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/15/09
(c) Date of acknowledgement of notification: 13/August/2015
(d) Title of the project

Phase III clinical trial, randomized, placebo-controlled study to evaluate the safety and the immunogenicity of three batches of stability and a lot of high doses of rVSV-ZEBOV-GP (vaccine against Ebola virus V920) in healthy adults

(e) Proposed period of release

From Sep/2015- until Jul/2016 (around 10 months from first patient recruited to last patient last visit)

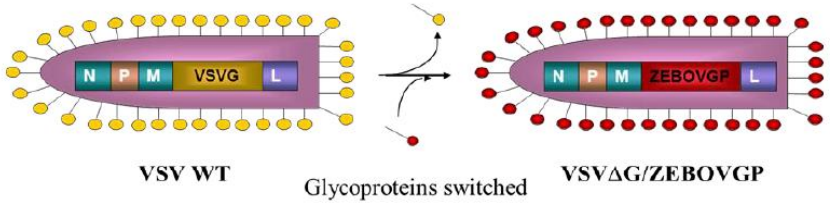
2. Notifier

Name of institution or company:

Cristina Alzina Fernandez-Figares
Merck Sharp & Dohme de España S.A
Calle Josefa Valcárcel n 38, 28027, Madrid

3. GMO characterisation

rVSVΔG-ZEBOV GP/ BPSC-1001/V920 is a live attenuated recombinant virus consisting of a single rVSV isolate (11481 nt, strain Indiana) with the gene for the ZEBOV GP, Kikwit strain, replacing the deleted VSV GP gene.



The basis for the attenuation of the rVSVΔG-ZEBOV GP virus has not been determined, but it is likely due to chimerization with replacement of the native envelope GP with a heterologous gene. In making rVSVΔG-ZEBOV GP, the VSV GP gene has been replaced with the EBOV GP gene. Wild-type VSV has a broad tropism (Hastie et al., 2013), whereas the rVSVΔG-ZEBOV GP has a different, and possibly narrower cellular tropism more reflective of ZEBOV. Hepatocytes, endothelial cells, dendritic cells, monocytes, and macrophages, all of which express C-type lectins, are thought to be preferred target cells of filoviruses (Hoenen et al., 2006). It is possible that this somewhat more restricted tropism contributes to the attenuation of the vaccine virus, including the absent neurovirulence attributed to vaccine vectors expressing the wild-type VSVG envelope GP.

(a) Indicate whether the GMO is a:

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

specify phylum, class …

rVSVΔG-ZEBOV GP/ BPSC-1001/V920 was generated by deleting the VSV GP gene and inserting the ZEBOV-GP gene in the new transcriptional unit after the VSV M gene.

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

The names of the GMO are: BPSC-1001 (initial code for preliminary studies), rVSVΔG-ZEBOV GP (generic name) or V920 (actual product code). All three codes identify the same product.

This rVSVΔG-ZEBOV GP/ BPSC-1001/V920 vaccine was derived from the VSV-Indiana serotype. As stated above, the rVSV-based vector used in this vaccine lacks the VSV GP, the viral determinant for neurotropism and pathogenicity. The vaccine with ZEBOV GP replacement has approximately 33% slower growth kinetics than wild-type VSV in Vero cell cultures, contributing to attenuation. The rVSVΔG-ZEBOV GP0 virus is apathogenic for hamsters, whereas wild-type VSV is lethal after peripheral inoculation (Feldmann H., unpublished data, 2014).

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

Two private companies and the Special Pathogens Department (SP) at the National Microbiology Laboratory (NML) of the Public Health Agency of Canada (PHAC) were involved in manufacturing this vaccine. The plasmids were produced according to cGMP specifications. Given technical difficulties with transfection of the plasmids in Vero cells alone, SP transfected the plasmids into a mixed culture containing a 1:1 ratio of HEK293 to

Vero cell in order to rescue the rVSVΔG-ZEBOV GP virus. Then a limiting dilution plaque purification was performed through sequential rounds of plaque purification and one clone was selected for manufacture of the Master Seed Virus, as it was free from any mutations in the original EBOV GP plasmid sequence. Each vaccine batch is tested using molecular genetic methods to ensure the presence of the Ebola GP insert and is tested in a cell-based assay for the ability to infect cells and produce the Ebola virus glycoprotein, using a Western blot approach.

rVSVΔG-ZEBOV GP/ BPSC-1001/V920 vaccine intended for clinical use was placed on concomitant stability testing according to International Conference on Harmonization (ICH) guidelines to ensure that it met specifications during the course of clinical trials. The final filled containers are being tested for stability at the intended storage temperature for 36 months. The biological drug substance before formulation is stable in long term storage at ≤ -60°C and for at least 4 weeks at 2-8°C. The vaccine in the final filled container is stable after thawing at 2-8ºC and room temperature to enable use in the clinic for a work-day. The vaccine should, however, preferably be held at 2-8ºC after thawing. This was shown for both the post-exposure (1×10^8 pfu/mL) dosage formulation, as well as for vaccine diluted for use in clinical trials to 3×10^6, 3×10^5, 3×10^4, and 3×10^3 pfu/mL. At 37ºC the virus loses infectivity after 2 hours.

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) …

rVSVΔG-ZEBOV GP/ BPSC-1001/V920 is a leading Ebola vaccine candidate that is currently under investigation in several clinical trials phase I/Ib/II/III. The main objective of the clinical development program is to achieve the vaccine registration and to impact the ongoing epidemic of Ebola Virus is West Africa.

At present there are eight clinical trials of phase I/Ib ongoing in the United States, Canada, Germany, Switzerland and two African countries not affected by the epidemic active Ebola virus (Gabon and Kenya). In these trials, more than 500 volunteers have received the vaccine in varying doses. V920 is also being studied in three Phase II/III trials of safety and efficacy in countries in West Africa affected by the active epidemic of Ebola virus (Liberia, Guinea and Sierra Leone, Africa). All these trials are sponsored by international organizations foreign, including the National Institutes of Health in the United States (Liberia), the World Health Organization (Guinea) and the Centers for Disease Control and Prevention United States (Sierra Leone). Additional information about the clinical trials is described in the section 5 of the Investigation Brochure (Annex 1). The studies are summarized in the following table.

Study Sponsors and Study Phase	Location
US National Institutes of Health, Walter Reed Army Institute of Research, University of Dalhousie, and NewLink Sponsored Phase I/Ib trials (All studies conducted under US IND that was held by NewLink and has just transferred over to MSD as of 05AUG2015)	North America (US and Canada)
WHO Sponsored VEBCON Phase I trials	EU (Geneva and Hamburg)
WHO Sponsored VEBCON Phase I trials	Africa (Gabon and Kenya)
US National Institutes of Health Sponsored Phase II PREVAIL Trial Safety	Liberia
US Centers for Disease Control Sponsored Phase II/III STRIVE Trial	Sierra Leone
WHO sponsored Phase II/III Frontline worker trial	Guinea
MSD Phase III Safety and Lot Consistency Trial (V920-P012)	North America, EU
WHO sponsored Phase III Ring Vaccination Trial	Guinea

The present international phase III clinical trial is planned to be ran by Merck Sharp & Dohme Corp., filial de Merck & Co., Inc. as sponsor, in 3 countries within the European Community: Spain, United Kingdom and Denmark.

It is worth of mention that Spain is the first country performing the submission process for GMO voluntary release for this phase III clinical trial.

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

rVSVΔG-ZEBOV GP/ BPSC-1001/V920 is a leading Ebola vaccine candidate that is currently under investigation in eight clinical trials phase I/Ib/II/III.

According to sponsor information there is no GMO release notification in the Community by the same notifier, although some clinical trials (phase I) have been run under WHO sponsorship in Germany and Switzerland. It is possible that WHO has previously filed the appropriate paperwork but it has never been filed by Merck Sharp & Dohme Corp. In the other hand, a search done in the "GMO Register of Deliberate Release and Placing on the EU Market of GMOs" in the JRC webpage, found a SNIFF published on the 10th of August of 2015, related to a Phase I clinical trial using the experimental Ebola Vaccine VSVΔG-ZEBOV. The notification number is B/DE/14/2247.
At this time and in regard to the present phase III study, Spain is the first country performing the submission process for GMO voluntary release.

6. Has the same GMO been notified for release or placing on the market outside the Community by the same or other notifier?

Yes (.) No (X)

If yes:

- Member State of notification …
- Notification number B/../../…

rVSVΔG-ZEBOV GP/ BPSC-1001/V920 is a leading Ebola vaccine candidate that is currently under investigation in several clinical trials phase I/Ib/II/III.

According to sponsor information and JRC register there is no GMO release notification outside the Community performed until now by the same or other notifier, although several clinical trials have been run in United States, Canada, Germany, Switzerland and five African countries.

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

A draft environmental risk assessment has been developed based on the Guidance on Environmental Risk Assessments for Medicinal Products Containing, or Consisting of, Genetically Modified Organisms (GMOs) (EMEA/CHMP/BWP/135148/ 2004). The Applicant is of the position that the risk of the Ebola vaccine, V920, to the environment and to humans is low to negligible due to the short duration of viremia in vaccinated individuals, minimal shedding and lack of stability on solid surfaces.

The rVSVΔG-ZEBOV GP/ BPSC-1001/V920 construct used in this clinical trial was derived from the VSV-Indiana serotype, and the vaccine lacks the VSV GP, the viral determinant for neurotropism and pathogenicity. The vaccine with ZEBOV GP replacement has approximately 33% slower growth kinetics than wild-type VSV in Vero cell cultures, contributing to attenuation. The rVSVΔG-ZEBOV GP virus is apathogenic for hamsters, whereas wild-type VSV is lethal after peripheral inoculation (Feldmann H., unpublished data, 2014).

The clinical trial planned to be started on September 2015, with title " Phase III clinical trial, randomized, placebo-controlled study to evaluate the safety and the immunogenicity of three batches of stability and a lot of high doses rVSV-ZEBOV-GP (vaccine against Ebola virus rVSVΔG-ZEBOV GP) in healthy adults" and EudraCT number 2015-001658-14, is planned to be conducted in different countries with a competitive recruitment of 1125 healthy subjects. The three countries in Europe are Spain, United Kingdom and Denmark. The trial in Spain is planned for a single center at the Hospital La Paz, located in Madrid. The sponsor estimates around 9 months to complete the trial in the site, starting with the first informed consent signed by a subject and finalizing with the last phone call or visit related to the study of the last subject.

On the selection/randomization day, each subject will receive a single dose of one of the four groups of study vaccine or placebo (see Annex 6 Lotes de vacuna for more detailed information about drug characterization and release), then each subject will be closely monitored for 42 days and long-term tracking will continue until 6 months after vaccination. The vaccines will be shipped frozen and stored in a limited-access area in the site according to pharmacy manual indications. The vaccine preparation is a simple process of thawing the vial for

15-30 minutes in a vertical flow cabinet and syringe loading. Use of an appropriate sterilization flow cabinet, labeling of the materials containing the GMO, and discard of exceess material will be performed according to GMO classification requirements of BSL-II. The vaccine is given by IM injection (1 mL) in the deltoid muscle and healthy subjects will be randomized to receive a single dose of vaccine one of three lots of stability rVSVΔG-ZEBOV GP, rVSVΔG-ZEBOV GP high dose or placebo (for further details see Anexo 4 Protocolo Clínico and Anexo 5 Manual de farmacia).

As previously mentioned, there are currently eight clinical studies of the rVSVΔG-ZEBOV GP/ BPSC-1001/V920 vaccine candidate ongoing in different countries. Therefore some viremia and vector shedding profile data from these human clinical trials are available. In general terms rVSVΔG-ZEBOV GP is a live, attenuated replicating vaccine, and viremia is an expected consequence of vaccination. Viremia following with vaccination was seen in most subjects but was transient (Days 1-3) and present at very low levels in some of the health subjects treated. The median vaccine copy number/mL of plasma was 328 (range <30-625); the ratio of genome equivalents to infectious units is ~100:1 in the PCR assay, so the levels in plasma represent minimal infectious virus. Attempts to isolate infectious virus from a sample of plasma specimens with the highest RNA levels, were unsuccessful, indicating that the risk of transmission by blood or excretions is minimal (for further details see Anexo 1 Manual del Investigador) In Phase I studies conucted in the U.S. virus was isolated from urine or saliva from a small number of vaccines in the first days following vaccinations (Regules et al 2015). This is not observed however in the Phase I trials conducted in Europe and Africa (Agnandji et al 2015) suggesting that it may ve a fairly rare event.

The intended clinical application presented for this vaccine is limited to one hospital site in Spain with a high expertise in the Ebola infection in humans. In addition, the results obtained in three international clinical trials with the same product, demonstrated a very limited number of particles measured in healthy volunteers serum/plasma samples and shedding appears to be rare. Therefore, the likelihood that any negative effects could occur is considered negligible. Nonetheless, the hospital site will be required to train the health care professionals involved in the study in the safe handling of this BSL-II genetic modified organism and to have best biosafety practices implemented in order to minimize any accidental exposure to the product, be it personnel, contact persons or the environment.

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

1. Recipient or parental organism characterisation:

(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 …

The drug substance or GMO of the proposed clinical trial, is a live recombinant viral vaccine derived from the Vesicular Stomatitis Virus (VSV) backbone. This virus is modified by a deletion of the VSV-G envelope glycoprotein and substitution with the envelope glycoprotein of the Ebolavirus-Zaire Kikwit strain.

VSV is s single-stranded, negative-sense RNA encoding 5 transcriptional units: N (nucleoprotein), P (phosphoprotein), M (matrix), GP (glycoprotein), and L (polymerase). VSV belong to the family Rhabdoviridae, genus Vesiculovirus. These are bullet-shaped, singlestranded, negative-sense RNA viruses containing 5 genes, 1 of which is the viral GP.

2. Name

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

VSV cause significant disease in pigs, cattle, and horses, primarily manifesting as crusting and vesiculation of the mucous membranes and skin, and lameness due to involvement of the coronary bands of the hoof.

The virus is maintained in nature in a cycle involving sandflies and rodent reservoirs. It is also transmitted between livestock by direct contact, likely including droplet spread and fomites, as well as mechanically by non-biting houseflies and face flies.

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 ..
Mediterranean ..
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)

Vesicular stomatitis is endemic in Mexico, Central America, northern South America and eastern Brazil, as well as in limited areas of the southeastern U.S. Occasional outbreaks are seen in other parts of the Western Hemisphere, both north and south of the endemic area.

The geographic distribution varies with the virus. VSV-NJ and VSV-IN outbreaks occur in North, Central and South America. In the U.S., VSV-NJ was once endemic in a large part of the Southeast, but it may now exist only in limited areas such as Ossabaw Island, Georgia. VSV-IN is not thought to be endemic in the U.S., but newly introduced viruses occasionally cause outbreaks. VSV-AV (Indiana–3) and Cocal virus (Indiana–2) have been seen only in parts of South America.

The virus is zoonotic and leads to a flu-like illness in infected humans. Pre-existing immunity to VSV is not prevalent in humans in the US, and VSV does not circulate outside the Western Hemisphere; therefore it is not expected to be a significant factor in a potential vaccination campaign in the USA or in parts of Africa with VSV-based vaccines. Moreover, the viral GP has been replaced in the investigational product, thus theoretically minimizing the impact of preexisting anti-VSV neutralizing antibodies.

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)

Vesicular stomatitis mainly affects horses, donkeys, mules, cattle and swine. South American camelids, sheep and goats occasionally have clinical signs. Serological evidence of infection has been found in many other animals including deer, pronghorn antelope, bighorn sheep, bats, raccoons, opossums, lynx, bobcats, bears, coyotes, foxes, dogs, non-human primates, rabbits, rodents, turkeys and ducks. In addition to livestock, guinea pigs, hamsters, mice, ferrets and chickens have been infected experimentally. Humans are also susceptible. The reservoir or amplifying hosts for VSV are unknown.

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

The transmission of vesicular stomatitis, and the relative importance of the different transmission routes for each virus, is incompletely understood. Insect vectors seem to introduce VSV into populations of domesticated animals. There is also some speculation that VSV could be a plant virus found in pastures, with animals at the end of an epidemiological chain.

Once it has been introduced into a herd, vesicular stomatitis can spread from animal to animal by direct contact. Broken skin or mucous membranes may facilitate entry of the virus. Infected animals shed VSV in vesicle material, saliva and to a lesser extent, in nasal secretions. In experimentally infected horses, VSV has been found in the saliva of animals with or without oral lesions. Fecal shedding has been reported occasionally in experimentally infected swine, but it has not been seen in horses. VSV does not appear to be shed in milk. Animals can also be infected by exposure to contaminated fomites including food, water and milking machines. VSV in saliva can survive for 3-4 days on fomites; however, this virus is inactivated by sunlight, and does not remain viable for long periods in the environment except in cool, dark places. Experimental infection of livestock by aerosols has been demonstrated, but this route did not result in skin lesions in most species. VSV does not appear to cross the placenta or cause fetal seroconversion.

Humans may be infected by contact with the lesions or secretions from infected animals, particularly vesicular fluid and saliva. Aerosol transmission occurs in laboratories. In addition, some people are probably infected through insect bites.

5. (a) Detection techniques

Serological testing for antibody at day 0 and day 7 and 14 (or 14 and 35 days after symptoms occur). is most commonly performed with an ELISA or complement fixation. Confirmation by viral isolation can also be attempted and PCR is available.

(b) Identification techniques

Q-PCR of viral RNA

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

Vesicular stomatitis virus (VSV) is a prototypic enveloped animal virus that has been used extensively to study virus entry, replication and assembly due to its broad host range and robust replication properties in a wide variety of mammalian and insect cells. BSL II practices and facilities are recommended for propagation of laboratory-adapted strains [HHS Publication No. (CDC) 938395]. See attached information on VSV virus (Annex 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)
animals	(X)
plants	(.)
other	(.)

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

Vesicular stomatitis is characterized by vesicles, papules, erosions and ulcers; these lesions are found particularly around the mouth but may also be present on the feet, udder and prepuce. Excessive salivation is often the first symptom. Closer examination may reveal the characteristic raised vesicles (blisters). Vesicles vary widely in size; while some are as small as a pea, others can cover the entire surface of the tongue. They rupture to become erosions or ulcers; this may happen before any vesicles are seen. A transient fever usually develops when the lesions appear. Vesicular stomatitis lesions are painful and can cause anorexia, refusal to drink, and lameness. Unless secondary bacterial infections or other complications develop, animals recover in approximately two to three weeks.

The virus is zoonotic and leads to a flu-like illness in infected humans. VSV virus 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. In very rare cases, humans can manifest vesicles on the oropharyngeal, nasal mucosa or skin. Human deaths secondary to infection have not been reported; however, encephalitis was reported in a single case of a 3-year old child secondary to VSV-Indiana infection (Quiroz et al., 1988). As there is only 1 case report in the literature, the risk of central nervous system infection following VSV infection is undoubtedly rare. In some areas of tropical America, a high seroprevalence to VSV has been reported without

recognized disease (Cline, 1976). Thus, it is likely that the ratio of infection to illness with VSV is high.

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 virus 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:

Not applicable

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

(c) 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 susceptible and so inactivated by: (1) Autoclave sensitive; (2) UV light; (3) Lipid solvents; (4) 1-10% bleach (500-5000 ppm sodium hypochlorite), 70% ethanol, 2% glutaraldehyde, 2.5% phenol, 0.4% HCL, 2% sodium carbonate, 4% sodium hydroxide, 2% iodophore disinfectants.

10. (a) Ways of dissemination

The VSV ways of transmission are aerosol, needlesticks, direct contact with skin abrasions, contact with animals, fomites, sand flies, and black flies. Broken skin or mucous membranes may facilitate entry of the virus. Infected animals shed VSV in vesicle material, saliva and to a lesser extent, in nasal secretions. Fecal shedding has also been reported occasionally in experimentally infected animals. Animals can also be infected by exposure to contaminated fomites including food, water and milking machines. VSV in saliva can survive for 3-4 days on fomites and infected saliva, however this virus is inactivated by sunlight, and does not remain viable for long periods in the environment except in cool, dark places. Experimental infection of livestock by aerosols has been demonstrated, but this route did not result in skin lesions in most species.

VSV virus can be transmitted to humans who come in close contact with infected animals. Humans may be infected by contact with the lesions or secretions from infected animals, particularly vesicular fluid and saliva. Aerosol transmission occurs in laboratories. In addition, some people are probably infected through insect bites.

(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)

According to sponsor information register there is no GMO release notification in the Community done by Merck, Sharp & Dohme Corp., although some clinical trials have been run in Germany and Switzerland under the auspices of the WHO who may have done the registration. In the other hand, a search done in the "GMO Register of Deliberate Release and Placing on the EU Market of GMOs" in the JRC webpage, found a SNIFF published on the 10th of August of 2015, related to a Phase I clinical trial using the experimental Ebola Vaccine VSVΔG-ZEBOV. The notification number is B/DE/14/2247.

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

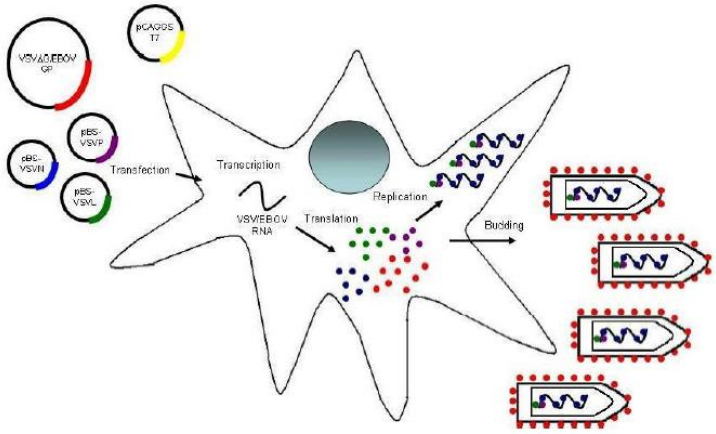
The cloning of the rVSV ZEBOV-GP virus has been described in the literature and consists of reverse genetics placing the ZEBOV envelope glycoprotein gene into the genome of the Indiana strain of Vesicular Stomatitis Virus as a substitution for the fusogenic VSV-G envelope glycoprotein (Properties of Replication-Competent Vesicular Stomatitis Virus

Vectors Expressing Glycoproteins of Filoviruses and Arenaviruses. Garbutt et al., JVirology 78(10):5458–5465, 2004). The reverse genetics system is based on 5 plasmids containing the VSV and EBOV genes:

- pCAGGS-T7 (helper plasmid containing the T7 promoter)
- pBS-N (helper plasmid containing VSV N protein gene)
- pBS-L (helper plasmid containing VSV L protein gene)
- pBS-P (helper plasmid containing VSV P protein gene)
- VSV XN2-ZEBOV GP (plasmid containing the entire VSV genome with the ZEBOV GP gene replacing the VSV GP gene)

This engineering strategy was taken from Lawson et al., (Recombinant vesicular stomatitis viruses from DNA PNAS (USA) 92:4477- 4481, 1995). All plasmids were sequenced prior to use and the sequence of the envelope glycoprotein gene within the product virus has been confirmed.

The rVSV expressing ZEBOV GP was rescued from cells transfected with VSVΔG/EBOV GP plasmids and VSV helper plasmids, and is fully infectious (See Annex 1 as Manual del Investigador for further information about this point).



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 (X) No (.)

If no, go straight to question 5.

The cloning of the rVSV ZEBOV-GP consists of reverse genetics placing the ZEBOV envelope glycoprotein gene into the genome of the Indiana strain of Vesicular Stomatitis Virus as a substitution for the fusogenic VSV-G envelope glycoprotein.

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

(a) Type of vector

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

(b) Identity of the vector

Vesicular Stomatitis Virus. VSV has eight main serotypes: Indiana, New Jersey, Cocal, Alagoas, Isfahan, Chandipura, Maraba, and Piry.

rVSVΔG-ZEBOV GP/ BPSC-1001/V920 is a live attenuated recombinant virus consisting of a single rVSV isolate (11481 nt, strain Indiana) with the gene for the ZEBOV GP, Kikwit strain, replacing the deleted VSV GP gene.

(c) Host range of the vector

Vesicular stomatitis mainly affects horses, donkeys, mules, cattle and swine. South American camelids, sheep and goats occasionally have clinical signs. Serological evidence of infection has been found in many other animals including deer, pronghorn antelope, bighorn sheep, bats, raccoons, opossums, lynx, bobcats, bears, coyotes, foxes, dogs, non-human primates, rabbits, rodents, turkeys and ducks. In addition to livestock, guinea pigs, hamsters, mice, ferrets and chickens have been infected experimentally.

Humans are also susceptible (except for Maraba and Cocal viruses).

The main reservoir is the sand fly, although arboreal rodents and non-human primates may also harbour VSV. Grasshoppers have also been implicated as a potential reservoir for VSV.

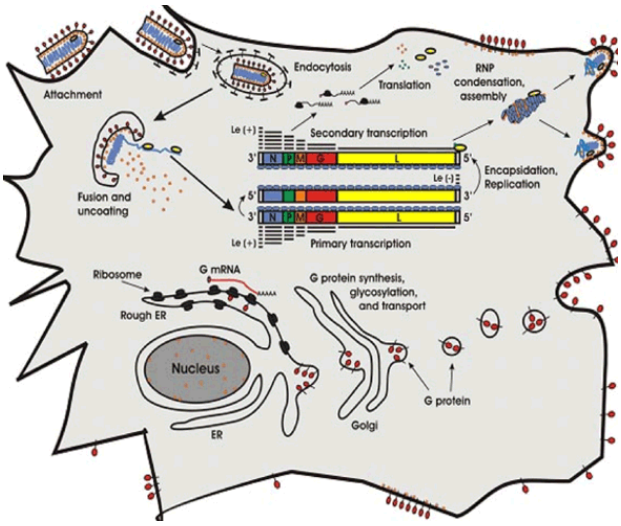
(d) Presence in the vector of sequences giving a selectable or identifiable phenotype

Yes () No (X)

antibiotic resistance (.)
other, specify …

Indication of which antibiotic resistance gene is inserted

(e) Constituent fragments of the vector



VSV belong to the family Rhabdoviridae, genus Vesiculovirus. These are bullet-shaped, singlestranded, negative-sense RNA viruses containing 5 genes, 1 of which is the viral GP.

The figure on the left describe the genome structure and the natural viral cycle when entering a host cell.

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

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

rVSVΔG-ZEBOV GP is a live attenuated recombinant virus consisting of a single rVSV isolate (11481 nt, strain Indiana) with the gene for the ZEBOV GP, Kikwit strain, replacing the deleted VSV GP gene.

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 final genetic modified organism named rVSVΔG-ZEBOV GP is a live attenuated recombinant virus based on the complete structure of a single rVSV isolate replacing its VSV GP gene by an exogenous gene. The exogenous gene is the ZEBOV GP protein from the Kikwit strain (11481 nt, strain Indiana). See Annex 3 for further information.

(b) Source of each constituent part of the insert

- The main constituent part is the complete genome of the VSV except the GP gene.
- The exogenous gene is the ZEBOV GP protein from the Kikwit strain (11481 nt, strain Indiana).

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

- The complete VSV genome, except the GP gene, is providing the ability to produce a recombinant VSV virus. The recombinant virus is recued on a cells transfected with VSVΔG/EBOV GP plasmids and VSV helper plasmids, and is fully infectious.

- The exogenous Kikwit strain ZEBOV GP protein gene will provide the genetically modified organism the property to produce Kikwit strain ZEBOV glucoprotein in a quimeric VSV that will make possible a recombinant virus to act as attenuated vaccine for Kikwit strain ZEBOV.

(d) Location of the insert in the host organism

- on a free plasmid ()
- integrated in the chromosome (.)
- other, specify …In the virus. rVSV expressing ZEBOV GP

(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	Filoviridae
(iii)	genus	Ebolavirus
(iv)	species	ZEBOV
(v)	subspecies	…
(vi)	strain	Kikwit strain (11481 nt, strain Indiana)
(vii)	cultivar/breeding line	…
(viii)	pathovar	…
(ix)	common name	Ebola virus

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	(X)
animals	(X)
plants	(.)
other	..

Ebola virus is a zoonotic pathogen. Intermediary hosts have been reported as several species of fruit bats throughout central and sub-Saharan Africa. Evidence of infection in bats has been detected through molecular and serologic means. End hosts are humans and great apes, infected through bat contact or through other end hosts. Pigs on the Philippine islands have

been reported to be infected with Reston virus, so other interim or amplifying hosts may exist.

(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):

As stated above, this GMO (vaccine) lacks the VSV GP, the viral determinant for neurotropism and pathogenicity of the parental organism from which the GMO is derived. Therefore, the tropism is determined by the ZEBOV GP that replaces the parental external glucoproteins. This replacement has approximately 33% slower growth kinetics than wild-type VSV in Vero cell cultures, contributing to attenuation (Garbutt et al., 2004).

ZEBOV Ebola virus begins its attack by attaching to host receptors through the glycoprotein (GP) surface peplomer and is endocytosed into macropinosomes in the host cell. To penetrate the cell, the viral membrane fuses with vesicle membrane, and the nucleocapsid is released into the cytoplasm. Encapsidated, negative-sense genomic ssRNA is used as a template for the synthesis (3'-5') of polyadenylated, monocistronic mRNAs and, using the host cell's ribosomes, tRNA molecules, etc., the mRNA is translated into individual viral proteins.

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

BSL IV practices and facilities are mandatory as a dangerous and exotic agent that pose a high individual risk of aerosol-transmitted laboratory infections, and cause severe to fatal disease in humans for which vaccines or other treatments are not available.

5. Do the donor and recipient organism exchange genetic material naturally?

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

As is typical of RNA-coded viruses, the VSV and Ebola viruses can mutate rapidly, both within a person during the progression of disease and in the reservoir among the local human population. The observed mutation rate of 2.0×10^{-3} substitutions per site per year for Ebola virus is as fast as that of seasonal influenza. Genetic analyses have shown that some Ebola strains had mutated hundreds of times since it diverged from an ancestral virus about ten years ago, but no one yet knows whether any of these mutations have altered important properties of the virus.

No understandable mechanism of natural exchange of genetic material between the parental VSV virus and the donor Ebola virus organism are 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 (X) No (.) Not known (.)

Specify …

The final genetic modified organism named rVSVΔG-ZEBOV GP is a live **attenuated** recombinant virus based on the complete structure of a single rVSV isolate replacing its VSV GP gene by an exogenous gene.

Both VSV and rVSVΔG-ZEBOV GP are susceptible and so inactivated by: (1) Autoclave sensitive; (2) UV light; (3) Lipid solvents; (4) 1-10% bleach (500-5000 ppm sodium hypochlorite), 70% ethanol, 2% glutaraldehyde, 2.5% phenol, 0.4% HCL, 2% sodium carbonate, 4% sodium hydroxide, 2% iodophore disinfectants.

(b) is the GMO in any way different from the recipient as far as mode and/or rate of reproduction is concerned?

Yes (X) No (.) Unknown (.)

Specify …

rVSVΔG-ZEBOV GP is a live **attenuated** recombinant virus. The basis for the attenuation of the rVSVΔG-ZEBOV GP virus has not been determined, but it is likely due to chimerization with replacement of the native envelope GP with a heterologous gene. In vitro replication of the chimeric rVSVΔG-ZEBOV GP is slightly slower than the parental VSV strain.

(c) is the GMO in any way different from the recipient as far as dissemination is concerned?

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

Specify …

In making rVSVΔG-ZEBOV GP, the VSV GP gene has been replaced with the EBOV GP gene. Wild-type VSV has a broad tropism (Hastie et al., 2013), whereas the rVSVΔG-ZEBOV GP has a different, and possibly narrower cellular tropism more reflective of ZEBOV.

Hepatocytes, endothelial cells, dendritic cells, monocytes, and macrophages, all of which express C-type lectins, are thought to be preferred target cells of filoviruses (Hoenen et al., 2006). It is possible that this somewhat more restricted tropism contributes to the attenuation of the vaccine virus, including the absent neurovirulence attributed to vaccine vectors expressing the wild-type VSVG envelope GP.

(d) is the GMO in any way different from the recipient as far as pathogenicity is concerned?

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

Specify …

rVSVΔG-ZEBOV GP is a live **attenuated** recombinant virus. The basis for the attenuation of the rVSVΔG-ZEBOV GP virus has not been determined, but it is likely due to chimerization with replacement of the native envelope GP with a heterologous gene.

Vesicular stomatitis (VS) is a vesicular disease of horses, cattle and pigs caused by vesiculoviruses of the family Rhabdoviridae. This disease is clinically indistinguishable from foot and mouth disease (FMD), vesicular exanthema of swine (VES), or swine vesicular disease (SVD) when horses are not involved. Sheep, goats and many other wild species can be infected. Humans are also susceptible. The disease is limited to the Americas; however, it was previously described in France and in South Africa.

Treatment with the vaccine rVSVΔG-ZEBOV GP has just demonstrated in eight clinical trials that vesicular lesions of the skin appearing in the first 2 weeks after vaccination are a potential source of virus infection; care should be taken to avoid contact spread from such lesions to others by use of a dressing or other means until healing occurs. As there is limited clinical experience with rVSVΔG-ZEBOV GP, only the profile of common AEs can be assessed (for further information Annex 1 or Manual del Investigador and Annex 4 or Protocolo Clinico)

2. Genetic stability of the genetically modified organism

Two private companies and the Special Pathogens Department (SP) at the National Microbiology Laboratory (NML) of the Public Health Agency of Canada (PHAC) were involved in manufacturing this vaccine. The plasmids were produced according to cGMP specifications. Given technical difficulties with transfection of the plasmids in Vero cells alone, SP transfected the plasmids into a mixed culture containing a 1:1 ratio of HEK293 to Vero cell in order to rescue the rVSVΔG-ZEBOV GP virus. Then a limiting dilution plaque purification was performed through sequential rounds of plaque purification and one clone was selected for manufacture of the Master Seed Virus, as it was free from any mutations in the original EBOV GP plasmid sequence. Each vaccine batch is tested using molecular genetic methods to ensure the presence of the Ebola GP insert and is tested in a cell-based assay for the ability to infect cells and produce the Ebola virus glycoprotein, using a Western blot approach.

rVSVΔG-ZEBOV GP vaccine intended for clinical use was placed on concomitant stability testing according to International Conference on Harmonization (ICH) guidelines to ensure that it met specifications during the course of clinical trials. The final filled containers are being tested for stability at the intended storage temperature for 36 months. BPSC-1001 (the biological drug substance) before formulation is stable in long term storage at ≤ -60°C and for at least 4 weeks at 2-8°C. The vaccine in the final filled container is stable after thawing at 2-8ºC and room temperature to enable use in the clinic for a work-day. The vaccine should, however, preferably be held at 2-8ºC after thawing. This was shown for both the post-exposure (1×10^8 pfu/mL) dosage formulation, as well as for vaccine diluted for use in

clinical trials to 3×10^6, 3×10^5, 3×10^4, and 3×10^3 pfu/mL. At 37ºC the virus loses infectivity after 2 hours.

3. Is the GMO significantly pathogenic or harmful in any way (including its extracellular products), either living or dead?

Yes (.) No (X) Unknown (X)

(a) to which of the following organisms?

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

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

The rVSVΔG-ZEBOV GP vaccine is currently under investigation in eight clinical trials phase I/Ib/II/III. The main objective of the clinical development program is to achieve a vaccine registration and to impact the ongoing epidemic of Ebola virus in West Africa. BSL II practices and facilities are recommended for manipulation of the vaccine in all the clinical trials.

At present there are eight clinical trials of phase I/Ib BPSC-1001 ongoing in the United States, Canada, Germany, Switzerland and two African countries not affected by the active Ebola virus epidemic (Gabon and Kenya). In these trials, more than 500 volunteers received V920 in varying doses. V920 is also being studied in three trials of safety and efficacy Phase II / III of V920 in countries affected by the Ebola virus outbreak (Liberia, Guinea, and Sierra Leone). The present clinical trial is planned to be ran by Merck Sharp & Dohme Corp., filial de Merck & Co., Inc. as sponsor, in 3 countries within the European Community: Spain, United Kingdom and Denmark. Spain is the first country performing the submission process for GMO voluntary release for this Phase III clinical trial. As a safety practicum, the protocol excluded subjects with house hold contact with pregnant or lactating women and house hold contact with subjects with impaired immunological functions.

Treatment with the vaccine rVSVΔG-ZEBOV GP has been demonstrated to result in vesicular lesions of the skin appearing in the first 2 weeks after vaccination that are a potential source of virus infection; care should be taken to avoid contact spread from such lesions to others by use of a dressing or other means until healing occurs. As there is limited clinical experience with BPSC-1001, only the profile of common AEs can be assessed (for further information Annex 1 or Manual del Investigador and Annex 4 or Protocolo Clinico).

4. Description of identification and detection methods

(a) Techniques used to detect the GMO in the environment

A real time qRT-PCR method is developed for detecting and quantifying recombinant virus specifically rVSVΔG-ZEBOV GP in human samples. The amplification target is directed to RNA sequences located at the junction of the

ZEBOV GP sequences and VSV vector, such that the method is specific for the vaccine rVSVΔG-ZEBOV GP and does not detect the VSV or ZEBOV wild types.

(b) Techniques used to identify the GMO

A real time qRT-PCR method is developed for detecting and quantifying recombinant virus specifically rVSVΔG-ZEBOV GP in human samples. The amplification target is directed to RNA sequences located at the junction of the ZEBOV GP sequences and VSV vector, such that the method is specific for the vaccine rVSVΔG-ZEBOV GP and does not detect either the VSV or ZEBOV wild types.

F. Information relating to the release

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

The rVSVΔG-ZEBOV GP vaccine is currently under investigation in several phase I/Ib/II/III clinical trials. The main objective of the clinical development program is to achieve BPSC-1001 vaccine registration and to impact the ongoing epidemic of Ebola virus in West Africa. At present there are eight clinical trials of phase I/Ib BPSC-1001 ongoing in the United States, Canada, Germany, Switzerland and two African countries not affected by the Ebola virus epidemic (Gabon and Kenya). In these trials, more than 500 volunteers have received rVSVΔG-ZEBOV GP in varying doses. V920 is also being studied in three Phase II/III trials of safety and efficacy of rVSVΔG-ZEBOV GP in Ebola affected countries (Liberia, Guinea, and Sierra Leone).

The present clinical trial is planned to be run by Merck Sharp & Dohme Corp., filial de Merck & Co., Inc. as sponsor, in 3 countries within the European Community: Spain, United Kingdom and Denmark. Spain is the first country performing the submission process for GMO voluntary release for this Phase III Clinical Trial.

Transmission of the vaccine virus appears to represent a minimal risk, and if transmitted the vaccine virus would retain its attenuated phenotype. As a safety practicum, the protocol excluded subjects with house hold contact with pregnant or lactating women and house hold contact with subjects with impaired immunological functions.

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 …

The rVSVΔG-ZEBOV GP vaccine will be administered in an international phase III clinical trial, with a unique site in Spain at University Hospital La Paz. The clinical team leading the clinical trial is expert in Ebola disease management and control (leaded by Dr. José Ramón Arribas). Neither Ebola nor VSV is typically present in Europe.

The vaccines will be shipped open-label; therefore, an unblinded pharmacist or qualified trial site personnel will be used to maintain the blind of the clinical supplies. Frozen vaccine vials will be

shipped to the site on dry ice. Placebo will be shipped separately to the site under ambient conditions. Study vaccine/placebo will be stored in a limited-access area. The site will comply with only one of the two options provided below: 1) One -70˚ C freezer with a temperature monitoring device to house both the serum storage and a non-transparent lock box for the study vaccine; 2) Two separate -70˚ C freezer with temperature monitoring devices: one for the serum storage and the other for the study vaccine storage (for further details see Annex 5 or Manual de Farmacia).

3. Information concerning the release and the surrounding area

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

The rVSVΔG-ZEBOV GP vaccine will be administered in an international phase III clinical trial, with a unique site in Spain at University Hospital La Paz (Madrid).

(b) Size of the site (m^2): University Hospital La Paz - IdiPAZ
(i) actual release site (m^2): Central Clinical Research Unit
(ii) wider release site (m^2): Central Clinical Research Unit

The vaccine will be received and stored by the coordinator of the Central Clinical Research Unit at the University Hospital La Paz - IdiPAZ.

The vaccine preparation for subject administration is performed by a very simple process: 1) stored vials should be removed from the freezer and thawed at room temperature in a vertical flow cabinet; 2) Once the vial is thawed, vial should then be gently inverted several times prior to withdrawal with the syringe; 3) Once the syringe is prepared, it is preferred that the unblinded study staff member administer the vaccine/placebo to the subject. The vaccine preparation is a simple process of thawing the vial contents for 15-30 minutes in a vertical flow cabinet and syringe loading. Appropriate sterilization flow cabinet, labeling of the materials containing the GMO, and discard of exceess material will be performed according to GMO classification requirements for BSL-II (for further details see Annex 5 or Manual de Farmacia).

(c) Proximity to internationally recognised biotopes or protected areas (including drinking water reservoirs), which could be affected:

Not applicable

(d) Flora and fauna including crops, livestock and migratory species which may potentially interact with the GMO

Vesicular lesions of the skin appearing in the first 2 weeks after vaccination are a potential source of virus infection; care should be taken to avoid contact spread from such lesions to others by use of a dressing or other means until healing occurs. Shedding of virus in secretions or urine is infrequent, at low levels, and appears to pose minimal if any risk of transmission to other persons. Due to the low level of viremia and other factors, it is highly unlikely that the vaccine virus can infect or be transmitted by blood-feeding arthropods limiting the potential risk of exposure to livestock.

The risk of infection or disease in domestic animals is unknown, but is a theoretical concern due to the nature of the parental VSV virus. Transmission is unlikely due to the minimum levels of virus shedding by human vaccines and disease in animals is unlikely due to the

attenuated nature of the virus. In a study in pigs experimentally infected with rVSVGZEBOV GP, no disease was observed (DeWitt E et al., 2015).

4. Method and amount of release

(a) Quantities of GMOs to be released:

The vaccine is given by intramuscular injection in the deltoid muscle and healthy subjects will be randomized to receive a single dose of vaccine one of three lots of stability rVSVΔG-ZEBOV GP vaccine, rVSVΔG-ZEBOV GP vaccine high dose or placebo. Study vaccine/placebo will be administered at visit 1 for all subjects. At this visit, subjects will receive a 1.0-mL intramuscular injection in the deltoid muscle of the non-dominant arm at a 90º angle into the muscle tissue using a needle long enough to ensure IM deposition of the study vaccine/placebo.

Partial or empty vaccine vials should be properly discarded as biohazardous waste (BSL-II). Clinical supplies that are affected by a temperature excursion and determined to be unacceptable for future use will be returned to the Sponsor or discarded per local guidance. At Last Patient Last Visit but no later than the end of the study, the site personnel will return all unused clinical supplies to the sponsor or discard the clinical supplies per local guidance. Where local discard and destruction is appropriate, the investigator is responsible for ensuring that a local discard/destruction procedure is documented.

Transmission of the vaccine virus appears to represent a minimal risk, and if transmitted the vaccine virus would retain its attenuated phenotype. Few healthy vaccinated volunteers of the eight clinical trials on going had low levels of virus in their blood for up to 2 week after vaccination. As for other live viral vaccines, vaccinated persons should not donate blood or plasma within 30 days after vaccination. Care should be taken to avoid direct blood contact, e.g. by sharing needles or razor blades. Vesicular lesions of the skin appearing in the first 2 weeks after vaccination are a potential source of virus infection; care should be taken to avoid contact spread from such lesions to others by use of a dressing or other means until healing occurs. Shedding of virus in secretions or urine is infrequent, at low levels, and appears to pose minimal if any risk of transmission to other persons. Due to the low level of viremia and other factors, it is highly unlikely that the vaccine virus can infect or be transmitted by blood-feeding arthropods. Additionally, as a safety practiques, protocol excluded subjects with house hold contact with pregnant or lactating women and house hold contact with subjects with impaired immunological functions.

(b) Duration of the operation:

The sponsor estimates around 10 months to complete the trial in the site, starting with the first informed consent signed subject and finalizing with the last call phone or visit related to the study of the last subject.
The selection/ randomization day, each subject will receive a single dose of one of the four groups of study vaccine or placebo. The vaccine is given by intramuscular injection in the deltoid muscle and healthy subjects will be randomized to receive a single dose of vaccine one of three lots of stability vaccine, vaccine high dose or placebo. Study vaccine/placebo will be administered at visit 1 for all subjects. At this visit, subjects will receive a 1.0-mL intramuscular injection in the deltoid muscle of the non-dominant arm at a 90º angle into the muscle tissue using a needle long enough to ensure IM deposition of the study vaccine/placebo.

At visit 1, all subjects will remain under observation for 30 minutes after vaccination for signs of hypersensitivity reactions or other adverse events. Then each subject will be closed monitored for 42 days and long-term tracking will continue until 6 months after vaccination.

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

Study vaccine/placebo will be stored in a limited-access area. All involved personnel will use best biosafety practices during transport, prior to and after administration and disposal.

In this study, as the previous eight trials on going by WHO, NIH, CDC, etc safety vaccinated subjects will not be isolated during the trial. As a safety practiques, protocol excluded subjects with house hold contact with pregnant or lactating women and house hold contact with subjects with impaired immunological functions.

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

The treatment will be performed at the hospital in an independent room, ambient indoor conditions for the intramuscular injection in the deltoid muscle and healthy subjects. Receiving environment for potential shed vector particles is most likely waste water and ambient temperature.

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.

Transmission of the rVSVΔG-ZEBOV GP vaccine appears to represent a minimal risk, and if transmitted the vaccine virus would retain its attenuated phenotype. Few healthy subjects vaccinated with rVSVΔG-ZEBOV GP vaccine had low levels of virus in their blood for up to 2 weeks after vaccination. As for other live viral vaccines, vaccinated persons should not donate blood or plasma within 30 days after vaccination. Care should be taken to avoid direct blood contact, e.g. by sharing needles or razor blades. Shedding of virus in secretions or urine is infrequent, at low levels, and appears to pose minimal if any risk of transmission to other persons.

Due to the low level of viremia and other factors, it is highly unlikely that the vaccine virus can infect or be transmitted by blood-feeding arthropods. The risk of infection or disease in domestic animals is unknown, but is a theoretical concern due to the nature of the parental VSV virus. Transmission is unlikely due to the minimum levels of virus shedding by human vaccines and disease in animals is unlikely due to the attenuated nature of the virus. In a study in pigs experimentally infected with rVSVGZEBOV GP, no disease was observed (DeWitt E et al. 2015).

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 program is to achieve rVSVΔG-ZEBOV GP vaccine registration and to impact the ongoing epidemic of Ebola virust in West Africa. The GMO generates a self-limiting infection followed with an adaptive immune response (antibody and T lymphocytes) against virus GP Ebola.

The GMO will be administered to healthy humans as a Ebola vaccine with the main objective to determine whether vaccination with rVSVΔG-ZEBOV GP from three batches of stability independent holds an equivalent immunogenicity; and also determine the safety and tolerability of the vaccine into three groups of batches stability and a high dose group during the 42 days after vaccination.

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

None expected

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

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

Give details

As stated above, the rVSV-based vector used in this vaccine lacks the VSV GP, the viral determinant for neurotropism and pathogenicity (Marzi et al., 2011; Mire et al., 2012; Marzi et al., 2014). rVSV with ZEBOV GP replacement has approximately 33% slower growth kinetics than wild-type VSV in Vero cell cultures, contributing to attenuation (Garbutt et al., 2004). The rVSVΔG-ZEBOV GP virus is apathogenic for hamsters, whereas wild-type VSV is lethal after peripheral inoculation (Feldmann H., unpublished data, 2014).

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

Even in the event of shedding in waste water no establishment in such a system can be expected due to the low volumes released, destruction of the virus by wastewater treatment techniques (e.g., temperature, chlorination).

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:

Horizontal gene transfer to bacteria highly unlikely but cannot be excluded.

(b) from other organisms to the GMO:

Not likely.

(c) likely consequences of gene transfer:

No advantage or disadvantage.

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.):

None available. rVSVΔG-ZEBOV GP vaccine 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 waste water.

9. Possible environmentally significant interactions with biogeochemical processes (if different from the recipient or parental organism)

None

H. Information relating to monitoring

1. Methods for monitoring the GMOs

The international clinical trial planned to be started on September 2015, will be conducted in different countries with a competitive recruitment of 1125 subjects. The three countries in Europe are Spain, United Kingdom and Denmark; the trial in Spain will be conducted at a single site at the Hospital La Paz, located in Madrid. The sponsor estimates around 10 months to complete the trial in the site, starting with the first informed consent signed by the subject and finalizing with the last phone call or visit related to the study of the last subject.

The management of the drug within the clinical trial is mainly focused on three phases:

(1) The vaccines will be shipped open-label and stored in a limited-access area in a -70˚ C freezer with a temperature monitoring device.

(2) The vaccine preparation is a simple process of thawing the vials for 15-30 minutes in a vertical flow cabinet and syringe loading. Use of an appropriate sterilization flow cabinet, labeling of the materials containing the GMO, and discard of exceess material will be performed according to GMO classification requirements (BSL-II). The vaccine is given by intramuscular injection (1 mL) in the deltoid muscle and healthy subjects will be randomized to receive a single dose of vaccine one of three lots of stability, high dose or placebo. At visit 1, all subjects will remain under observation for 30 minutes after vaccination for signs of hypersensitivity reactions or other adverse events.

(3) Shedding of virus in secretions or urine is infrequent, at low levels, and appears to pose minimal if any risk of transmission to other persons. Serum samples for future research will be obtained from each healthy participant at three time points: visit 1 (day 1), visit 2 (day 28) and visit 4 (6 months).

2. Methods for monitoring ecosystem effects

Transmission of the vaccine virus appears to represent a minimal risk, and if transmitted the vaccine virus would retain its attenuated phenotype. Few of the healthy volunteers vaccinated with rVSVΔG-ZEBOV GP vaccine (in the eight clinical trials on going) had low levels of virus in their blood for up to 2 weeks after vaccination. Care should be taken to avoid direct blood contact, e.g. by sharing needles or razor blades.

Shedding of virus in secretions or urine is infrequent, at low levels, and appears to pose minimal if any risk of transmission to other persons. Due to the low level of viremia and other factors, it is highly unlikely that the vaccine virus can infect or be transmitted by blood-feeding arthropods. Serum samples for future research will be obtained from each healthy participant at three time points: visit 1 (day 1), visit 2 (day 28) and visit 4 (6 months).

The risk of infection or disease in domestic animals is unknown, but is a theoretical concern due to the nature of the parental VSV virus. Transmission is unlikely due to the minimum levels of virus shedding by human vaccinees and disease in animals is unlikely due to the

attenuated nature of the virus. In a study in pigs experimentally infected with rVSVGZEBOV GP, no disease was observed (DeWitt E et al. 2015, Annex 7).

3. Methods for detecting transfer of the donated genetic material from the GMO to other organisms

Not applicable

4. Size of the monitoring area (m^2)
… m^2

University Hospital La Paz - IdiPAZ. Central Clinical Research Unit

5. Duration of the monitoring

The sponsor estimates around 10 months to complete the trial in the site, starting with the first informed consent signed subject and finalizing with the last call phone or visit related to the study of the last subject.

The proposed period of release is from Sep/2015- until Jul/2016.

6. Frequency of the monitoring

At Visit 1, all subjects will remain under observation for 30 minutes after vaccination for signs of hypersensitivity reactions or other adverse events. Then all participants will be followed up by a total of 4 follow up visits at 28 days, 42 days, 3 and 6 months at the University Hospital of La Paz, by the clinical team headed by Dr. José Ramón Arribas.

Serum samples for future research will be obtained from each healthy participant at three time points: visit 1 (day 1), visit 2 (day 28) and visit 4 (6 months).

I. Information on post-release and waste treatment

1. Post-release treatment of the site

Decontamination with virucidal disinfectants according to local biosafety guidelines BSL-II.

2. Post-release treatment of the GMOs

All equipment used during the procedure will either be disposed of in line with current biological hazard procedures or decontaminated with virucidal agents as dictated by the local biological hazard waste management plan for BSL-II.

3. (a) Type and amount of waste generated

Empty vials and used vials and the used delivery system components (injection needle and syringe), gauzes and personal protective equipment and components used for collecting body fluids samples after administration.

3. (b) Treatment of waste

Delivery system components (injection needle and syringe) will be disposed of in a manner consistent with the standard practice of the institution for biohazardous sharps. In addition any disposable surgical instruments or other materials used during the administration procedure or collection of body fluids will be disposed according to standard biosafety practice of the institution.

All non-disposable surgical equipment will be cleaned using a chemical disinfectant with proven virucidal activity and then sterilized by autoclaving according to standard practice of the institution.

J. Information on emergency response plans

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

The rVSVΔG-ZEBOV GP vaccine will be prepared in the Central Clinical Research Unit of the University Hospital La Paz - IdiPAZ. The vaccine preparation is a simple process of thawing vials for 15-30 minutes in a vertical flow cabinet and syringe loading. Use of an appropriate sterilization flow cabinet, labeling of the materials containing the GMO, and discard of excess material will be performed according to GMO classification requirements. The vaccine is given by IM injection (1 mL) in the deltoid muscle and healthy subjects will be randomized to receive a single dose of vaccine one of three lots of stability rVSVΔG-ZEBOV GP, rVSVΔG-ZEBOV GP high dose or placebo.

In case of unexpected spread (e.g. spills) the affected area, lineated with absorbing material, will be decontaminated using appropriate disinfectants. In case of injury, the injured site will be disinfected appropriately according to the best biosafety practice standards (BSL-II).

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

Wear protective clothing and gently cover spill with paper towels. Apply chemical disinfectant such as hypoclorite 1% solutions. Start at the perimeter of the spill and work towards the centre. Allow disinfectant a minimum of 30 minutes contact time before clean up.

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

No undesirable effects are expected, however, if an undesirable effect occurs then the use of rVSVΔG-ZEBOV GP vaccine would be put on hold until the effects are fully assessed and measures are put in place to mitigate further risks. All areas and facilities that had been used to administer the product would be cleaned and decontaminated using virucidal agents.