RSV-PED-011 Clinical study

ChAd155-RSV

Directive 2001/18/EC – Annex II

PRINCIPLES FOR THE ENVIRONMENTAL RISK ASSESSMENT (ERA)

Notifier: Glaxo SmithKline Biologicals

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Abbreviations

Ad5	Adenovirus 5
AE	Adverse Event
BAC	Bacterial Artificial Chromosome
BGHpA	Bovine Growth Hormone Polyadenylation Signal
ChAd	Chimpanzee adenoviruses
ChAd155	Chimpanzee Adenovirus Type 155
ChAd155-RSV	Investigational Recombinant Chimpanzee Adenovirus Type 155-vectored RSV Vaccine
CMI	Cell-Mediated Immunity
CPE	Cytopathic effect
DNA	Deoxyribonucleic Acid
EC	Regulation
eCRF	electronic Case Report Form
EEC	European Economic Community
EU	European Union
F	RSV Fusion Protein
F0ΔTM	RSV Fusion Protein deleted from its transmembrane domain
FMDV	Foot and mouth disease virus
GLP	Good Laboratory Practices
GMO	Genetically modified organism
GMP	Good Manufacturing Practices
GSK	GlaxoSmithKline
HCV	Hepatitis C Virus
HEK293	Human Embryonic Kidney 293
HERVs	Human endogenous retroviruses
huCMV	Human Cytomegalovirus
IB	Investigator Brochure
IDMC	Independent Data Monitoring Committee
IM	Intramuscular
iSRC	internal Safety Review Committee
IU	infectious units
LLOQ	Lower Limit Of Quantification
MUSCLE	Multiple Sequence Comparison by Log-Expectation
MS	Master Seed
MVA	modified Vaccinia Ankara
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
TFF	Tangential flow filtration

UK	United Kingdom
US	United States
UV	Ultraviolet
vp	Viral particles
VSMB	Vaccine safety monitoring board
VSS	Virus Seed Stock

I. Introduction

1. Context of the release

As per the requirements of Directive 2001/18/EC, this document presents an assessment of the possible risks to the environment following deliberate release of a genetically modified organism.

The proposed release will occur during a clinical trial 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"

The EudraCT number of the study is: 2018-000431-27 and the company code is: RSV-PED-011.

The study will be initiated in April/May 2019; duration of the study will be 24 months per patient.

2. Characteristics of the GMO

a. Description of the GMO and doses

The GMO ChAd155-RSV is a viral suspension of a live recombinant, replication-defective simian (chimpanzee-derived) group C adenovirus serotype 155 (ChAd155) viral vector construct engineered to express three proteins from the Respiratory Syncytial Virus (RSV): the fusion F protein (deleted of the transmembrane region, named F0ΔTM), the nucleocapsid protein N and the transcription anti-termination protein M2-1. The ChAd155 vector is derived from the wild type chimpanzee Ad type 155 genome and was isolated from a healthy young chimpanzee housed at the New Iberia Research Centre facility (New Iberia Research Centre; The University of Louisiana at Lafayette, LA, USA) using standard procedures. The viral genome was then cloned in a plasmid vector and subsequently modified to carry the deletion into viral genome of E1 and E4 regions and the insertion of E4orf6 derived from human Ad5.

The vaccine is presented as a single-dose (monodose) for intramuscular (IM) administration. The volume per nominal dose is 0.5 ml. The vaccine is stored at <-60°C. One dose of $1.5x10^{10}$ viral particles or two dose of $5x10^{10}$ viral particles, administered with a 4-week interval between vaccinations will be evaluated. during the study.

b. Detection and identification of the GMO

The following techniques are used to detect and identify the GMO:

- A specific polymerase chain reaction (PCR);
- Full genome sequencing;
- restriction analysis

- In addition, western blots developed by the Applicant are relevant to detect and identify the GMO insert since they use specific antibodies: targeting either the RSV F protein or the RSV N-M2-1 fusion one.

3. Characteristics of the release

a. Previous release of the GMO

The present ChAd155-RSV vaccine has been released in

1. the ongoing phase I trial conducted in RSV-seropositive infants aged 12 to 23 months (EudraCT: 2016-000117-76) 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 23 months'.

   No adverse event of concern has been reported to the sponsor to date.

2. a completed Phase 1 trial in healthy adults aged 18 to 45 years (EudraCT: 2014-005333-31) entitled: "A phase I, randomised, observer-blind, controlled study to evaluate the safety, reactogenicity and immunogenicity of a GlaxoSmithKline Biologicals Respiratory Syncytial Virus (RSV) investigational vaccine based on 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 in healthy adults aged 18 to 45 years." There were no significant safety concerns identified up to Day 60 in this study.

b. Characteristics of the planned release

The planned released of the GMO will occur during a clinical study (RSV-Ped-011) to be held at several investigational sites under the responsibilities of local principal investigators.

This is a Phase I/II clinical trial, observer-blind, randomized, controlled, multi-centric study with three parallel groups. It will be conducted on infants aged 6 and 7 months (having a low chance of natural exposure to RSV before inclusion in the study). The target will be to enroll approximately 150 infants. In the same investigational center, infants will be vaccinated sequentially, separated by a minimum interval of 60 minutes.

The GMO will be administrated using a vaccination regimen based on one IM injection of 1.5×10^{10} viral particles (vp) of the ChAd155-RSV vaccine or two IM injections of 5×10^{10} viral particles (vp) of the ChAd155-RSV vaccine. IM injections are performed in the anterolateral thigh.

II. Identification of GMO characteristics which may cause adverse effects

1. Possible negative effects including allergenic and toxic effects in Humans

a. Allergenic effects

The ChAd155-RSV vaccine is not expected to have any toxic nor allergenic effects beyond transient local or systemic reactions associated with vaccination (see section III.1.a.).. However as in the standard Phased approach to clinical development a careful approach will be taken progressively increasing the exposure whilst monitoring the accumulating safety date.

b. Toxicity/Pathogenicity

Comprehensive toxicity studies (single-dose, repeat-dose, and mechanistic toxicology studies) performed in a GLP compliant environment failed to demonstrate any toxic effect of the ChAd155-RSV. These toxicity studies ruled out any toxicity either from the vector itself and the product of transgene expression; therefore, no harmful effects are envisaged.

c. Replication competent adenovirus

The GMO is derived from the simian ChAd155 strain and has been rendered replication deficient thanks to deletion of the E1 and E4 sequence. The presence of replication competent adenovirus (RCA) is assessed on the master virus seed MVA and each batch of DS with a specification < 1 RCA / 3 x 10e10 viral particles tested. The RCA detection assay is an extended in vitro test using A549 cells as the indicator cell line to determine the presence of virus-induced cytopathic effect (CPE) after 28 days in culture. The confirmation of absence of RCA has been demonstrated in all manufacturing lots to date.

d. Shedding/Survivability

The GMO is replication deficient and is not expected to survive, multiply or disperse following its release during the proposed clinical study. The presence of replication competent adenovirus is assessed on the drug substance and is a release test. Moreover, it will be administrated intramuscularly, a route which limits its potential shedding.

e. Reactivation of endogenous retroviruses

As up to 8% of the human genome is of retroviral origin (Hurst et al. 2017, Meyer et al. 2017). Potential interaction of the GMO with human endogenous retroviruses (HERVs) must be envisaged. The evaluation of the potential reactivation of HERVs by the GMO is covered in sections III.1.e. and IV.1.e.

2. Possible negative effects including allergenic and toxic effects in animals and plants

Comprehensive toxicity studies (single-dose, repeat-dose, and mechanistic toxicology studies) performed in animals failed to demonstrate any toxic effect of the ChAd155-RSV. Since no interaction or survival in the environment can be expected, no specific studies on the potential ecological impact of the GMO have been performed.

3. Effects on the dynamics of populations of species in the receiving environment and the genetic diversity of these populations

The GMO will be administered to infants aged 6 and 7 months (likely to be previously unexposed to RSV) in a highly controlled clinical trial setting. No effects on the dynamics of populations of species and/or modification of the genetic diversity of this population is foreseen.

4. Altered susceptibility to pathogens facilitating the dissemination of infectious diseases and/or creating new reservoirs or vectors

The GMO is a replication-defective simian-derived adenovirus vector that is incapable of establishing a propagative infection and so is not pathogenic to animals or humans. The intended function of the GMO is to induce an antigen-specific immune response against HBV in an effort to control the infection. The GMO vector carries no information that would serve to facilitate the dissemination of infectious disease.

5. Compromising prophylactic or therapeutic medical, veterinary, or plant protection treatments

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.

The GMO is not capable to transduce plant cells and therefore this section is not applicable for plan protection treatments.

6. Effects on biogeochemistry

The GMO is an investigational medicinal product and its release is not expected to have any effect on biogeochemical cycles.

III. Evaluation of the magnitude of the potential consequences and likelihood of occurrence of adverse effects

1. Possible negative effects including allergenic and toxic effects in Humans

a. Allergenic effects

Allergenic reaction post-vaccination are 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 gelatin or egg protein (Zent, Arras-Reiter et al. 2002). In order to be able to treat infants with an immediate systemic allergic reaction to vaccination, all infants across all steps will need to remain under observation (visual follow-up as well as measurement of vital signs) at the study site for at least 60 minutes after vaccination.

There is no basis to indicate the ChAd155-RSV GMO will provoke allergenic effects in the vaccinated study population.

b. Toxicity/Pathogenicity

- Clinical data

Safety results from the Phase I clinical trial performed with ChAd155-RSV (RSV-Ped-001) indicate acceptable safety of the candidate ChAd-RSV vaccine.

RSV ped-001 was a Phase I, randomised, observer-blind, controlled study to evaluate the 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), when administered intramuscularly according to a 0, 1 month schedule to healthy adults aged 18 to 45 years.

The 72 subjects enrolled were allocated into 4 study groups with N = the number of subjects vaccinated:

- RSV vaccine Low dose: $5x10^9$ viral particles (vp) (RSV-Ld), [N=8];
- RSV vaccine High dose: $5x10^{10}$ vp (RSV-Hd), [N=32];
- Placebo (Pbo), [N=16];
- Active Control (AC): Bexsero, [N=16].

There were no significant safety concerns identified up to Day 60 in this study. Overall, the RSV-Hd seems to be more reactogenic (local & general) than the RSV-Ld, however, the reactogenicity profile was less than that observed in the licensed active comparator.

No safety signal from the assessed haematology parameters (Hemoglobin, Platelet count, Prothrombin Time and APTT) was observed in subjects receiving the RSV vaccine. No significant reductions in platelet count or clinically significant changes in coagulation parameters were observed up to 30 days post dose 2.

These data are consistent with observations from other clinical trials performed with simian adenovectors (i.e., ChAd63 PanAd3 and ChAd3) demonstrating an acceptable safety profile in the study populations with no reported vaccine related SAEs (Sheehy et al. 2011; Barnes et al. 2012; O'Hara et al. 2012; Colloca and Folgori 2013; de Barra et al. 2014; Hodgson et al. 2015; Ledgerwood et al. 2015).

- Preclinical GMO toxicity data

Comprehensive toxicity studies performed in a GLP compliant environment failed to demonstrate any toxic effect of the ChAd155-RSV. These toxicity studies ruled out any toxicity either from the vector itself and the product of transgene expression; therefore, no harmful effects are envisaged. These data are summarized in the section below.

In the single dose and local tolerance studies, no unscheduled mortality or clinical signs of toxicity were noted, and no grossly visible local reactions were observed after ChAd155-RSV injection. Furthermore, body weight or body weight gain did not differ significantly between controls and test item-treated animals. There were no macroscopic findings related to ChAd155-RSV. Minimal infiltrate of mononuclear cells was observed in the interstitium of the muscle injected with ChAd155-

RSV candidate vaccine. Under the conditions of this study, the ChAd155-RSV candidate vaccine was considered to be well tolerated locally.

Repeated IM injection of the ChAd155-RSV candidate vaccine produced no unexpected mortality, systemic clinical signs, local reactions, ophthalmological changes, or remarkable differences in body weight and food consumption. In animals administered the candidate vaccine, lower platelet counts (-53% in males and -27% in females compared to pretest values and -59% in males and -40% in females compared to control values) and prolonged APPT (+19% in males and +20% in females compared to pretest values) were noted 24 hours after the first injection in both sexes. However, these effects were transient and were not associated with systemic clinical signs, reduced body weight or food consumption, or signs of hemorrhage at necropsy. Furthermore, an additional rabbit study was initiated to evaluate the kinetics and recovery of the hematological parameters following IM exposure to the ChAd155-RSV candidate vaccine. Preliminary results from this study confirmed the observation in the repeated dose toxicity study that the point of maximal platelet reduction was 24 hours after IM injection of ChAd155-RSV. A small increase in body temperature was recorded after the first dosing, however, similar body temperature values in the controls were observed at other time points, or before dosing in the treated animals, so the toxicological significance is uncertain.

Transient increases in CRP were recorded after dosing in both sexes. Three days after the last injection, changes suggestive of an immunogenic response were noted in the spleen and draining lymph nodes. At the injection sites, mild inflammatory changes were observed, along with minimal muscular degenerative changes. Twenty eight days after the last injection, minor changes were still present in the spleen of males and in the iliac lymph nodes. At the injection sites only minor remnants of the previous inflammatory reactions were seen, indicating that a recovery process was well underway.

c. Replication competent adenovirus

RCA testing is performed as a release test at the level of the MVS and on each batch of bulk drug substance (specification <1 RCA / 3 x 10e10 vp tested). To date, no single RCA event has been detected during the manufacture of toxicology or clinical batches.

d. Shedding/Survivability

The GMO will be administered by intramuscular (IM) injection. With this route of administration, previous studies conducted with other adenovirus vectors showed there is limited virus shedding and limited spread to other tissues, as the virus vector remains mainly localized to the site of injection (Sheets et al. 2008).

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 lymphnodes, 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.

In the unlikely event of shedding or accidental spills, although adenoviruses can survive for long periods on environmental surfaces, they are inactivated by heat and are susceptible to different disinfectants shown to be active against non-enveloped viruses.

e. Reactivation of endogenous retroviruses

The likelihood of reactivation of HERVs following intramuscular administration of the GMO is considered neglible since the vector DNA remains epichromosal.

2. Possible disease including allergenic and toxic effects in animals and plants

Toxicity studies have ruled out the potential pathogenicity of the GMO in rodents (see § IIIb above). The GMO is therefore not expected to be allergenic or toxic in animals and is not capable of transducing plant cells.

3. Effects on the dynamics of populations of species in the receiving environment and the genetic diversity of these populations

There is no identified potential for competitive increase in the human population as patients (or volunteers) who have received the GMO will have no selective advantage over non-treated humans.

The recipient has been modified from the parental organism to be replication defective following deletion of the E1A gene, and there is no basis to consider that addition of the RSV transgene to the GMO would promote any post-release selection for increased invasiveness. Therefore, no competitive advantage was conferred to the GMO in relation to the recipient and parental organism.

The only non-target organism that may potentially receive the GMO is hospital personnel following a needle-stick injury. As for the patients enrolled in the proposed clinical study, there is no identified risk and therefore non-target organisms will not be adversely affected by the release of the GMO.

The overall diversity of the human population is not expected to be altered since the GMO is not integrative and is intended for treatment of RSV-positive patients only.

4. Altered susceptibility to pathogens facilitating the dissemination of infectious diseases and/or creating new reservoirs or vectors

The administration of the GMO should not increase the dissemination of infectious disease. Neither should GMO administration into the target host alter susceptibility to pathogens in a negative sense. In fact, the role of the GMO is to induce an antigen-specific immune response designed to control RSV infection. The GMO is not expected to create new reservoirs since it is non-integrative and will be cleared from the host due to the host immune response.

5. Compromising prophylactic or therapeutic medical, veterinary, or plant protection treatments

Plasmids used for construction of the GMO bear antibiotic resistance genes (ampicillin and kanamycin). However, the final GMO does not encompass any of these antibiotic resistance genes. In addition, benzonase treatment is performed during manufacturing of the drug substance (prior to final formulation), allowing digestion of any remaining DNA sequences. Therefore, the probability of transfer of antibiotic resistance gene compromising future prophylactic or therapeutic treatments to the target host (Human) is negligible.

6. Effects on biogeochemistry

The GMO is an investigational medicinal product to be released in a highly controlled clinical setting and should have no impact on biogeochemical cycles.

IV. Estimation of the risk posed by each identified characteristic of the GMO.

1. Possible negative effects including allergenic and toxic effects in Humans

a. Allergenic effects

Based on the nonclinical data generated with the GMO and clinical data obtained with both identical and similar ChAd vector backbones containing different transgenes, the risk of allergenic effect following administration of the GMO during the planned release is considered low.

b. Toxicity/Pathogenicity

Preclinical toxicity studies demonstrated absence of toxicity of the GMO. Clinical studies using other simian adenovirus strains showed adverse events that were only transient; no SAE related to the experimental product. In addition, in the completed Phase I study assessing safety, reactogenicity and immunogenicity of the GMO, no SAE has been reported to date. The risk of toxicity following GMO administration in the planned release is therefore expected to be low.

c. Replication competent adenovirus

Quality controls are performed routinely during the manufacturing process and each batch of GMO toxicology and clinical material is tested for the presence of RCA at the level of the bulk drug substance. Each batch of material must meet the pre-defined specifications for the absence of RCA prior to release.

The remaining risk of RCA formation would be in case of recombination of the GMO with human adenovirus in case the host is infected. However, the probability of 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. The use of codon-optimized transgene sequence reduces further the probability of recombination with wild-type sequences. The risk of RCA is therefore considered low.

d. Shedding/Survivability

The potential for shedding of infectious GMO particles into the environment is considered negligible especially when administered by the intramuscular injection route. There is no indication that the RSV transgene could influence the shedding behaviour of recombinant ChAd vectors.

Preventive measures implemented during the conduct of the clinical trial will also minimize inadvertent dissemination from spills or accidents.

There is also minimal risk of persistence or survivability of the GMO vector in the environment. Survivability of the GMO is not expected to be different from the parental virus strain and the risk of increased survivability is considered negligible.

e. Reactivation of endogenous retroviruses

The overall risk of reactivation of endogenous retroviruses is considered negligible.

2. Possible disease including allergenic and toxic effects in animals and plants

The potential negative effects to animals is similar to those identified for humans although their likelihood of occurrence is almost null since animals are not the target organism and the release will occur during a clinical study. Therefore, the overall risk to animals is considered negligible.

The risk to plants is null since the GMO is not capable of transducing plant cells.

3. Effects on the dynamics of populations of species in the receiving environment and the genetic diversity of these populations

The risk of modification of the population dynamics and of the genetic diversity is considered negligible.

4. Altered susceptibility to pathogens facilitating the dissemination of infectious diseases and/or creating new reservoirs or vectors

The risk of alteration of the susceptibility to pathogens is considered null. Neither facilitation of infectious diseases nor creation of new reservoirs is therefore expected.

5. Compromising prophylactic or therapeutic medical, veterinary, or plant protection treatments

The risk of compromising prophylactic or therapeutic medical protection treatments is considered negligible since no antibiotic resistance gene will be transferred to the host. The risk to animals is also considered negligible since they are not targeted hosts and accidental transmission is considered null (the release will occur during a clinical study and shedding has never been noticed during prior release of adenovirus based vaccines). Finally, the risk to plants is null since the GMO is not capable of transducing plant cells.

6. Effects on biogeochemistry

The risk of effects on biogeochemistry is null.

V. Application of management strategies for risks from the deliberate release of GMO

1. Management strategies for vaccinated patients

The investigational ChAd155-RSV vaccine has never been administered to RSV- potentially naïve infants. Safety precautions such as limited vaccination, safety evaluations by an iSRC and an IDMC, and study holding rules have therefore been defined.

In the same investigational center, all the infants should be vaccinated sequentially and at least 60 minutes apart to allow monitoring of any acute events (e.g., hypersensitivity reaction). All infants should be closely observed (visual follow-up as well as measurement of vital signs) for at least 60 minutes after vaccination. Vital signs are body temperature, HR and RR. Vital signs are preferably measured when the infant is calm.

This study will be overseen by an iSRC operating under a charter. An iSRC was already involved in study RSV PED-002 (204838). Core members of the iSRC will include a GSK Biologicals' safety physician, a CRDL, and a biostatistician who are not otherwise involved in the conduct of the project. The iSRC safety reviews will be conducted using unblinded data. The iSRC has access to the subject randomization and reviews unblinded data.

This study will be overseen by an IDMC operating under a charter. The IDMC involved in the present study was already involved in study RSV PED-002 (204838). Overall, the role of the IDMC includes the review and protection of data integrity and rights and safety of study participants throughout the study period. It will provide initial, regular, and closing advice to GSK Biologicals on medical, ethical, scientific and safety-related issues. Its advice will be based on the interpretation of study data with reference to the study protocol.

The safety holding rules which will be assessed by the investigator are defined in Table 1 and the safety holding rules which will be assessed by the iSRC or IDMC during the safety evaluation are defined in Table 2.

Table 1 Holding rules assessed by the investigator

Holding Rule	Event	Number of infants/group
1a	Death or any life-threatening serious adverse event (SAE) that can be causally related to vaccination, according to investigator's assessment.	≥ 1
1b	Any withdrawal from the study (by investigator or parent(s)/LAR(s) of the subject request) following a Grade 3 AE that cannot reasonably be attributed to a cause other than vaccination.	≥ 1
1c	Any local or general solicited AE leading to hospitalization that cannot reasonably be attributed to a cause other than vaccination.	≥ 1
1d	Within 30 days post-vaccination, Any spontaneous local or general bleeding AND Thrombocytopenia < 50000/mm^3	≥ 1

If an investigator detects one of the holding rules mentioned above, he/she will immediately put the enrolment or the vaccination on hold and he/she will immediately inform the sponsor and enter the

data in the eCRF. It is sponsor's responsibility to put the enrolment or the vaccination on hold at all sites.

Table 2 Holding rules during the planned iSRC or IDMC evaluation (Amended XX June 2018)

Holding Rule	Event	Number of infants
2a	Any Grade 3 solicited local AE lasting 48 hours or more following administration of an investigational RSV vaccine, within the 7-day (Days 1-7) post-vaccination period.	≥ 25% & ≥ 2 infants in ***any of two*** pooled RSV vaccine group***s***
2b	Any Grade 3 solicited general AE lasting 48 hours or more following administration of an investigational RSV vaccine, that cannot reasonably be attributed to a cause other than vaccination, within the 7-day (Days 1-7) post-vaccination period.	≥ 25% & ≥ 2 infants in ***any of two*** pooled RSV vaccine group***s***
2c	Any ≥ Grade 3 unsolicited AE following administration of an investigational RSV vaccine, that cannot reasonably be attributed to a cause other than vaccination, within the 7-day (Days 1-7) post-vaccination period	≥ 25% & ≥ 2 infants in ***any of two*** pooled RSV vaccine group***s***
2d	Infants hospitalized for RSV-LRTI	≥ ***6*** (in the combination of ***two*** pooled RSV vaccine and the pooled comparator/Placebo groups)

2. Management strategies for workers protection

Clinical study staff involved in the storage, preparation and administration of the GMO will be appropriately trained. To minimize exposure, all personnel handling the GMO will be required to wear appropriate personal protective equipment, according to institutional procedures established for handling GMO's classified as BSL1 organisms.

Accidental exposure of health care professionals to the vaccine or biological samples possibly containing the vaccine is also possible via (1) close contact with the injection site or with the needle stick, or (2) indirect contact with contaminated surfaces or objects. All staff will be instructed on the procedures to act in case of accidental release per their site's Standard Operating Procedure SOP and any other local regulations that may apply.

3. Management strategies in case of accidental spillage

Accidental spillages will be reported according to local procedures. Key staff members of the clinical study team including the Principal Investigator will be contacted immediately. A report of the spillage will be documented and the clean-up procedure will be monitored according to local procedures.

VI. Determination of the overall risk of the GMO

The active substance of the ChAd155-RSV is a recombinant replication-defective simian (chimpanzee-derived) adenovirus group C vector engineered to express three 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. Sequences of the insert derived from human RSV sequences and have been codon-optimized for human use and are under a huCMV

promoter. The genome has been modified to generate a replication deficient adenovirus and deleted from the E1 and E4 sequences. The GMO is intended for triggering an immune response against RSV.

All potential pathogenic sequences of the wild-type ChAd155 virus have been removed to construct the BAC ChAd155 RSV.

There are no risks associated with the genetic insert. The product expression will only be transient since the GMO is not integrative and will be cleared by the immune system. The GMO is replication deficient and probability of *in vivo* recombination leading to RCA formation is low.

Genetic stability is assessed at several steps of the manufacturing process to ensure that there is no genetic modification during GMO manufacturing. Genetic stability assessments include: identity by PCR, genetic characterization by full genome sequencing, identity by restriction analysis, and transgene expression by Western blot analysis.

The GMO is replication deficient, is not integrative and is not expected to survive, multiply or disperse following its release during the proposed clinical study. The presence of replication competent adenovirus is assessed on the drug substance and is a release test. Moreover, it will be administrated intramuscularly, a route which limits its potential shedding.

The present GMO has been released in the UK during a Phase 1 FTIH (EudraCT: 2014-005333-31) and in an ongoing Phase 1/2 in Spain & Italy (EudraCT: 2016-0001117-76). No safety issue following the use of the GMO has been reported; as well as no safety issues in any of the previous released use of the GMOs derived from other simian adenoviruses.

All available nonclinical and clinical data suggest that the ChAd155-RSV vaccine candidate has acceptable immunogenicity, efficacy, biodistribution, tolerability/toxicity profiles for conducting the clinical trial and is not expected to pose risks during the proposed release.

VII. Conclusion on the potential environmental impact from the release of the GMO

A. Likelihood of the GMO to become persistent and invasive in natural habitats under the conditions of the release

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.
- 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 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. It is important to highlight that in any case adenovirus detected had replicative capacity that suggested occurrence of in vivo recombination phenomena (Zhu, Grace et al. 1999). 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 and will be disposed as GMO waste by autoclaving or in accordance with current standard European Union (EU) guidelines.

B. Any selective advantage or disadvantage conferred to the GMO and the likelihood of this becoming realised under the conditions of the proposed release

No selective advantage or disadvantage will be conferred by the GMO to the target host since the vector is non-integrative to the host's genome, and being replication-defective the GMO remains temporally in the host.

C. Potential for gene transfer to other species under conditions of the proposed release of the GMO and any selective advantage or disadvantage conferred to those species

The GMO will be released during a clinical study conducted according to the Good Clinical Practices, at identified investigational sites and under the responsibility of principal investigators. Therefore and since no shedding is expected, there is no expected gene transfer to other species than the target ones (i.e. patients enrolled in the clinical study).

The only non-target organism that might receive gene transfer from the GMO is hospital personnel following a needle-stick injury. If an accidental administration, or transfer of the GMO to a non-target host, were to occur there is no selective advantage or disadvantage conferred by the GMO.

D. Potential immediate and/or delayed environmental impact of the direct and indirect interactions between the GMO and target organisms (if applicable)

The expected biological activity of the GMO following intramuscular injection is the induction of an immune response against the RSV proteins that constitute the transgene: the fusion (F) protein, the nucleocapsid (N) protein and the transcription anti-termination protein (M2-1) protein.

This should translate into a clinical benefit since the target host is RSV-positive infants. The elicited immune response is expected to be sufficient to clear the RSV from the vaccinated subjects.

E. Potential immediate and/or delayed environmental impact of the direct and indirect interactions between the GMO with non-target organisms, including impact on population levels of competitors, prey, hosts, symbionts, predators, parasites and pathogens

The release will take place during a clinical study; there is therefore no expected interaction between the GMO with non-target organism.

F. Possible immediate and/or delayed effects on human health resulting from potential direct and indirect interactions of the GMO and persons working with, coming into contact with or in the vicinity of the GMO release

Following its administration into humans, the expected biological effect is the triggering of an immune response targeting the RSV proteins encoded by the GMO. Since there is no expected shedding of the GMO from vaccinated patients, no immediate and/or delayed effect resulting from potential indirect interactions with persons working with or coming into contact with or in the vicinity of the GMO release is expected.

G. Possible immediate and/or delayed effects on animal health and consequences for the feed/food chain resulting from consumption of the GMO and any product derived from it, if it is intended to be used as animal feed

There is no expected effect on animal health or consequences for the feed/food chain; the GMO is an investigational medicinal product intended for treatment of RSV and will be released during a clinical study.

H. Possible immediate and/or delayed effects on biogeochemical processes resulting from potential direct and indirect interactions of the GMO and target and non-target organisms in the vicinity of the GMO release

No immediate or delayed effects on biogeochemical processes are expected.

I. Possible immediate and/or delayed, direct and indirect environmental impacts of the specific techniques used for the management of the GMO where these are different from those used for non-GMOs

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 effect will be performed by performing blood sampling at several timepoints to assess the humoral and cell-mediated immunity. There are no expected immediate and/or delayed direct and indirect environmental impacts of these.

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