site.

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.

5. ENVIRONMENTAL RISK ASSESSMENT

Comment 1

P33/43 in CAF Hazard identification: data from animal studies strongly suggest that VSV-GP128 is rapidly controlled and eliminated, and that spreading of infectious virus is not expected (see Section 2.18), however, it concerns a different 'host' and the virus is made to replicate in the cancer cells of the patient, which would imply that the titers would be significantly higher following administration in a colorectal cancer patient.

Coordinator Comment:
Yes the virus may replicate to higher titres in the tumour cells AND the liberated virus may accumulate to higher levels in tissues and blood because these patients do not have fully competent immune responses due to their disease and treatment regimes.

For point 5.2, the expert would indicate for clarity that the non-clinical studies for VSV-GP128 were not performed in human (and thus are not relevant but at best indicative). Consequences of unintended transmission are in his opinion difficult to judge now.

SBB Comment:
As it has been mentioned in the SNIF, section B.7.b (p5/16), there are no clinical data currently available for VSV-GP, meaning that this study corresponds to a first in-human study for the VSV-GP128 viral vector. As no clinical shedding data in humans are available yet and as VSV-GP128 presence in biological fluids cannot be excluded, biosafety precautions according to local procedures and legislation will be implemented to avoid any potential transmission to Health Care Professionals, patient's close contacts and to the environment (CAF, section 2, p8/43).

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.

Additional SBB Comment:
The life cycle of VSV involves sandflies and rodent reservoirs. VSV-NJ and VSV-I can be transmitted between livestock by direct contact, likely including droplet spread and fomites, as well as mechanically by non-biting houseflies and face flies. Mechanical transmission by flies and animal-to-animal or animal-to-human transmission may occur.
Referring to the vector-borne properties of the wt-VSV, the notifier could be requested to discuss the probability of transmission by blood-feeding arthropods in view of the observed blood –levels in animal studies. Are there any replication data available of VSV-GP128 in arthropods (e.g. replication data in relevant arthropod cell cultures or live mosquitoes) that can support the minimal risk of transmission through arthropod vectors.

6. OTHER INFORMATION

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

Comment 1

Typo
In section 5.5. it states 'control of control of spread and unintended release'. The expert assumes this should be 'control of spread…'.

SBB Comment:
This comment could be added together with other typo errors.

Comment 2
None

Comment 3
None

Comment 4
None

References

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S.A.Felt *et al.*, Recent advances in vesicular stomatitis virus-based oncolytic virotherapy: a 5-year update, J Gen Virol. 2017 Dec; 98(12): 2895–2911

P. Rozo-Lopez *et al.*, Vesicular Stomatitis Virus Transmission: A Comparison of Incriminated Vectors, Insects 2018,9, 190

Pathogen Safety Data Sheets: Infectious Substances – Vesicular stomatitis virus (VSV) : https://www.canada.ca/en/public-health/services/laboratory-biosafety-biosecurity/pathogen-safety-data-sheets-risk-assessment/vesicular-stomatitis-virus.html