ChAd155-RSV

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

Notifier: Glaxo SmithKline Biologicals

EudraCT number: 2016-000117-76

V1.0 - 05 August 2016 (Spain)

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List of Abbreviations

Ad5	Adenovirus 5
AE	Adverse Event
BAC	Bacterial Artificial Chromosome
BGHpA	Bovine Growth Hormone Polyadenylation Signal
ChAd155	Chimpanzee Adenovirus Type 155
ChAd155-RSV	Investigational Recombinant Chimpanzee Adenovirus Type 155-vectored RSV Vaccine
CMI	Cell-Mediated Immunity
DNA	Deoxyribonucleic Acid
EEC	European Economic Community
F	RSV Fusion Protein
F0ΔTM	RSV Fusion Protein deleted from its transmembrane domain
GLP	Good Laboratory Practices
GMO	Genetically modified organism
GMP	Good Manufacturing Practices
GSK	GlaxoSmithKline
HEK293	Human Embryonic Kidney 293
HCMV	Human Cytomegalovirus
IB	Investigator Brochure
IM	Intramuscular
IMPD	Investigational Medicinal Product Dossier
LLOQ	Lower Limit Of Quantification
LRTI	Lower respiratory tract infection
MUSCLE	Multiple Sequence Comparison by Log-Expectation
MS	Master Seed
MVS	Master Virus Seed
M2-1	RSV Matrix Protein
N	RSV Nucleocapsid Protein
NHP	Non-Human Primates
PCR	Polymerase Chain Reaction
qPCR	Quantitative Polymerase Chain Reaction
RCA	Replication Competent Adenovirus
RNA	Ribonucleic Acid
RSV	Respiratory Syncytial Virus
SAE	Serious Adverse Event
SOP	Standard operating procedure
TetO	Tetracycline Operon Operator Sequence
TetR	Tetracycline Repressor
vp	Viral particles
VSS	Virus Seed Stock

A. General information

1. Details of notification

(a) Member State of notification Spain
(b) Notification number B/ES/16/07
(c) Date of acknowledgement of notification 17/08/2016
(d) Title of the project:

Clinical trial RSV PED-002 entitled: "A Phase 1/2, randomized, observer-blind, controlled, multi-center, dose-escalation study to evaluate safety, reactogenicity and immunogenicity of GSK Biologicals' respiratory syncytial virus (RSV) investigational vaccine based on the RSV viral proteins F, N and M2-1 encoded by chimpanzee-derived adenovector (ChAd155-RSV) (GSK3389245A), when administered intramuscularly according to a 0, 1-month schedule to RSV-seropositive infants aged 12 to 17 months".

A short title is also provided for convenience: "A study to evaluate safety, reactogenicity and immunogenicity of GSK Biologicals' RSV investigational vaccine based on viral proteins encoded by chimpanzee-derived adenovector (ChAd155-RSV) (GSK3389245A) in RSV-seropositive infants".

(e) Proposed period of release:

The proposed period of release for the complete clinical trial RSV PED-002 is from sept 2016 to jan 2018. Duration of the clinical trial will be approximately 16 months, from first patient recruited to last patient last visit (date of study completion).

2. Notifier

Name of institution or company:
GlaxoSmithKline Biologicals
Rue de l'Institut, 89
1330 Rixensart, Belgium

3. GMO characterisation

The name of the GMO is: ChAd155-RSV.

This recombinant vector is a genetically modified organism (GMO) based on a simian (chimpanzee-derived) group C adenovirus serotype 155 (ChAd155) without replication capacity, that was constructed by deletion of E1 and E4 region of the viral genome and engineered to express three RSV proteins: the fusion F protein (deleted of the transmembrane region and endodomain F0ΔTM), the Nucleocapsid protein N and the transcription anti-termination protein M2-1. These RSV antigen genes have been inserted in place of the E1 deleted gene.

(a) Indicate whether the GMO is a:

viroid (.)
RNA virus (.)
DNA virus (X) rAdV- recombinant gene therapy product replication-deficient
bacterium (.)
fungus (.)
animal
- mammals (.)

- insect (.)
- fish (.)
- other animal (.) specify phylum, class

other, specify (kingdom, phylum and class)

(b) Identity of the GMO (genus and species)
Family: Adenoviridae
Genus: Mastadenovirus
Species: this is a recombinant adenoviral vector (replication deficient) derived from simian adenovirus subgroup C serotype 155

The identity of the GMO is ChAd155-RSV, as unique name for this recombinant vector. ChAd155-RSV is a genetically modified organism derived from recombinant replication-defective simian (chimpanzee-derived) group C adenovirus serotype 155 (ChAd155). This adenovirus is a member of the genus *Mastadenovirus*, which lies within the *Adenoviridae* family.

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

The GMO (ChAd155-RSV vector) is a simian adenovirus that upon administration to target organism localizes in the nucleus of the host cell, however does not integrate its DNA into the host cell genome. Integration of adenovirus DNA into the host cell genome has been observed as an extremely rare event in some human primary cell line cultures. Also according to EMA guidelines adenoviral vectors are considered as non-integrating vectors.

The GMO is a replication incompetent vector and therefore genetically stable by default, considering that it does not present any internal mechanism for genetic variation/transference. As the GMO is no able to replicate its genome, alterations in the genome are not expected.

The genetic structure of the GMO vaccine is verified at different steps of the process of production to demonstrate the integrity of the vector and identity of the insert (such as restriction pattern and sequencing of the full genome). All genetic characterization analyses on tested products showed conformity to predicted sequences.

Adenoviruses have a duplex DNA genome, a sturdy capsid, and no lipid membrane, and therefore they are expected to be stable. Other factors that may affect genetic stability are temperature, although adenovirus are only inactivated at temperatures higher than 56°C, and UV light, both absent from usual environment.

One of the factors that may affect genetic stability is the occurrence of replication competent adenoviruses (RCA). Formation of RCA from homologous recombination between the ChAd155 viral vector and the huAd5 E1 region of the host cell could arise. Although the risk of occurrence of this event is considered very low, the ChAd155-RSV is tested for the presence of RCA using a RCA assay [specification: <1 RCA / $3x10^{10}$ vp; although EMA does not recommend a specific level, this range is within recommended range by FDA (meeting#30). The confirmation of absence of RCA has been demonstrated in all manufacturing lots to date.

The GMO is stored at <-60°C until use. Long term stability was shown for up to 12 months when stored at <-60°C, showing the material meets product stability specifications throughout this period of time. Analytical procedures test to ensure genomic stability include potency by vector particle concentration or genome quantitation by Q-PCR, infectious virus titer by hexon immuno staining, identity by PCR and potency by transgene expression by Western Blot.

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): IT

5. Has the same GMO been notified for release elsewhere in the Community by the same notifier?

   Yes (X) No (.)

   If yes:

   - Member State of notification GB
   - Notification number GM462.15.81; EudraCT number: 2014-005333-31; REC: 15/SC/0133.

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 Non Member states: US
   - Notification number Not applicable. According to USA legislation there is no need to notify the GMO release

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

There is no available data on the environmental impact of the release of the ChAd155-RSV, as the first in Human clinical trial using the same GMO is being undergone at this time in GB (protocol RSV PED-001).

The GMO of this voluntary release notification, ChAd155-RSV is replication-deficient and it incorporates an expression cassette based on DNA for the RSV antigens (fusion F protein, the Nucleocapsid protein N and the transcription anti-termination protein M2-1) under the control of a human cytomegalovirus (HCMV) promoter. In addition, adenoviral genome remains epichromosomal thus avoiding the risk of integration of the viral DNA into the host genome after infection of host cells (Feuerbach and Crystal 1996). Therefore, ChAd155-RSV is incapable of surviving outside a host (animal) cell.

The ChAd155-RSV is planned to be administered in a multicenter and international phase I/II gene therapy trial in USA, Italy and Spain, treating a total of 96 RSV-seropositive infants aged 12 to 17 months at the time of first vaccination in a 1:1 randomized dose-escalation study; therefore, the GMO will be administered to 48 subjects in total. Vaccination will be limited to a maximum of 10 infants per day. In the same investigational center, infants will be vaccinated sequentially, separated by a minimum interval of 60 minutes to allow monitoring of any acute events (e.g. hypersensitivity reaction). The study is designed in a staggered manner to allow dose escalation from 5×10^{9} vp to 5×10^{10} vp dose (similar to the Phase I adult study RSV PED-001). A third intermediate dose of 1.5×10^{10} vp will be tested in this first time in pediatric population study to allow a more progressive escalation of the dose-related risk in this vulnerable population. 32 infants will be enrolled per step, aged 12 to 17 months to ensure that 96 infants receive at least one dose of study vaccine. Moving to a next step will be conditional to the favourable outcome of

the evaluation of safety data obtained up to 7 days after administration of the second vaccine dose in the previous step (for all subjects), performed by an Independent Data Monitoring Committee (IDMC).
The clinical trial in Spain is going to be multi-centric [Hospital Universitario La Paz; Hospital Clínico Universitario de Santiago; Area de Vacunas, Centro de Investigación en Salud Pública, Valencia; Hospital Universitari i Politècnic La Fe; Hospital Universitario de Móstoles]
The GMO will be stored at the respective label storage temperature conditions in a safe and locked place. The storage space to be used for the IMPs should be clearly delimited and physically separated from all other medicinal products, samples/items contained in the storage facility. The access to the storage equipment (refrigerator/cold room/freezer) must be restricted to authorized personnel. The storage temperature will be continuously monitored with calibrated (if not validated) temperature monitoring device(s) and recorded.

The GMO will be administered at hospital via intramuscular injection in the deltoid. The site of administration of Dose 2 should preferably remain the same as the site of administration of Dose 1. The subjects will be observed closely (visual follow-up as well as measurement of resting vital signs) for at least 60 minutes following the administration of the vaccines. Then patient will be followed up for a total of 12 months within the clinical trial with a total of 10 site visits.

Distribution and persistence of ChAd155-RSV vaccine for up to 70 days post-dose was evaluated in a GLP rat biodistribution study. ChAd155-RSV was undetectable in brain, heart, kidney, lung, ovary or testis samples on Days 2 and 8, or in blood samples assayed on Day 8 only. ChAd155-RSV was quantified at slightly higher than the LLOQ in the liver of one animal and in the spleen of four animals on Day 2, but was no longer detected in either organ by Day 8. ChAd155-RSV was detected from Day 2 at the injection site and the draining lymph nodes, including the iliac, inguinal, and to some extent, the popliteal lymph node. The highest levels were noted on Day 2, and levels progressively decreased over time, with undetectable levels in the muscle by Day 29 in some animals and most animals by Day 70, and in lymph nodes in most animals by Day 70. The quantities noted in the sampled tissues/organs at all timepoints were lower than 30,000 copies/µg host cell DNA, which is below the threshold level for which further studies would be required. Therefore, it does not disseminate inside the target host. The distribution and persistence of the ChAd155-RSV vaccine candidate is consistent with other similar adenovector-type vaccines.

There is a theoretical possibility of shedding of infectious particles into the environment and potentially to the public during the proposed release. Defective recombinant adenoviruses have been used extensively in clinical trials, either through direct administration or cell therapy strategies (contained in the cells). The majority of the studies have not detected viral release in biological samples (sputum, saliva, urine, feces) and whenever detected through urine or saliva, it disappears in few days from administration. Besides, GMO will be administered by intramuscular (IM) injection, a route which limits its potential shedding. Using this route of administration, studies show there is limited virus shedding and limited spread to other tissues, as the virus vector remains localized to the site of injection.
Data from a similar E1/E4-deleted simian adenovirus (ChAD3) but expressing a Hepatitis C Virus gene, no viral vector shedding (in urine and throat swabs) was observed after intramuscular chimpanzee or human adenovirus immunization (clinical study HCV001, EudraCT Number: 2007-004259-12). No shedding using GMO derived from other simian adenovirus strain was also observed during other clinical studies (Wold and Toth 2013). Therefore, as distribution and persistence of the ChAd155-RSV vaccine candidate is consistent with other similar adenovector-type vaccines (Sheets et al. 2008), it is expected similar vector shedding from this GMO.

There are no non clinical/clinical shedding data available for this GMO, based on the fact that this is not mandatory at this stage of the development according to FDA and EMA guidelines. According to FDA shedding studies should be performed on the target population during Phase II studies (after dose and regimen have been determined) (http://www.fda.gov/downloads/biologicsbloodvaccines/guidancecomplianceregulatoryinformation/guidances/cellularandgenetherapy/ucm404087.pdf), while according to EMA, the exact timing should be discussed with authorities (http://www.ema.europa.eu/docs/en_GB/document_library/Scientific_guideline/2009/09/WC500002680). However, studies regarding vector shedding are planned to be performed in a near future to confirm these observations.

The likelihood of ChAd155-RSV becoming persistent and invasive in natural habitats is low for the following reasons:

- The risk of occurrence of the formation of replication competent adenovirus (RCA) from homologous recombination between the ChAd155 viral vector and the human Ad5 E1 region of the host cell is considered very low, due to the lack of sequence homology between the human E1 flanking regions of human Ad5 and chimpanzee adenovirus E1. It has been shown that recombination and production of RCA does not occur when they are propagated in HEK-293 cells (Colloca and Folgori 2013), thus eliminating the problem of RCA generation during the adenovector manufacture. To control this issue, the ChAd155-RSV drug substance material is tested for the presence of RCA during different steps of the manufacture's process [see section A.3.(c)].
- Horizontal gene transfer is unlikely and due to Ch155Ad-RSV sequence characteristics there is no possibility to confer a selective advantage to bacteria or other microorganisms, because it does not contain any prokaryotic promoters, any antibiotic or other types of resistance genes which would enhance or constrain their growth.
- In regard to humans, the infection risk with this GMO is almost null due to its release by patients is limited on time and at very low doses. Toxicity studies were performed and ruled out any toxicity either from the vector itself and the product of transgene expression; therefore, no harmful effects are envisaged.

The likelihood that any negative effects could occur is considered negligible, there is no risk for people, animals, microorganisms and the environment as the GMO release is planned during a clinical trial, and will be administered intramuscularly to the patients.

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

The recipient or parental organism is the AdCh155, as the main backbone where the main genetic modification is performed. However it must be considered that for construction purposes of the GMO, an artificial bacterial chromosome has been used as a tool for the process.

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 (.)
(specify phylum, class)

other, specify

Recipient organism contains deletions in the E1 and E4 regions and substitution of the native E4 by the E4ORF6 from human adenovirus 5 (Ad5).

Chimpanzee-derived adenoviruses exhibit sequence homology to human adenoviruses within the hexon protein in particular, which is a major capsid protein used for subgroup classification of adenoviruses (Roy et al. 2011; Colloca et al. 2012). Most of the simian adenoviruses known to date were detected in or isolated from captive individuals and propagated in cell culture. Unfortunately, little is known about them in wild animals. They are not known to cause pathological illness in humans and antibodies against chimpanzee adenoviruses have low or no seroprevalence ranging from 0-4% in Europe and in the United States, up to 20% in the developing countries, which is far less than the most common human adenovirus serotype 5 (Ad5), with seroprevalence rates of 40–45% in the United States and up to 90% in sub-Saharan Africa (Capone et al. 2013).

For these reasons simian adenoviral vector are been used as a vector for gene therapy clinical trials being currently registered 22 human clinical trials using this kind of vector (clinicaltrials.gov).

2. Name

(i)	order and/or higher taxon (for animals)	Adenoviridae
(ii)	genus	Mastadenovirus
(iii)	species	Simian Adenovirus Subgroup C
(iv)	subspecies	N/A
(v)	strain	serotype 155 from chimpanzee adenovirus. Recipient organism contains deletions in the E1 and E4 regions and

		substitution of the native E4 by the E4ORF6 from human adenovirus 5 (Ad5)
(vi)	pathovar (biotype, ecotype, race, etc.)	N/A
(vii)	common name	Chimpanzee adenovirus type 155 (ChAd155)

3. Geographical distribution of the organism

(a) Indigenous to, or otherwise established in, the country where the notification is made:
Yes (.) No (X) Not known (.)

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

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

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

(ii) No (X).
(iii) Not known (..)

(c) Is it frequently used in the country where the notification is made?
Yes (.) No (X)

(d) Is it frequently kept in the country where the notification is made?
Yes (.) No (X)

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
The natural host of ChAd155 adenovirus is the chimpanzee. The parental ChAd155 adenovirus is not found in natural ecosystem outside of its natural host.
Parental organism was isolated from a healthy young chimpanzee housed at the New Iberia Research Center facility (New Iberia Research Center; The University of Louisiana at Lafayette).

(b) If the organism is an animal: natural habitat or usual agroecosystem:
Not applicable

5. (a) Detection techniques

Wild type simian adenoviruses are detected using PCR according to general simian adenoviral sequences or specific for the 155 serotype of analysis. They could also be detected by immunocytochemistry assay with anti-hexon antibody that has shown reactivity against ChAd155 hexon protein.

(b) Identification techniques

Wild type simian adenoviruses are detected using PCR according to general simian adenoviral sequences or specific for the serotype of analysis. DNA sequencing is also used for the identification of simian adenoviruses.

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

In terms of classification of hazard, human adenovirus is considered as a group 2 biological agent as per the European Economic Community classification for the protection of workers with biological agents (Directive 2000/54/EC). The group 2 designation applies to agents that can cause human disease and might be a hazard to workers, that are unlikely to spread to the community and for which there is usually effective prophylaxis or treatment available.

However, many adenoviral vectors as our parental organism have been constructed by deletion of E1 region which encodes key genes required for viral growth. These E1 deleted adenoviral vectors are disabled and incapable of establishing a productive, transmissible infection in humans. Therefore, these E1 deleted vectors can be considered to be avirulent and may be handled safely at Containment Level 1 (http://www.hse.gov.uk/biosafety/gmo/acgm/acgmcomp/part2.pdf).

The ChAdV155 is a chimpanzee species that is not specifically classified by the EEC directive. Based on the inability of simian adenovirus to cause human disease and results of toxicity studies that demonstrated safety and tolerability, the GMO is not considered to pose a risk to human health. It has been manipulated under a containment level 1 (BSL1) up to date as the ChAdV155 is deleted in the E1 region allowing handling the parental organism under BSL1 rules.

Similar kind of vector is used on a previous notification for voluntary release of recombinant simian adenovirus in the contest of human clinical trials such (B/ES/12/09) that has been presented to CIOMG with BSL1 biosafety classification.

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

Adenoviruses are classified as Class 2 under Directive 2000/54/EC due their limited pathogenicity. Human adenoviruses commonly cause asymptomatic infection in humans, although they can also cause respiratory tract infections, gastrointestinal discomfort or eye infections with severity being variable. They are more common in children and in immunocompromised population. The incubation period ranges from 1 to 10 days. The majority of the population is seropositive for more than one subspecies of adenovirus and can quickly produce neutralizing antibodies. The principal host is human, and the minimal infectious dose is >150 plaque-forming units intra-nasally. Normally, the virus enters the respiratory tract or the eyes through aerosols produced by infected individuals. Most infections are minor in nature and self-limiting. Adenoviruses are usually not integrated into the host cells genome and do not persist in lymphoid tissues. Adenovirus may be transmitted between individuals via fecal-oral route, respiratory droplets, hand-to-eye and venereal transfer.

Adenovirus infections in non-human primates (NHP) are also predominantly subclinical, except for some cases of pneumonia in immunosuppressed Simian immunodeficiency viruses (SIV)-infected animals. Defective recombinant adenoviruses have been widely used in clinical trials, both as direct administration or cell therapy strategies (contained in the interior of cells). The non-replicative adenoviruses lacking the E1 were classified as biosafety level class 1 for R&D purposes.

8. Information concerning reproduction

(a) Generation time in natural ecosystems:

The wild type adenovirus infection cycle, from infecting a cell to producing new infectious particles consists on the following steps: (1) attachment to the cell membrane; (2) receptor-mediated endocytosis; (3) endosomal trafficking; (4) escape from the late endosome or lysosome; (5) translocation to the nucleus; (6) uncoating; (7) transcription of viral DNA; (8) expression of rep genes; (9) genome replication; (10) expression of cap genes, synthesis of progeny DNA particles; (11) assembly of complete virions, and (12) release from the infected cell.

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

AdV DNA replication is a very efficient process. Within 40 hours, an infected cell produces approximately one million copies of viral DNA.

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

(c) Factors affecting reproduction:

Adenoviruses are able to infect both dividing and non-dividing cells. The outcome of an adenovirus infection depends on the animal species, cell type, and virus type involved. Cells differ in their permissivity for adenovirus types as in permissive cells the virus multiplies productively, while in semipermissive cells replication is allowed at low efficiency, and in others replication is blocked and the infection is abortive.

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
Adenoviral vectors do not form survival structures

(b) relevant factors affecting survivability:
Adenoviruses lose bioactivity at room temperature with falls in levels on a logarithmic scale and although adenoviruses can survive for long periods on environmental surfaces and are resistant to lipid disinfectants because they are non-enveloped, they are inactivated by heat, and susceptible to different chemical agents such as sodium hypochlorite 1% and 2% glutaraldehyde, commonly used as disinfectants. A completely effective elimination is achieved by autoclaving at 121 ° C for 15 minutes.

10. (a) Ways of dissemination
Adenoviruses are transmitted effectively by direct contact via contaminated aerosols and water droplets, and indirectly via contact with objects contaminated with respiratory secretions from an infected person. The minimal infectious dose of adenovirus is 150 plaque forming units when administered intra-nasally. Adenovirus may also be spread via the faecal-oral route.

(b) Factors affecting dissemination
Factors affecting the dissemination of adenoviruses include the administered dose, formation of aerosols, and proximity of susceptible uninfected hosts to immunized subjects.

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)
Not applicable. This is the first use in Spain. However there is a voluntary release notificacion of recombinant simian adenoviruses with different serotype, ChAdV63 (B/ES/12/09).

C. Information relating to the genetic modification

1. Type of the genetic modification

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

2. Intended outcome of the genetic modification

The intended outcome of the genetic modifications described below is to develop a replication-defective recombinant simian adenoviral vector capable of expressing the 3 RSV antigens: the fusion (F) protein deleted of the transmembrane and cytoplasmic regions (F0ΔTM), the nucleocapsid (N) protein and the transcription anti-termination protein (M2-1) protein in infected cells and to activate a RSV antigen-specific immune response in the host.
For GMO preparation, codifying sequences from parental organism not needed for GMO generation are replaced by a complete expression cassette containing the RSV antigen sequences and additional regulatory sequences needed for the protein expression such as human cytomegalovirus promoter and bovine growth hormone polyadenylation signal. The purpose of these genetic modifications is the preparation of an efficient vehicle to deliver the RSV antigens.

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.
In the final GMO will be included as genetic sequence the recombinant expression cassette with the RSV antigens and the genes for the GMO capsid (fiber and hexon). Other auxiliary genes will only be used for the correct packing of the GMO.

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

(a) Type of vector

Two vectors are considered here:

plasmid	(X) (plasmid carrying the RSV transgene)
bacteriophage	(.)
virus	(.)
cosmid	(.)
transposable element	(.)
other, specify	Bacterial Artificial Chromosome (BAC) vector

(b) Identity of the vector

The DNA of RSV transgene was cloned into a shuttle vector (plasmid) under the control of HCMV promoter with the TetO inserted downstream the HCMV promoter TATA box and bovine growth hormone polyadenylation signal (BGHpA).

Standard DNA manipulation techniques in *E.coli* (direct cloning and homologous recombination) were used to clone the ChAd155 viral genome into a Bacterial Artificial Chromosome (BAC) vector and to modify the plasmid vector in order to introduce the deletion of native E1 and E4 regions as well as the introduction of Ad5E4orf6 region. RSV transgene was then inserted in the plasmid BAC/ChAd155 (DE1, DE4, Ad5E4orf6) by homologous recombination. The resulting ChAd155-RSV virus was rescued in a HEK293 derivative cell line expressing the Tet repressor, designated as Procell-92.S by transfection and was then further amplified by serial passaging.
(For detailed information regarding constituent fragments of vector, please referred to IMPD section S.1.2 and S.2.3).

(c) Host range of the vector
There are no host identified for any of the plasmids, since they are cloning products that are not available in the natural environment and do not have infective capacity.

- The genetic content of RSV plasmid construction are the respiratory syncytial virus antigens, therefore the hypothetic host would be humans.
- The ChAd155 vector that codifies for simian adenoviral genome except for E1 and E4 regions, would have the same host as wild type.
- Ad5E4orf6 vector would have humans as potential host because of its adenoviral nature.
- BAC is a DNA construct, based on a functional fertility plasmid (or F-plasmid), used for transforming and cloning in bacteria, usually E. coli. Therefore, E.coli would be its host range.

(d) Presence in the vector of sequences giving a selectable or identifiable phenotype
Yes (X) No (.)

antibiotic resistance (X) Kanamicin-resistance gene in the plasmid containing transgene cassette, and a ampicillin-resistance gene in the BAC vector
other, specify Amp-LacZ-SacB selection cassette in the BAC vector

Indication of which antibiotic resistance gene is inserted
A Kanamicin resistance gene is inserted in the RSV plasmid expressing transgene cassette. However, after homologous recombination with the vector containing the modified ChAd155 viral genome, the Kanamicin resistance gene is not present in the final recombined adenovirus vector.

A selection cassette including the suicide gene SacB, ampicillin–resistance gene and lacZ (Amp-LacZ-SacB selection cassette) is inserted in the BAC vector during the process. However, this Amp-LacZ-SacB selection cassette is substituted by the RSV transgene cassette and is therefore not present in the final GMO.

(e) Constituent fragments of the vector
The DNA fragment inserted as the transgene in the Chimpanzee Adenovirus vector (ChAd155) encodes sequences derived from three RSV proteins and it contains the HCMV promoter with the TetO inserted downstream the HCMV promoter TATA box to provide transcriptional control of the transgene while in the packaging cell line. The ChAd155 viral genome was cloned into a BAC vector by homologous recombination, in E. coli strain BJ5183, between ChAd155 viral DNA and Subgroup C BAC Shuttle. Subsequent genetic

modifications were performed for deletion of E1 and E4 and replacement of native E4 by ORF 6 of the human adenovirus type 5 (Ad5). (For detailed information regarding constituent fragments of vector, please referred to IMPD section S.1.2 and S.2.3).

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

(i)	transformation	(X) homologous recombination in *E.coli*
(ii)	electroporation	(X)
(iii)	macroinjection	(.)
(iv)	microinjection	(.)
(v)	infection	(.)
(vi)	other, specify	

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

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

6. Composition of the insert

(a) Composition of the insert

The DNA fragment inserted as transgene in the Chimpanzee Adenovirus vector (ChAd155) contains:

- RSV transgene contains consensus codon optimized sequences of: F0ΔTM followed by 2A, a translational cleavage site and then by the N and M2-1 consensus sequences fused by a flexible linker sequence (N-M2-1). The RSV transgene is under the transcriptional control of human Cytomegalovirus (HCMV) promoter and bovine growth hormone poly-adenylation signal (BGHpA). The construct contains the aphtovirus Foot and Mouth Disease Virus 2A region between the soluble F protein F0ΔTM and the other two RSV antigens, which mediates polyprotein processing by a translational effect known as ribosomal skip (Donnelly et al. 2001). After transfection into mammalian cells, cleavage occurs and the soluble F protein is detected in the cell culture supernatant as expected. The fusion protein N-M2-1 is instead expressed and detected in the intracellular fraction;
- strong human cytomegalovirus (HCMV) promoter;
- bovine growth hormone polyadenylation signal (BGHpA), a specialized termination sequence for protein expression in eukaryotic cells;
- tetracycline operon operator sequence (TetO) localized downstream of the HCMV transgene promoter in the viral construct that acts as a repressor of transgene expression;
- VAI and VAII RNAs;
- structural adenoviral genes, fiber and hexon.

The DNA fragment inserted as the RSV transgene in the Chimpanzee Adenovirus vector (ChAd155) encodes sequences derived from three RSV proteins : F, the fusion F protein deleted of the transmembrane region (F0DTM or F0ΔTM, 528 amino acids), the nucleocapsid protein (N) and the transcription anti-termination protein (M2-1). The F protein is expressed as a single protein whereas the N and M2-1 proteins are expressed as a fusion protein (N-M2-1, 594 amino acids).

(b) Source of each constituent part of the insert
See above.

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

- Both the CMV promoter and the BGH polyadenylation sequences are dedicated to promote the expression of the gene of interest, allowing the recognition by the RNA polymerase for transcription and increasing the stability of mRNA molecules synthesized.
- The tetracycline operon operator sequence (TetO) inserted downstream of the CMV transgene promoter in the viral construct is intended to help repression of transgene expression during viral propagation. Transcriptional control is reversible, and in the host where there is no expression of repressor protein, the HCMV promoter is not blocked and normal transcription of the transgene resumes.
- VAI and VAII RNAs: about 160 nucleotides long, which are not translated but regulate the translation of viral mRNAs
- Structural adenoviral genes, fiber and hexon that will constitute the viral capsid.

Rationale for antigens selected for the vaccine:

- The fusion (F) protein deleted from the transmembrane and cytoplasmic regions (F0ΔTM). The F protein is a major surface antigen of the RSV virus that is well conserved among RSV-A and RSV-B subgroups. In addition, it is the main target of the neutralizing antibody response to RSV, which is considered essential for protection against RSV-associated severe disease (Magro et al. 2012).
- The nucleocapsid (N) protein is an internal (non-exposed) antigen, highly conserved between RSV strains and known to be a source of many T-cell epitopes (Anderson, Huang, and Langley 2010; Townsend and Skehel 1984). The N protein is essential for the replication and transcription of the RSV genome. Its primary function is to encapsidate the virus genome protecting it from ribonucleases.
- The matrix (M2-1) protein is a transcription anti-termination factor that is important for the efficient synthesis of full-length messenger ribonucleic acid (RNA) as well as for the synthesis of polycistronic readthrough messenger RNA, which are characteristic of non-segmented negative-strand RNA viruses. M2-1 is an internal (non-exposed) antigen, highly conserved between RSV strains and known to be a source of many T-cell epitopes (Anderson, Huang, and Langley 2010; Townsend and Skehel 1984).

(d) Location of the insert in the host organism

- on a free plasmid (.)
- integrated in the chromosome (.)

- other, specify integrated in the genome of the adenovirus replacing part of the genome of the parental organism.

(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

The following information is related to the organism from which the inserted transgene (F, N, and M2-1 proteins) belongs, i.e. the human Respiratory Syncytial Virus (RSV).

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)	Mononegavirales
(ii) family name for plants	Paramyxoviridae
(iii) genus	Pneumovirus
(iv) species	human
(v) subspecies	…
(vi) strain	…
(vii) cultivar/breeding line	…
(viii) pathovar	…
(ix) common name	Respiratory Syncytial Virus (RSV)

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 (.)
plants (.)
other ..

(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):

RSV is a highly contagious human pathogen that causes respiratory tract infections (RTI) in people of all ages. During the first year of life, 50-70% of infants are infected with RSV and essentially all children have had an RSV infection by their second birthday. The risk for severe RSV-associated lower respiratory tract infections (LRTI) is highest in infants below 6 months of age and is a leading cause for hospitalization. Although RSV hospitalization rates substantially decrease after 6 months of age, a considerable number of RSV infections in children of 6-15 months of age, still lead to bronchiolitis or (broncho)pneumonia requiring medical attention (Fisher et al. 1997). Previous infection with RSV does not prevent subsequent infections. Therefore, re-infection with RSV occurs throughout an individual's lifetime and is common in all age groups (Krilov 2011; Simoes 1999). These re-infections generally go undiagnosed because they usually present as common acute upper respiratory tract infections. In more vulnerable persons (e.g. immunocompromized subjects or elderly), re-infections can however also lead to severe disease (Graham 2011).
The RSV antigens used in GMO correspond to three RSV proteins: fusion (F) protein deleted of the transmembrane and cytoplasmic regions (F0ΔTM), nucleocapsid (N) protein and transcription anti-termination (M2-1) protein.

The RSV F protein is a major surface antigen and mediates viral fusion to target cells. The F protein is a known protective antigen, which is highly conserved among RSV subgroups and strains. The F protein is a target for neutralizing antibodies, including the prophylactic RSV-neutralizing monoclonal antibody Synagis. It is considered essential for protection against RSV-associated severe disease (Magro et al. 2012). Deletion of the transmembrane region and cytoplasmic tail permits secretion of the F0ΔTM protein.

The N protein is an internal (non-exposed) antigen, highly conserved between RSV strains and known to be a source of many T cell epitopes (Townsend and Skehel 1984). The N protein is essential for the replication and transcription of the RSV genome. The primary function of the N protein is to encapsulate the virus genome for the purposes of RNA transcription, replication and packaging and protects it from ribonucleases.

The M2-1 protein is a transcription anti-termination factor that is important for the efficient synthesis of full-length messenger RNAs (mRNAs) as well as for the synthesis of polycistronic readthrough mRNAs, which are characteristic of non-segmented negative-strand RNA viruses. M2-1 is an internal (non-exposed) antigen, which is highly conserved between RSV strains and known to be a source of many T cell epitopes (Townsend and Skehel 1984).
The N and M2-1 antigens are included in the GMO as source of T cell epitopes for the induction of cell-mediated immunity (CMI) in the context of the clinical trial where this release will take place.

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

   In terms of classification of hazard, Respiratory Syncytial Virus is considered as a group 2 biological agent as per the European Economic Community classification for the protection of workers with biological agents (Directive 2000/54/EC). The group 2 designation applies to agents that can cause human disease and might be a hazard to workers, that are unlikely to

spread to the community and for which there is usually effective prophylaxis or treatment available.

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

E. Information relating to the genetically modified organism

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

(a) is the GMO different from the recipient as far as survivability is concerned?
Yes (.) No (X) Not known (.)
Specify
The GMO should have survival or stability similar to the wild type virus. The genetically modified organism is replicative defective (E1 E4 deleted). The GMO will enter a cell and remains epichromosomal thus avoiding the risk of integration of the viral DNA into the host genome after infection of host cells. The GMO cannot replicate and as it is non integrative, only the proteins can pass from one cell to another due to a bystander effect.

(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
The final GMO is a recombinant virus, completely deficient for replication due to E1 and E4 replacement of adeno viral genes by the cassette of expression for human RSV antigens.

(c) is the GMO in any way different from the recipient as far as dissemination is concerned?
Yes (X) No () Not known (.)
Specify
As the GMO cannot replicate, the dissemination of the organism is limited to the administration of the GMO to the patients in the clinical study.

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

2. Genetic stability of the genetically modified organism
See section A.3.(c)
Specifically for this GMO, its genome has been constructed by recombinant DNA in the lab through a DNA plasmid. The gene expression cassette content in the plasmid is analyzed during the manufacturing process by several tests to assess its identity (Q-PCR, DNA sequencing and restriction analysis). Considering that the GMO is containing the genome of the plasmid used for its production and cannot replicate further, the GMO is considered as genetically stable.

3. Is the GMO significantly pathogenic or harmful in any way (including its extracellular products), either living or dead?
Yes (.) No (X) Unknown (.)
The GMO is replicative-deficient and is not considered pathogenic to the host or to non-target organisms.

(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)

For relevant information specified under Annex III A, point II(A)(11)(d), refer to sections B.7.(b) and D.3.(b).

The GMO is not expected to have any toxic nor allergenic effects. As with all injectable vaccines, immediate systemic allergic reactions to vaccination can occur. These are however very rare and are estimated to occur once per 450 000 to once per 1 000 000 vaccinations for vaccines which do not contain allergens such as gelatine or egg protein (Zent et al. 2002). During the study, all infants across all steps will remain under observation (visual follow-up as well as measurement of vital signs) at the study site for at least 60 minutes after vaccination.
The proposed study is conducted in RSV-seropositive infants who are assumed to have been previously infected with RSV and therefore not at risk for enhanced RSV disease. Intramuscular (IM) vaccination commonly precipitates a transient and self-limiting local inflammatory reaction. This may typically include redness, swelling and tenderness. Most systemic symptoms observed in a clinical trial with a similar product carried out in healthy adults (chimpanzee adenovirus-based vaccine with ChAd3 as vector for vaccination against hepatitis C) at doses to be used in this trial did not exceed mild severity (clinical study HCV001, EudraCT Number: 2007-004259-12). Fatigue, headache and malaise were the most commonly reported systemic AEs overall.

Due to the lack of data in human subjects to date, there is no information available about adverse events following administration of the ChAd155-RSV candidate vaccine. However, in Ebola Phase I studies in adults investigating a similar adenoviral vectored vaccine (ChAd3-EBO-Z), transient decreases in thrombocyte counts were also observed. These decreases occurred mostly on Day 1 after vaccination and generally returned to baseline by Day 7. Although most of these decreases remained within the normal range, the per protocol criteria for thrombocytopenia (i.e. thrombocyte count of < 150 x 103/µL) were met for 2.6% (7 out of 270) of the vaccinated subjects. None of the decreases in thrombocyte counts or the cases of thrombocytopenia was clinically significant (i.e. no clinical signs or symptoms suggestive of increased tendency to bleeding were reported in any of the subjects). Although the mechanism underlying these decreases currently remains unclear, it is well described in literature that, post intravenous administration, adenovirus activates platelets and induces platelet-leukocyte aggregate formation, causing an associated increase in platelet and leukocyte-derived microparticles (Othman et al. 2007; Stone et al. 2007).
All available nonclinical data suggest that the ChAd155-RSV vaccine candidate has acceptable immunogenicity, efficacy, biodistribution, tolerability/toxicity profiles for conducting the clinical trial.
Although human adenoviruses may cause more infections in children and in immunocompromised population, ChAd155-RSV is replication-deficient and derived

from a chimpanzee species, it is therefore considered not to be pathogenic. Furthermore, extreme precaution will be taken at patient screening, where infants with any confirmed or suspected immunosuppressive or immunodeficient condition or presenting any medical condition that in the judgment of the investigator would make GMO injection unsafe will be excluded from the study.

In addition, the GMO does not present a risk of integration or activation of latent provirus. Since it is replication deficient, the GMO is not expected to display capacity for colonization. No probability of generating RCA has indeed being detected as evaluated at different steps of the vaccine manufacturing process.

Finally, no antibiotic-resistance genes are expressed in the GMO further excluding antibiotic resistance patterns.

4. Description of identification and detection methods

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

GMO could be detected using PCR according to general simian adenoviral sequences or specific for the 155 serotype of analysis. They could also be detected by immunocytochemistry assay with anti-hexon antibody that has shown reactivity against ChAd155 hexon protein.

(b) Techniques used to identify the GMO

Same as in 4.a

F. Information relating to the release

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

The release will be the administration of the product, in hospital rooms, by intramuscular (IM) injections to patients as part of an international, multicenter clinical trial.
This clinical trial is a phase 1/2 trial in RSV-seropositive infants aged 12 to 17 months.
The GMO ChAd155-RSV has been developed as a preventive vaccine candidate for active immunization of infants for the prevention of any lower respiratory tract infections (LRTI; bronchiolitis and [broncho]pneumonia) associated with RSV [subtypes A and B]. The investigational ChAd155-RSV vaccine will be administered to 48 subjects in total. As a control, 48 subjects will be vaccinated with placebo. The purpose of this trial is to evaluate the safety, reactogenicity and immunogenicity of this RSV candidate vaccine when first administered via IM injection according to a 0, 1-month schedule. Duration of the study will be approximately 16 months.

The release will be performed by dedicated and trained personnel. Detailed instructions on how to prevent contamination by the vaccine will be provided to all personnel involved in handling of the product.
The handling of this study vaccine follows work practices for a BioSafety Level 1 contaminant (Risk Group 1).

As study vaccine is part of GMO, all efforts should be done to avoid contamination of the environment as for any medicinal material (even if the adenovector is non replicative with no risk of disease for humans). Therefore:

- The personnel will use gloves and protective clothes during handling of the study vaccine.
- The vaccine will be prepared in a biosafety cabinet type 2 (vertical flow for complete protection of environment and hospital staff) that should be decontaminated before and after manipulation with a 1% solution of sodium hypochlorite or equivalent. In order to measure the correct volume of the vaccine into the syringe, a needle will be placed on the syringes and syringe will be hold upright. The syringe will be gently tap to bring all air bubbles to the top. Excess volume will be expelled together with any remaining air bubble onto a towel that was previously treated with 1% solution of sodium hypochlorite or equivalent, so that a volume of 0.5 mL is left in the syringe. The towel should be discarded into the biohazard waste container.
- The syringe is to then be placed in a sealed rigid container (to prevent spills) to be transferred to clinic room for administration labelled with biohazard symbol and the statement “CONTAINS GMO”.
- After the administration of the vaccine, all empty vaccine vials, needles and syringes are to be discarded in biohazard waste containers after vaccine preparation is completed for each subject. Keep the secondary containers for vaccine accountability by the monitor.
- After each vaccination, the injection site will be covered with a dressing in order to absorb any virus that may leak out through the needle track. After 30 minutes, the dressing will be treated with a 1% solution of sodium hypochlorite and then disposed of in the biohazard waste container.

Clinical sites will follow their institutional procedures for the disposal of biohazard material. There are no significant environmental benefits expected following the GMO release during this clinical trial.

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

   Simian adenoviruses are not found naturally in the environment of the geographical locations surrounding the clinical study sites where the administration of the ChAd155-RSV GMO will take place.

3. Information concerning the release and the surrounding area

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

   ChAd155-RSV will be administrated at the following clinical sites:

   - Paseo de la Castellana 261, Hospital Universitario La Paz, Madrid, Madrid, Spain, 28046.
   - C/ Travesia da Choupana s/n, Servicio de Críticos, Intermedios y Urgencias Pedi, Hospital Clínico Universitario de Santiago de Compostela, Santiago de Compostela, Spain, 15706.
   - Av. Cataluña 21, Area de Vacunas, Centro de Investigación en Salud Pública, Valencia, Spain, 46020.
   - Avinguda Fernando Abril Martorell, 106, Hospital Universitari i Politecnic La Fe, Valencia, Spain, 46026.
   - Unidad de Neonatologia, Río Júcar s/n, Hospital de Mostoles, Móstoles/Madrid, Spain, 28935.

   (b) Size of the site (m^2):

   (i) actual release site (m^2):

   No specific site size is required for the release. The release of the GMO will take place in a designated standard-sized hospital within each of the designated clinical institutions.

   (ii) wider release site (m^2):

   No specific size is required for the clinical site.

   The study vaccine must be stored at the respective label storage temperature conditions in a safe and locked place. The storage space to be used for the IMPs should be clearly delimited and physically separated from all other medicinal products, samples/items contained in the storage facility. The access to the storage equipment (refrigerator/cold room/freezer) must be restricted to authorized personnel. GMO will be transferred from their storage site to clinic room for patient's administration in sealed, rigid containers labelled with the biohazard symbol and the statement "Contains GMO", to ensure that the likelihood of accidental spillage is reduced to a minimum.

   Environmental surfaces and medical devices should be routinely cleaned with a hospital-grade disinfectant. Any and all waste should be discarded according to local process for biohazard waste.

(c) Proximity to internationally recognised biotopes or protected areas (including drinking water reservoirs), which could be affected:
Release will occur during a clinical trial held in hospital settings.

- La Paz: Located At 12 km of the Hospital la Paz, The Monte de El Pardo This protected area is listed as a Special Protection Area. Natura 2000 (Dir. 79/409 EEC) with the number ES0000011.It is close to other green areas (Monte de la Zarzuela, Dehesa de la Villa and Casa de Campo).
- Hospital Universitario Santiago de Compostela: There are no protected areas located within 50 km from the hospital.
- Centro de investigación en salud publica, área de vacunas, Valencia: The Albufera de Valencia is 10 km far from Valencia City.
- Hospital la Fe de Valencia: Please, see Centro de investigación en salud pública, área vacunas, Valencia.
- Hospital de Móstoles: At 60 km, the Reserva del Regajal-Mar de Ontígola is a wetland located in the town of Aranjuez, Madrid (Spain). Declared in 1994 as a natural reserve..

(d) Flora and fauna including crops, livestock and migratory species which may potentially interact with the GMO
The wild type and recombinant virus can infect mammal cells but not plant cells. A limited release of the GMO to waste water is expected after the administration to the patients. Due to the GMO replication incapacity, no interaction of the GMO with the flora, fauna, livestock or migratory species is expected.

4. Method and amount of release

(a) Quantities of GMOs to be released:
A vaccination regimen based on two intramuscular (IM) injections (in the deltoid [if the muscle size is adequate; otherwise injections might be done in the anterolateral thigh]) of 5×10^{9}, 1.5×10^{10}, or 5×10^{10} viral particles (vp) of the ChAd155-RSV vaccine administered according to a 0, 1-month schedule (4-week interval between vaccinations) will be evaluated in this study.

The investigational ChAd155-RSV vaccine will be administered to 48 subjects in total, 16 will be receiving a dose of 5×10^{9} (0.5ml), another 16 patients will be receiving 1.5×10^{10} (0.15ml), and the last 16 a dose of 5×10^{10} (0.5ml) vp of the GMO vaccine. Vaccination will be limited to a maximum of 10 infants per day. In the same investigational center, infants will be vaccinated sequentially, separated by a minimum interval of 60 minutes to allow monitoring of any acute events (e.g. hypersensitivity reaction).

In total, it is estimated to administer 22.4×10^{11} vp, corresponding to a maximum of 96 vials (extra patients counting for loss), in this study (in all countries concerned). Taking into account the formulations that are 5×10^{9} (in 0.5ml) or 5×10^{10} (in 0.5ml), the expected release will be $33.6\ 10^{11}$ vp.

(b) Duration of the operation:
Each vaccination takes about few minutes. The recruitment period (and dosing of ChAd155-RSV) of the 48 participants is estimated to be made over a year. Total study for each subject will last approximately 12 months.

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

The GMO is intended for clinical use only according to the provisions of the clinical protocol. The amount of ChAd155-RSV supplied to the site at any one time is limited to that needed for patient administration and access to the product is restricted to authorized personnel.

The vials will be imported from Belgium and stored in freezers < -60°C at the clinical site until use.
The GMO is to be provided to clinical sites in sealed vials that are appropriately labelled and packaged. The preparation and administration of the product is to be performed by trained personnel, under the responsibility of the investigator, according to a clinical protocol and respecting the rules of Good Clinical Practice. Please see section F.1 for the detailed measures taken for the management of the vaccine.
It is noted that needle puncture of the stoppered vial containing the viral vector is a potential source of aerosols. Therefore, the preparation of the product is done in a Biosafety cabinet type 2. The cabinet is decontaminated before and after manipulation with a 1% solution of sodium hypochlorite. All transfers of the GMO preparation should be undertaken using a sealed container (to avoid spills) clearly labelled with biohazard symbol and "contains GMO". Furthermore, employees will follow the hospital policy recommended for biohazard handling.
Also, after each vaccination, the injection site will be covered with a dressing in order to absorb any virus that may leak out through the needle track. After 30 minutes, the dressing will be treated with a 1% solution of sodium hypochlorite and then disposed of in the biohazard waste container.

Any contaminated materials will be removed from the room and maintained in sealed containers or in special bags that are clearly labelled as biohazard medical waste. Some institutions may require the biohazard waste to be destroyed separately from other Virology laboratory waste.

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

Not applicable. All GMO administrations are to be performed in conventional hospital rooms at the clinical institutions listed.

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.

The same GMO is being used at present time in the first Phase I in healthy adult subjects aged 18 to 45 years (study 201974 [RSV PED-001]) in United Kingdom. In UK, the use of the GMO has been listed as contained use. RSV PED-001 is a phase I, randomized, observer-blind, controlled study which aims to evaluate the safety, reactogenicity and immunogenicity of the ChAd155-RSV vaccine, when administered intramuscularly according to a 0, 1 month schedule in healthy adults aged 18 to 45 years. The RSV PED-001 trial is currently conducted in a 3-step staggered design similarly to the present study (RSV PED-002). Subjects in the RSV PED-001 will receive a vaccination regimen based on 2 IM injections of $5x10^9$ or $5x10^{10}$ viral particles. A placebo and a vaccine against meningitis serogroup B (Bexsero) are used as controls. Recruitment is still ongoing at the date of release of this document.

To date, several clinical trials have been completed or are ongoing with Chimpanzee adenovirus-based vaccines with vectors similar to ChAd155. The immunization schedule based on two IM injections of the same chimpanzee Ad-based vaccine has been evaluated in the HCV001 (with the vector ChAd3) and RSV001 (with the vector PanAd3) clinical trials for vaccination against Hepatitis C and RSV infections, respectively. Several Phase I studies have been conducted with a ChAd3-based vaccine against Ebola (ChAd3-EBOZ). The candidate vaccine is currently being further evaluated as a single IM dose in phase II studies. There are currently no known risks associated with ChAd3-EBO-Z which would preclude further development. So far, three different simian adenoviruses have been used in clinical trials:

- The ChAd63 adenovirus (Biswas et al. 2011) belongs to serotype E (Colloca et al. 2012) and has been mainly used in malaria trials (de Barra et al. 2014; Hodgson et al. 2015; O'Hara et al. 2012; Sheehy et al. 2011) where more than a thousand healthy volunteers have been vaccinated, including two month old babies.
- The ChAd3 (Peruzzi et al. 2009) and PanAd3 (Vitelli et al. 2013) adenoviruses belong to serotype C (Colloca et al. 2012) and have been used in Hepatitis C Virus (HCV) and Ebolavirus trials with more than 1500 vaccines and in a recent phase I clinical RSV trial enrolling 42 volunteers, respectively.

All simian adenovectors tested so far in the clinic (i.e., ChAd63 PanAd3 and ChAd3) showed an acceptable safety profile in the study populations with no reported vaccine related SAEs (Colloca et al. 2012; Colloca and Folgori 2013; de Barra et al. 2014; Hodgson et al. 2015; Ledgerwood, Sullivan, and Graham 2015; O'Hara et al. 2012; Sheehy et al. 2011).

In particular, a similar simian adenoviral vector-based RSV vaccine with the same insert as the ChAd155-RSV candidate vaccine (PanAd3-RSV) has been used previously in humans (RSV001 trial). Forty-two healthy subjects received the PanAd3-RSV vaccine at least once during the RSV001 trial. This study evaluated different adenovector-based RSV vaccine prime/ boost regimens including a heterologous boosting (with a recombinant modified Vaccinia Ankara [MVA] virus-vectored RSV vaccine) and priming by Intranasal administration. In the RSV001 study, there were no medically significant adverse events up to Week 28. However, a transient drop in hemoglobin, total white cell count and neutrophils were noted following vaccination. These were non-clinically significant to this study population and levels restored over time. The most commonly reported adverse events concerned injection site reactions. Most adverse events within one week of vaccination resolved within a few days. There were no significant events considered related to the vaccine. Two SAEs were reported (serological confirmed scrub typhus and hypnopompic flaccid paralysis with visual hallucinations); none were considered related to the vaccine.

The expectation for ChAd155-RSV is to mirror the previous clinical experience with closely related vectors.

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)	…
(ii)	family name for plants	…
(iii)	genus	*Homo*
(iv)	species	*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 ChAd155-RSV GMO is a vaccine based on a recombinant viral vector carrying relevant RSV antigens which is expected to mobilize both humoral and cellular arms of the immune response in the host.
Immunity induced by natural RSV infection is not able to fully prevent RSV re-infection and re-infections are common throughout life. ChAd155-RSV GMO is considered as an adequate solution to induce a balanced and more effective immune response against the RSV virus in a naïve population.

The ChAd155-RSV GMO encodes 3 RSV proteins which have been previously shown to trigger particular immune responses in the host:

- The fusion (F) protein deleted from the transmembrane and cytoplasmic regions (F0ΔTM) which is a major surface antigen of the RSV virus and the main target of the neutralizing antibody response to RSV, which is considered essential for protection against RSV-associated severe disease (Magro et al. 2012).
- The nucleocapsid (N) protein is essential for the replication and transcription of the RSV genome. It is an internal (non-exposed) antigen, highly conserved between RSV strains and known to be a source of many T-cell epitopes (Anderson, Huang, and Langley 2010; Townsend and Skehel 1984).
- The matrix (M2-1) protein is a transcription anti-termination factor that is important for the efficient synthesis of particular RNA molecules which are characteristic of non-segmented negative-strand RNA viruses. M2-1 is an internal (non-exposed) antigen, highly conserved between RSV strains and known to be a source of many T-cell epitopes (Anderson, Huang, and Langley 2010; Townsend and Skehel 1984).

The N and M2-1 antigens were included in the vaccine construct as source of T-cell epitopes for the induction of cell-mediated immunity.

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

The possibility of interactions to other species is minimal under the conditions of the proposed clinical release of the GMO. As mentioned in section F, the GMO will be administered to subjects in an enclosed hospital and is unlikely to come in contact with any non-target organisms in the ecosystem.

See section G.5 for further details about the interaction with non-target species.

Regarding microorganisms interactions, the viral genes to be exchanged to or from the GMO, to the genome of other wild type species of adenovirus, susceptible cells would need to be simultaneously infected with wild type simian adenovirus, which is extremely unlikely. Although transmission of adenoviruses between species, particularly between humans and non-human primates is still not well established, possible horizontal transmission events between humans and NHPs of such viruses is most likely to occur at places with close physical contact between NHPs and humans, such as zoos and other animal facilities (Wevers et al. 2011).

Further limiting any chance of gene transfer are the phenotypic characteristics of the ChAd155 vector in that it is replication-defective and as such is non-pathogenic.

Furthermore, genetic information carried by ChAd155 remains epichromosomal in infected cells thus eliminating any risk of integration of the viral DNA into the host genome.

4. Is post-release selection such as increased competitiveness, increased invasiveness for the GMO likely to occur?
Yes (.) No (X) Not known (.)
Give details
Compared to the parental simian adenovirus, the GMO has been modified to be replication defective, and there is no basis to believe that addition of the RSV transgene to the GMO would promote any post-release selection for increased invasiveness.

Plasmids used for construction of the GMO (the ChAd155 RSV) bear antibiotic resistance genes (ampicillin and kanamycin). However, the final GMO does not encompass any of these antibiotic resistance genes.

5. Types of ecosystems to which the GMO could be disseminated from the site of release and in which it could become established
Due to the context of the proposed GMO release, where the GMO is administered to subjects in an enclosed hospital, it is unlikely the GMO will come into contact with any non-target organisms in the ecosystem.

The likelihood of ChAd155-RSV becoming persistent and invasive in natural habitats is low for the following reasons:

- The ChAd155 vector to be used in the proposed clinical study is replication-deficient and only capable of transducing animal cells. In addition, adenovector genome remains epichromosomal thus avoiding the risk of integration of the viral DNA into the host genome after infection of host cells (Feuerbach and Crystal 1996).
- The risk of occurrence of the formation of replication competent adenovirus (RCA) from homologous recombination between the ChAd155 viral vector and the human Ad5 E1 region of the host cell is considered very low, due to the lack of sequence homology between the human E1 flanking regions of human Ad5 and chimpanzee adenovirus E1. It has been shown that recombination and production of RCA does not occur when they are propagated in HEK-293 cells (Colloca and Folgori 2013), thus eliminating the problem of RCA generation during the adenovector manufacture. To

control this issue, the ChAd155-RSV drug substance material is tested for the presence of RCA during different steps of the manufacture's process.
- The ChAd155-RSV is incapable of surviving outside a host (animal) cell since the vector is replication-defective.
- There is a theoretical possibility of shedding of infectious particles into the environment and potentially to the public during the proposed release. Defective recombinant adenoviruses have been used extensively in clinical trials, either through direct administration or cell therapy strategies (contained in the administered cells). The majority of the studies have not detected viral release in biological samples (sputum, saliva, urine, feces) and whenever detected through urine or saliva, it disappears in few days from administration. In the proposed study, arrangements will be made to minimize dissemination and inadvertent transmission (such as use of disinfectants). In addition, after each vaccination, the injection site will be covered with a dressing in order to absorb any virus that may leak out through the needle track. The dressing will be removed only after 30 minutes and will be disposed as GMO waste by autoclaving or in accordance with current standard European Union (EU) guidelines.

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 …

Not applicable. However, the possibility that hospital staff may be injected by accident may occur. As the administration of the vaccine will be performed by dedicated and trained medical personnel, this risk is considered minimal. Secondary transmission to patients' family members is considered unlikely as well. Indeed, the injection site will be covered with a dressing in order to absorb any virus that may leak out through the needle track and properly decontaminated and discharged (see section J1).

7. Likelihood of genetic exchange in vivo

(a) from the GMO to other organisms in the release ecosystem:
This is highly unlikely for the same reasons as described above in Section G.3. However it cannot be excluded.

(c) from other organisms to the GMO:
This is highly unlikely for the same reasons as described above in Section G.3. However it cannot be excluded.

(d) likely consequences of gene transfer:
No data are available. However, toxicity studies ruled out any toxic or pathogenic effect of the GMO. It is likely that gene transfer does not offer any 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.):
No data are available.

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

H. Information relating to monitoring

1. Methods for monitoring the GMOs

Defective recombinant adenoviruses have been widely used in clinical trials, both as direct administration or through cell therapy strategies (contained in the interior of cells). Most studies have not detected viral release in biological samples (sputum, saliva, urine, feces) and the few that have detected the GMO in the urine or saliva, usually disappear after two days of administration.

The GMO will be administered by intramuscular (IM) injection. With this route of administration, studies in animals show there is limited virus shedding and limited spread to other tissues, as the virus vector remains localized to the site of injection. Distribution and persistence of ChAd155-RSV vaccine for up to 70 days post-dose were evaluated in a GLP rat biodistribution study. ChAd155-RSV was undetectable in brain, heart, kidney, lung, ovary or testis samples on Days 2 and 8, or in blood samples assayed on Day 8 only. ChAd155-RSV was quantified at slightly higher than the LLOQ in the liver of one animal and in the spleen of four animals on Day 2, but was no longer detected in either organ by Day 8. ChAd155-RSV was detected from Day 2 at the injection site and the draining lymph nodes, including the iliac, inguinal, and to some extent, the popliteal lymph node. The highest levels were noted on Day 2, and levels progressively decreased over time, with undetectable levels in the muscle by Day 29 in some animals and most animals by Day 70, and in lymph nodes in most animals by Day 70. The quantities noted in the sampled tissues/organs at all timepoints were lower than 30,000 copies/µg host cell DNA, which is below the threshold level for which further studies would be required. The distribution and persistence of the ChAd155-RSV vaccine candidate is consistent with other similar adenovector-type vaccines.

It has not been planned in the current proposal or in the study already undergoing, any specific viral detection of ChAd155-RSV in biological fluids or blood.

The GMO will be released in a clinical study with the intent of triggering an immune response against the three RSV proteins encoded by the GMO genome. Hence, monitoring of the GMO effects will be performed by assessing the humoral and cell-mediated immunity at several timepoints post GMO administration.

There will be monitoring of side effects of treatment during the trial by physical examination, blood tests, nasal swab and communication of adverse events. The safety assessment will be made over the participation of patients in the clinical trial and up to the final visit in the study (see details in attached protocol).

2. Methods for monitoring ecosystem effects

No additional methods have been developed to monitor the effects of the GMO in ecosystems, as it is not naturally found in the environment and it is not expected it realises to the environment under the conditions of management of this GMO.

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

The probability for a transfer of the donated genetic material to other organisms is negligible (refer to section G.3. and G.7. above).

No additional methods will be performed to detect any transfer of genetic material from the GMO to others organisms during the proposed release.

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

Not applicable: The GMO is administered only to patients by intramuscular injection in the hospital rooms, as described in Section F. Furthermore, all biologics sampling will be performed at the same place.

5. Duration of the monitoring
Safety assessments will be performed all along the patient's participation in the clinical trial and up to study conclusion (16 months).

6. Frequency of the monitoring
According to the schedule provided in the clinical study protocol, subjects will be monitored routinely for safety and clinical outcome during planned visits throughout the period of GMO release (FPFV until LPLV) (see details in attached clinical protocol).

10 monitoring visits have been scheduled over the one year participation: at D0 (vaccination); D1; D3; D7; D30; D31; D33; D37 then at D60 and D365. Hence, they are more frequent just after each GMO administration with a visit scheduled the day after, then 3 days and 7 days post-vaccination.

I. Information on post-release and waste treatment

1. Post-release treatment of the site

The hospital room(s) used to prepare and administer the GMO vaccine will be cleaned with a 1% sodium hypochlorite solution immediately after the administration.

After each vaccination, the injection site will be covered with a dressing in order to absorb any virus that may leak out through the needle track. The dressing will be removed only after 30 minutes and treated with a 1% solution of sodium hypochlorite and then disposed of in the biohazard waste container decontamined by autoclaving or in accordance with the applicable guidelines/SOPs at the investigator's site.

All post-release treatment of the site will be performed by personnel who are trained to dispose of biohazard waste.
In case of spillover, the perimeter of the spill will be limited with paper towels and appropriate virocidal agent will be use to clean the area.

2. Post-release treatment of the GMOs

Following administration, used study vaccine materials will be placed immediately into biohazard bin. It refers to all empty vaccine vials, needles and syringes. The secondary containers for vaccine are retained for accountability (not in contact with the GMO).
After each vaccination, the injection site will be covered with a dressing in order to absorb any virus that may leak out through the needle track. After 30 minutes, the dressing will be treated with a 1% solution of sodium hypochlorite and then disposed of in the biohazard waste container.

Upon reconciliation and accountability, used study materials and unused study vaccine will be destroyed by the clinical site following institutional procedures for the disposal of biohazard material.

3. (a) Type and amount of waste generated

Based on the current protocol, 48 subjects will be enrolled in ChAd155-RSV treatment cohort. A total of 96 IM administrations of the GMO will be performed during the study. As this is a multicentre trial that will be conducted in several countries, subjects may be enrolled at any of the clinical sites listed in Section F.3.(a).

ChAd155-RSV vaccine is supplied in a glass vial, stoppered with grey rubber stoppers and capped with aluminium seals. Based on the packaging configuration, generated waste will include an estimated 96 vaccine vials and associated needles, syringes, packaging, gloves, masks, goggles, disposable gowns, bandages, and tape for the 48 subjects recruited into this cohort.

3. (b) Treatment of waste

Waste generated during the course of the study will be destroyed on site, following accepted institutional or clinical site procedures (e.g. autoclave, incineration or treated with sodium hypochlorite solution) by personnel trained in the disposal of biohazard waste.

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.

At the conclusion of the treatment phase of the study, all study vaccine will be either destroyed on site, following accepted institutional or clinical site procedures by personnel trained in the disposal of biohazard waste; or destroyed by a licensed facility contracted by the site.

At the conclusion of the treatment phase of the study, each participating study site will prepare an overall summary listing all study vaccine received, unused, partially used and destroyed.

J. Information on emergency response plans

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

   In case of contamination (skin contamination, needle stick or other puncture injury, eyes contamination) the personnel involved in the preparation, packaging or product management will notify the principal investigator and others as required by institutional policy (including CNB, MAGRAMA). All staff will be instructed on the procedures to act in case of accidental release.

   In the unlikely event of accidental spills, although adenoviruses can survive for long periods on environmental surfaces and are resistant to lipid disinfectants as all non-enveloped viruses, they are inactivated by heat, and susceptible to different chemical agents such as sodium hypochlorite 1% and 2% glutaraldehyde. A completely effective elimination is achieved by autoclaving at 121 °C for 15 minutes.

   In case of accidental spread, an absorbent tissue will be immediately placed over the spill to absorb the liquid containing the GMO, and then the tissue and contaminated surface should be decontaminated with a standard disinfectant active against the GMO (e.g., 1% solution of sodium hypochlorite). The disinfectant solution should be left in contact with the spill area for at least 30 minutes. Practical spill training sessions will be provided to all staff prior to working on the study. Records of staff training and competency will be documented.

   Accidental exposure in the form of a needle-stick injury will be minimised by the completion and demonstration of competency in the trial specific requirements for every member of staff involved with the study. All relevant standard and study specific operating procedures must be followed in the event of such as accident or incident occurring. No accidental inoculation is expected of staff but if it was to occur it would be considered as being safe and followed up by existing local guidance.

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

   See Section J.1.

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

   See Section J.1.

   Patients included in the clinical trial will be monitored for the occurrence of adverse events and serious adverse events (SAE) according to the clinical protocol. Each SAE will be recorded and assessed by the hospital staff and the study sponsor, and Health Authorities will be notified when applicable. Adverse events will be registered and reported according to detailed procedures in the clinical study protocol.

   In case of an undesirable effect, this medicine will be put on hold until the effects are fully assessed and measures are taken to mitigate further risks. All areas and facilities that had been used to administer the product would be cleaned and decontaminated using virucidal agents.

Due to the extensive procedural controls in place for the transport, storage, administration, disposal and monitoring of the administration of the GMO, the risk of an accidental environmental release, or a resulting undesirable effect from such an accidental release, is considered very low.

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