PART 1 (COUNCIL DECISION 2002/813/EC)

SUMMARY NOTIFICATION INFORMATION FORMAT FOR THE RELEASE OF GENETICALLY MODIFIED ORGANISMS OTHER THAN HIGHER PLANTS IN ACCORDANCE WITH ARTICLE 11 OF DIRECTIVE 2001/18/EC

In order to tick one or several possibilities, please use crosses (meaning x or X) into the space provided as (.)

A. General information

1. Details of notification

(a)	Member State of notification	Spain
(b)	Notification number	B/ES/22/12
(c)	Date of acknowledgement of notification	16th June 2022
(d)	Title of the project	Phase I open-label, dose escalation trial of BI 1831169 monotherapy and in combination with ezabenlimab in patients with advanced or metastatic solid tumors
(e)	Proposed period of release	October 2022 (depending on when the resolution is received) to June 2025

2. Notifier

Name of institution or company: Boehringer Ingelheim España, S.A

3. GMO characterisation

VSV-GP is a recombinant Vesicular Stomatitis Virus (VSV) carrying the glycoprotein (GP) of Lymphocytic Choriomeningitis Virus (LCMV) instead of the native VSV-G glycoprotein. It is designed to be replication competent, and its intended purpose is to infect, replicate in and kill interferon-deficient cancer cells. VSV-GP is not designed to express foreign gene products to alter the infected cells permanently; expression ceases if the cell is killed or the virus is eliminated by the host immune response.

(a) Indicate whether the GMO is a:

viroid	(.)
RNA virus	(X)
DNA virus	(.)
bacterium	(.)
fungus	(.)
animal	
- mammals	(.)

- insect (.)
- fish (.)
- other animal (.)

specify phylum, class …

(b) Identity of the GMO (genus and species)

Genus*: Vesiculovirus*
Species*: Vesicular stomatitis virus*

(c) Genetic stability – according to Annex IIIa, II, A(10)

The clinical vector is genetically stable.

4. Is the same GMO release planned elsewhere in the Community (in conformity with Article 6(1)), by the same notifier?
Yes (X) No (.)
If yes, insert the country code(s) AT, BE, DE, FR

5. Has the same GMO been notified for release elsewhere in the Community by the same notifier?
Yes (.) No (X)
If yes:
- Member State of notification …
- Notification number B/../../…

Please use the following country codes:
Austria AT; Belgium BE; Germany DE; Denmark DK; Spain ES; Finland FI; France FR; United Kingdom GB; Greece GR; Ireland IE; Iceland IS; Italy IT; Luxembourg LU; Netherlands NL; Norway NO; Portugal PT; Sweden SE

6. Has the same GMO been notified for release or placing on the market outside the Community by the same or other notifier?
Yes (X) No (.)
If yes:
- Member State of notification USA
- Notification number IND 027886

7. Summary of the potential environmental impact of the release of the GMOs.

The overall risk of the clinical vector for humans is considered to be low. VSV-GP is a recombinant VSV virus expressing the LMCV glycoprotein instead of VSV-G native glycoprotein. Therefore, it is not classified as a human pathogen. Viral integration into the host genome (viral replication of the RNA virus occurs in cytoplasm) as well as recombination with other viruses is highly unlikely. Moreover, it has low ability to survive outside the host as it is inactivated by sunlight and does not remain viable for long periods in the environment.

The risk of infection or disease in livestock animals and rodents is a theoretical concern due to the nature of the wild-type (wt)VSV, with its natural host population including domesticated cattle, horses, and swine. LCMV glycoprotein does not pose an additional risk.
In conclusion, risks to humans and the environment from exposure to the VSV-GP are expected to be very low.

B. Information relating to the recipient or parental organism from which the GMO is derived

1. Recipient or parental organism characterisation:

VSV-GP is derived from the published vesicular stomatitis virus sequence (VSV-Indiana serotype). This wtVSV will be described in the following sections.

(a) Indicate whether the recipient or parental organism is a:

(select one only)

viroid		(.)
RNA virus		(X)
DNA virus		(.)
bacterium		(.)
fungus		(.)
animal		
-	mammals	(.)
-	insect	(.)
-	fish	(.)
-	other animal	(.)

(specify phylum, class) …

other, specify …

2. Name

(i)	order and/or higher taxon (for animals)	*Rhabdoviridae family*
(ii)	genus	*Vesiculovirus*
(iii)	species	*Vesicular stomatitis virus*
(iv)	subspecies	…
(v)	strain	Indiana
(vi)	pathovar (biotype, ecotype, race, etc.)	VSV
(vii)	common name	…

3. Geographical distribution of the organism

(a) Indigenous to, or otherwise established in, the country where the notification is made:

Yes (.) No (X) Not known (.)

(b) Indigenous to, or otherwise established in, other EC countries:

(i) Yes (.)

If yes, indicate the type of ecosystem in which it is found:

Atlantic	..
Mediteranean	..
Boreal	..
Alpine	..
Continental	..
Macaronesian	..

(ii) No (X)

(iii) Not known (.)

(c) Is it frequently used in the country where the notification is made?

Yes (.) No (X)

(d) Is it frequently kept in the country where the notification is made?

Yes (.) No (X)

WtVSV is reported to exist exclusively in the western hemisphere. It is maintained in stable ecologic niches in Central and South America and Mexico and emerges from tropical areas to cause sporadic epidemics in cooler climates during the summer months.

4. Natural habitat of the organism

(a) If the organism is a microorganism

water	(.)
soil, free-living	(.)
soil in association with plant-root systems	(.)
in association with plant leaf/stem systems	(.)
other, specify …	(X)

The natural hosts of wtVSV primarily include domesticated cattle, horses and swine, and rarely sheep, goats, and camelids. Under laboratory as well as field conditions, it was demonstrated that infection by wtVSV is possible in other animal species, such as rodents and rabbits. A definitive natural host reservoir remains unclear and transmission cycles between vectors and wildlife have not been established.

(b) If the organism is an animal: natural habitat or usual agroecosystem:

Not applicable

5. (a) Detection techniques

RT-PCR

(c) Identification techniques

Sequencing

6. Is the recipient organism classified under existing Community rules relating to the protection of human health and/or the environment?

Yes (X) No (.)

If yes, specify

- VSV is assigned to risk group 2 according to DIRECTIVE 2000/54/EC OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 18 September 2000 on the protection of workers from risks related to exposure to biological agents at work (seventh individual directive within the meaning of Article 16(1) of Directive 89/391/EEC).
- According to position statement by the ZKBS (BVL, Germany): VSV is assigned to risk group 2 (BSL 2).
- According to Austrian GTG § 6 VSV is assigned to risk group 2 (BSL 2).

7. Is the recipient organism significantly pathogenic or harmful in any other way (including its extracellular products), either living or dead?

Yes (X) No (.) Not known (.)

If yes:

(a) to which of the following organisms:

humans	(X) generally apathogenic, disease is considered mild and self-limiting
animals	(X) may affect livestock with a self-limiting, non-lethal disease
plants	(.)
other	(.)

(b) give the relevant information specified under Annex III A, point II. (A)(11)(d) of Directive 2001/18/EC

WtVSV is not considered a human pathogen, however, it was reported that humans living in enzootic areas have a high seroprevalence rate and that intimate contact with infected animals may lead to infection of humans with flu-like symptoms. It is believed that transmission to humans occurs through direct contact with active lesions or saliva containing infective wtVSV. There are no reports of humans transmitting the infection to other humans or to animals, although transmission via contaminated equipment, hands, gloves, and clothing probably occurs. Veterinarians, animal health technicians, livestock handlers, laboratory personnel and others working closely with infected animals or live virus are at increased risk. Nevertheless, most seropositive people have not had clinical disease, or have had mild disease symptoms (usually a mild flu-like illness).

8. Information concerning reproduction

(a) Generation time in natural ecosystems:

The incubation period in animals is usually two to eight days; however, longer or shorter incubation periods have also been reported. In contrast, lesions or fever develop in 1-3 days in some experimentally infected horses and swine.
VSV can be transmitted to humans who come in close contact with infected animals. The incubation period is most commonly 3 to 4 days. The most common clinical manifestation is a limited, 3- to 5-day flu-like illness.

(b) Generation time in the ecosystem where the release will take place:

Generation time is supposed to be comparable in ecosystem.
…

(c) Way of reproduction: Sexual .. Asexual X..

Factors affecting reproduction:

Not applicable

9. Survivability

(a) ability to form structures enhancing survival or dormancy:

(i)	endospores	(.)
(ii)	cysts	(.)
(iii)	sclerotia	(.)
(iv)	asexual spores (fungi)	(.)
(v)	sexual spores (funghi)	(.)
(vi)	eggs	(.)
(vii)	pupae	(.)
(viii)	larvae	(.)
(ix)	other, specify	…

(b) relevant factors affecting survivability:

VSV is inactivated by sunlight and does not remain viable for long periods in the environment except in cool, dark places. Common disinfections agents (alcohols, aldehydes, and detergents) appear to be highly efficient for virus inactivation, as well as the temperature higher than 55°C.
VSV is susceptible to all disinfectants for enveloped viruses and is inactivated by 1% cresylic acid, phenolics, chlorinated phenol, 2.5% phenol, 0.4% HCl, 2% sodium orthophenylphenate 14, and 1% sodium hypochlorite. They are inactivated by heating (60°C, 30min), and can survive temporarily on contaminated surfaces.

10. (a) Ways of dissemination

VSV transmission between natural hosts occur through the bite of sand flies and may also occur via direct contact with an active lesion that contains high concentration of infectious virus, but this is unlikely to result in widespread dissemination. Moreover, the virus may spread through water troughs, milking equipment, feed and hands within livestock animal husbandry. The virus is inactivated by sunlight and does not remain viable for long periods in the environment except in cool, dark places. However, intimate contact with infected animals may lead to infection of humans with flu-like symptoms. A definitive natural host reservoir remains unclear and transmission cycles between vectors and wildlife have not been established.

(b) Factors affecting dissemination
Not known

11. Previous genetic modifications of the recipient or parental organism already notified for release in the country where the notification is made (give notification numbers)

A search done in the "GMO Register of Deliberate Release and Placing on the EU Market of GMOs" in the JRC webpage, discovered two SNIFFs published related to the experimental Ebola Vaccine VSVΔG-ZEBOV. The notification numbers are B/DE/14/2247 and B/ES/15/09.

C. Information relating to the genetic modification

1. Type of the genetic modification

(i)	insertion of genetic material		(X)
(ii)	deletion of genetic material		(X)
(iii)	base substitution		(.)
(iv)	cell fusion		(.)
(v)	others, specify	…	reverse genetics system

2. Intended outcome of the genetic modification

VSV-GP is a recombinant VSV carrying the GP of LCMV instead of the native VSV-G glycoprotein. The GP of the LCMV abrogates neurotoxicity even after direct injection of high VSV-GP doses directly into the brain.

3. (a) Has a vector been used in the process of modification?
Yes (X) No (.)

If no, go straight to question 5.

(b) If yes, is the vector wholly or partially present in the modified organism?
Yes (.) No (X)

If no, go straight to question 5.

4. If the answer to 3(b) is yes, supply the following information

(a) Type of vector

plasmid	(.)
bacteriophage	(.)
virus	(.)
cosmid	(.)
transposable element	(.)
other, specify	…

(b) Identity of the vector
(c) Host range of the vector
(d) Presence in the vector of sequences giving a selectable or identifiable phenotype

Yes (.) No (.)

antibiotic resistance	(.)
other, specify	…

Indication of which antibiotic resistance gene is inserted

(e) Constituent fragments of the vector

(f) Method for introducing the vector into the recipient organism

(i)	transformation	(.)
(ii)	electroporation	(.)
(iii)	macroinjection	(.)
(iv)	microinjection	(.)
(v)	infection	(.)
(vi)	other, specify	(.)

5. If the answer to question B.3(a) and (b) is no, what was the method used in the process of modification?

(i)	transformation	(.)
(ii)	microinjection	(.)
(iii)	microencapsulation	(.)
(iv)	macroinjection	(.)
(v)	other, specify …	

6. Composition of the insert

(a) Composition of the insert

The gene encoding for LCMV WE-HPI glycoprotein was inserted without any other regulatory sequences.

(b) Source of each constituent part of the insert

See under 6 (a).

(c) Intended function of each constituent part of the insert in the GMO

The function of the glycoprotein is the viral attachment to the host cell to mitigate viral entry into the cell. The replacement of the wtVSV glycoprotein by the LCMV glycoprotein abrogates neurotoxicity.

(d) Location of the insert in the host organism

- on a free plasmid (.)
- integrated in the chromosome (.)
- other, specify … Integration into (-)ssRNA genom

(e) Does the insert contain parts whose product or function are not known?
Yes (.) No (X)
If yes, specify …

D. Information on the organism(s) from which the insert is derived

1. Indicate whether it is a:

viroid (.)
RNA virus (X)
DNA virus (.)
bacterium (.)
fungus (.)
animal
- mammals (.)
- insect (.)
- fish (.)
- other animal (.)
(specify phylum, class) …

other, specify

2. Complete name

(i) order and/or higher taxon (for animals) …
(ii) family name for plants …
(iii) genus *Mammarenavirus*
(iv) species
Lymphocytic choriomeningitis mammarenavirus
(v) subspecies …
(vi) strain …
(vii) cultivar/breeding line …
(viii) pathovar …
(viii) common name

lymphocytic choriomeningitis virus (LCMV)

3. Is the organism significantly pathogenic or harmful in any other way (including its extracellular products), either living or dead?
Yes (X) No (.) Not known (.)
If yes, specify the following:

(b) to which of the following organisms:

humans (.)
animals (X)
plants (.)
other …

The natural host of LCMV is the house mouse (*Mus musculus*) but infections of pet rodents and humans have also been reported (Lymphocytic choriomeningitis - Information from the CDC).

(b) are the donated sequences involved in any way to the pathogenic or harmful properties of the organism
Yes (X) No (.) Not known (.)

If yes, give the relevant information under Annex III A, point II(A)(11)(d):

The LCMV WE-HPI glycoprotein is known to bind to surface receptors, including but not limited to α-dystroglycan (αDG), which is a ubiquitously expressed cell-surface receptor. The binding properties of the glycoprotein of LCMV WE-HPI and the availability of multiple alternative receptors confer a broad range of susceptible host cells and organisms.

4. Is the donor organism classified under existing Community rules relating to the protection of human health and the environment, such as Directive 90/679/EEC on the protection of workers from risks to exposure to biological agents at work?
Yes (X) No (.)

If yes, specify
- LCMV is assigned to risk group 2 according to DIRECTIVE 2000/54/EC OF THE EUROPEAN PARLIAMENT AND OF THE COUNCIL of 18 September 2000 on the protection of workers from risks related to exposure to biological agents at work (seventh individual directive within the meaning of Article 16(1) of Directive 89/391/EEC).
- According to position statement by the ZKBS (BVL, Germany): LCMV is assigned to risk group 2 (BSL 2).
- According to Austrian GTG § 6 LCMV is assigned to risk group 2 (BSL 2).

5. Do the donor and recipient organism exchange genetic material naturally?
Yes (.) No (X) Not known (.)

E. Information relating to the genetically modified organism

1. Genetic traits and phenotypic characteristics of the recipient or parental organism which have been changed as a result of the genetic modification

(a) is the GMO different from the recipient as far as survivability is concerned?
Yes (.) No (X) Not known (.)
Specify wtVSV, VSV-GP is susceptible to all disinfectants for enveloped viruses and is inactivated by 1% cresylic acid, phenolics, chlorinated phenol, 2.5% phenol, 0.4% HCl, 2% sodium orthophenylphenate 14, and 1% sodium hypochlorite. They are inactivated by heating (60°C, 30min), and can survive temporarily on contaminated surfaces.

(b) is the GMO in any way different from the recipient as far as mode and/or rate of reproduction is concerned?
Yes (.) No (.) Unknown (X)
Specify …

(c) is the GMO in any way different from the recipient as far as dissemination is concerned?
Yes (X) No (.) Not known (.)
Specify …

Due to the replacement of the VSV wild-type glycoprotein with the GP from LCMV the tropism is altered. VSV-GP is incapable of infecting neurons.

(d) is the GMO in any way different from the recipient as far as pathogenicity is concerned?
Yes (X) No (.) Not known (.)
Specify …

VSV tropism is mediated by the VSV glycoprotein allowing the infection of a wide variety of eukaryotic cell types from a broad range of host species. This pantropism is due to the widespread expression of the LDL receptor, which serves as the major cellular entry port for the virus. Moreover, VSV G glycoprotein allows the virus to enter neurons, were the lack of Interferon response leads to uncontrolled viral replication and neurotoxicity. The LCMV WE-HPI glycoprotein was chosen to replace the VSV-G protein as it had been described not to allow entry to neurons. In fact, it was experimentally demonstrated that VSV containing the LCMV glycoprotein instead of VSV-G leads to abrogation of neurotoxicity. Furthermore, non-clinical studies in both mice and pigs showed that the exchange of the glycoproteins removed the risk of pathogenicity.

2. Genetic stability of the genetically modified organism

Genetically stable

3. Is the GMO significantly pathogenic or harmful in any way (including its extracellular products), either living or dead?
Yes (.) No (X) Unknown (.)

(a) to which of the following organisms?

humans (.)
animals (.)
plants (.)
other …

(b) give the relevant information specified under Annex III A, point II(A)(11)(d) and II(C)(2)(i)
…

4. Description of identification and detection methods

(a) Techniques used to detect the GMO in the environment
RT-PCR

(b) Techniques used to identify the GMO
Sequencing

F. Information relating to the release

1. Purpose of the release (including any significant potential environmental benefits that may be expected)

The purpose of the release of VSV-GP in a clinical trial investigation is to evaluate its dosage range, safety, tolerability, and anti-tumor activity in patients with advanced or metastatic solid tumors.

2. Is the site of the release different from the natural habitat or from the ecosystem in which the recipient or parental organism is regularly used, kept or found?
Yes (X) No (.)
If yes, specify Controlled release in phase I clinical site.

3. Information concerning the release and the surrounding area

(a) Geographical location (administrative region and where appropriate grid reference):

(a 1) START Madrid – Fundación Jiménez Díaz
Hospital Fundación Jiménez Diaz
Avenida Reyes Católicos, 2
Phase I Clinical Trials, Floor 1
28040 Madrid, Spain

(a 2) Clínica Universidad de Navarra
Unidad Central de Ensayos Clínicos
7ª planta – II fase
Avenida Pio XII, 36
31008 Pamplona

(b 1) Size of the site (m^2):
(i) actual release site (m^2):
Private hospital room (25 m^2)
(ii) wider release site (m^2):
It is not anticipated to be needed.

(b 2) Size of the site (m^2): …
(i) actual release site (m^2):
Day hospital: 5 m^2
Hospital floor: 15 m^2
(ii) wider release site (m^2):
It is not anticipated to be needed.

(c) Proximity to internationally recognised biotopes or protected areas (including drinking water reservoirs), which could be affected:
Not applicable as administration will only occur in the clinic.

(d) Flora and fauna including crops, livestock and migratory species which may potentially interact with the GMO
With the procedures implemented to reduce spread of VSV-GP to the environment, it is highly unlikely that any animals will come into direct contact with the virus. Data from animal studies has indicated that viral shedding poses a negligible risk. However, patients are still given extensive biosafety advice including contact with livestock and rodents before being discharged from the clinical facility, to further decrease the possibility of the GMO spreading to other species.

4. Method and amount of release

(a) Quantities of GMOs to be released:

Part 1: BI 1831169 will be tested at doses of $5x10^7$, $5x10^8$, $5x10^9$ and $5x10^{10}$ $TCID_{50}$.
Part 2: BI 1831169 will be tested initially at one dose level less than the RP2D dose for the corresponding arm in Part 1 in addition to ezabenlimab.
In both part 1 and part 2, BI 1831169 will be administered 5 times.

(b) Duration of the operation:
October 2022 (Depending on the resolution date) - June 2025

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

Appropriate risk management strategies are in place to communicate and minimise the risks of exposure to live animal stock, including:

- Design of the viral construct (abrogated neurotoxicity)
- Control of virus spread or unintended release
- Transportation precautions
- Administration precautions
- Cleaning and waste management
- Communication of risks and precautions to health care providers and patients
- Appropriate activities are proposed to monitor the release of VSV-GP

The specific measures to ensure the minimizing the risk of virus transfer to livestock animals, rodents and the environment:

- Isolation of the patient during and after the treatment, surgical grade mask wearing for the 10 following days to each treatment. Note isolation during and after treatment covers the time the patient is in the hospital for observations by keeping them in a private room. Patients will also be expected to avoid close contact with vulnerable populations for 10 days after administration, including those people they live with, or wear a mask if close contact is unavoidable.
- Providing the clear instructions to the subject of treatment to avoid the contact with livestock animals (e.g. pigs, cows, horses, etc.) and rodents during 10 days after the administration.

Regular measures related to biohazard waste will be applied per site-specific practices. Measures at the clinical site will be taken to minimize dissemination of the genetically modified organisms to the environment during patient administration, control after administration, patient sample handling and disposal of infectious waste, all as per local protocols and regulations.

5. Short description of average environmental conditions (weather, temperature, etc.)

Not applicable (the risk of release is unrelated to the Spanish climate).

6. Relevant data regarding previous releases carried out with the same GMO, if any, specially related to the potential environmental and human health impacts from the release.

Not applicable.

G. Interactions of the GMO with the environment and potential impact on the environment, if significantly different from the recipient or parent organism

1. Name of target organism (if applicable)

(i)	order and/or higher taxon (for animals)	Primate
(ii)	family name for plants	…
(iii)	genus	Homo
(iv)	species	Homo sapiens

(v)	subspecies	…
(vi)	strain	…
(vii)	cultivar/breeding line	…
(viii)	pathovar	…
(ix)	common name	human

2. Anticipated mechanism and result of interaction between the released GMOs and the target organism (if applicable)

The main objective of the clinical development programme is to assess the safety and tolerability of VSV-GP. VSV-GP is used in this to increase the strength of the host immune response against tumor cells.

3. Any other potentially significant interactions with other organisms in the environment

Due to the decreased transmission risk, VSV-GP is unlikely to be transmitted to other organisms in the environment. Nevertheless, patients are given biosafety advice to follow in the 10 days after treatment, such as avoiding contact with livestock and rodents, and using appropriate waste disposal measures, e.g. for plasters that were in direct contact with the VSV-GP injection site.

4. Is post-release selection such as increased competitiveness, increased invasiveness for the GMO likely to occur?

Yes (.) No (X) Not known (.)

5. Types of ecosystems to which the GMO could be disseminated from the site of release and in which it could become established

Unknown

6. Complete name of non-target organisms which (taking into account the nature of the receiving environment) may be unintentionally significantly harmed by the release of the GMO

(i)	order and/or higher taxon (for animals)	…
(ii)	family name for plants	…
(iii)	genus	…
(iv)	species	…
(v)	subspecies	…
(vi)	strain	…
(vii)	cultivar/breeding line	…
(viii)	pathovar	…
(ix)	common name	…

None.

7. Likelihood of genetic exchange in vivo

(a) from the GMO to other organisms in the release ecosystem:

VSV-GP is a single-stranded RNA virus, which does not use DNA to replicate. Since replication occurs in the cytoplasm, the RNA genome and the human host DNA do not come into close contact. Consequently, risk of genes being transferred from the virus to humans is considered negligible.

(b) from other organisms to the GMO:

The VSV-GP genome is tightly bound by the nucleoprotein to a structure called nucleocapsid. For this reason, it is unlikely to come into contact with or swap genetic information with other viruses.

(d) likely consequences of gene transfer:

Recombination is only likely between VSV-GP viruses. However, recombination in this case leads to production of similar VSV-GP that exhibits equal infectious or pathogenic properties. In consequence, VSV-GP gene transfer is considered to be low risk.

8. Give references to relevant results (if available) from studies of the behaviour and characteristics of the GMO and its ecological impact carried out in stimulated natural environments (e.g. microcosms, etc.):
VSV-GP is expected to be degraded after administration to humans by endogenous protein and DNA catabolic pathways. Shed virus or vector RNA is not expected to be stable in wastewater. In pig studies it could be shown that VSV-GP was apathogenic.

9. Possible environmentally significant interactions with biogeochemical processes (if different from the recipient or parental organism)
Not applicable.

H. Information relating to monitoring

1. Methods for monitoring the GMOs

Shedding of the virus will be assessed in the following samples, collected from all patients that have received VSV-GP:

- Buccal swabs,
- Nasal swabs,
- i.v./i.t. administration site swabs,
- Urine

The collected samples described above will be assessed by qPCR and viral culture.

The management of the GMO within the clinical trial is described below:

(1) Transport and storage:

<u>Site 1: START Madrid – Fundación Jiménez Díaz:</u>

Once the diluted or undiluted syringes have been prepared, they will be stored in Phase I Pharmacy, at room temperature (if the administration is going to be in the next minutes) or in fridge. We will dispense the prepared syringes to the treatment

nurses in a disposable impermeable plastic container through the window that communicates Phase I Pharmacy with treatment room of the Unit.

Site 2: Clínica Universidad de Navarra:

Once prepared, the medicinal product will be placed in a sterile bag (secondary packaging material), which will be identified with patient's details, drug, route of administration and storage conditions. In all cases, the safety aspects specified in procedures FTC.MIV.OF.002 ("Handling of cytostatics and other hazardous medicinal products") and FTC.MIV.OF.003 ("Systematic processing of cytostatics and other hazardous medicinal products") will be followed.

For storage until dispensing and for transport through the clinic, the preparation will be placed in an airtight container, labelled with the biohazard label. The temperature conditions required by the sponsor will be maintained.

Dispensing will be carried out individually for each preparation. Dispensing will be as close as possible (in time) to the moment when the drug is to be administered. The aim is to avoid storage at the site of administration and thus to keep the number of sites exposed to contamination to a minimum.

(2) Preparation of the VSV-GP solution:

Site 1: START Madrid – Fundación Jiménez Díaz:

Preparation of solution takes place in Phase I Pharmacy and will be done using a Class IIB Biosafety Cabinet following the Pharmacy Manual instructions.

Before and after the GMO preparation, the cabinet has to be cleaned with disinfectant and alcohol 70ºC. The cabinet will be without preparing other treatments for 30 minutes. The material and used vials will be disposed in class 3 biosanitary containers for biohazardous waste.

Site 2: Clínica Universidad de Navarra:

The preparation will take place in the Pharmacotechnical Unit. Access to the room is limited to authorized personnel. During the entire process of preparation, the door of the room will be marked with the pictogram "biohazard" and the warning "restricted access". All drug dilution processes will be carried out in the Class II biological safety vertical flow cabinet (CFLV-SB) with external air extraction. The cabinet disinfection and decontamination procedures specified in the relevant document (procedure FTC.MIV.GE.005) will be followed. The Operator will at all times follow the general working rules of the Pharmacotechnics and Aseptic Preparations Unit (procedure FTC.MIV.OF.001). He/she will use the personal protective equipment (PPE) provided for the handling of cytostatics and other hazardous medicinal products: gown, impermeable on the front and sleeves, fitted with elastic cuffs; gloves; hat, mask and leggings.

2. Methods for monitoring ecosystem effects

Not applicable as VSV-GP is not expected to disseminate in the environment or ecosystem. No environmental samples are analysed to check for environmental dissemination.

3. Methods for detecting transfer of the donated genetic material from the GMO to other organisms
Not applicable as VSV-GP cannot be integrated into the genome as replication takes only place in the cytoplasm.

4.1 Size of the monitoring area (m^2): Not applicable. A specific liberation site size cannot be set as the VSV-GP will be administered to patients in the hospital within the framework of a clinical trial.

4.2 Size of the monitoring area (m^2): Not applicable. A specific liberation site size cannot be set as the VSV-GP will be administered to patients in the hospital within the framework of a clinical trial.

5. Duration of the monitoring
The monitoring of the GMO will be from production until either destruction at the site or depot. The patient will be monitored for shedding of the virus from the day of the first treatment administration until the end of treatment visit.

6. Frequency of the monitoring
After administration of the GMO the patient will be regularly monitored for viral shedding at every visit between the start of treatment until the end of treatment.

I. Information on post-release and waste treatment

1. Post-release treatment of the site
START Madrid – Fundación Jiménez Díaz:

Waste is disposed of in black containers. An exhaustive cleaning is done with bleach.

Clínica Universidad de Navarra:

All waste generated shall be managed as biological waste.

Disinfection shall be performed in accordance with established internal procedures.

2. Post-release treatment of the GMOs

(a) Type and amount of waste generated

Empty vials and used vials and the used delivery system components (e.g. injection needle, catheter, and syringe), gauzes and personal protective equipment and components used for collecting body fluids samples after administration. Equipment used in the preparation of the material (luer-lock connectors, stopper, needles).

(b) Treatment of waste
START Madrid – Fundación Jiménez Díaz:

The waste is deposited in black containers and then the biological waste system is followed, through the hospital's Waste Management System.

Clínica Universidad de Navarra:

The waste will be managed as biological waste and will be destroyed by the external company Consenur.

J. Information on emergency response plans

1. Methods and procedures for controlling the dissemination of the GMO(s) in case of unexpected spread

In case of self-accidental injection of medical personnel, the injection site will be disinfected, and personnel will be followed up in case of symptoms related to immune reaction against VSV-GP.

2. Methods for removal of the GMO(s) of the areas potentially affected

After patient's discharge, potentially contaminated surfaces (e.g. bathroom equipment: faucet, toilet, sink, etc.), room furniture (nightstand, table, chair, floor, handrails etc.) should be disinfected following applicable local cleaning procedures.

Any spills or soiled material handled per standard procedures for infectious/contaminated material.

- Inactivation: BI 1831169 is susceptible to all disinfectants for enveloped viruses and is inactivated by 1% cresylic acid, phenolics, chlorinated phenol, 2.5% phenol, 0.4% HCl, 2% sodium orthophenylphenate 14, and 1% sodium hypochlorite. Physical inactivation: BI 1831169 is inactivated by heating (60°C, 30min). BI 1831169 survives temporarily on contaminated surfaces.

- Handling of spills: Inform and warn colleagues in direct proximity. Allow aerosols to settle and, wearing protective clothing, gently cover spill with paper towels and apply appropriate disinfectant, starting at the perimeter and working towards the centre. Allow sufficient contact time before cleaning up (30 min).

3. Methods for disposal or sanitation of plants, animals, soils, etc. that could be exposed during or after the spread

Not applicable.

4. Plans for protecting human health and the environment in the event of an undesirable effect

Even though the release of the GMO from the patients to a third party is highly unlikely, preventive measures are defined in order to counteract in such case; a fast and accurate diagnosis is possible by PCR measurements, and symptomatic treatment can be given in the case that flu-like symptoms appear, when a positive diagnosis is confirmed.