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: 2018-000431-27

V1.0 - 28 August 2018 (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
huCMV	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/18/33
(c)	Date of acknowledgement of notification	16/11/2018
(d)	Title of the project:	

Clinical trial RSV PED-011 entitled: "A Phase 1/2, randomized, observer-blind, controlled, multi-center 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 as a single dose or as two doses according to a 0, 1-month schedule, to infants aged 6 and 7 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 infants.".

(e) Proposed period of release:

Duration of the clinical trial RSV PED-0011 will be approximately 24 months, from the first patient recruited to last patient last visit (December 2019).

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
The 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)
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)

Order of the parental organism: Adenoviridae
Genus of the parental organism: Mastadenovirus
Species of the parental organism: Simian Adenovirus Subgroup C

The identity of the GMO is ChAd155-RSV, a 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.

Adenovirus have a duplex DNA genome, a sturdy capsid, and no lipid membrane, and therefore they are expected to be stable. Some factors that may affect genetic stability are temperature, UV light and cleaning procedures as adenoviruses are commonly susceptible to those. However, adenoviruses can survive for long periods on environmental surfaces; they are resistant to lipid disinfectants (because they are non-enveloped), but are inactivated by heat (>56°C), and disinfectants including: 1% sodium hypochlorite, 2% glutaraldehyde, 0.25% sodium dodecyl sulphate.

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 material (MVS and DS) is tested for the presence of RCA using a RCA assay (specification: <1 RCA / 3x1010 vp). The confirmation of absence of RCA has been demonstrated in all manufacturing lots to date.

The working virus stock is stored at <-60°C until use. Long term stability for final drug product was shown for up to 48 months when stored at < 60°C, showing the material meets product stability specifications throughout this period of time.

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, BE, FI, Poland

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.

- Member State of notification ES
- Notification number B/ES/16/07; EudraCT number: 2016-000117-76.

- Member State of notification IT
- Notification number Not applicable; EudraCT number: 2016-000117-76.

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 US, Canada, Taiwan, Panama, Australia, Hong Kong
- Notification number Not applicable

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

- Lack of prolonged transgene expression has made replication incompetent adenoviruses attractive viral vectors for vaccine development. They possess a stable virion, allowing

inserts of foreign genes to remain intact and they can infect many different cell types. 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 (see section A.3.(c)).
- The Chad155-RSV is incapable of surviving outside a host (animal) cell since the vector is replication-defective.
- To minimize release of the recombinant vectored vaccine virus into the environment, each vaccine is produced under good manufacturing practice (GMP) conditions with the handling of live material in appropriate laboratory facilities. This is to ensure that any release of modified organism is contained, inactivated and incinerated, using single use equipment as much as possible; to avoid release of modified genetic material into the environment. Furthermore, administration of the vaccine will be performed in access-restricted clinical room where any waste/materials used for the GMO administration will be autoclaved and incinerated after vaccination.
- 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. Following administration of 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). 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 in the hospital and will be disposed as GMO waste by autoclaving.

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.

Finally, as the release is planned during a clinical trial, and will be administered intramuscularly to the patients, it is highly unlikely that the GMO will come into contact with the environment.

As a conclusion, the potential environmental impact of the release of the GMO is negligible.

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

Please note that in the following section, information relating to both the recipient organism (the engineered vector "empty" (ie without the transgene)) and the parental organism (the organism from which the engineered vector is derived) are provided when appropriate.

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

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 (parental organism). 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) The recipient organism is engineered in a laboratory, and is then not naturally found in the environment.
(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.

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

5. (a) Detection techniques
The infectivity of ChAd155 adenovirus is assessed using real-time quantitative polymerase chain reaction (Q-PCR) to quantify viral particles in the host cells, and an immunocytochemistry assay with anti-hexon antibody that has shown reactivity with ChAd155 hexon protein.

(b) Identification techniques
See section B.5.(a) above.

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 safety at Containment Level 1.

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

The recipient of the vaccine, ChAd155 is replication-deficient and a chimpanzee-derived species and therefore considered not to be pathogenic to humans or other non-target organisms. Moreover, adenoviruses are not integrated in the host genome and do not present a risk to activate latent provirus.

8. Information concerning reproduction

(a) Generation time in natural ecosystems:
Not applicable. The replication-defective recipient organism does not generate in natural ecosystems.

(b) Generation time in the ecosystem where the release will take place:
Not applicable. The replication-defective recipient organism will not generate in the ecosystem where the release will take place.

(c) Way of reproduction: Sexual .. Asexual ..
Not applicable.

(c) Factors affecting reproduction:
Not applicable.

9. Survivability

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

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

The adenoviral vectors do not form structures enhancing survival.

(b) relevant factors affecting survivability:

Adenoviruses 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. Identical factors are expected to apply to the GMO.

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)

Please refer to section A 4,5 and 6

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 pf 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.

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 (recipient organism)

(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.

(c) Host range of the vector

There are no host identified 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 E-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 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.

(d) Constituent fragments of the vector

The DNA fragment inserted as the transgene in the Chimpanzee Adenovirus vector (ChAd155) encondes sequences derived from three RSV protein and it contains the HCMV promoter with the TeO inserted downstream the HCMV promoter TAT box to provide transcriptional control of the transgene while in the packing cell line. The ChAd155 viral genome was cloned into the 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 replacement of native E4 by ORF 6 of the human adenovirus type 5 (Ad5)

(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 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.

(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 repression protein, the HCMV promoter is not blocked and normal transcription of the transgene resumes.

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, Mas 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 (Townsend and Skehel 1984; Anderson, Huang et al. 2010). 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 (Townsend and Skehel 1984; Anderson, Huang et al. 2010).

(e) 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

(f) 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, Gruber 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 (Simoes 1999; Krilov 2011). 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 the investigational RSV vaccine 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, Mas 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).

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 and 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 (.) No (X) Unknown (.)
Specify

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

(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)

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, Arras-Reiter 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 infants aged 6 and 7 months, likely to be unexposed to RSV. 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 [**¡Error! No se encuentra el origen de la referencia.**, 2011]. Fatigue, headache and malaise were the most commonly reported systemic AEs overall.

The immunogenicity, safety and reactogenicity of the ChAd155-RSV vaccine have been evaluated in healthy adults aged 18 to 45 years (study 201974 [RSV PED-001; NCT02491463]) There were no significant safety concerns identified up to Day 60 in study RSV PED-001. Overall, the ChAd155-RSV vaccine high dose (5×10^{10} vp) seems to be more reactogenic (local and general) than the ChAd155-RSV vaccine low dose (5×10^{9} vp), however, the reactogenicity profile was less than that observed in the *Bexsero* group. No safety signal from the assessed hematology parameters (hemoglobin, platelet count, prothrombin time and APTT) was observed in subjects receiving the ChAd155-RSV vaccine. No significant reductions in platelet count or clinically significant changes in coagulation parameters were observed up to 30 days post Dose 2. An approximately 2.4-fold increase in RSV-A neutralizing antibody titers (geometric mean titer [GMT] from baseline) was observed in both RSV low dose and RSV high dose after Dose 1. No booster effect was evident after Dose 2. An anti-F IgA and IgG antibody secreting B-cells response and an RSV F, N and M2-1 specific IFN-γ secreting T-cells response in RSV high dose group after the first dose were observed with ELISpot. There was no booster response after the second vaccination. There was no specific vaccine-induced CD4 T-cellular response observed with intracellular staining (ICS). For CD8 T-cells only a weak CD8 IFN-γ response to N was shown with ICS for some subjects.

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

There are no techniques planned to detect and identify the GMO in the environment in the context of the proposed clinical trial.

(b) Techniques used to identify the GMO

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.

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 operating rooms, by intramuscular (IM) injections to patients as part of an international, multicentre clinical trial.

This clinical trial is a phase I/II trial in infants aged 6 and 7 months (having a low chance of natural exposure to RSV before inclusion in the study).

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 approximately 100 subjects in total. As a control, 50 subjects will be vaccinated with placebo or active comparator (*Menveo* or *Bexsero*, or *Nimenrix*, or *Synflorix)*. The choice of control between an active comparator or Placebo will be done at country level. In Spain, *Bexsero* (GSK's multicomponent meningococcal B vaccine (recombinant, adsorbed)) is selected as active comparator.

The purpose of this trial is to provide critical information on the safety, reactogenicity and immunogenicity profile of the ChAd155-RSV vaccine in infants likely to be unexposed to RSV before moving to a proof-of-concept trial in infants that will compare the requirement for a one or two dose regimen. This study will also assess if there is a risk of 'vaccine-induced enhanced RSV disease' after vaccination of infants aged 6 and 7 months, likely to be unexposed to RSV, with the ChAd155-RSV vaccine.

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.

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 clinical sites provided in the table below. It is possible that other sites will be added in future if the study does not recruit as planned.

Clinical Study Site Address	Principal Investigator
Spain	
Hospital La Paz Edificio Materno-Infantil, Servicio de Neonatología. Planta 2ª Paseo de la Castellana, 261	Dr. Fernando Baquero Artigao
Hospital Clínico Universitario de Santiago (CHUS) Servicio de Críticos, Intermedios y Urgencias pediátricas. 2º Planta. Travesía da Choupana s/n 15706 - Santiago de Compostela (A CORUÑA)	Dr. Federico Martinon-Torres
Centro Superior de Investigaciones en Salud Pública - Generalitat Valenciana (CSISP). Área de Vacunas. Sótano -1 Av. Cataluña, 21.	Dr. Javier Díez Domingo
Hospital 12 de Octubre Edificio Materno-Infantil. Servicio de Pediatría. Sección de Infectología Pediátrica. 6ª Planta. Av. De Córdoba s/n	Dr. Pablo Rojo Conejo
Hospital Universitario Puerta de Hierro Servicio de Pediatría. 2ª Planta. C/ Manuel de Falla, 1. 28222 Majadahonda (MADRID)	Dr. Miguel Ángel Marín Gabriel
Hospital Clínico San Carlos. Servicio de Pediatría C/ Dr. Martin Lagos, s/n	Dr. José Tomás Ramos Amador
Hospital Universitario de Burgos Unidad de Investigación. Planta 0 Bloque G Av. Islas Baleares s/n 09006 BURGOS	Dr. José Manuel Merino
International Hospitality Projects (IHP) Jardín de la Isla, nº6 - Accesoria nº 1 Edificio Expolocal 41014 Sevilla	Dr. Ignacio Salamanca de la Cueva
Hospital Universitario HM Montepríncipe Servicio de Pediatría Av. de Montepríncipe, 25 28660 - Boadilla del Monte Madrid	Dr. Jose García Sicilia
Hospital Universitario HM Puerta del sur Servicio de Pediatría Av. de Carlos V, 70 28938 – Móstoles Madrid	Dr. Jose García Sicilia

(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 or clinic examination room 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 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:

Not applicable since the release will occur during a clinical trial held in hospital settings.

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

Not applicable since the release will occur during a clinical trial held in hospital settings.

4. Method and amount of release

(a) Quantities of GMOs to be released:

This study will evaluate two vaccine regimens: either one dose of 1.5 x 10^{10} viral particles (vp) or two doses of 5 x 10^{10} viral particles (vp) of the ChAd155-RSV vaccine will be administered intramuscularly (in the anterolateral thigh]). For the two doses schedule an interval of approximately one month between dosing will be used.

The investigational ChAd155-RSV vaccine will be administered to approximately 100 subjects, 50 will be receiving one dose of 1.5 x 10^{10} vp ChAd155 vaccine, and 50 subjects will be receiving two doses of the 5x10^{10} vp ChAd155 vaccine. 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 18 x 10^{11} vp, corresponding to a maximum of 150 vials (extra patients counting for loss), in this study (in all countries concerned).

(b) Duration of the operation:

Each vaccination takes about few minutes. Total study for each subject will last approximately 24 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 route of administration has been chosen in order to minimise potential shedding from the patient.

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.

The vials will be imported from Belgium and stored in freezers < -60°C at the clinical site until use.

It is noted that needle puncture of the stoppered vial containing the viral vector is a potential source of aerosols.

The bench top used to prepare the GMO for injection should be decontaminated before and after manipulation with a 1% solution of sodium hypochlorite. All transfers of the GMO preparation should be undertaken using a closed container clearly labelled with biohazard symbol. Furthermore, employees will follow the clinic or hospital policy recommended for handling of viral vector GMO vaccines.

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 GMO contaminated waste to be destroyed separately from other Virology laboratory waste.

There is no biological test designed specifically to determine if personnel handling this GMO have been infected.

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 immunogenicity, safety and reactogenicity of the pediatric candidate RSV vaccine (ChAd155-RSV vaccine) have been evaluated in healthy adults aged 18 to 45 years (study 201974 [RSV PED-001; NCT02491463]) There were no significant safety concerns identified up to Day 60 in study RSV PED-001. Overall, the ChAd155-RSV vaccine high dose (5 x 1010 vp) seems to be more reactogenic (local and general) than the ChAd155-RSV vaccine low dose (5 x 109 vp), however, the reactogenicity profile was less than that

observed in the Bexsero group. No safety signal from the assessed hematology parameters (hemoglobin, platelet count, prothrombin time and APTT) was observed in subjects receiving the ChAd155-RSV vaccine. No significant reductions in platelet count or clinically significant changes in coagulation parameters were observed up to 30 days post Dose 2. An approximately 2.4-fold increase in RSV-A neutralizing antibody titers (geometric mean titer [GMT] from baseline) was observed in both RSV low dose and RSV high dose after Dose 1. No booster effect was evident after Dose 2. An anti-F IgA and IgG antibody secreting B-cells response and an RSV F, N and M2-1 specific IFN-γ secreting T-cells response in RSV high dose group after the first dose were observed with ELISpot. There was no booster response after the second vaccination. There was no specific vaccine-induced CD4 T-cellular response observed with intracellular staining (ICS). For CD8 T-cells only a weak CD8 IFN-γ response to N was shown with ICS for some subjects.

A clinical study is currently being conducted in RSV-seropositive infants aged 12 to 23 months (study 204838 [RSV PED-002]). At the condition of satisfactory safety profile of the ChAd155-RSV vaccine in RSV-seropositive infants, as evaluated by an IDMC on Day 60 data (i.e., 30 days post-Dose 2 of the highest dose level) of the study RSV PED-002, the present study will be performed.

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, Mas 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 (Townsend and Skehel 1984; Anderson, Huang et al. 2010).
- 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 (Townsend and Skehel 1984; Anderson, Huang et al. 2010).

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 gene transfer 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 a standard hospital room and is unlikely to come in contact with other animal species.

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.

In order for 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, Metzger et al. 2011).

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.

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 or clinical examination room, it is unlikely the GMO will come into contact with any non-target organisms in the ecosystem.
In the event of inadvertent administration to non-target organisms, further dissemination is unlikely because GMO is unable to complete a viral replication cycle and as such is non-virulent and unable to disseminate in target or non-target organisms.

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 (see section I).

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.

(c) from other organisms to the GMO:

This is highly unlikely for the same reasons as described above in Section G.3.

(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. It is important to note that in no case were detected adenoviruses able to replicate to indicate the occurrence of recombination events *in vivo* (Zhu, Grace et al. 1999).
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 realised 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 H.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 (day 365).

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).

I. Information on post-release and waste treatment

1. Post-release treatment of the site

The hospital or clinic 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 disposed as genetically modified organism waste 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.

2. Post-release treatment of the GMOs

Following administration, used study vaccine materials will be placed immediately into biohazard bin and retained for accountability (not in contact with the GMO).

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, 100 subjects will be enrolled in ChAd155-RSV treatment cohort. A total of 150 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 150 vaccine vials and associated needles, syringes, packaging, gloves, disposable gowns, bandages, and tape for the 100 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 viricidal 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 Centro Nacional de Bioseguridad (CNB) and Consejo Interministerial de Organismos MOdificados genéticamente (CIOMG)). 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 121ºC 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 session 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.

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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