

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/17/19 |
| (c) | Date of acknowledgement of notification | 20/11/2017 |
| (d) | Title of the project | "Double-Blind, Randomized, Placebo-Controlled Phase 2b, Multicenter Study to Evaluate the Safety, Tolerability, Efficacy, and Immunogenicity of a 2-Dose and a 3-Dose Regimen of V160, (Cytomegalovirus [CMV] Vaccine) in Healthy Seronegative Adolescent and Adult Women 16 to 35 Years of Age" |
| (e) | Proposed period of release (estimated) | From 15/03/2018 until 15/09/2018 |

2. Notifier

Name of institution or company:
Lourdes López Bravo
Clinical Research Director
Merck Sharp&Dohme de España S.A.
Calle Josefa Valcárcel nº38, 28027, Madrid

3. GMO characterisation

V160 is a vaccine being developed for the prevention of congenital HCMV (cHCMV) infection, and by extension, cHCMV disease, in infants born to women. Congenital HCMV is a frequent and yet significantly under-appreciated cause of disease in newborns and young children, including the most common non-genetic cause of permanent hearing loss among children in developed countries.

V160 is a whole virus vaccine composed of purified human cytomegalovirus (HCMV) expressing the pentameric glycoprotein H (gH) complex (gH/gL/pUL128/pUL130/pUL131). The pentameric gH complex has been shown important for generating potent neutralizing antibodies. V160 was engineered from the attenuated AD169 strain, evaluated as a live vaccine in 1970s. The V160 genome has the same ~15-kb genome deletion present in AD169. In addition, V160 was engineered to be conditionally replication-defective by fusion of a "destabilization domain" (ddFKBP) to two essential, nonstructural viral proteins (IE1/2 and UL51). Replication of V160 is dependent on the presence of a synthetic molecule called Shield-1 which must be added to culture medium during production. During vaccine purification, the Shield-1 concentration is reduced below the level required to support productive virus replication. As expected, no evidence of V160 shedding was detected in the

Phase 1 clinical study. Based on the available data, V160 is classified as a BSL-1 organism. It is considered that V160 must be handled under biosafety practices level 1. The parental AD169 virus was demonstrated to be non-pathogenic in two human trials and is classified as a biosafety level 1 (BSL-1) organism by American Type Culture Collection (ATCC).

(a) Indicate whether the GMO is a:

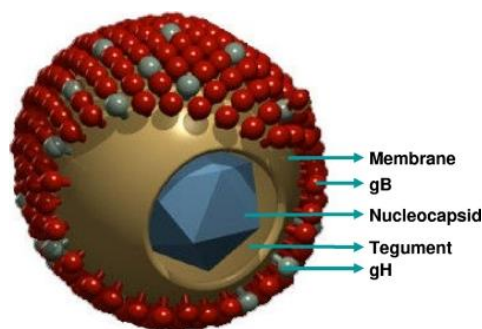
- | | |
|----------------|-----|
| viroid | (.) |
| RNA virus | (.) |
| DNA virus | (X) |
| bacterium | (.) |
| fungus | (.) |
| animal | |
| - mammals | (.) |
| - insect | (.) |
| - fish | (.) |
| - other animal | (.) |

specify phylum, class

(b) Identity of the GMO (V160)

The name of the GMO is V160, also called ddIE1/2-ddUL51 (Wang et al, Sci Transl Med 2016). The GMO was modified from an attenuated human cytomegalovirus (HCMV) laboratory strain, AD169, which was previously tested in clinical studies. V160 belongs to the herpesviridae family, betaherpesviridae subfamily, and Cytomegalovirus genus.

V160 is a human cytomegalovirus (HCMV) with a genome of ~231-kb that includes the ~15-kb deletion present in the parental virus, AD169. Virions consists of a double-stranded linear DNA genome packaged into an icosahedral nucleocapsid that is surrounded by a proteinaceous tegument. This tegument is enveloped by a lipid bilayer containing a variety of viral glycoproteins. Virions have a diameter of ~200 nm.



Three-dimensional structure of HCMV virions (Crough and Khanna, Clin Microbiol Rev. 2009)

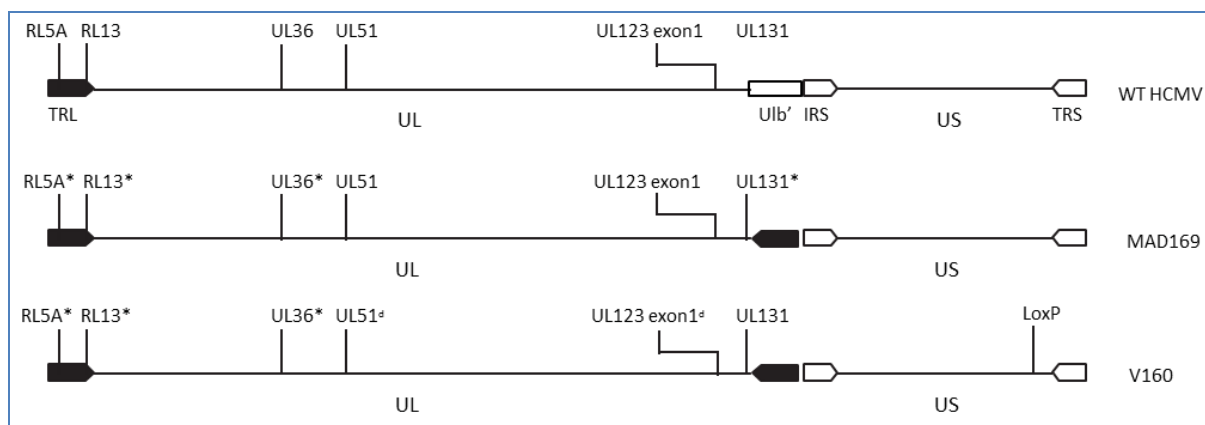
Cytomegaloviruses are ancient viruses, each co-evolved with its respective host species over millions of years (McGeoch et al, Virus Research 2006). Each animal species has its own

cytomegalovirus, and the species barrier prevents CMV from productively infecting other animal species (McGeoch et al, Virus Research 2006). For example, even between closely related rhesus and cynomolgus monkeys that share considerable genetic traits (<1% different in single copy sequence (Rogers, Nat Rev Genet 2014)), and a high genetic homology of each CMV (about 89% at the nucleotide level), interspecific infection is not possible (Marsh et al., J. Virol 2011). Due to the species barrier, functions for the majority of HCMV genes are not well understood, especially in regard to their roles in viral pathogenesis in humans. Virus replication *in vitro* is tightly regulated in temporal fashion with different sets of viral genes being expressed at different kinetic phases.

A variety of human cells, including epithelial and endothelial cells, muscle and fibroblast cells, and leukocytes and myeloid lineage cells, are susceptible to HCMV infection *in vitro*. Entry of HCMV requires multiple viral glycoprotein complexes. Glycoprotein B (gB) is a class III fusion protein (Burke and Heldwein, PLoS Path 2005). Its fusogenic activity is triggered via interaction with complexes containing glycoproteins H (gH) and L (gL). The pentameric gH complex, comprised of gH/gL bound with pUL128, pUL130, and pUL131, enables HCMV to infect epithelial cells, endothelial cells, and leukocytes, most likely through a receptor-mediated endocytosis pathway (Wang and Shenk, 2005; Ryckman et al., 2008).

V160 was constructed on the backbone of AD169 strain. AD169 vaccine strain was originally reported by Elek and Stern (Lancet 1974). Merck & Co., Inc. received the material from Dr. H. Stern at passage 53 and passaged five additional times in WI-38 human fibroblasts. Merck AD169 (MAD169) is a passage variant of AD169 and therefore has similar gene constitution and protein compositions as the parental AD169 virus. The genome of MAD169 was used to construct an infectious bacterial artificial chromosome (BAC) clone used for engineering V160.

V160 was constructed from the backbone of MAD169 with two new features: 1) restoration of the expression of the HCMV gH/gL/pUL128-131 pentameric glycoprotein complex, a dominant target for neutralizing antibodies following naturally acquired HCMV infection; and 2) fusion of the destabilization domain of FK506 binding protein (ddFKBP) to the N-termini of viral immediate early proteins IE1/2 and a terminase subunit pUL51 rendering the virus conditionally replication-defective (Wang et al., Sci Transl Med 2016). The terminase complex is a viral specific enzyme complex that includes pUL51 as one of its subunits. The enzyme complex is responsible for cleavage of concatamerized copies of the viral genome during viral replication. Like all herpesvirus, HCMV conducts its genome replication using “rolling circle” replication which produces a concatamer containing multiple copies of the viral genome. The concatamer must be cleaved by the terminase complex in order to produce single copies of viral genome, suitable for packaging into progeny virions. In the absence of Shield-1, the ddKFBP-pUL51 fusion protein is degraded thereby blocking terminase function. Therefore, in the absence of Shield-1, viral genomes of V160 cannot be packaged into viral capsids thus preventing the production of infectious virions. The genomic structure of V160 compared to wild-type HCMV and MAD169 is shown in Figure below.



Genetic map of V160, MAD169 and wild-type HCMV. Notable changes in MAD169 include deletion of UL/b' and duplication of RL fragment, point mutations in genes RL5A, RL13, UL36 and UL131. These mutations have been reported in all AD169 derived variants (Bradley et al., 2009), causing frame-shift and premature termination of RL5A, RL13 and UL131, and a substitution mutation in UL36 which inactivates its function. V160 was engineered using MAD169 as parental virus, in which the UL131 frame-shift mutation was repaired and ddFKBP was fused in frame to the N-termini of UL123 (IE1) exon1 and UL51. One copy of LoxP sequence (34 nt in length) was inserted between US28 and US29, and LoxP does not encode any polypeptide. Symbols: * mutation, ^d ddFKBP

The published mutations in RL5A, RL13, UL36, and the deletion of UL/b' (Bradley et al., 2009) have been confirmed in the sequence of V160.

The destabilizing domain FK506 binding protein (ddFKBP) gene encodes a protein prone to degradation (Banaszynski et al., Cell 2006). The ddFKBP gene was synthetically made based on the known amino acid sequence of ddFKBP protein. The amino acid sequence of ddFKBP is shown in Figure below. (Banaszynski et al, Cell 2006).

MGVQVETISPGDGRTFPKRGQTCVVHYTGMLEDGKKVDSSRDRNKPFFKMLGKQE
VIRGWEEGVAQMSVGQRAKLTISPDYAYGATGHPGIIPPHATLVFDVELLKPE

A LoxP sequence was introduced during construction of self-excised BAC constructs and is not expressed as a polypeptide. The 34 nucleotides LoxP sequence (ataacttcgtatagcatattacgaagttat) was based on a previous report (Yu et al, JV 2002). It is located between US28 and US29 in the V160 genome and serves as a genetic marker for differentiation of V160 versus wild-type HCMV. The fragment was made synthetically by overlapping oligonucleotide fragments.

- (c) Genetic stability – according to Annex IIIa, II, A(10)
V160 is a double stranded DNA virus with genome size of approximately 230-kb. The viral polymerase has 3' exonuclease activity which helps maintain genome fidelity by enabling error correction during DNA synthesis. To date, deep sequencing data has confirmed genetic stability across multiple viral passages of V160.

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)

Finland

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

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 United States ...
- Notification number

V160 has been evaluated in a Phase 1 study in the US (<https://clinicaltrials.gov/ct2/show/NCT01986010>). The study demonstrated that the vaccine was tolerated and there was no vaccine virus detected by a sensitive qPCR method in the urine or saliva in study participants. The details are summarized in Investigator Brochure (IB).

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

Human cytomegalovirus is ubiquitous in human populations. Approximately 50-90% of adult populations worldwide are seropositive to HCMV, indicating that most adults have been exposed to the virus. Like other herpesviruses, wild-type (wt) HCMV establishes latent infections, therefore, worldwide more than half of all adults are latently infected with wt HCMV. HCMV cannot replicate or survive in the environment outside of a human host and must be transmitted within human populations through close personal contact.

V160 was developed from parental virus AD169, an attenuated strain of HCMV having a 15-kb deletion in its genome. V160 was further attenuated by genetic engineering to make its replication dependent on a synthetic molecule called Shield-1 that is not found in nature. During manufacture of the vaccine, Shield-1 is provided in the culture medium to support V160 replication. During vaccine purification, the Shield-1 concentration is reduced below the level required to support productive virus replication. As expected, no evidence of V160 shedding was detected in the Phase 1 clinical study. Together with the strict species barrier of cytomegaloviruses, the requirement for Shield-1 to enable V160 replication makes the potential for environmental impact negligible.

Starting on the selection/randomization day, each subject will receive the first of two or three doses of study vaccine or placebo at month 0 (day 1), month 2 and month 6. Each subject will be closely monitored for adverse events (AEs) related with vaccination and monitored for viral shedding in saliva and/or urine by qPCR for up to 36 months after vaccination. If

there is a viral shedding, a secondary PCR will be conducted to differentiate the GMO V160 vs. wild-type HCMV.

V160 is manufactured at a facility in the US, and classified as a BSL-1 organism. Transportation and storage of V160 is in accordance with human vaccine industry standard. The final drug product is lyophilized and stored at 2-8°C. Each dose of V160 is provided in a two-vial image, with one vial containing lyophilized V160 (100 antigen units per dose) and the other containing Merck Aluminum Phosphate Adjuvant (MAPA) diluent (450 µg aluminum per mL). The lyophilized V160 product and the MAPA diluent are both stored at 2-8°C. The expected expiry date is three years after the date of manufacture. Both the lyophilized vaccine and the diluent are shipped at 2-8°C. Vaccine (lyophilized product) is reconstituted with 0.7 mL of the MAPA diluent using a disposable syringe.

Vaccine will be administered intramuscularly at deltoid muscle in a 0.5 mL dose as a two or three dose regimen. Each 0.5 mL dose contains 100 units of V160 antigen and 225 µg of MAPA. Each vaccine contains approximately 1×10^7 viral particles when cultured with Shield-1.

The intended clinical application presented for this vaccine is limited to four hospital sites in Spain with high expertise in vaccination studies in humans. In addition, the results obtained in the US Phase 1 study with the same product demonstrated no vaccine viral shedding in 170 study participants who received V160 active vaccine. Therefore, the likelihood for any negative effects to occur is considered negligible. Nonetheless, the Sponsor will be responsible to train the healthcare professionals that are involved in the study about the safety handling of this GMO in order to reduce to minimum level any possible accidental exposure to this product, either personal, contact to other people or 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	(.)
DNA virus	(X)
bacterium	(.)
fungus	(.)
animal	
- mammals	(.)
- insect	(.)
- fish	(.)
- other animal	(.)
...	

V160 was engineered using the genome of an attenuated strain of HCMV called AD169. The AD169 strain was developed using HCMV virus isolated from a throat swab of a healthy 7-year old girl. During extended passaging in human fibroblast cells, AD169 accumulated attenuating mutations including a ~15-kb genome deletion. AD169 was evaluated in two studies in healthy volunteers, and shown to be safe, well-tolerated, and immunogenic in inducing HCMV antibodies. No viral shedding was detected. (Elek and Stern, Lancet 1974; Neff et al, Proc. Soc. Prog. Bio. Med. 1979).

Wild-type HCMV is ubiquitous in human populations with the majority of adults worldwide being seropositive and latently infected. HCMV cannot replicate or survive in the environment outside of a human host. Wild-type HCMV is transmitted among human populations through close personal contact. Primary infection is rarely associated with any specific clinical symptoms in a healthy host, however, viremia may last for several weeks, and the individual may shed virus through saliva and urine for months. HCMV enters a new host through infection of mucosal epithelial cells. Virus spreads from the local infected epithelial cells to leukocytes and endothelial cells. The leukocytes and circulating endothelial cells will disseminate the virus into the blood stream, leading to viral infection of organ- and tissue-specific cells, including the vascular endothelial cells and epithelial cells of liver, lung, kidney and salivary gland. Progeny virions from infected kidneys or salivary glands are shed in urine or saliva, respectively. Virions shed in the bodily fluids are the source for new infections (Mocarski et al., Fields Virology 2013).

Comparison of the genomes of V160 with AD169 (the attenuated parental virus).

Feature	AD169	V160	Comment
~15-kb deletion of UL/b' region of genome	Yes	Yes	Relative to wild-type HCMV genomes
Mutations in RL5A, RL13, UL36	Yes	Yes	Relative to wild-type HCMV sequences
Frameshift mutation in UL131	Yes	No	Repaired in V160 to restore expression of the pentameric gH complex
ddFKBP sequences fused to IE1/2 and UL51	No	Yes	Makes V160 replication dependent on Shield-1
LoxP sequence	No	Yes	34 nucleotides between US28 and US29, does not encode a polypeptide

2. Name
- | | | |
|-------|---|-------------------|
| (i) | order and/or higher taxon (for animals) | Betaherpesviridae |
| (ii) | genus | Cytomegalovirus |
| (iii) | species | human |
| (iv) | subspecies | |
| (v) | strain | AD169 |
| (vi) | pathovar (biotype, ecotype, race, etc.) | ... |
| (vii) | common name | CMV |

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)

The parental AD169 strain was previously evaluated as a live viral vaccine in two studies in healthy volunteers, and shown to be safe, well-tolerated, and immunogenic in inducing HCMV antibodies. No viral shedding was detected.

Wild-type HCMV is prevalent worldwide in all known human populations. HCMV cannot replicate or survive in the environment outside of a human host.

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

HCMV is an obligatory human virus and only can infect and propagate in human beings.
There is no known animal reservoir for HCMV.

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

N/A

5. (a) Detection techniques

Exposure to HCMV can be confirmed with serological assays, designed to detect immunoglobins specific to viral antigens. In addition, viral shedding in urine and saliva or other body fluid can be detected by either sterile viral culture or qPCR using primers specific for HCMV viral sequences.

(b) Identification techniques

Viral culture, qPCR, viral genome 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

...As indicated in the Royal Decree 664/1997, of 12th May, in relation to the protection of workers against risks related to the exposure to biological agents during working time, the cytomegalovirus are classified as type 2. The parental AD169 virus was demonstrated to be non-pathogenic in two human trials and is classified as a biosafety level 1 (BSL-1) organism by American Type Culture Collection (ATCC).

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

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

If yes:

(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) of Directive 2001/18/EC

AD169 has a large 15-kb deletion of the UL/b' region which harbors 19 open reading frames and is believed to be a key genetic marker associated with the attenuation of AD169 (Cha et al, JV 1996). Additional mutations in the AD169 genome are found in RL5A, RL13, UL36 and UL131. The mutation in UL131 is known to be responsible for the inability of AD169 to infect epithelial cells (Wang and Shenk, JV 2005).

The AD169 strain was previously evaluated as a live viral vaccine in two studies in healthy volunteers, and shown to be safe, well-tolerated, and immunogenic in inducing HCMV antibodies. No viral shedding was detected.

8. Information concerning reproduction

(a) Generation time in natural ecosystems:

AD169 is an attenuated laboratory strain of HCMV not found in nature and the available data indicate that this virus is not capable of replicating in nature. AD169 was evaluated in two studies in healthy volunteers, and shown to be safe and well-tolerated. No viral shedding was detected. (Elek and Stern, Lancet 1974; Neff et al, Proc. Soc. Prog. Bio. Med. 1979).

In vitro HCMV viruses take approximately 48-72 hours to complete the viral life cycle. However, generation time of HCMV in a human host has not been definitively determined. In a human challenge study in which healthy volunteers were given a pathogenic strain of HCMV, mild symptoms and laboratory abnormalities did not develop until 5-6 weeks post inoculation (Plotkin et al, JID 1989).

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

AD169 is an attenuated laboratory strain of HCMV not found in nature. AD169 was evaluated in two studies in healthy volunteers, and shown to be safe and well-tolerated. No viral shedding was detected. (Elek and Stern, Lancet 1974; Neff et al, Proc. Soc. Prog. Bio. Med. 1979). The available data suggest that AD169 is not capable of replicating in humans.

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

(c) Factors affecting reproduction:

AD169 is a laboratory strain, adapted for growth in human fibroblast cells. AD169 has a large 15-kb deletion of the UL/b' region which harbors 19 open reading frames and is believed to be a key genetic marker associated with the attenuation of AD169 (Cha et al, JV 1996). Additional mutations in the AD169 genome are found in RL5A, RL13, UL36 and UL131. The mutation in UL131 is known to be responsible for the lack of expression of the pentameric gH complex and thus the inability of AD169 to infect epithelial cells (Wang and Shenk, JV 2005).

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 | not known able to survive or achieve dormancy outside human host |

(b) relevant factors affecting survivability:

Like wild-type HCMV, the AD169 laboratory strain requires human cells for replication and survivability outside of human cells has been shown to be limited. To assess survival time, live AD169 virus was diluted with saliva and phosphate-buffered saline (10^5 infectious particles/mL) then applied to human hands. Live AD169 virus was recovered from 18 of 20 hands after 1 minute but from only 4 of 20 hands after 15 minutes. To assess survival/transferability, AD169 virus was applied to human hands which then touched plastic, metal, glass, wood, rubber, cotton cloth, cracker, or another hand surface. Viable AD169 virus could be recovered after 15 minutes from plastic, 5 minutes from crackers and glass, 1 minute from metal, and less than 1 minute from cloth. No viable virus was recovered at any timepoint from wood, rubber, or another hand. To evaluate the effectiveness of hand-cleansing methods to remove HCMV, AD169 virus was applied to the hand surface prior to cleansing with water, antibacterial soap, or hand sanitizer (65% ethyl alcohol). No viable virus was recovered from hands after any of these treatments (Stowell, 2014).

Like other viruses, HCMV can effectively be inactivated by: (1) Autoclave; (2) UV light; (3) Lipid solvents; (4) 1-10% bleach, 70% ethanol, 2% glutaraldehyde, and 2.5% phenol.

10. (a) Ways of dissemination

No viral shedding of AD169 was detected in either of two studies in healthy volunteers (Elek and Stern, Lancet 1974; Neff et al, Proc. Soc. Prog. Bio. Med. 1979). Therefore, the available data suggest that the parental AD169 virus is not capable of replicating in humans, nor of being disseminated.

Wild-type HCMV is known to be disseminated by contact with the bodily fluids of an infected human being. Other transmission modes for wild-type HCMV include organ transplantation from donors who are HCMV seropositive, maternal-fetal transmission, and transfusion of blood from HCMV seropositive donors.

(b) Factors affecting dissemination

AD169 is a laboratory strain, adapted for growth in human fibroblast cells. AD169 has a large 15-kb deletion of the UL/b' region which harbors 19 open reading frames and is believed to be a key genetic marker associated with the attenuation of AD169 (Cha et al, JV 1996). Additional mutations in the AD169 genome can found in RL5A, RL13, UL36 and UL131. The mutation in UL131 is known to be responsible for the lack of expression of the pentameric gH complex and thus the inability of AD169 to infect epithelial cells (Wang and Shenk, JV 2005).

Wild-type HCMV can be disseminated by poor hygiene practice such as sharing food and drink, and kissing with exchange of saliva, close personal contact with the individuals actively shedding virus.

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)

N/A (parental virus, AD169, was not a GMO)

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 | |

Multiple genetic changes exist in AD169 (compared to wild-type HCMV) following extended passaging in human fibroblast cells in an attempt to generate a live attenuated vaccine. In addition, genetic modification was made to the Merck AD169 (MAD169) to generate V160. The genetic history of V160 is summarized below:

- MAD169 is a passage variant of AD169, and is an attenuated virus which contains genetic changes with multiple mutations, including RL5A, RL13, UL36 and the deletion of ~15Kb UL/b' (Bradley et al., JGV 2009)
- MAD169 virus has a frameshift mutation in UL131 which results in defective expression of the pentameric gH complex (gH/gL/pUL128-131) needed for infection of endothelial and epithelial cells (Wang and Shenk, JV 2005). Wild-type HCMV expresses this pentameric gH complex which contains key antigens for a potent neutralizing antibody response. The frameshift mutation in UL131 in MAD169 was repaired in V160 by deleting one adenosine nucleotide thus correcting the frameshift. This genetic repair enables V160 to induce more potent neutralizing antibodies than MAD169 (Wang et al, Sci Transl Med 2016).
- Two modified FK506 binding protein (ddFKBP) sequences based on a human FKBP sequence were inserted at two locations of MAD169 genome. By design, the modified ddFKBP sequence is unstable upon expression and rapidly degraded unless rescued by a small molecule called Shield-1 (molecular weight 750). This system has been used to regulate protein function when the protein of interest fused to ddFKBP (Banaszynski

et al, Cell 2006). In V160, the ddFKBP sequence is fused to two essential viral genes. As a result, two nonstructural viral proteins (IE1/2 and UL51) are degraded upon expression unless Shield-1 is provided. This modification results in a vaccine strain that cannot productively replicate unless Shield-1 is provided.

- A LoxP sequence (34 nucleotides in length) is inserted between US28 and US29 ORFs.

2. Intended outcome of the genetic modification

Previous vaccine candidates such as AD169 and Towne vaccines lacked the pentameric gH complex as comparing to the wild-type HCMV. These viruses, although capable of modifying the disease outcome, were not capable of preventing HCMV infection. Restoring expression of the pentameric gH complex on V160 improved vaccine immunogenicity in preclinical studies.

Because two essential nonstructural viral proteins (IE1/2 and UL51) are fused to ddFKBP, productive virus replication is prevented unless Shield-1 is provided in the virus culture. (Wang et al, Sci Transl Med 2016).

The LoxP sequence in V160 is noncoding but serves as a genetic marker for differentiation of V160 versus wild-type HCMV.

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
...

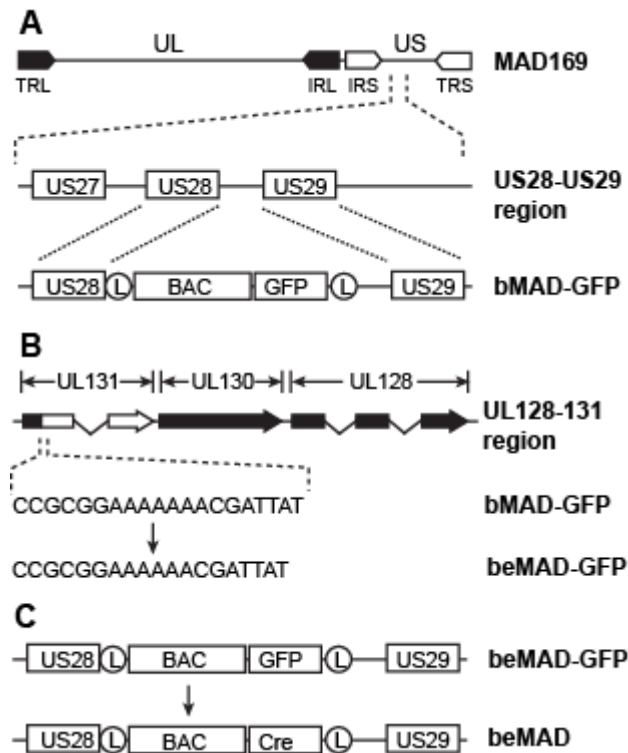
- (f) Method for introducing the vector into the recipient organism
- (i) transformation (.)
 - (ii) electroporation (.)
 - (iii) macroinjection (.)
 - (iv) microinjection (.)
 - (v) infection (.)
 - (vi) other, specify ...

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

The genetic construction of V160 was enabled by bacterial artificial chromosome (BAC) technology. The entire viral genome of MAD169 was cloned with a DNA fragment containing BAC element along with selection and screening markers; this fragment inserted between US28 and US29 ORFs in the viral genome subsequently allowed the entire viral genome to be preserved and propagated as a single copy in *E. coli*. It is also enabled genetically manipulation of viral genome in *E. coli*. BACmid is considered a vector, which upon insertion into a viral genome would enable propagation and preservation of the viral DNA in *E. coli*.

Figure below outlines the modification strategy



Schematic diagram of the construction strategy of epithelial tropic AD169 strain. (A) Generation of hCMV infectious BAC clone of MAD169. A DNA fragment containing the BAC element and a GFP expression cassette flanked by two LoxP sites was integrated into MAD169 genome between US28 and US29 ORFs by homologous recombination to generate infectious BAC clone bMAD-GFP. (B) Rescue of the epithelial tropism of bMAD-GFP. One adenine nucleotide was removed from the first exon of UL131 in bMAD-GFP to repair the frame shift mutation of UL131, creating infectious BAC clone beMAD-GFP. (C) Construction of self-excisable BAC. The GFP ORF in beMAD-GFP was subsequently replaced by a Cre recombinase ORF to generate infectious BAC clone beMAD. The infectious BAC clone beMAD was used to construct ddfKBP mutants including V160.

Using the self-excisable BAC clone of beMAD, ddfKBP was fused to a panel of selected viral genes required for viral replication (Wang et al, Sci Transl Med, 2016). These essential genes were selected largely based on the fact that they cannot be detected in HCMV virions by Liquid chromatography tandem -mass spectrometry (LC-MS/MS) (Varnum et al, JV 2004). For V160, ddfKBP was fused to two viral essential proteins IE1/2, and UL51. As viral IE1 and IE2 were expressed from alternatively spliced major immediate early transcripts that share the first three exons and differ in their 3' exons, thus, as designed, both IE1 and IE2 proteins are produced as a ddfKBP fusion protein. The recombinant BAC clones were electroporated into ARPE-19 cells, and cultured in the medium containing 2 μ M of Shield-1 to rescue infectious virus. The progeny virions were plaque purified and sequence of the ddfKBP gene insertion was confirmed by sequencing analysis.

6. Composition of the insert

(a) Composition of the insert

- V160 has two identical ddFKBP inserts fused in frame to the 5' end of UL123 (IE1) exon1 and the 5' UL51 ORF to create fusion proteins between ddFKBP and IE1/2, and ddFKBP and pUL51. The amino acid sequence of ddFKBP is shown below.

MGVQVETISPGDGRTFPKRGQTCVVHYTGMLEDGKKVDSSRDRNKPFKFMLGK
QEVIRGWEEGVAQMSVGQRAKLTISPDYAYGATGHPIPPHATLVFDVELLKPE

This insert sequence represents a modified human FKBP sequence selected based on its ability to destabilize the expressed fusion protein (Banaszynski et al, Cell 2006).

- One copy of a LoxP sequence (ataacttcgtatagcatatcgaagttaa) was inserted between US28 and US29 during genetic engineering. This LoxP sequence does not encode a polypeptide.

(b) Source of each constituent part of the insert

ddFKBP: genetically engineered human protein FKBP12 using artificial codons, referenced in Banaszynski et al, Cell 2006.

LoxP: referenced in Yu et al, JV, 2002.

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

ddFKBP: encode a ddFKBP protein which is destined for rapid degradation unless rescued by Shield-1.

LoxP: no coding capacity and no function.

(d) Location of the insert in the host organism

- on a free plasmid (.)
- integrated in the chromosome (.)
- other, specify inserted at V160 viral genome at specific location.

(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 (.)

DNA virus (.)
 bacterium (.)
 fungus (.)
 animal
 - mammals (X)
 - insect (.)
 - fish (.)
 - other animal (.)
 (specify phylum, class) modified version of human gene
 other, specify ...

The insert was modified from a human gene encoding a protein called FK506-binding protein 12. The modifications from the human gene have been reported and are (1) deepened the binding pocket (F36V) which improved its specificity to Shield-1 over FK506 (Clarkson et al, PNAS 1999); and (2) amino acid sequence changes that made the protein more unstable without Shield-1 (Banaszynski et al, Cell 2006).

2. Complete name

(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

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

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

If yes, specify the following:

(b) to which of the following organisms:

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

(b) are the donated sequences involved in any way to the pathogenic or harmful properties of the organism

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

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

...

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 (.) No (X)

If yes, specify ...

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

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

E. Information relating to the genetically modified organism

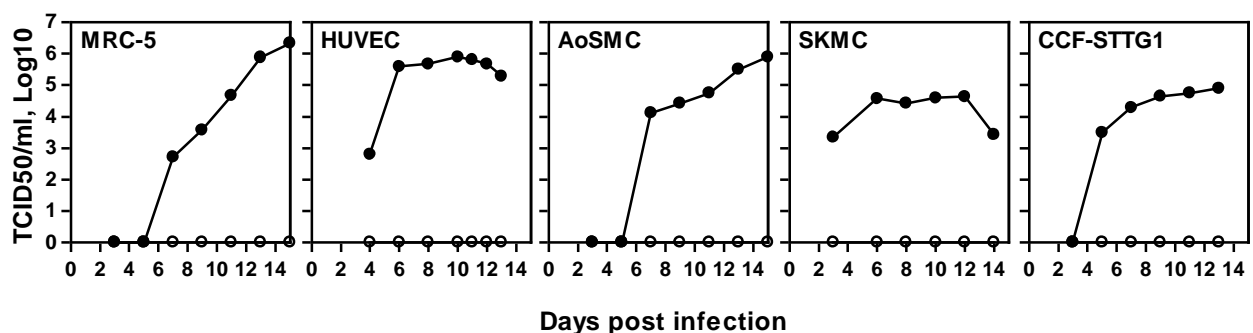
1. Genetic traits and phenotypic characteristics of the recipient or parental organism which have been changed as a result of the genetic modification

- (a) is the GMO different from the recipient as far as survivability is concerned?

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

Specify

V160 will have *decreased* survivability relative to wild-type HCMV (or parental AD169) since V160 is conditionally replication-defective and cannot productively replicate without Shield-1. Shield-1 is an artificial molecule not found in nature therefore survivability *in vivo* or the natural environment is negligible. The results below demonstrate the dependence of V160 replication on the presence of Shield-1 using five different human cell types.



Cultures of cells, originated from human cell types such as fibroblasts (MRC-5), endothelial cells (HUVEC), smooth and skeletal muscle cells (AoSMC and SKMC) or neuronal cells (CCF-STTG1), were infected with V160 virus at a multiplicity of 0.1 PFU/cell, except for CCF-STTG1 at 5 pfu/cell. After one hour, the cells were washed twice with fresh medium, and incubated in the absence (○) or presence (●) of 2 μ M Shield-1. Cell-free virus was collected at the indicated time points after infection, and infectious virus unit was quantified by TCID50 assay on ARPE-19 cells in medium containing 2 μ M of Shield-1.

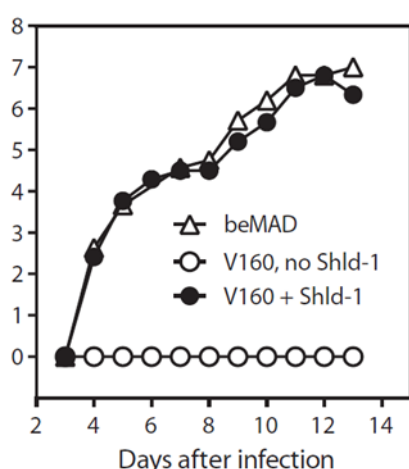
- Additionally, the frameshift mutation present in UL131 of MAD169 was repaired in the genome of V160 by deleting one adenosine nucleotide to correcting the frameshift. This frameshift repair enables V160 to express the pentameric gH complex (missing from MAD169) and thereby to induce more potent neutralizing antibodies than MAD169 (Wang et al, Sci Transl Med 2016). Wild-type HCMV expresses this pentameric complex which is required for infection of epithelial and endothelial cells.

- (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

Because it does not express the pentameric gH complex, the parental MAD169 virus cannot infect epithelial and endothelial cells. Repair of the frameshift mutation in UL131 enables V160 to infect epithelial and endothelial cells provided that Shield-1 is present. The figure below shows that, in the presence of Shield-1, V160 replicates at a similar rate to “beMAD” which is a virus made by repairing the UL131 frameshift mutation in MAD169. The beMAD virus is not Shield-1 dependent and was used in this experiment only for comparison to V160.



Effect of Shield-1 on V160 replication in comparison with beMAD virus, a parental MD169 virus but restored of the pentameric complex expression. ARPE-19 cells were infected with V160 at an MOI of 0.01 PFU per cell and incubated in the absence (○) or presence (●) of 2 μM Shield-1. Cells infected with beMAD at an MOI of 0.01 PFU/cell were included as a control (Δ). Progeny virus was collected at the indicated time points after infection and quantified by TCID50 assay on ARPE-19 cells supplemented with 2 μM Shield-1.

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

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

Specify

V160 is not able to replicate productively in the absence of Shield-1 and is therefore unable to disseminate from human-to-human. Within vaccine recipients, V160 is not able to disseminate from the vaccine site (deltoid muscle) because no Shield-1 is present in the human body. Consistent with this, no evidence of V160 shedding was detected in the Phase 1 clinical study.

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

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

Specify

V160 is unable to replicate without Shield-1 thus is not expected to cause any pathology. This assessment is supported by the Phase 1 safety data.

2. Genetic stability of the genetically modified organism

HCMV is a double stranded DNA virus with genome size of approximately 230-kb. The viral polymerase has 3' exonuclease activity which helps maintain genome fidelity by enabling error correction during DNA synthesis. To date, deep sequencing data has confirmed genetic stability across multiple viral passages of V160.

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

Viral shedding is virological evidence of active viral replication in a human host. With no active viral replication expected for V160 due to the lack of Shield-1, there is minimal chance that V160 will be detected in bodily fluids.

Detection of viral shedding by qPCR is the field standard, based on throughput, precision, and ease of handling of specimens. Real Time qPCR assays with high sensitivity and specificity have been developed to evaluate shedding of both wild-type (WT) HCMV and the V160 vaccine strain in saliva and urine specimens. The WT-qPCR assay detects UL54, which is present and highly conserved among all HCMV, including WT and vaccine strain viruses. WT positive samples are subject to vaccine strain-specific qPCR assay, where additional viral targets, including those unique to V160, are measured in a multiplexed assay. The qPCR assays have demonstrated to be sensitive and specific, with the limit of detection determined at 200 copies/mL, and have the ability to distinguish the vaccine virus, and other HCMV including both WT strains and laboratory strains.

The vaccine strain-specific qPCR was qualified using virus spiked into human saliva and human urine matrices. The assay characteristics, including the limit of detection, analytical specificity and assay precision, were determined in a formal qualification

study. The limit of detection was at least 100 copies/mL. There is high assay specificity for V160 vs. WT HCMV or other herpesviruses, as designed. Acceptable assay performance has been demonstrated (Internal report).

(b) Techniques used to identify the GMO

qPCR using primers specific for the V160 vaccine strain

F. Information relating to the release

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

The GMO V160 will be investigated as an experimental vaccine for prevention of HCMV infection in human subjects. The vaccine is planned in several clinical trials to test its safety, immunogenicity and efficacy.

Transmission of the vaccine virus appears to represent a minimal risk, because it is a conditional replication-defective virus dependent on Shield-1 which is not found in nature.

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 (.) No (X)

If yes, specify ...

3. Information concerning the release and the surrounding area

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

The clinical trial will be run in the next hospital facilities and by the next medical doctors:

- Hospital de Santiago de Compostela, Dr. Martínón. Service of Pediatrics. City: Santiago de Compostela

- Hospital 12 de octubre, Dr Blazquez. Service of Pediatrics. City: Madrid

- Hospital Clinic, Dra. Aldea. Service of Preventive medicine and Epidemiology. City: Barcelona

- Hospital La Paz, Dra. Mellado. UCICET (Central Unit of Clinical Investigation and Clinical Trials) of IdiPaz. City: Madrid

(b) Size of the site (m²): ... m²

(i) actual release site (m²): ... m²

(ii) wider release site (m²): ... m²

The release of the vaccine is done in hospital facilities located in urban environment. The areas in which the vaccine is prepared and released are access controlled. The study staff that could potentially receive an accidental administration will be properly trained, in order to avoid it.

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

The hospitals in which the vaccine will be released are located in urban environment. They are not located close to significant biotopes, protected areas or drinking water supplies.

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

This section is not applicable, as the vaccine will be release in hospitals located in urban environment.

4. Method and amount of release

- (a) Quantities of GMOs to be released:

V160 is manufactured at a facility in the US and classified as a BSL-1 organism. Vaccine (lyophilized product) is reconstituted with the attached diluent (MAPA) using a disposable syringe to a final volume of 0.7 mL. Reconstituted V160 will be administered intramuscularly in the deltoid muscle in a 0.5 mL dose as a two or three dose regimen. Each 0.5 mL dose contains 100 units of V160 antigen (HCMV antigen in V160 particles). The “V160 antigen” is measured by ELISA method using monoclonal antibodies specific for) and 225 µg of MAPA. In Spain 127 participants will be randomized. One third will receive a 3-dose regimen, and the other one-third will receive the 2-dose regimen.

The total vaccines release in Spain will be 215 vaccines in a time period of 7 months.

- (b) Duration of the operation:

The study is estimated to be around 36-42 months. The study starts with the first subject recruited at the clinical site with the informed consent signed, and closes with the last subject completing month 36 visit or the study reaching the pre-defined endpoint, whichever comes first. After enrolled into the study, each subject will receive 2 or 3 doses of injection of study vaccine or placebo at month 0, 2 and 6 respectively. The vaccine is given by intramuscular injection in the deltoid muscle. Study vaccine/placebo will be administered for all subjects a 0.5-mL intramuscular injection in the deltoid muscle at a 90° angle into the muscle tissue using a needle long enough to ensure intramuscular deposition of the study vaccine/placebo.

Vaccination is by intramuscular injection and is no more than 1 minute in duration. All subjects will remain under observation for 30 minutes after vaccination for signs of hypersensitivity reactions or other adverse events. Each subject will be monitored for safety and viral shedding for up to 36 months.

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

Transmission of the vaccine virus appears to represent a minimal risk. V160 was built on the backbone of AD169 virus, an attenuated vaccine which was shown safe in human studies without evidence of viral shedding. In addition, V160 is constructed as

a conditionally replication-defective virus that cannot replicate productively in the absence of Shield-1. The results of a Phase 1 study in the US confirmed that V160 is incapable of replicating in study participants and there was no vaccine virus detected in urine or saliva samples during study period of 18 months. The safety data are summarized in the investigator's brochure (IB).

Intramuscular injection is designed to minimize spill or accidental spread of the GMO V160. Study vaccine/placebo will be stored in a limited-access area. All involved personnel will use appropriate biosafety practices for a BSL-1 organism during transport, prior to and after administration and disposal. Used syringes, needles, empty or partially empty vaccine and diluent vials will be discarded as biohazard medical waste per local regulation.

Precautionary notes will be provided to clinical sites. As for other live viral vaccines, vaccinated recipients should not donate blood or plasma within 30 days after each vaccination. Care should be taken to avoid direct blood contact, e.g. by sharing needles or razor blades, all of this information is included in the Patient Guideline Form.

Apart from the training at the site about the saliva and urine sample collection provided by the investigator, the participant will be provided by a guideline of the sample collection procedures that the participant could use it as a reference. This guideline is established by the Sponsor. The participants can contact to the site anytime they need it in order to answer any question that they may have in relation to the sample self-collection at home. The site will contact to the participants monthly to remind them how the collection must be performed and how to deliver them to the site. The receive of the samples will be reviewed by the sites at real time. The site will contact and re-train the participant if needed.

Commercially available, validated kits for the self-collection urine and saliva will be packaged by the central lab and will be shipped to clinical sites. Upon randomization to the trial, the sites will provide comprehensive written and verbal instructions to the participant on how to collect and safely handle each sample. Kits will be distributed to the participants and are to be stored at room temperature.

On a monthly basis participants will self-collect a urine and saliva samples. Upon completion of the saliva sample, participants will be asked to remove the collection funnel and secure the cap to lock in the sample. Upon completion of the urine sample participants will secure the sample by ensuring the cap seals tightly. Participants will complete the lab requisition form and store and mail samples at ambient temperature in the secure packaging provided. Samples will be mailed at ambient temperature, in accordance with local regulations, from the participant to the clinical sites. As the study participants are healthy women, there is no reason to believe that risk of presence of CMV in these samples will be higher than any specimens mailed by healthy subjects for any other purpose (i.e. genotyping for ancestry determination).

Once samples have been received at the clinical site the study coordinator will review the requisition, record the sample requisition information in the study database, and store the samples at -20 Celsius. Samples will then be shipped on a bi-weekly basis from the site to the central lab on dry ice where the central lab will log samples into central lab database. Sample reconciliation between the Sponsor database and the central lab basis will be ongoing during the life of the study.

Only participants enrolled in the study may receive study treatment and only authorized site staff may supply or administer study treatment. All study treatments must be stored in a secure, environmentally controlled, and monitored (manual or automated) area in accordance with the labeled storage conditions with access limited to the investigator and authorized site staff.

The investigator, institution, or the head of the medical institution (where applicable) is responsible for study treatment accountability, reconciliation, and record maintenance (ie, receipt, reconciliation, and final disposition records).

For all trial sites, the local country Sponsor personnel or designee will provide appropriate documentation that must be completed for drug accountability and return, or local discard and destruction if appropriate. Where local discard and destruction is appropriate, the Sponsor must inform the site how to perform this local destruction and the investigator is responsible for ensuring that a local discard/destruction is documented.

The trial site is responsible for recording the lot number, manufacturer, and expiry date for any locally purchased product as per local guidelines unless otherwise instructed by the Sponsor.

The investigator shall take responsibility for and shall take all steps to maintain appropriate records and ensure appropriate supply, storage, handling, distribution and usage of study treatments in accordance with the protocol and any applicable laws and regulations.

The V160 GMO vaccine will be shipped and stored at 2 to 8°C, protected from light.

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 V160 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.

V160 was evaluated in a Phase 1 human study in the United States, in which 170 subjects including both HCMV seropositive and seronegative volunteers were inoculated with three doses of varying amounts of V160 active vaccine. (<https://clinicaltrials.gov/ct2/show/NCT01986010>). The subjects were vaccinated three times at day 1, month 1 and month 6, and they were followed for viral shedding for up to 18 month. No V160 virus was detected in the urine and saliva samples from these study participants using a sensitive qPCR method. The details of the study are summarized in IB.

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)

V160 is intended as a human vaccine, and is under development to prevent infant disability caused by congenital CMV infection. The immunization of V160 is expected to induce immune responses in the target organism, i.e., humans, which can protect these vaccine recipients and their offspring against future infection of HCMV.

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

Not likely. V160 is unable to proliferate in nature since it is obligatory pathogen and can only replicate in human cells under specific culturing condition, i.e., with Shield-1. Shield-1 is an artificial molecule not found in the environment or the human body.

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

None. V160 could not survive in any ecosystem, including human host, since there is no Shield-1 available to support its replication. Even in the worst case of scenario that V160 were shed in vaccine recipients' urine and released into waste water, there is no reason that V160 particles would be more stable than wild-type HCMV present in sewage.

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:

None is expected. Genetic exchange between herpesvirus and other microorganism has never been reported.

- (b) from other organisms to the GMO:

None is expected.

- (c) likely consequences of gene transfer:

No advantage for GMO could be envisioned with such gene transfer.

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. V160 vaccine is expected to be degraded after administration to humans by endogenous protein and DNA catabolic pathways. The GMO is not expected to be released from vaccine recipients.

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 start in early 2018 will be conducted in different countries including the US, Canada, Finland, and Spain; the trial in Spain will be conducted at:

- Hospital de Santiago de Compostela, Dr. Martínón. Service of Pediatrics. City: Santiago de Compostela
- Hospital 12 de octubre, Dr Blazquez. Service of Pediatrics. City: Madrid
- Hospital Clinic, Dra. Aldea. Service of Preventive medicine and Epidemiology. City: Barcelona

- Hospital La Paz, Dra. Mellado. UCICEC (Central Unit of Clinical Research and Clinical Trials) of IdiPaz. City: Madrid

The sponsor estimates around 36-42 months to complete the trial in Spain, 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 vaccine within the clinical trial is mainly focused on three phases:

(1) Each dose of V160 is provided in a two-vial image, with one vial containing lyophilized V160 (100 antigen units per dose) and the other containing Merck Aluminum Phosphate Adjuvant (MAPA) diluent. Both the lyophilized vaccine and the diluent are shipped at 2-8°C according to international standards and the lyophilized V160 product and the MAPA diluent are both stored at 2-8°C in a limited-access area with a temperature monitoring device.

(2) Vaccine (lyophilized product) is reconstituted with 0.7 mL of the MAPA diluent using a disposable syringe. A final volume of 0.5 mL will be loaded into a syringe ready for administration. Each 0.5 mL dose contains 100 units of V160 antigen and 225 µg of MAPA. The vaccine is given by intramuscular injection (0.5 mL) in the deltoid muscle. Used syringes, needles, empty or partially empty vaccine and diluent vials will be discarded as biohazard medical waste per local regulation as biohazard waste classification requirements (BSL-1).

(3) Subjects will be enrolled and randomized into 2-dose, 3-dose or placebo groups, and receive 2- or 3-dose injection of vaccine/placebo at day 1, month 2 and month 6. Subjects will be followed either by phone calls or visits for adverse events associated with vaccination through the study period (36 months).

(4) Commercially available, validated kits for the self-collection urine and saliva will be packaged by the central lab and will be shipped to clinical sites. Upon randomization to the trial, the sites will provide comprehensive written and verbal instructions to the participant on how to collect and safely handle each sample. Kits will be distributed to the participants and are to be stored at room temperature.

On a monthly basis participants will self-collect a urine and saliva samples. Upon completion of the saliva sample, participants will be asked to remove the collection funnel and secure the cap to lock in the sample. Upon completion of the urine sample participants will secure the sample by ensuring the cap seals tightly. Participants will complete the lab requisition form and store and mail samples at ambient temperature in the secure packaging provided (a crush-proof container for mailing). Samples will be mailed at ambient temperature, in accordance with local regulations, from the participant to the clinical sites. As the study participants are healthy women, there is no reason to believe that risk of presence of CMV in these samples will be higher than any specimens mailed by healthy subjects for any other purpose (i.e. genotyping for ancestry determination).

Once samples have been received at the clinical site the study coordinator will review the requisition, record the sample requisition information in the study database, and store the samples at -20°C. Samples will then be shipped on a bi-weekly basis from the site to the central lab on dry ice where the central lab will log samples into central lab database. Sample reconciliation between the Sponsor database and the central lab basis will be ongoing during the life of the study.

(4) Shedding of virus in saliva and/or urine will be monitored. Subjects will be asked to collect their own saliva and/or urine samples at monthly intervals with phone call reminders, and deliver by mail the specimens in secure containers to the sites, and then the sites will deliver them to a central lab where the viral qPCR will be conducted for any shedding of

HCMV. If there is any positive reading on HCMV detection, a secondary PCR will be performed which can differentiate the GMO V160 vs. wild-type HCMV. Serum and peripheral blood mononuclear cell (PBMC) samples for future research will be obtained from each participant at pre-defined time-points to monitor immune responses by vaccination.

2. Methods for monitoring ecosystem effects

Transmission of the vaccine virus appears to represent a minimal risk, since the GMO V160 is a replication defective virus. The results from the US Phase 1 study further support this assumption as no participant was found shedding V160 during 18-month study period (IB). Nonetheless, it would be recommended that study participants be educated about avoidance of direct blood contact including sharing needles or razor blades. Serum samples for future research will be obtained from each participant at predefined time points.

The risk of V160 dissemination into environment is low as HCMV is an obligatory intracellular pathogen and cannot survive in waste water. In addition, HCMV is not known to cause productive infection in animal species other than humans. Lastly, the GMO V160 is a conditionally replication-defective virus depending on the artificial chemical, Shield-1, for productive replication.

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

N/A

4. Size of the monitoring area (m²)
... m²

The release of the vaccine will be done in hospital facilities limited to specific areas with access restrictions.

The reception, storage and preparation of the vaccine will be done in the Pharmacy Service, where the access is also controlled.

Transportation of the vaccine will be done in a sealed box labelled as “contains GMO”.

The administration of the vaccine will be also done in a restricted area.

Only the study staff will have access to the vaccine.

The study staff will be trained in biosafety measures type 1 along the reception, management, transport, administration and disposal of the vaccine. Biosafety measures type 1 applicable to facilities and equipment will also be followed.

5. Duration of the monitoring

Each participant will be enrolled and followed for up to 36 months. The sponsor anticipates that this study will last 36-42 months.

6. Frequency of the monitoring

After enrollment, each subject will be trained for self-collection of saliva and/or urine samples. The samples will be collected on monthly basis with phone reminders from clinical sites, and the samples will be mailed to a central laboratory where a viral specific qPCR will be conducted. Month monitoring will be conducted on self-collected saliva and/or urine

samples will last for up to 36 months. In addition, serum and PBMC samples will be collected for all study participants at predefined time-points for monitoring immune responses to vaccination.

I. Information on post-release and waste treatment

1. Post-release treatment of the site

Decontamination with disinfectants (alcohol disinfectants, halogen and aldehydes disinfectants, and sodium hypochlorite) according to local biosafety guidelines for BSL-I.

2. Post-release treatment of the GMOs

The syringes and vaccine vials used will be discharged according to the local guidelines of biological materials handling. These procedures are the removal of the waste in the containers of hazardous waste, being inactivated by autoclave.

In addition, any material used for the administration or collection of the body fluids will be discharged according to the usual biosafety procedures in the sites, also in the containers of biohazardous waste that are inactivated by autoclave.

The guidelines of the sites describe the management of this waste; however, the Sponsor will train the investigator team in order to ensure that the discharge of the vaccine is performed properly.

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 (gloves, lab coat) 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 site for biohazardous sharps. In addition, other materials used for the administration or collection of the body fluids will be discharged according to the usual biosafety procedures in the sites, in the appropriate containers for this purpose that are labelled with the pictogram of biohazard this waste will be decontaminated by autoclave before its removal.

J. Information on emergency response plans

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

The V160 vaccine will be prepared in the Pharmacy of the hospital, except in La Paz where the vaccine preparation will be done in the UCICEC (IdiPaz, Central Unit for Clinical Research). The vaccine preparation is a simple process of reconstitution of lyophilized vaccine with the provided diluent using syringe, and syringe loading. The vaccine is given by IM injection (0.5 mL) in the deltoid muscle and healthy subjects will be randomized to receive 2 or 3 doses of vaccine or placebo at month 0, 2 and 6.

In case of unexpected spread (e.g. spills) the affected area, lineated with absorbing material, will be decontaminated using appropriate disinfectants (alcohol disinfectans, halogen and aldehydes disinfectants as well as sodium hypochorite). In case of injury, the injured site will be disinfected appropriately.

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

Wear protective personal equipment (nitrile/latex gloves, lab coat) and cover spill with paper towels. Apply chemical disinfectant such as 70% ethanol solutions. Start at the perimeter of the spill and work towards the center. 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

N/A.

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

No undesirable effect is expected, however, if an undesirable effect occurs then the use of V160, the 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 disinfectants.