Th HBV VV-001 Clinical study

ChAd155-hIi-HBV

Directive 2001/18/EC – Annex II

PRINCIPLES FOR THE ENVIRONMENTAL RISK ASSESSMENT (ERA)

Notifier: GlaxoSmithKline Biologicals

EudraCT number: 2017-001452-55

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Abbreviations

Ad5	Adenovirus 5
AE	Adverse Event
ChAd155	Chimpanzee Adenovirus Type 155
ChAd155-hIi-HBV	Recombinant Chimpanzee Adenovirus Type 155 Vectored HBV Vaccine
DNA	Deoxyribonucleic Acid
FTIH	First-Time-In Human
GLP	Good Laboratory Practices
GMO	Genetically Modified Organism
GMP	Good Manufacturing Practices
GSK	GlaxoSmithKline
HBc	Hepatitis B Core Protein
HBs	Hepatitis B Surface Protein
HBV	Hepatitis B Virus
HCV	Hepatitis C Virus
hIi	Human MHC class II-associated invariant chain p35 isoform
IB	Investigator Brochure
IDMC	Independent Data Monitoring Committee
IM	Intramuscular
iSRC	Internal Safety Review Committee
MHC	Major Histocompatibility Complex
MVA	Modified Vaccinia Virus Ankara
MVA-HBV	Modified Vaccinia Ankara-Hepatitis B Virus
MVS	Master Virus Seed
NA	Nucleo(S)Tides Analogues
PCR	Polymerase Chain Reaction
Q-PCR	Quantitative Polymerase Chain Reaction
RCA	Replication Competent Adenovirus
RSV	Respiratory Syncytial Virus
SAE	Serious Adverse Event
vp	Viral Particles

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 first-time-in human (FTIH), Phase I, randomized, multi-centric, single-blind, controlled dose-escalation study to evaluate the reactogenicity, safety, immunogenicity and efficacy of GSK Biologicals' HBV viral vectored vaccines given in a prime-boost schedule with sequential or co-administration of adjuvanted proteins therapeutic vaccine (GSK3528869A) in chronic Hepatitis B patients (18-65 years old) well controlled under nucleo(s)tides analogues (NA) therapy".*

The EudraCT number of the study is: 2017-001452-55 and the Applicant's study code number is: 204852 / abbreviated title is: TH HBV VV-001.

Duration of the vaccination phase of the clinical trial Th HBV VV-001 will be approximately 2.5 years starting from Q3-2018 to the date of last dose of the study in Q1-2021. With safety follow-up the total duration of the study is 4.5 years with study completion in Q1-2023.

2. Characteristics of the GMO

a. Description of the GMO and doses

The ChAd155-hIi-HBV candidate vaccine consists of a recombinant replication-defective simian (chimpanzee-derived) adenovirus group C vector (ChAd155) encoding a fusion of sequences derived from two hepatitis B virus (HBV) protein antigens. The two HBV proteins include: the truncated core nucleocapsid protein antigen (HBc) and the full-length small surface antigen (HBs), separated by the self-cleaving 2A region of the foot-and-mouth disease virus (FMDV), that allows processing of the HBc-HBs fusion into separate protein antigens. In addition, the N-terminus of the gene encoding the HBc protein has been fused to the gene encoding the human Major Histocompatibility Complex (MHC) class II-associated invariant chain p35 isoform (hIi).

The investigational ChAd155-hIi-HBV vaccine is manufactured in a GMP compliant environment. The vaccine is presented as a sterile liquid suspension filled in 3 mL glass vials , closed by a rubber stopper and sealed with an aluminium tear-off cap. The vaccine is formulated in buffer without addition of a preservative.

The ChAd155-hIi-HBV final container vials are filled at a nominal volume of 0.7 mL per vial. The ChAd155-hIi-HBV final container vials are stored at <-60°C.

Two concentrations will be assessed in the proposed FTIH study as shown in Table 1.

Table 1: GMO doses in the Th HBV VV-001

Vaccine candidate	Dose
ChAd155-hIi-HBV (low dose)	5 x 10e10 vp/dose

ChAd155-hIi-HBV (high dose)	5 x 10e9 vp/dose

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 pattern analysis."
- Transgene expression and identity is assessed by Western blot analysis using polyclonal antibodies targeting HBc and HBs.

3. Characteristics of the release

a. Previous release of the GMO

This will be a First-Time-In-Human study with the proposed GMO, the ChAd155-hIi-HBV.

Another GMO with an identical ChAd155 backbone but encoding an RSV antigen (ChAd155-RSV) has been assessed for safety, reactogenicity and immunogenicity in a 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." The same GMO (ChAd155-RSV) is also currently assessed in a Phase 1/2 study in pediatric population (EudraCT: 2016-0001117-76) in Spain & Italy under the protocol entitled "A Phase 1/2, randomized, observer-blind, controlled, multi-center, dose-escalation study to evaluate safety, reactogenicity and immunogenicity of GSK Biologicals' respiratory syncytial virus (RSV) investigational vaccine based on the RSV viral proteins F, N and M2-1 encoded by chimpanzee-derived adenovector (ChAd155-RSV) (GSK3389245A), when administered intramuscularly according to a 0, 1-month schedule to RSV-seropositive infants aged 12 to 17 months".

Three different simian adenoviruses have been used in clinical trials sponsored by the Applicant: The ChAd63 adenovirus (Biswas et al. 2011) used in malaria trials (Sheehy et al. 2011, O'Hara et al. 2012, de Barra et al. 2014, Hodgson et al. 2015) where > 1,000 healthy adults and children have been vaccinated, including two month old babies. The ChAd3 (Peruzzi et al. 2009) and PanAd3 (Vitelli et al. 2013) adenoviruses belong to serotype C (Colloca et al. 2012) and have been used in hepatitis C virus (HCV) and Ebolavirus trials with more than 1,500 vaccinees, and also in a Phase I clinical RSV trial enrolling 42 volunteers, respectively.

All simian adenovectors tested so far in the clinic showed an acceptable safety profile with no reported vaccine related SAEs (Sheehy et al. 2011, Barnes et al. 2012, O'Hara et al. 2012, Capone et al. 2013, de Barra et al. 2014, Hodgson et al. 2015, Ledgerwood et al. 2015).

b. Characteristics of the planned release

The planned released of the GMO will occur during a clinical study to be conducted at multiple investigational sites in several countries under the responsibilities of local principal investigators.

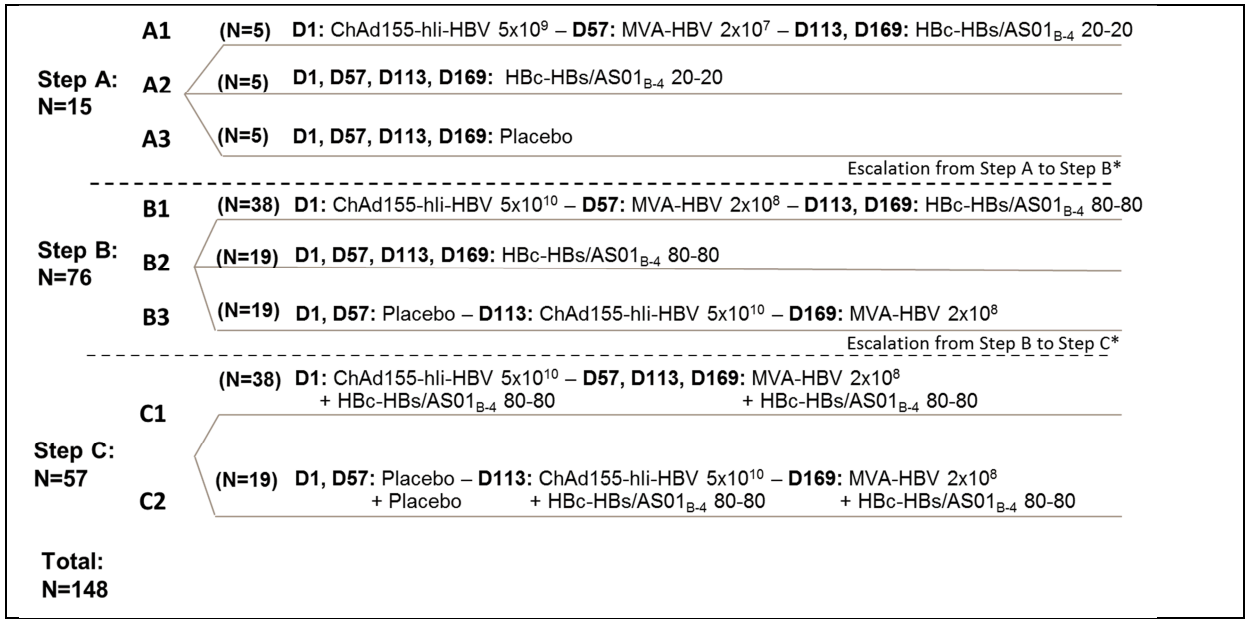
Two concentrations of the GMO will be evaluated during the proposed clinical study including: a lower potency dose of 5 x 10e9 viral particles (vp) per dose or a higher potency dose of 5 x 10e10 vp/dose. The candidate vaccine will be administered by the intramuscular route of administration.

The study cohort is planned to consist of 148 enrolled patients. The GMO will be administered in steps according to the schedule provided in Figure 1. For the GMO ChAd155-hIi-HBV vaccine the administrations are as follows:

- Step A: Three randomized groups. The first group (N=5) will receive one dose of ChAd155-hIi-HBV vaccine at the lower dose of 5 x 10e9 vp at Day 1.
- Step B: Three randomized groups. The first group (N=38) will receive 1 dose of ChAd155-hIi-HBV vaccine at the higher dose of 5 x 10e10 vp at day 1. The third group (N=19) will receive one dose of the ChAd155-hIi-HBV higher dose vaccine at day 113.
- Step C: Two randomized groups. The first group (N=38) will receive 1 dose of ChAd155-hIi-HBV vaccine at the higher dose of 5 x 10e10 vp at day 1. The second group (N=19) will will receive 1 dose of ChAd155-hIi-HBV vaccine higher dose at day 113.

Based on the dose levels of 5 x 10e9 vp/dose and 5 x 10e10 vp/dose, the planned number of patients per group, and the number of doses to be administered (5 low dose and 114 high dose), it is estimated that a total of 5.725 x 10e12 vp will be administered (released) during the proposed study (all investigational sites pooled).

Figure 1 Study steps and study groups



II. Identification of GMO characteristics which may cause adverse effects

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

a. Allergenic effects

As with all injectable vaccines/medicinal products, immediate local and/or delayed systemic allergic reactions to vaccination can occur. However, there is no scientific evidence available that may indicate that the ChAd155-hIi-HBV GMO will provoke adverse allergenic effects in the vaccinated study population beyond transient local or systemic reactions associated with vaccination (see section III.1.a.).

b. Toxicity/Pathogenicity

The most likely reaction to occur following IM administration of the GMO is a transient and self-limiting local inflammatory reaction. This may typically include redness, swelling and tenderness at the site of injection. Mild systemic reactions following vaccination may include: fatigue, fever, headache, stomach or intestinal troubles, muscle pain or feeling cold (chills).

c. Replication competent virus

As a replication-defective adenovirus vector, another risk that should be evaluated is the potential for a reversion event leading to a replication-competent adenovirus (RCA) which could enhance it's ability to survive in the environment. Evaluation of this risk is covered in sections III.1.c. and IV.1.c.

d. Shedding/Survivability

One aspect to be considered in an environmental risk assessment, for the release of a viral vector GMO in a clinical study, is the potential for shedding of the GMO following its administration to study subjects. The evaluation of shedding of the GMO is covered in sections III.1.d. and IV.1.d.

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

The ChAd155 vector as a subgroup C-type adenovirus is capable of transducing animal cells exhibiting coxsackievirus and adenovirus receptors (CAR). Based on GLP toxicology studies performed in animals evaluating the ChAd155-hIi-HBV candidate and on safety follow-up of the release of the identical ChAd155 backbone, and other similar ChAd vectors, in human clinical studies, there is no indication the ChAd backbone nor the HBV insert will cause toxic effects. 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 GMO will be administered to chronic HBV patients 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 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 plant 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 et al. 2002).

To provide emergency care to any study subjects who might experience an immediate systemic allergic reaction to GMO vaccination, all patients across all steps will need to remain under observation (visual follow-up, no specific procedure) at the study site for at least 60 minutes after vaccination.

Recombinant adenovirus vectors have been used as vaccine candidates for decades, since they are efficient at presenting the transgene they encode and have shown immunogenicity in humans. However, concerns were raised following the use of human adenovirus with a potential risk

associated with the presence of pre-existing immunity to the viral vector. Chimpanzee adenoviruses (ChAd) have negligible to null seroprevalence in human populations and hence, the likelihood of occurrence of negative effects associated with the presence of pre-existing immunity to the viral vector is negligible.

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

b. Toxicity/Pathogenicity

- Clinical data

Most systemic symptoms observed in clinical trials with similar products (ChAd3-HCV, ChAd3-Ebola, ChAd155-RSV) carried out in healthy adults and children using doses similar to that proposed for the ChAd155-hIi-HBV GMO FTIH study, did not exceed mild severity. Fatigue, headache and malaise were the most commonly reported systemic AEs.

A transient non-clinically significant drop in platelets was noted post IM vaccination in a preclinical study with the ChAd155-vector. Furthermore, in Ebola Phase I studies in adults investigating a similar adenoviral vectored vaccine (ChAd3-EBO-Z), transient decreases in thrombocyte counts were also observed. These decreases occurred mostly on Day 1 after vaccination and generally returned to baseline by Day 7. Although most of these decreases remained within the normal range, the per protocol criteria for thrombocytopenia (i.e. thrombocyte count of < 150 x 10e3/µL) were met for 2.6% (7 out of 270) of the vaccinated subjects. None of the decreases in thrombocyte counts or the cases of thrombocytopenia was clinically significant. Although the mechanism underlying these decreases currently remains unclear, it is well described in literature that, post intravenous administration, adenovirus activates platelets and induces platelet-leukocyte aggregate formation, causing an associated increase in platelet and leukocyte-derived microparticles (Othman et al. 2007, Stone et al. 2007).

The ChAd155-hIi-HBV vaccine includes a DNA sequence coding for CD74, also called hIi (human invariant chain) that acts as a genetic adjuvant to optimize the CD8+ T-cell immune response to the HBc antigen. Since hIi is a self-antigen expressed throughout the immune system by B cells, activated T-cells, dendritic cells, monocytes and macrophages and widely expressed in the thymus, it should be highly tolerated. As hIi genetic adjuvant has never been administered in human, the risk that the ChAd155-hIi-HBV vaccine induces an immune response against the hIi and a potential immune-mediated disease (pIMD) cannot be entirely ruled out.

- Preclinical GMO toxicity data

Two GLP-compliant toxicity studies were performed using GMO batches comparable to the clinical trial materials including: a single-dose local tolerance and a repeat-dose toxicity studies. The toxicology studies were performed using the same dose and volume, number of administrations-plus-one, and route of administration, as will be used in the clinical study.

In the repeat-dose rabbit study, 5 doses of each vaccine were administered i.e. 1 more dose than the clinical dosing regimen that anticipates 4 vaccinations. Three different schedules of vaccination (adjuvanted recombinant proteins HBc-HBs/AS01B-4 alone; combination of ChAd155-hIi-HBV, MVA-

HBV and HBc-HBs/AS01B-4; co-administrations of HBc-HBs/AS01B-4 with ChAd155-hIi-HBV or MVA-HBV) were used. The frequency of administration was compressed (every 2 weeks) vs. the clinical regimen (every 8 weeks).

Results of the single dose toxicity studies demonstrated that administration of ChAd155-hIi-HBV simultaneously to HBc-HBs/AS01B-4 (in the opposite leg, both via intramuscular route), did not induce safety concerns. Only one animal had a transient and non-adverse local reaction (erythema) after the injection of the GMO. No sign of systemic toxicity was reported.

In the repeated dose toxicity study, all vaccination schedules were clinically well tolerated, and all vaccinated animals had anti-HBc and anti-HBs antibodies at the end of the treatment and recovery periods. The in-life findings were all consistent with the inflammatory reaction and the immune response that may occur after administration of vaccines. Hematology findings mainly consisted in increased neutrophil counts, which were accompanied by increased fibrinogen and CRP levels, and by decreased albumin/globulin ratio at blood biochemistry. All these parameters were returned to normalcy within 7 days after dosing. 3 days after the last injection, the administration of HBV therapeutic candidate vaccines given alone or in combination/co-administration induced inflammatory reaction at the injection sites along with slight changes indicative of an immune stimulation in draining lymph nodes and spleen. Similar changes but of lower severity were seen 28 days after the last injection, suggesting that the recovery was ongoing. When compared to controls, the severity and/or incidence of the changes were more pronounced in animals injected sequentially with ChAd155-hIi-HBV and MVA-HBV in the right site and HBc-HBs/AS01B-4 in the left site, then in animals treated sequentially with the 3 HBV candidate vaccines ChAd155-hIi-HBV, MVA-HBV and HBc-HBs/AS01B-4 in the right site, and finally in animals given HBc-HBs/AS01B-4 alone in the right site. Overall, the HBV therapeutic candidate vaccines were considered to be well tolerated since the microscopic findings indicative of an inflammatory reaction/immune response are those expected after an antigenic stimulation by the intramuscular route.

In another study evaluating the immunogenicity and safety of ChAd155-hIi-HBV in combination/co-administration with MVA-HBV/HBc-HBs/AS01B-4 vaccine regimens in AAV2/8-HBV transduced HLA.A2/DR1 mice. Vaccine-associated liver inflammation was assessed by measuring serum activities of AST and ALT and by performing liver histopathological evaluation. The study results showed no increase in liver enzymes were detected in the vaccine groups when compared with the non-vaccinated group and no microscopic findings could be related to the vaccine treatments. Reassuring results showing safety of the GMO in a model mimicking the targeted and more fragile study population.

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 the GMO was assessed from Day 2 to Day 48 post-injection (intramuscular administration) of a dose of 10e10 vp in male and female Sprague-Dawley rats in a GLP compliant environment. Results demonstrated that vector sequences were detected at the injection site and in the draining iliac lymph node. Sequences were also detected in the inguinal lymph node and spleen as well as in blood and popliteal lymph node for 24 hours only. The GMO was undetectable in brain, heart, kidney, liver, lung, ovary or testis samples on Days 2 and 8.

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.

The Applicant will perform an ancillary shedding study (TH HBV VV-031 HBS:001) following a subset of subjects from the proposed FTIH clinical study (TH HBV VV-001) to evaluate viral shedding of the ChAd155-hIi-HBV vaccine.

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

Toxicology studies have ruled out the potential pathogenicity of the GMO in rodents. The GMO is therefore not expected to be allergenic or toxic in animals. In addition, 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

There is no identified potential for competitive increase in the human population as organisms 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 due to the E1A gene deletion, and there is no basis to indicate the insert will promote post-release selection for increased invasiveness. Therefore, no competitive advantage has been conferred to the GMO in relation to the recipient and parental organism.

The only non-target organism that may potentially receive the GMO is clinical study staff following a needle-stick injury. As for the study subjects 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 patients chronically infected with the hepatitis B virus.

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 HBV 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. However, the GMO does not encompass any of these antibiotic resistance genes. Therefore, the probability of transfer of antibiotic resistance gene compromising future prophylactic or therapeutic treatments to the target host is negligible. Identical rational applies for veterinary treatments. The GMO is not capable of transducing plant cells.

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 and no SAE related to the experimental product. In addition, in the ongoing Phase I study assessing safety, reactogenicity and immunogenicity of the ChAd155-RSV GMO (an identical ChAd155 vector backbone), 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 post-release in the event of recombination of the GMO with human adenovirus where the study subject receiving the vaccine is co-infected by human adenovirus. 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 HBV 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 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 treatments in humans 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). 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 release will occur during a First-Time-in-Human clinical study in HBV chronically infected patients. Particular attention will be paid following GMO administration to monitor any immediate or delayed adverse event. Dosage and administration of study vaccines will be performed in steps as described in Figure 1.

Patients will receive a prime-boost administration of the GMO (i.e. ChAd155-hIi-HBV) and another GMO (MVA-HBV), both encoding the hepatitis B core (HBc) and surface (HBs) antigens, and a recombinant protein vaccine containing HBc and HBs antigens adjuvanted with the adjuvant system $AS01_{B\text{-}4}$ (HBc-HBs/$AS01_{B\text{-}4}$) administered either sequentially or concomitantly to the prime-boost. All vaccines will be administered intramuscularly.

Prior to administration of the next consecutive dose or to escalation to the subsequent step, the safety data will be reviewed by an internal Safety Review Committee (iSRC) independent from the study team. The iSRC will conduct unblinded reviews of all available safety data and will escalate any safety signal to the Applicant which may lead to the modification or suspension of the study.

The safety holding rules which will be assessed by the iSRC are displayed in Table 2:

- Holding rules 1, 2 and 3 will be assessed by the iSRC during the safety evaluation.
- Holding rules 1 and 3 will also be monitored by the investigator on a continuous basis irrespective of the number of patients enrolled. If an investigator detects one of the holding rules mentioned above, he/she will immediately put the enrolment or the vaccination on hold and will immediately inform the Sponsor and enter the data in the eCRF. It is the Sponsor's responsibility to put the enrolment or the vaccination on hold at all sites.

Table 2: Holding rules during the planned iSRC

Holding Rule	Event	Number of patients
1a	Death or any life-threatening SAE	≥ 1
1b	Any SAE that is considered as related to the vaccine in an investigational group	≥ 1
1c	Any withdrawal from the study (by investigator or patient request) following a Grade 3 AE that cannot reasonably be attributed to a cause other than vaccination	≥ 1
1d	Any local or general solicited AE leading to hospitalization, or fever > 40°C (104°F) that cannot reasonably be attributed to a cause other than vaccination, or necrosis at the injection site, within the 7-day (days 1-7) post-vaccination period	≥ 1
2a	Any Grade 3 solicited local AE (lasting 48h or more) in an investigational group, within the 7-day (day 1-7) post-vaccination period	At least 25% AND ≥ 2 in a vaccine group
2b	Any Grade 3 solicited general AE (lasting 48h or more) in an investigational group, that cannot reasonably be attributed to a cause other than vaccination, within the 7-day (day 1-7) post-vaccination period	At least 25% AND ≥ 2 in a vaccine group
2c	Any Grade 3 unsolicited AE in an investigational group, that cannot reasonably be attributed to a cause other than vaccination, within the 7-day (day 1-7) post-vaccination period or Any Grade 3 abnormality in pre-specified hematological or biochemical laboratory parameters in an investigational group within the 7-day (day 1-7) post-vaccination period	At least 25% AND ≥ 2 in a vaccine group
3a	Any acute exacerbation or severe hepatitis flare (intermittent elevation of ALT to more than 10 times the ULN)*	≥ 1
3b	Any acute exacerbation or moderate hepatitis flare for more than 2 weeks (intermittent elevation of ALT to > 5 to < 10 X ULN)*	≥ 1
3c	Any ALT flare (ALT > 3XULN) with other substantial liver biochemical change defined as an increase in serum bilirubin to ≥2 x ULN and/or international normalized ratio (INR) >1.5*	≥ 1
3d	Any hepatic decompensation defined as the occurrence of 1 or more of the following events: ascites, spontaneous bacterial peritonitis, hepatorenal syndrome, variceal bleeding, or hepatic encephalopathy	≥ 1
3e	Any reactivation of chronic hepatitis B as characterized by HBV-DNA breakthrough accompanied with 1 or more of the following: ALT elevation to > 3 X ULN, substantial biochemical changes, or hepatic decompensation as defined above	≥ 1
3f	Any AE related to spontaneous local or general bleeding AND Thrombocytopenia < 50,000/mm3	≥ 1

* The abnormal value should be confirmed by an additional testing preferably within 48-72 hours; if no additional value is available within one week, the initial value will be considered as confirmed.

2. Management strategies for workers protection

Personnel who will handle, store, prepare or administer the GMO will be appropriately trained. All personnel handling the GMO will be required to wear appropriate personal protective equipment.

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.

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 will be contacted immediately. A report of the spillage will be documented and the clean-

up procedure will be monitored according to local procedures. Records of staff training and competency will be documented.

VI. Determination of the overall risk of the GMO

The GMO is a recombinant replication-defective simian (chimpanzee-derived) adenovirus group C vector engineered to express a transgene derived from HBV under control of the human CMV promoter.

Preclinical studies have not identified potential safety risks associated with the genetic insert. The transgene product expression will be transient since the GMO DNA is not integrative into the host genome and the virus vector will be cleared by the immune system. The GMO is replication-defective 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-defective, is not integrative and is not expected to survive, multiply or disperse following its release during the proposed clinical study. The presence of RCA is assessed on the bulk drug substance and is a release test. Moreover, it will be administrated intramuscularly, a route which limits its potential shedding.

Finally, GLP single dose and repeated dose toxicology studies did not demonstrate any toxic or pathogenic effect of the GMO when administered at the same doses and using the same route than the proposed clinical ones.

All available nonclinical and supportive clinical data generated with identical or similar simian-derived vector backbones, encoding different transgenes, suggest that the GMO 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 the GMO 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-defective 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 et al. 1996).

- There is a very low risk of shedding of the GMO into the environment. Defective recombinant adenoviruses have been used extensively in clinical trials, either through direct administration or cell therapy strategies. Where shedding was evaluated, 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 a few days from administration. Following administration of a similar E1 and E4-deleted recombinant simian adenovirus (ChAd3) expressing the HCV transgene NSmut, no viral vector shedding (in urine and throat swabs) was observed after intramuscular immunization (clinical study HCV001, EudraCT Number: 2007-004259-12).
- 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 during manufacture is considered very low, due to the lack of sequence homology between the *E1* flanking regions of human Ad5 and chimpanzee adenovirus *E1*. To ensure the absence of RCA during manufacture, each batch of material is tested for RCA at the level of the bulk drug substance.
- The GMO is incapable of surviving outside a host (animal) cell since the vector is replication-defective.
- The release of the GMO will occur during the conduct of a highly controlled clinical study setting via the intrasmuscular route of administration to study subjects, where procedures will minimize dissemination and inadvertent transmission.
- Any GMO waste generated during the conduct of the clinical study will be managed according to procedures specific to the GMO as approved by the local institutional policy and the clinical study protocol.

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. Since no shedding is expected, there is no expected gene transfer to other species than the target host (i.e. patients enrolled in the clinical study). The only non-target organism that might accidently receive an administration of the GMO is clinical study 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 HBV proteins that constitute the transgene. This should translate into a clinical benefit to the study subjects who are chronically infected with HBV.

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 organisms.

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

The GMO will be released during a clinical study via the intramuscular route of administration. Human studies with recombinant adenovirus constructs administered by this route have been unable to detect vector shedding from study subjects. Following its administration into humans, the expected biological effect is the triggering of an immune response targeting the hepatitis B proteins encoded by the GMO. Since there is no basis to expected shedding of the GMO from the vaccinated cohort in the proposed study, 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. Furthermore the GMO cannot cause disease in animals or humans.

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

Not applicable. The GMO is an investigational medicinal product 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 setting with a neglible likelihood of the GMO coming into contact with the environment. The only techniques foreseen to manage the GMO upon release (upon administration to study subjects), is a decontamination of the immediate area and to manage biohazard waste according to study protocol and local or institutional procedures specified for the GMO. These procedures will serve to further limit any contact or spread of the GMO to the environment. There are no expected immediate and/or delayed direct and indirect environmental impacts of these procedures.

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