TH HBV VV-001 Clinical study

Annex IIIA according to Directive 2001/18/EC

Information Required in Notifications Concerning Releases of Genetically Modified Organisms Other than Higher Plants

Notifier: GlaxoSmithKline Biologicals

EudraCT number: 2017-001452-55

Version 1.0 – February-2018

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Abbreviations

Ad5	Adenovirus 5
AE	Adverse Event
BAC	Bacterial Artificial Chromosome
BGHpA	Bovine Growth Hormone Polyadenylation Signal
BSL1	Biosafety Level 1
CAR	Coxsackievirus and Adenovirus Receptor
ChAd155	Chimpanzee Adenovirus Type 155
ChAd155-hIi-HBV	Recombinant Chimpanzee Adenovirus Type 155-Vectored HBV Vaccine
CHB	Chronic Hepatitis B
CMI	Cell-Mediated Immunity
DNA	Deoxyribonucleic Acid
DS	Drug Substance
FMDV	Foot And Mouth Disease Virus
FTIH	First-Time-In Human
GLP	Good Laboratory Practices
GMO	Genetically Modified Organism
GMP	Good Manufacturing Practices
GSK	GlaxoSmithKline
HBc	Core HBV Nucleocapsid
HBs	Small HBV Surface Antigen
HEK-293	Human Embryonic Kidney 293 Cells
hIi	Human MHC class II-associated invariant chain p35 isoform
HBV	Human Hepatitis B Virus
IB	Investigator Brochure
IDMC	Independent Data Monitoring Committee
IM	Intramuscular
ITR	Inverted Terminal Repeat
MHC	Major Histocompatibility Complex
MVS	Master Virus Seed
NA	Nucleo(S)Tides Analogues
NHP	Non-Human Primates
PVS	Primary Virus Seed
Q-PCR	Quantitative Polymerase Chain Reaction
RCA	Replication Competent Adenovirus
RSV	Respiratory Syncytial Virus
SAE	Serious Adverse Event
TetO	Tetracycline Operon Operator Sequence
TetR	Tetracycline Repressor
vp	Viral Particles

I. GENERAL INFORMATION

A. Name and address of the notifier (company or institute)

GlaxoSmithKline Biologicals
Rue de l'Institut, 89
1330 Rixensart, Belgium

B. Name, qualifications and experience of the responsible scientist(s)

The GSK sponsor of the study
Lan Lin, MD
Clinical Research & Development Lead
GlaxoSmithKline Biologicals
Avenue Fleming 20,
1300 Wavre, Belgium

Principal investigators

Clinical Study Site Address	Principal Investigator
Belgium	
Hôpital Erasme; Route de Lennik 808, Brussels	Christophe Moreno
UZ Gent; De Pintelaan 185, Gent	Hans Van Vlierberghe
UZ Antwerpen, Wilrijkstraat 10; Edegem	Thomas Vanwolleghem
SGS Life Science Services, Lange Beeldekensstraat 267, Antwerpen	Stefan Bourgeois
UZ Leuven, Herestraat 49, Leuven	Frederik Nevens
Cliniques Universitaires Saint-Luc, Avenue Hippocrate 10, Brussels	Yves Horsmans
Germany	
Klinikum der J. W. Goethe-Universitaet-Med. K. I, Theodor-Stern-Kai 7, Frankfurt	Stefan Zeuzem
Universitaetsklinikum Aachen-Med. Klinik III, Pauwelsstr. 30, Aachen	Christian Trautwein
Medizinische Hochschule Hannover-Gastroenterologie, Carl-Neuberg-Str. 1, Hannover	Markus Cornberg
Universitaetsklinikum Tuebingen-Innere Medizin I, Otfried-Mueller-Str. 10,Tuebingen	Christoph Berg
Universitaetsklinikum Essen-Gastroenterologie, Hufelandstr. 55, Essen	Guido Gerken
Universitaetsklinikum Bonn-Med. Klinik I, Sigmund-Freud-Str. 25, Bonn	Ulrich Spengler
Johannes-Gutenberg-Universitaet Main, Langenbeckstr. 1, , Mainz	Martin Sprinzl
Universitaetsklinikum Eppendorf-Ambulanzzentrum, Martinistr. 52, Hamburg	Julian Schulze zur
United Kingdom	
Royal London Hospital NHS Foundation, Whitechapel road, London	Patrick Kennedy
John Radcliffe Hospital; Headley Way; Oxford	Paul Klenerman
Southampton General Hospital, Tremona Road, Southampton	Salim Khakoo
Kings College Hospital 1; Denmark Hill, London	Kaushik Agarwal
Queens Medical Centre, Derby Road, Nottingham	Stephen Ryder

C. Title of the project

The release of the GMO will take place during a clinical study 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".

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

II. INFORMATION RELATING TO THE GMO

A. Characteristics of (a) the donor, (b) the recipient or (c) (where appropriate) parental organism(s):

Please find below the proposed nomenclature of terms used in this document:

- The name of the GMO: ChAd155-hIi-HBV.
- The donor organism: the organism(s) from which sequences encoded by the GMO are derived.
- The recipient organism: the "empty" (i.e. without the transgene) replication-defective ChAd155 simian-derived adenovirus vector backbone.
- The parental organism: the replication-competent simian-derived ChAd155 adenovirus isolate from which the engineered vector backbone is derived.

1. scientific name;

(a) Donor

The transgene contains genetic information from three donor organisms as follows:

- The truncated core protein (HBc) and the full length surface antigen of the human hepatitis B virus (HBV).
- The human Major Histocompatibility Complex (MHC) class II-associated invariant chain p35 isoform (hIi).
- The 2A cleavage sequence from the foot-and-mouth disease virus (FMDV).

(b) Recipient

Chimpanzee-derived adenovirus 155 (ChAd155) vector with the E1 and E4 regions deleted and replaced by the insertion of the E4 region open reading frame 6 (E4orf6) from human adenovirus type 5 (Ad5).

(c) Parental

The parental organism is the replication-competent chimpanzee-derived group C adenovirus serotype 155 (ChAd155) isolate from which the engineered vector backbone is derived.

2. taxonomy;

(a) Donor

HBV: Family: Hepadnaviridae; Genus: Orthohepadnaviruses; Species: Human hepatitis B virus

FMDV: Family: Picornaviridae; Genus: Aphthoviruses; Species: foot-and-mouth disease virus

Human: Family: Hominidae; Genus: Homo, Species: sapiens

(b) Recipient

Not applicable

(c) Parental

Family: Adenoviridae; Genus: Mastadenovirus; Species: Simian Adenovirus subgroup C

3. other names (usual name, strain name, etc.);

(a) Donor

HBV, FMDV, human

(b) Recipient

ChAd155 backbone vector

(c) Parental

ChAd155 virus isolate

4. phenotypic and genetic markers;

(a) Donor

Hepatitis B virus (HBV) is a highly contagious DNA virus restricted to Humans, transmitted via infected blood and semen, which affects the liver. Infection with Hepatitis B virus can cause both acute resolving infection and chronic hepatitis B (CHB) and accounts for about 780,000 related deaths per year. More than 240 million people worldwide are chronically infected with hepatitis B.

Foot and mouth disease virus (FMDV) is one of the most contagious animal diseases with cattle as the main host and potentially all domestic and wild cloven-hoofed animals susceptible to infection. Humans can also harbor FMDV in their respiratory tract for up to 48 hours.

The transgene is constituted by a sequence derived from two HBV proteins: the core nucleocapsid (HBc) and the small surface antigen (HBs), separated by the self-cleaving 2A region of the foot-and-mouth disease virus (FDMV), that allows processing of the HBc-HBs fusion into separate protein antigens. In addition, the N-terminal part 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 HBV transgene is under the transcriptional control of huCMV promoter and bovine growth hormone poly-adenylation signal (BGH pA). The construct contains the 2A region from the aphtovirus FMDV between the HBc protein and the HBs protein, which mediates polyprotein processing by a translational

effect known as ribosomal skip (Donnelly et al. 2001). After transfection into mammalian cells, cleavage occurs.

The region 2A-mediated protease cleavage occurs at the C-terminus of 2A just ahead of the last proline in the amino acid sequence. The proline remains at the N-terminus of the HBs protein, while the 23 amino acids preceding the proline cleavage site remain with the HBc-2A polypeptide. The 2A region (18 amino acids) has been supplemented with a spacer of 6 amino acids at its N-terminus; spacers of this nature have been reported to increase the efficiency of 2A mediated cleavage.

The expression of the transgene, following protease processing, thereby results in the production of two separate polypeptides: HBc-spacer-2A and HBs. For brevity the HBc-spacer-2A polypeptide will be referred to as the HBc protein throughout the dossier.

Each antigenic sequence was codon-optimised for expression in eukaryotic cells, chemically synthesised and assembled.

(b) Recipient

The recipient is a recombinant, replication-defective, chimpanzee-derived adenovirus group C vector (ChAd155) that has been developed by the Applicant and is used as vector backbone for prophylactic and therapeutic vaccination in several disease indications.

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 (The University of Louisiana at Lafayette, USA) using standard procedures. The viral genome was then cloned in a plasmid vector and subsequently modified to increase its safety for human use. The E1 and E4 gene regions have been deleted which renders it replication-defective and decreases the production of late gene products thereby reducing induction of vector-specific immune responses. The recipient virus has been further modified with the insertion of Ad5E4orf6, to facilitate enhanced replication of the ChAd construct in human E-1 complementing cell lines expressing human Ad5 E1.

The ChAd155 belongs to the subgroup type C of adenoviruses based on the hexon protein sequence. ChAd155 genome sequence analysis shows very high similarity with other subgroup C adenoviruses, suggesting that it uses the same coxsackie virus and adenovirus receptor (CAR) to enter into the host cell.

(c) Parental

Adenoviruses have been used as vaccine candidates for decades, since they are immunogenic and efficient at presenting the transgene they encode without integrating into the host genome. 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 low seroprevalence in human populations and have been therefore used in the development of safe and effective human vaccines.

Adenoviruses are double-stranded DNA viruses with a genome of 34-43 kb. They are species-specific and different serotypes have been isolated from a variety of mammalian species such as humans, simians including chimpanzee (Tatsis et al. 2004). One of their features is that they encode polypeptides from both DNA strands and use alternative splicing along with different poly(A) sites. ChAd155 is classified within subgroup C of human adenoviruses. A common characteristic among subgroup C adenoviruses, is that they require the CAR receptor to effect host cell entry.

5. degree of relatedness between donor and recipient or between parental organisms;

The donors do not have any relatedness with the recipient or parental organisms.

The recipient is derived from the parental organisms using the methods described in section II.C.1.

6. description of identification and detection techniques;

(a) Donor

Several assays are now commercially available for identification and detection of HBV. HBV is most commonly detected in serum or whole blood although the virus can also be detected in dried blood, hepatocytes and other tissues such as renal tissues.

Serological methods are the most widely used methods since they are rapid and cost effective to detect different markers such HBsAg, anti-HBcAg, HBeAg, and anti-HBeAg. Amongst these assays, ELISA and chemiluminescent enzyme immunoassay are dominant methods for HBV detection and quantification followed by molecular techniques such as RT PCR.

(b) Recipient

A specific polymerase chain reaction (PCR) has been developed using 4 sets of primers (2 primers for each set): 2 sets annealing in the hexon portion of the adeno DNA and 2 sets annealing in the transgene region and immediately out of the transgene region. The pattern is then visualized by electrophoresis on agarose gel. This PCR is performed at several steps of the manufacturing process and including drug substance and the drug product.

In addition, full genome sequencing has also been developed and is performed on the master virus seed in its final container and on the drug substance. The viral DNA is first isolated from purified virus bulks by SeqWright Genomic Services that uses the full-length shotgun approach for sequencing adenovirus. A quantity of 20 µg of pure DNA is used for the sequencing. Editing and alignment of resulting sequences is performed and final consensus sequence is produced.

An identity test using restriction analysis enables the assessment of the integrity of the GMO vector DNA. GMO DNA is isolated from purified virus bulks and restricted with endonucleases, selected based on the DNA sequence, to show a restriction pattern with separate and distinguishable bands. A positive control, the plasmid used to generate the vector, is included in each assessment. The digests are visualized on agarose gels after staining followed by ultraviolet imaging. The resulting restriction fragment patterns of each sample digest are compared to their respective control digest restriction patterns for a qualitative confirmation of sample identity. Results are reported as “as predicted.”

(c) Parental

Hexon is the most abundant protein in the icosahedral capsid and the hexon gene has been analyzed to characterize and determine adenovirus types.

7. sensitivity, reliability (in quantitative terms) and specificity of detection and identification techniques;

(a) Donor

See above section 6a above.

(b) Recipient

- Polymerase chain reaction

The PCR assay is specific to the adenoviral vector and the transgene sequences since the primers are designed based on the sequences to be identified. The assay has not been validated yet.

- Full genome sequencing

The genetic structure of the GMO is verified in order to confirm its integrity and identity. Comparison is made with the theoretical sequence and the result should be reported as “conform to predicted sequence”.

- Identity by restriction analysis

Restriction fragment analysis checks the genetic structure of the sample to confirm integrity and identity.

The test is considered as suitable for the purpose since a control sample is included in each assay: This control material is the HBV pre-adeno plasmid used to generate the virus, previously digested with the enzyme that releases the plasmid portion and gives an additional band of about 7,523 base pairs. The resulting restriction fragment patterns are compared to their respective control digest restriction patterns for a qualitative confirmation of sample identity: the pattern has to be congruent with the expected genetic structure without indication of difference or additional bands. Sensitivity as well as limit of detection and of quantification vary from one laboratory to another.

(c) Parental

Identity can be determined by appropriate restriction fragment analysis.

8. description of the geographic distribution and of the natural habitat of the organism including information on natural predators, preys, parasites and competitors, symbionts and hosts;

(a) Donor

HBV is a highly contagious pathogen that infects mostly humans but can also infect related species such as primates (usually for R&D purposes).

FMDV is an animal (cloven-hoofed) pathogen which rarely causes infections in humans.

(b) Recipient

The recipient is an adenoviral vector engineered by molecular techniques and maintained in laboratories; there is therefore no natural habitat.

(c) Parental

The natural habitat of ChAd155 is chimpanzees.

9. organisms with which transfer of genetic material is known to occur under natural conditions;

(a) Donor

In the case of chronic HBV infection, integrated HBV DNA can be detected in patients with hepatocellular carcinomas. Otherwise FMDV DNA is not known to integrate in their natural hosts.

(b) Recipient

The recipient is a recombinant vector system engineered by molecular techniques and maintained in laboratories; there is therefore no natural habitat.

(c) Parental

The parental virus ChAd155 is chimpanzee-derived adenovirus strain, there is no data available about transfer of genetic material to other organisms.

10. verification of the genetic stability of the organisms and factors affecting it;

(a) Donor

Not relevant here

(b) Recipient and (c) Parental

Parental adenoviruses are stable in nature. The GMO (ChAd155-hIi-HBV) is a simian adenovirus that upon administration to the 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 only been observed as an extremely rare event in some human primary cell line cultures.

The genetic structure of the GMO vaccine is verified at different stages of the manufacturing process to demonstrate the integrity and identity of the vector and of the transgene insert. Tests are performed at the level of the pre-GMP primary virus seed (PVS), the GMP-grade master virus seed (MVS), virus harvest, bulk drug substance and final vaccine. Tests include identity by PCR, full genome DNA sequencing, restriction pattern analysis, analysis of the replicative competent adenoviruses (RCA).

The long-term stability of the ChAd155-hIi-HBV MVS, DS and vaccine when stored frozen at temperatures <-60°C will be followed according pre-defined stability plans. Long-term stability data for the GMO vaccine has been obtained for up to 24 months when stored at < -60°C, showing the material meets product stability specifications throughout this period of time.

In summary, testing performed at different stages of the production process provides phenotypic and genotypic verification of the genetic stability of the GMO material as compared to reference standards.

11. pathological, ecological and physiological traits:

(a) classification of hazard according to existing Community rules concerning the protection of human health and/or the environment;

(a) Donors

HBV. Hepatitis B virus has been classified, as other Hepadnaviruses, as Class 3 under the Directive 2000/54/EC on the protection of workers from risks related to exposure to biological agents at work. With however a note stating that HBV presents a limited risk of infection for workers because it is not normally infectious by air-borne route.

FMDV. The Picornaviridae family has been classified as Class 2 under the Directive 2000/54/EC. FMDV has not been specifically classified under the Directive 2000/54/EC.

However, only a very small portion of DNA from the donor HBV and FMDV organisms is present in the transgene, not enough to create infectious HBV or FMDV particles.

(b) Recipient and (c) Parental organism

While human adenoviridae are classified as Class 2 under the EEC directive, neither the recipient organism nor the parental simian adenovirus are classified. As a replication-defective virus, the recipient is incapable of establishing a productive, transmissible infection in its target host. Therefore the recipient organism and the GMO cannot be pathogenic to humans and may be handled under biosafety containment level 1 (BSL1). Indeed, in previous clinical studies evaluating similar chimpanzee-derived adenoviral vectors with different transgenes, national and local authorities have classified the ChAd-based GMOs as BSL1 organisms.

(b) generation time in natural ecosystems, sexual and asexual reproductive cycle;

(a) Donors

For detailed information on the generation time of the hepatitis B virus please refer to (Lin et al. 2015). However, not relevant, as only a very small portion of DNA from the donor HBV and FMDV organisms is present in the transgene, not enough to create infectious HBV or FMDV particles.

(b) Recipient

The recipient is replication-defective due to deletion of the *E1* gene and incapable of completing a reproductive cycle.

(c) Parental organism

Adenovirus (subgroup C) entry into the host cell is mediated by the surface receptor CAR (Coxsackievirus and adenovirus receptor). After entry and unpacking inside the cytoplasm, the viral genome is transcribed in the nucleus, mRNA is translated in the cytoplasm, and virions self-assemble in the nucleus.

(c) information on survival, including seasonability and the ability to form survival structures;

(a) Donors

The transgene gene segments, which are based on donor sequences from HBV (HBc and HBs antigens), FMDV (2A region) and human (hIi), are unable to reverse the replication-defective genotype of vector. The recipient is incapable of completing a reproductive cycle and hence cannot survive in the natural ecosystem.

(b) Recipient

The recipient is an E1-deleted replication-defective vector incapable of completing a reproductive cycle and hence cannot survive in the natural ecosystem.

(c) Parental organism

No particular seasonality is seen for infection by human adenoviruses. Adenoviruses can survive for long periods on environmental surfaces, but are susceptible to heat and disinfectants active against enveloped viruses.

(d) pathogenicity: infectivity, toxigenicity, virulence, allergenicity, carrier (vector) of pathogen, possible vectors, host range including non-target organism. Possible activation of latent viruses (proviruses). Ability to colonise other organisms;

(a) Donors

While the wild type HBV and FMDV viruses are known to be pathogenic, the HBc, HBs and 2A region gene sequences extracted from them and compiled into the GMO transgene, are not known to alter the infectivity, toxigenicity, virulence or allergenicity of the GMO construct.

(b) Recipient

The ChAd155 vector is replication-deficient and only capable of transducing animal cells. It is devoid of any pathogenic sequences. Toxicity studies have ruled out the potential pathogenicity of the final GMO (please refer to section II.C.2(i).

(c)Parental organism

As a subgroup type C adenovirus that utilizes the coxsackievirus and adenovirus receptor (CAR) to effect entry into host cells, the parental ChAd155 virus isolate could theoretically infect humans.

(e) antibiotic resistance, and potential use of these antibiotics in humans and domestic organisms for prophylaxis and therapy;

(a) Donors and (c) Parental Organism

Not applicable. Neither the donor-based sequences nor the gene sequence of the parental ChAd155 virus encode antibiotic resistance genes.

(b) Recipient

Plasmids with antibiotic resistance genes (ampicillin, kanamycin, chloramphenicol) were used as selection markers during the construction of the recombinant virus construct. However these antibiotic selection markers are not present in the final construct. No antibiotic resistance markers are present in the MVS nor in the final GMO vaccine. In addition, benzonase treatment is performed during GMO manufacturing process, affecting digestion of any residual DNA sequences.

(f) involvement in environmental processes: primary production, nutrient turnover, decomposition of organic matter, respiration, etc.

Not applicable.

12. nature of indigenous vectors:

This section is not applicable since there are no indigenous sequences that might enhance the transfer of the genetic material.

(a) sequence;

(b) frequency of mobilisation;

(c) specificity;

(d) presence of genes which confer resistance.

13. history of previous genetic modifications.

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

Another GMO with an identical ChAd155 backbone encoding an RSV antigen (ChAd155-RSV) has been assessed for safety, reactogenicity and immunogenicity in a Phase 1 trial in adults (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.” It is also currently assessed in a Phase 1/2 study in a pediatric population (EudraCT: 2016-0001117-76) 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 17 months”.

Several other adenoviral vectors derived from subgroup C adenovirus have been produced using a similar manufacturing process and assessed for safety and efficacy in clinical trials: chimpanzee-derived ChAd3. Three different simian adenoviruses have been used in clinical trials including: ChAd63

adenovirus (Biswas et al. 2011) belongs to serotype E (Colloca et al. 2012) and has been mainly used in malaria trials (Sheehy et al. 2011, O'Hara et al. 2012, de Barra et al. 2014, Hodgson et al. 2015) where more than a thousand healthy volunteers have been vaccinated, including two month old babies. The ChAd3 (Peruzzi et al. 2009) and PanAd3 (Vitelli et al. 2013) adenoviruses belonging 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 in a Phase I clinical RSV trial enrolling 42 volunteers, respectively.

All simian-derived adenoviral vectors tested so far in the clinic showed 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, Capone et al. 2013, de Barra et al. 2014, Hodgson et al. 2015, Ledgerwood et al. 2015).

B. Characteristics of the vector:

1. nature and source of the vector;

The wild type chimpanzee adenovirus type 155 was isolated from a healthy young chimpanzee housed at the New Iberia Research Center facility (The University of Louisiana at Lafayette, USA) using standard procedures. 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 Ad5 E4orf6 region.

The DNA of the hIi-HBV transgene was synthesized by GeneArt (Life Technologies Corporation) and cloned into the shuttle vector under the control of human CMV promoter with the tetracycline operator sequence (TetO) inserted downstream of the huCMV promoter TATA box and bovine growth hormone polyadenylation signal (BGHpA). The hIi-HBV transgene was then inserted in the plasmid BAC/ChAd155 (DE1, DE4, Ad5E4orf6) by homologous recombination.

2. sequence of transposons, vectors and other non-coding genetic segments used to construct the GMO and to make the introduced vector and insert function in the GMO;

Confidential information.

3. frequency of mobilisation of inserted vector and/or genetic transfer capabilities and methods of determination;

A potential product-related impurity is the formation of replication competent adenovirus (RCA) resulting from homologous recombination between the ChAd155-hIi-HBV viral vector and the human Ad5 E1 region of the E1-complementing production cell line. The risk of occurrence of the formation of 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. Additionally, the absence of RCA generation is assessed routinely on the drug substance for each clinical batch. RCA testing is also performed at the level of the MVS.

4. information on the degree to which the vector is limited to the DNA required to perform the intended function.

The expected biological activity of the GMO is the induction of a robust antigen-specific immune response against the HBc and HBs proteins, an immune response that will be augmented by the presence of the hIi genetic adjuvant in the transgene. A listing of genes integrated into the GMO vector construct and the role of each is summarized as follows:

- Tet O: Working in concert with the expression of TetR by the packaging cell line, the TetO gene product effects transcriptional control of the transgene during manufacture.
- Human CMV promotor: A human promoter ensures high levels of transgene expression in targeted human host cells and decreases the probability of transgene expression in non-target organisms.
- Poly adenylation signal: The bovine growth hormone polyadenylation (bgh-PolyA) signal is a specialized termination sequence for protein expression in eukaryotic cells.
- Human Ad5 E4 orf6: The insertion of Ad5E4orf6 is to facilitate enhanced replication of the ChAd construct in human E-1 complementing cell lines expressing human Ad5 E1.
- HBc and HBs of HBV: Serves to induce an antigen-specific immune response.
- 2A region of FMDV: Serves to separate the HBc and HBs antigens.
- hIi of human: Serves as a genetic adjuvant.

C. Characteristics of the modified organism:

1. Information relating to the genetic modification:

(a) methods used for the modification;

Genetic modifications to generate the final GMO involve insertion and deletion of genetic material using molecular biology techniques (detailed below).

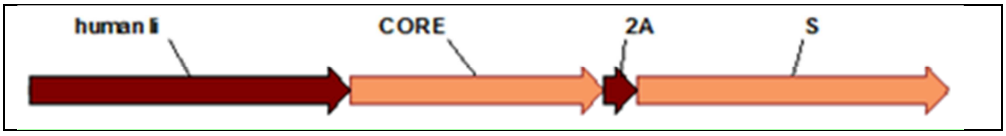
(b) methods used to construct and introduce the insert(s) into the recipient or to delete a sequence;

Confidential information.

(c) description of the insert and/or vector construction;

The transgene of the ChAd155-hIi-HBV GMO is under the transcriptional control of huCMV promoter. The construct contains a fusion of the HBc and HBs antigens of HBV separated by the 2A region of the FMDV. The 2A region of FMDV has been inserted to mediate a cleavage of the HBc and HBs antigens by a translational effect known as ribosomal skip (Donnelly et al. 2001). In addition, the N-terminal part 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). A schematic representation of the transgene is provided in Figure 1.

Figure 1 Schematic representation of the ChAd155-hIi-HBV insert



(d) purity of the insert from any unknown sequence and information on the degree to which the inserted sequence is limited to the DNA required to perform the intended function;

The insert has been described above. Its identity and purity are assessed at several steps of the manufacturing process of the GMO:

- Identity by PCR is performed on the master viral seed, and during the manufacture of each clinical batch of vaccine at the level of the virus harvest, bulk drug substance, and drug product (vaccine).
- Full genome sequencing is performed on the master virus seed and on each batch of bulk drug substance.
- Identity by restriction analysis is performed on each drug substance.
- Transgene expression and identity is assessed by Western blot analysis using monoclonal antibodies targeting HBc and HBs. This test is performed on the master viral seed, and during the manufacture of each clinical batch of vaccine at the level of the virus harvest, bulk drug substance, and drug product (vaccine).

(e) methods and criteria used for selection;

The GMO transgene encodes for the HBc and HBs antigens of HBV; as well as the human invariant chain (hIi) fused to the N-terminus of the HBc protein which serves as an adjuvant.

Rationale for choosing these proteins is detailed below:

HBc antigen. Several published studies compared the HBV antigen-specific T-cells in different segments of patients affected by HBV (post-acute infection, patients recovering from a chronic infection, active chronic infection and inactive carriers). The outcome of these studies highlighted that a strong, multi-specific T-cell response, particularly to the HBcAg, is essential for the clearance of HBV. When comparing T cells from patients with a chronic HBV resolving infection versus patients with unresolved chronic HBV infection, higher CD4+ T-cells and CD8+ T-cells specific to the core protein was evidenced in patients with resolving infection (Li et al. 2011, Liang et al. 2011, Boni et al. 2012). Further evidence of the role of T-cells targeting the HBc antigen in resolving HBV infection comes from data in bone marrow transplants. Bone marrow recipients with chronic hepatitis B effectively cleared their infection after they received bone marrow from donors naturally immune against HBV infection. The clearance of infection was associated with the transfer of core-specific CD4+ T-cells from the donor to the recipient and an increase

in CD4+ T-cells and CD8+ T-cells predominantly specific to the HBc antigen (Lau et al. 2002). In addition, the core protein is highly conserved across HBV genotypes and subtypes.

HBs antigen. HBs as the principle surface antigen of HBV, contains the key antigenic determinants (defining the genotype) as well as some of the key cross-genotype-preserved B-cell epitopes responsible for induction of broad neutralizing responses (Bhatnagar et al. 1982, Ryu et al. 1997). Although the HBs sequence is variable across genotypes, the Applicant's licensed prophylactic hepatitis B vaccine, Engerix-B, using the same HBs sequence, is protective against HBV across genotypes.

Human Major Histocompatibility Complex (MHC) class II-associated invariant chain p35 isoform. The human invariant chain (hIi), also known as CD74 when expressed on the plasma membrane, is an evolutionarily conserved type II membrane protein which has several roles within the cell and throughout the immune system (Borghese et al. 2011). Enhanced and sustained CD8+ T-cells responses were demonstrated in mice and non-human primates using an adenoviral vector-based vaccine encoding a fusion protein of target antigen with hIi (Capone et al. 2014). In order to increase further the induction of antigen-specific CD8+T-cell responses from the candidate ChAd155-HBV vaccine, the DNA fragment coding for the hIi has been N-terminally fused to the DNA coding for the HBc antigen of the HBV transgene, thus aiming to act as a genetic adjuvant to the HBc antigen. Preclinical studies demonstrated that the immune response of the HBV insert is increased when fused to hIi sequence.

(f) sequence, functional identity and location of the altered/inserted/deleted nucleic acid segment(s) in question with particular reference to any known harmful sequence.

The DNA sequence of the transgene has been provided in **Error! Reference source not found.**. The transgene does not encode any known harmful or pathological sequences.

2. Information on the final GMO:

(a) description of genetic trait(s) or phenotypic characteristics and in particular any new traits and characteristics which may be expressed or no longer expressed;

The ChAd155-hIi-HBV GMO consists of a recombinant replication-defective chimpanzee-derived adenovirus serogroup C vector engineered to express two HBV antigens, the truncated core protein (HBc) and the full-length surface antigen (HBs) separated by the 2A region of the FMDV. The human MHC class II-associated invariant chain p35 isoform (hIi) is fused to the N-terminus of the HBc antigen. Sequences of the insert have been codon-optimized for human use and are under transcriptional control by the human CMV promoter. The ChAd155 vector backbone has been modified to generate a replication-defective adenovirus vector through the deletion of the wild type E1 and E4 gene sequences. The ChAd155-hIi-HBV GMO vaccine is intended for trigger an HBV antigen-specific immune response in patients suffering from chronic HBV infection.

(b) structure and amount of any vector and/or donor nucleic acid remaining in the final construction of the modified organism;

The structure of the GMO vaccine candidate construct is confirmed by full genome DNA sequencing at the level of the MVS and the bulk drug substance. The transgene in the GMO has been confirmed by DNA sequencing to conform to the desired transgene encoding hIi-HBc-Spacer-2A region-HBs, and is identical to the positive control transgene carried by the shuttle plasmid.

(c) stability of the organism in terms of genetic traits;

Genetic stability is assessed at several steps of the manufacturing process to ensure no genetic modification occurs during manufacture of the GMO candidate vaccine.

The genetic structure of the GMO vaccine is verified at different stages of the manufacturing process to demonstrate the integrity and identity of the vector and of the transgene insert. Tests are performed at the level of the pre-GMP primary virus seed (PVS), the GMP-grade master virus seed (MVS), virus harvest, bulk drug substance and final vaccine. Tests include identity by PCR, full genome DNA sequencing, restriction pattern analysis, analysis of the replicative competent adenoviruses (RCA).

The long-term stability of the ChAd155-hIi-HBV MVS, DS and vaccine when stored frozen at temperatures <-60°C will be followed according pre-defined stability plans. Long-term stability data for the GMO vaccine has been obtained for up to 24 months when stored at < -60°C, showing the material meets product stability specifications throughout this period of time.

In summary, testing performed at different stages of the production process provides phenotypic and genotypic verification of the genetic stability of the GMO material as compared to reference standards.

(d) rate and level of expression of the new genetic material. Method and sensitivity of measurement;

Confidential information.

(e) activity of the expressed protein(s);

The expected biological effect of the expressed HBc and HBs proteins is to trigger an antigen-specific immune response against the HBV proteins as the GMO is intended for HBV treatment.
Non-clinical immunogenicity studies with ChAd155-hIi-HBV were performed in several animal models such as CB6F1 (hybrid of C57Bl/6 and Balb/C mice) and HDD mice (transgenic for the human HLA-A2 and HLA-DR1). Since HBV infects only humans and chimpanzees, relevant animal models to assess efficacy of HBV treatments are limited.
Immunogenicity of ChAd155-hIi-HBV was assessed using different regimen. Indeed, in the proposed FTIH clinical trial, the Applicant intends to assess a vaccination strategy involving a prime vaccination with the ChAd155-hIi-HBV vaccine followed by a boost with an MVA-HBV vaccine, depending on the cohort this will include either concomitant or subsequent administrations of adjuvanted lyophilized HBc-HBs proteins. Consequently, the immunogenicity of ChAd155-hIi-HBV was assessed on its own then the full regimen immunogenicity was assessed.

- ChAd155-hIi-HBV administrated at 10e8 vp in HLA.A2/DR1 (HHD) transgenic mice induced a strong CD8+ T-cell response to HBc and to a lesser extent to the HBs antigen; the response to the HBc

antigen was clearly enhanced by the presence of the hIi in the construct (as assessed by a head-to-head comparison with a construct without hIi). Of note, the subsequent administration of MVA-HBV at a dose of 10e7 pfu further increases the CD8+ T-cell response to both antigens.

- When administered to HHD transgenic mice, ChAd155-hIi-HBV induced the highest CD8+ T-cell response against HBc when compared to ChAd155-HBV (similar vector without the hIi sequence) and this response was further increased after MVA-HBV boost (at Day 28).
- An immunogenicity study was conducted in CB6F1 to investigate T and B cell tolerance to the "invariant chain" sequence (Ii) in a homologous model using a ChAd155 construct coding for the mouse Ii sequence (mIi): ChAd155-mIi-HBV. Induction of autologous mIi-specific immune responses was evaluated after 2 immunisations (days 0 and 14) with a high dose (109 vp) of the ChAd155-mIi-HBV vector. No anti-mIi antibodies and no mIi-specific T-cells were detected in any animals at 2 weeks post-first or second immunisation, suggesting that the immune tolerance to the mIi sequence was preserved.
- As a first step prior to evaluating the full prime-boost/co-administration vaccine regimens in a mouse model of chronic HBV infection, a feasibility study was conducted in C57Bl/6 mice transgenic for the whole HBV viral genome (Guidotti et al. 1995, Buchmann et al. 2013) to investigate if various ChAd155-hIi-HBVF/MVA-HBVF vector prime-boost regimen (with the HBc encoding sequence bearing the Kb-restricted dominant Class I epitope MGLKFRQL "F", recognized in C57Bl/6 mice genetic background) were able to elicit HBc-specific CD8+ T-cells in HBVTg mice. The results show that no HBc MGLKFRQL "F"-specific CD8+ T-cells were induced with any of the regimen, while strong responses were detected in C57BL/6, suggesting a very strong T-cell tolerance to the HBc antigen in HBVTg mice. Since potential impact of vaccination with a slightly different form of the GMO could not be accurately evaluated, no further investigations on the full HBV vaccine regimen were performed in the HBVTg mice model.

(f) description of identification and detection techniques including techniques for the identification and detection of the inserted sequence and vector;

The techniques used to detect and identify the recipient relevant for the GMO are:

- A specific polymerase chain reaction (PCR) has been developed by the Applicant;
- Full genome sequencing has also been developed and is performed on the drug substance
- An identity test using restriction analysis enables the assessment of viral DNA.
- In addition, the Western blots developed by the Applicant are relevant to detect and identify the GMO, especially the insert.

(g) sensitivity, reliability (in quantitative terms) and specificity of detection and identification techniques;

The identity of ChAd155-hIi-HBV is confirmed by full genome DNA sequencing of the viral vector. Identity testing of both the vector and the insert are performed at several stages during manufacture of the product using various methods including: PCR and Western blot expression of the transgene. All these methods have been qualified.

(h) history of previous releases or uses of the GMO;

This will be a First-Time-In-Human study with the proposed GMO, the ChAd155-hIi-HBV.
A GMO with an identical ChAd155 backbone, encoding an RSV antigen (ChAd155-RSV) 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). Containment level during both trials, based on data submitted at the time of clinical trial application is BSL1 (the lowest containment level) since no specific risk associated to use (and release) of the GMO was identified.
In addition, three other simian adenoviruses have been used in clinical trials sponsored by the Applicant: The ChAd63 adenovirus (Biswas et al. 2011) mainly used in malaria trials (Sheehy et al. 2011, O'Hara et al. 2012, de Barra et al. 2014, Hodgson et al. 2015) where more than a thousand healthy volunteers have been vaccinated, including two month old babies. The ChAd3 (Peruzzi et al. 2009) and PanAd3 (Vitelli et al. 2013) adenoviruses belong to serotype C (Colloca et al. 2012) and have been used in Hepatitis C Virus (HCV) and Ebolavirus trials with more than 1,500 vaccinees and in a recent Phase I clinical RSV trial enrolling 42 volunteers, respectively. All simian adenovectors showed 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, Capone et al. 2013, de Barra et al. 2014, Hodgson et al. 2015, Ledgerwood et al. 2015).

(i) considerations for human health and animal health, as well as plant health:

(i) toxic or allergenic effects of the GMOs and/or their metabolic products;

The GMO is not expected to have any toxic nor allergenic effects.

Potential 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 gelatin or egg protein (Zent et al. 2002).

In order to be able to treat patients with an immediate systemic allergic reaction to vaccination in the proposed trial, patients will remain under observation (visual follow-up) at the study site for at least 60 minutes after vaccination.

Potential toxic effects

The proposed clinical study will be a First-In-Human of ChAd155-hIi-HBV.

Vaccination in general may lead to transient local reactions at injection site, such as pain, redness and swelling. Transient systemic reactions may also occur following any vaccination, such as fever, malaise, fatigue, gastro-intestinal symptoms or chills.

Several other chimpanzee-derived adenoviral vectors have been produced using a similar manufacturing process and assessed for safety and efficacy in clinical trials. Three different simian adenoviruses have been evaluated in clinical trials including: ChAd63 adenovirus (Biswas et al. 2011) belonging to serotype group E (Colloca et al. 2012) and has been used in malaria trials (Sheehy et al. 2011, O'Hara et al. 2012, de Barra et al. 2014, Hodgson et al. 2015), where more than 1,000 healthy volunteers have been vaccinated, including two month-old babies. The ChAd3 (Peruzzi et al. 2009) and PanAd3 (Vitelli et al.

2013) adenoviruses belonging 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 in a Phase I clinical RSV trial enrolling 42 volunteers, respectively.

ChAd155 is closely related to the ChAd3 vector (they both belong to serotype C). ChAd3-based vaccines using HCV and Ebola immunogens were evaluated in clinical trials in up to 2,800 subjects and were well tolerated. ChAd3-HCV administered as a prime dose followed by MVA-HCV boost vaccination was tested in 245 healthy subjects and 14 chronic HCV patients. Mild local and systemic reactions were observed that increased with dose but were short-lived (Barnes et al. 2012, Swadling et al. 2014). Fatigue, headache and malaise were the most commonly reported systemic adverse events. 270 subjects were vaccinated with a ChAd3 Ebola-based vaccines in 4 Phase I clinical trials (doses ranging 1x10e10- 1x10e11 vp).

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 micro-particles (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 by B cells, activated T-cells, dendritic cells, monocytes and macrophages and widely expressed in the thymus, it should be highly tolerated. However, 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.

Two recent publications have shown that autoantibodies targeting the CLIP peptide (a region of the hIi) may be a highly specific biomarker for inflammatory spondyloarthritis (SpA), which highlights potential safety concerns (Baerlecken et al, 2014; Barliakos et al, 2014)**Error! Reference source not found.**.

However several arguments suggest the risk for vaccine-induced SpA or other pIMD is minimal:

- A humanized anti-CD74 monoclonal antibody (milatuzumab) targeting a cell surface expressed epitope of the molecule CD74 which is expressed on monocytes, macrophages, and B cells but not T cells was assessed in patients with myeloma multiple or B-cell lymphoma. No spondyloarthritis cases were reported in these patients. The anemia, lymphopenia, neutropenia, thrombocytopenia reported in these patients could be due to the patient's underlying disease as similar AEs have been observed with other monoclonal antibody therapies. Single dose studies of milatuzumab in monkeys

showed no adverse effects but did decrease circulating B and T lymphocytes and natural killer cells (Kaufman, 2013; Christian, 2015; Martin, 2015; Stein, 2007).

- The immunopathogenesis of SpA is not entirely clear but current hypotheses do not suggest a causative role for antibodies, nor B-cells, but the involvement of MHC-class I cells: 80% of all cases of SpA, and 94% of ankylosing spondylitis (a subgroup of SpA) occur in HLA-B27+ subjects. Two hypotheses for immunopathogenesis of SpA relates to the B27 protein forming dimers with the heavy chain and such dimers interact with MHC-class I rich cells (monocytes, dendritic cells), leading either to inhibition of intracellular signaling or Unfolded Protein Response (UPR) activation and UPR-mediated apoptosis of these cells. Further, SpA therapy relies on non- steroidal anti-inflammatory drugs and anti-TNFα therapy, the latter being effective in approximately half of patients: TNFα is mostly produced by dendritic cells and monocytes. A B-cell-directed therapy (rituximab) was safely tested but ineffective in patients with SpA failing to respond to TNFα blockers, further ruling out a role for B-cells in SpA pathogenesis (Song, 2010; Bowness, 2015).
- In mice, after repeated administrations of ChAd155-mIi-HBV, that includes murine invariant chain (mIi) as genetic adjuvant, no immune responses targeted against the mIi were induced. In the malaria and HCV programmes, the analysis of T-cell and antibody response against the invariant chain in several experiments in mice and non-human primate (NHP) vaccinated with various ChAd and MVA-vectored vaccines including the invariant chain as genetic adjuvant did not induce a break in tolerance to the autologous invariant chain self-antigen.
- Genetic adjuvants, typically immune genes that encode fully human cytokines or lymphokines, have previously been evaluated in healthy adults as well as in CHB patients and appear to be safe and modestly effective in driving CD4+ and CD8+ T-cell responses. The major experience comes from DNA vaccination strategies and studies in HIV and cancer that have used IL-12 and GM-CSF (Flingai, 2013). Similar approach was used in CHB patients with DNA-based vaccines including IL-2 and INF-γ or IL-12 as genetic adjuvants (Michel, 2015).

Toxicity studies in animals

Two toxicity studies were performed in a GLP compliant environment using GMO batches that are comparable to the clinical trial materials and using the same administration route: pivotal single-dose New Zealand White rabbits local tolerance and pivotal repeat-dose New Zealand rabbit toxicity studies. The clinical dosage of antigens/adjuvant, and volume were administered. In the repeat-dose rabbit study, 5 doses of each vaccine (N+1) were administered vs. the clinical dosing regimen (4 vaccinations). Three different schedules of vaccination (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 in the rabbit study (every 2 weeks) vs. the clinical regimen (every 8 weeks). Additionally, supportive toxicology data were gathered on the adjuvants and potential enhancers.

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 induced

safety concerns. Only one animal had a transient and non-adverse local reaction (erythema) after the injection of ChAd155-hli-HBV. The GMO was well tolerated without sign of systemic toxicity.

In the repeat 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. Histologically 3 days after then 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-hli-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-hli-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.

Conclusions
All available nonclinical data suggest that the GMO has acceptable tolerability/toxicity profiles for conducting the clinical trial.

(ii) comparison of the modified organism to the donor, recipient or (where appropriate) parental organism regarding pathogenicity;

The parental organism, the ChAd155 adenovirus was isolated from chimpanzees. Adenoviral disease in non-human primates (NHP) is predominantly subclinical, except for some cases of pneumonia in immunosuppressed SIV-infected animals. The GMO transgene insert is derived from gene sequences of the HBV. While HBV can infect humans and chimpanzees in laboratory settings, the HBc and HBs transgene sequences encode structural proteins and are not in themselves pathogenic. Importantly, as detailed above, the GMO is replication-defective and is not capable of establishing a propagative infection and as such is not pathogenic.

(iii) capacity for colonisation;

The GMO is not expected to display capacity for colonization since it is replication-defective. The RCA assay is performed as a routine quality control check on the drug substance to confirm the lack of replication competent adenoviruses.

(iv) if the organism is pathogenic to humans who are immunocompetent:
- diseases caused and mechanism of pathogenicity including invasiveness and virulence,

- communicability,

- infective dose,
- host range, possibility of alteration,
- possibility of survival outside of human host,
- presence of vectors or means of dissemination,
- biological stability,
- antibiotic-resistance patterns,
- allergenicity,
- availability of appropriate therapies.

Infections with human adenovirus are common worldwide, and occur throughout the year. These infections are frequent during childhood, when they tend to be self-limited and to induce serotype-specific immunity. Adenoviruses are endemic in the pediatric population; epidemics and outbreaks with higher morbidity and mortality can also occur. Clinical manifestations in immunocompromised patients include pneumonia, hepatitis, hemorrhagic cystitis, colitis, pancreatitis, meningoencephalitis, and disseminated disease, depending on the underlying disease, affected organ system, patient age, and virus serotype.

However the GMO recipient is a simian-derived adenovirus backbone, and the GMO itself is not expected to be pathogenic in immunocompetent or immunocompromised humans since the encoded transgene is not pathogenic.

(v) other product hazards

None known

III. INFORMATION RELATING TO THE CONDITIONS OF RELEASE AND THE RECEIVING ENVIRONMENT

A. Information on the release

1. description of the proposed deliberate release, including the purpose(s) and foreseen products,

The GMO will be released during a clinical study 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 GMO ChAd155-hIi-HBV is intended to be used in a prime-boost vaccine regimen for the treatment of HBV. It is aimed at restoring patients’ immunity to HBV, leading to clearance of HBsAg or reduction of HBsAg concentration, in order to allow patients to safely discontinue nucleos(t)ide analogue (NA) therapy without virological or clinical relapse.

The GMO is a recombinant, replication-defective, simian (chimpanzee-derived) adenovirus group C vector (ChAd155) engineered to express HBV antigens, the truncated core protein (HBc) and the full-length surface antigen (HBs) separated by the 2A region of FMDV. The N-terminus of the HBc gene insert is fused to the human Major Histocompatibility Complex (MHC) class II-associated invariant chain p35 isoform (hIi).

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

The ChAd155-hIi-HBV final container vials are filled at a nominal volume of 0.5 mL per vial. The ChAd155-hIi-HBV final container vials are stored at <-60°C.

Two separate doses of the ChAd155-hIi-HBV investigational vaccine will be evaluated in the Phase 1 clinical study: a higher potency dose and a lower potency dose.

Table 1 GMO doses to be evaluated in 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

2. foreseen dates of the release and time planning of the experiment including frequency and duration of releases,

The study is expected to be initiated in Q3-2018 with an enrolment of approximately 148 patients. The duration of the study will be roughly 28 months (120 weeks) per patient with a 6 month vaccination phase followed by an 22 month safety follow-up.

3. preparation of the site previous to the release,

The GMO release will be performed in designated rooms within a hospital or clinical setting. There is therefore no specific preparation of the site previous to the GMO release other than gathering study materials required to prepare and administer the vaccine, and disinfectants to clean surfaces post-release.

4. size of the site,

The GMO will be administered in clinical rooms.

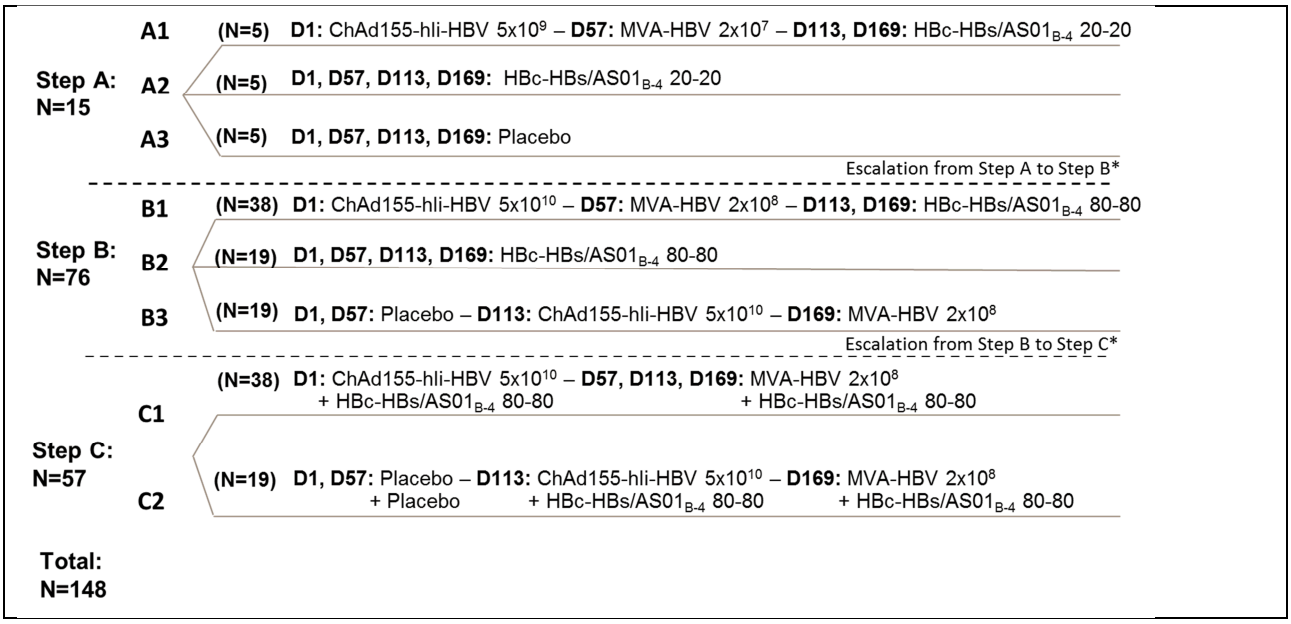
5. method(s) to be used for the release,

The GMO will be administered to study subjects by intramuscular injection. The vaccine will be prepared and administered by trained clinical study staff following procedures outlined in the clinical study protocol. 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 after 30 minutes and will be disposed as GMO waste by autoclaving or in accordance with the applicable guidelines/standard operating procedures at the investigator's site.

6. quantities of GMOs to be released,

The Th HBV VV-001 is a three step dose-escalating study, randomised within each step to evaluate the safety, immunogenicity and efficacy of different therapeutic HBV vaccination regimens. It will include 8 study groups totaling approximately 148 patients. Each of the three dose-escalating steps are described in Figure 2.

Figure 2 Study steps and study groups



Based on the number of patients in each study group, the number of doses administered, potency of each dose administered, a summary of the total amount of GMO ChAd155-hIi-HBV to be released during Th HBV VV-001 is provided in Table 2.

Table 2 Calculation of released GMO on basis of administered quantities

Step	Dose group	Number of patients	Number of vaccinations	Total quantities administered (viral particles)
A	Low dose 5 x 10e9 vp	5	5 total doses 1 dose (N=5 at D1)	Total number of low dose administrations =5 2.5 x 10e10
B	High dose 5 x 10e10vp	57	57 total doses 1 dose (N=38 at D1) + 1 dose (N=19 at D113)	Total number of high dose administrations 57+57=114 5.7 x 10e12
C		57	57 total doses 1 dose (N=38 at D1) + 1 dose (N=19 at D113)	
Total quantity of GMO released				**5.725 x 10e12**

7. disturbance on the site (type and method of cultivation, mining, irrigation, or other activities),

Not applicable

8. worker protection measures taken during the release,

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. During previous clinical studies involving the release of ChAd GMOs that contained other transgenes, regulatory authorities have viewed the use of ChAd GMOs as belonging to BSL1 category of organisms given that ChAd cannot cause disease and has an established human safety record in clinical research.

9. post-release treatment of the site,

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 after 30 minutes and will be disposed as biohazard waste in accordance with institutional procedures. A subsequent dressing applied to cover the site of injection may be disposed of by the study subject in household trash without special precautions.

For post-release treatment of the clinical rooms where preparation and administration of the GMO takes place see section V.B.

10. techniques foreseen for elimination or inactivation of the GMOs at the end of the experiment,

All empty vaccine vials, needles and syringes are to be discarded in biohazard waste containers after vaccine preparation/administration is completed for each subject. Keep the secondary containers for vaccine reconciliation by the monitor

Upon reconciliation and accountability, used study materials and unused study vaccine will either be destroyed following institutional procedures for the disposal of biohazard material, or will be returned to the sponsor for destruction.

11. information on, and results of, previous releases of the GMOs, especially at different scales and in different ecosystems.

The GMO has been released at the same dosage and route of administration during the conduct of two toxicology studies performed in rabbits. Otherwise, study TH HBV-001 will be the first release of the GMO in the targeted human host.

B. Information on the environment (both on the site and in the wider environment):

1.geographical location and grid reference of the site(s) (in case of notifications under part C the site(s) of release will be the foreseen areas of use of the product),

The ChAd155-hIi-HBV GMO will be administered during the proposed clinical trial at the following sites:

Clinical Study Site Address	Principal Investigator
Belgium	
Hôpital Erasme; Route de Lennik 808, Brussels	Christophe Moreno
UZ Gent; De Pintelaan 185, Gent	Hans Van Vlierberghe
UZ Antwerpen, Wilrijkstraat 10; Edegem	Thomas Vanwolleghem
SGS Life Science Services, Lange Beeldekensstraat 267, Antwerpen	Stefan Bourgeois
UZ Leuven, Herestraat 49, Leuven	Frederik Nevens
Cliniques Universitaires Saint-Luc, Avenue Hippocrate 10, Brussels	Yves Horsmans
Germany	
Klinikum der J. W. Goethe-Universitaet-Med. K. I, Theodor-Stern-Kai 7, Frankfurt	Stefan Zeuzem
Universitaetsklinikum Aachen-Med. Klinik III, Pauwelsstr. 30, Aachen	Christian Trautwein
Medizinische Hochschule Hannover-Gastroenterologie, Carl-Neuberg-Str. 1, Hannover	Markus Cornberg
Universitaetsklinikum Tuebingen-Innere Medizin I, Otfried-Mueller-Str. 10,Tuebingen	Christoph Berg
Universitaetsklinikum Essen-Gastroenterologie, Hufelandstr. 55, Essen	Guido Gerken
Universitaetsklinikum Bonn-Med. Klinik I, Sigmund-Freud-Str. 25, Bonn	Ulrich Spengler
Johannes-Gutenberg-Universitaet Main, Langenbeckstr. 1, , Mainz	Martin Sprinzl
Universitaetsklinikum Eppendorf-Ambulanzzentrum, Martinistr. 52, Hamburg	Julian Schulze zur
United Kingdom	
Royal London Hospital NHS Foundation, Whitechapel road, London	Patrick Kennedy
John Radcliffe Hospital; Headley Way; Oxford	Paul Klenerman

Southampton General Hospital, Tremona Road, Southampton	Salim Khakoo
Kings College Hospital 1; Denmark Hill, London	Kaushik Agarwal
Queens Medical Centre, Derby Road, Nottingham	Stephen Ryder

2. physical or biological proximity to humans and other significant biota,

The GMO will be released during a clinical study. Except the study subject and the required clinical study staff, no other person is authorized to be present during GMO preparation and administration; thus limiting the proximity to humans.

3. proximity to significant biotopes, protected areas, or drinking water supplies,

Not applicable since the GMO will be administered in a clinical setting.

4. climatic characteristics of the region(s) likely to be affected,

Not applicable since the GMO will be administered during a clinical study.

5. geographical, geological and pedological characteristics,

Not applicable since the GMO will be administered during a clinical study.

6. flora and fauna, including crops, livestock and migratory species,

Not applicable since the GMO will be administered during a clinical study.

7. description of target and non-target ecosystems likely to be affected,

Not applicable since the GMO will be administered during a clinical study.

8. a comparison of the natural habitat of the recipient organism with the proposed site(s) of release,

The recipient organism is an engineered adenoviral vector maintained in laboratories; there is therefore no natural habitat.

9. any known planned developments or changes in land use in the region which could influence the environmental impact of the release.

Not applicable since the GMO will be administered during a clinical study.

IV. INFORMATION RELATING TO THE INTERACTIONS BETWEEN THE GMOs AND THE ENVIRONMENT

A. Characteristics affecting survival, multiplication and dissemination

1. biological features which affect survival, multiplication and dispersal,

The GMO is replication-defective 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, the GMO will be administered intramuscularly in patients, a route which limits its potential shedding.

2.known or predicted environmental conditions which may affect survival, multiplication and dissemination (wind, water, soil, temperature, pH, etc.),

Adenoviruses are readily inactivated by a number of disinfectants active against non-enveloped viruses, and have shown sensitivity to heat inactivation. A completely effective elimination is achieved by autoclaving at 121°C for 15 minutes. They are however resistant to lipid disinfectants because they are non-enveloped. The GMO being derived from the ChAd155 strain is sensitive to the same agents.

3. sensitivity to specific agents.

Adenoviruses are readily inactivated by a number of disinfectants active against non-enveloped viruses.

B. Interactions with the environment

1. predicted habitat of the GMOs,

Following its intramuscular administration, the GMO is expected to remain mainly at the site of injection with some vectors migrating to peripheral draining lymph nodes. ChAd155 genome sequence analysis shows very high similarity with other subgroup C adenoviruses, suggesting that it can use the same Coxsackievirus and Adenovirus Receptor(CAR) to enter into the host cell. As a non-integrative virus, following host cell infection, the adenovector genome remains epichromosomal and does not integrate into the host genome.

2. studies of the behaviour and characteristics of the GMOs and their ecological impact carried out in simulated natural environments, such as microcosms, growth rooms, greenhouses,

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. genetic transfer capability

(a) post-release transfer of genetic material from GMOs into organisms in affected ecosystems;

The GMO viral vector is an effective vehicle to enable the transfer of the transgene into the targeted host cell for the transient expression of the transgene, as delivered by the intramuscular route of administration. But because adenoviruses are epichromosomal, they do not integrate their viral DNA into the host cell genome.

The possibility of gene transfer to other species is minimal under the conditions of the proposed clinical release of the GMO. The GMO will be administered to subjects in a clinical setting and is unlikely to come in contact with other animal species.

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 immunization (clinical study HCV001, EudraCT Number: 2007-004259-12).

In addition, the GMO is replication, does not integrate into genome of the hosts since it remains epichromosomal.

(b) post-release transfer of genetic material from indigenous organisms to the GMOs;

No such post release transfer from indigenous organisms to the GMO is expected since the GMO will be provided in a sealed vial and the release will occur during a clinical trial fulfilling the GCP with traceability of the GMO during the whole study, and therefore the GMO will not come into contact with indigenous organisms in the environment.

4. likelihood of post-release selection leading to the expression of unexpected and/or undesirable traits in the modified organism,

The likelihood of post-release selection leading to the expression of unexpected and/or undesirable traits in the modified organism is negligible.

5. measures employed to ensure and to verify genetic stability. Description of genetic traits which may prevent or minimise dispersal of genetic material. Methods to verify genetic stability,

Techniques to detect and identify the GMO and its genetic stability have been described in section II.2.(f). Genetic stability is assessed throughout the GMO manufacturing process which includes full genome sequencing of the master viral seed and drug substance. In addition, expression and identity of the transgene are assessed on the drug substance and drug product using Western blots with specific antibodies.

The GMO is replication-defective which minimizes further the genetic instability and probability of dispersal of the genetic material. Finally, formation on replication competent adenovirus is also assessed to ensure that no RCA is present.

6. routes of biological dispersal, known or potential modes of interaction with the disseminating agent, including inhalation, ingestion, surface contact, burrowing, etc.,

The GMO is a laboratory-developed viral vector that being replication-defective, and incapable of producing virus particles in the targeted human host, has lost its biological modes of transmission.

Accidental exposure in the form of a needle-stick injury will be minimized 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.

7. description of ecosystems to which the GMOs could be disseminated,

Not applicable since the GMO will be released in the context of a clinical study and administered by appropriately trained personnel.

8. potential for excessive population increase in the environment,

Not applicable since the GMO will be released in the context of a clinical study.

9. competitive advantage of the GMOs in relation to the unmodified recipient or parental organism(s),

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 transgene insert 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.

10. identification and description of the target organisms if applicable,

The release will be performed in the context of a clinical trial that will enroll patients with the following main eligibility criteria:

- Male or female between, 18-65 years old (at the time of the first study vaccination)
- Chronically Hepatitis B infected subjects* adherent to entecavir or tenofovir treatment given as per approved label/dosage as a first course of HBV oral therapy for least 30 months
- Specific criteria with regards to serology have been established as well as the criteria to document the medical history
- Stabilized liver disease for at least 24 months (specific criteria implemented).

11. anticipated mechanism and result of interaction between the released GMOs and the target organism(s) if applicable,

The expected biological activity of the GMO following intramuscular injection is the induction of an immune response against HBV. More specifically, a strong CD8+ T-cell responses as well as strong antigen-specific CD4+ T-cell and antibody responses are expected following the proposed heterologous prime-boost regimen.

12. identification and description of non-target organisms which may be adversely affected by the release of the GMO, and the anticipated mechanisms of any identified adverse interaction,

The only non-target organism that may potentially receive the GMO is clinical study personnel in the unlikely event a needle-stick injury occurs. Even if a needle-stick injury were to occur, the risk remains the same as for enrolled study subjects receiving the GMO in the proposed clinical study, there is no identified safety risk.

13. likelihood of post-release shifts in biological interactions or in host range,

Not applicable since the GMO is replication-defective and remains epichromosomal post administration.

14. known or predicted interactions with non-target organisms in the environment, including competitors, preys, hosts, symbionts, predators, parasites and pathogens,

Not applicable since the GMO is an investigational medicinal product

15. known or predicted involvement in biogeochemical processes,

Not applicable since the GMO is an investigational medicinal product

16. other potential interactions with the environment.

None identified

V. INFORMATION ON MONITORING, CONTROL, WASTE TREATMENT AND EMERGENCY RESPONSE PLANS

A. Monitoring techniques

1. methods for tracing the GMOs, and for monitoring their effects,

The GMO will be released in a clinical study. As reported from clinical studies where recombinant adenovirus vectors containing different transgenes were administered by intramuscular injection, the potential for shedding of virus particles is limited. No environmental monitoring of the clinical sites for the released GMO is planned during the conduct of the study.

Monitoring of the functional effects resulting from GMO vaccination will be performed to assess the antigen-specific cell-mediated and humoral immunity from blood samples collected at several time points following vaccination. The tables below lists the cell-mediated immunity and humoral immunity assays, respectively.

Table 3 Cell-mediated immunogenicity

System	Component	Challenge	Method	Unit
PBMC	HBs-specific (CD4/CD8) T-cells	HBs peptide pool	ICS	Events per million T-cells
	HBc-specific (CD4/CD8) T-cells	HBc peptide pool	ICS	Events per million T-cells
	hIi-specific (CD4/CD8) T-cells	hIi peptide pool	ICS	Events per million T-cells
	Adenovirus-specific (CD4/CD8) T-cells	Adenovirus Hexon peptide pool	ICS	Events per million T-cells

ICS: intracellular staining

Table 4 Humoral immunogenicity

System	Component	Method	Kit / Manufacturer	Unit	Cut-off
Serum	Anti-HBs IgG	CLIA	ADVIA Centaur anti-HBs2 (Siemens Healthcare)	mIU/ml	6.2 mIU/ml
	Anti-HBc IgG	TBD	TBD	TBD	TBD
	Anti-hIi IgG	ELISA	TBD	TBD	TBD
	Anti-ChAd155 neutralization	Neutralization	In-house	TBD	TBD

2. specificity (to identify the GMOs, and to distinguish them from the donor, recipient or, where appropriate, the parental organisms), sensitivity and reliability of the monitoring techniques,

No environmental detection and identification of the GMO following administration is foreseen during the proposed trial. See Sections II.A.6. and II.A.7.

3. techniques for detecting transfer of the donated genetic material to other organisms,

In case of a suspicion of accidental transfer from the target host (patient enrolled in the clinical study) to a non-targeted organism, antigen-specific humoral immunity testing could be performed on blood samples.

4. duration and frequency of the monitoring.

The study duration per patient is 120 weeks (48 weeks of vaccination phase plus 72 weeks of follow-up). Safety and immunogenicity will be monitored during 26 monitoring visits which have been scheduled as follows: at Day 1 (pre-vaccination); Day 3; Day 8; Day 15; Day 31; Day 57; Day 64; Day 71; Day 87; Day 113; Day 120; Day 127; Day 143; Day 169; Day 176; Day 183; Day 199; Day 225; Day 253; Day 281; ; Day 309 Day 337; Day 421; Day 505; Day 673; Day 841. Hence, they are more frequent just after each GMO administration than outside these “windows”.

B. Control of the release

1. methods and procedures to avoid and/or minimise the spread of the GMOs beyond the site of release or the designated area for use,

The GMO will be stored in a secured and dedicated storage area with restricted access to authorized personnel. Personal protective equipment appropriate to the containment level will be worn at all times during handling and vaccine preparation/administration. All clinical study staff will receive GMO-specific training in all study-specific and GMO-associated procedures.

2. methods and procedures to protect the site from intrusion by unauthorised individuals,

The GMO will be released during the conduct of a highly controlled multi-centre international clinical study. Access to areas within each study site where GMO material is stored, prepared and administered will be accessible only by trained clinical study staff.

3. methods and procedures to prevent other organisms from entering the site.

The GMO will be released during the conduct of a highly controlled multi-centre international clinical study. Access to areas within each study site where GMO material is stored, prepared and administered will be accessible only by trained clinical study staff.

C. Waste treatment

1. type of waste generated,

Waste generated following the IM administration of the GMO vaccination to each study subject will be minimal and consists mostly of GMO vials, cotton swabs, and material used to perform the IM administration (needle + syringe).

2. expected amount of waste,

One (1) vial of GMO vaccine (plus syringe and needle) will be used per study subject per injection. In addition, disposable personnel protective equipment used by clinical study staff will be treated as biohazardous waste.

3. description of treatment envisaged.

All empty vaccine vials, needles and syringes are to be discarded in biohazard waste containers after vaccine preparation/administration is completed for each subject. Keep the secondary containers for vaccine reconciliation by the monitor

Upon reconciliation and accountability, used study materials and unused study vaccine will either be destroyed following institutional procedures for the disposal of biohazard material, or will be returned to the sponsor for destruction.

D. Emergency response plans

1. methods and procedures for controlling the GMOs in case of unexpected spread,

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.

2. methods for decontamination of the areas affected, for example eradication of the GMOs,

The GMO vector is replication-defective and susceptible to most common disinfectants. All surfaces will be disinfected using appropriate means. Practical spill training sessions will be provided to all staff prior to working on the study. Record of staff training and competency will be documented.

3. methods for disposal or sanitation of plants, animals, soils, etc., that were exposed during or after the spread,

This is not applicable since the release will occur during a clinical study held at hospitals sites. Therefore, no contact with plants, animals or soils with the GMO is foreseen.

4. methods for the isolation of the area affected by the spread,

In case of accidental spread, an absorbent tissue will be immediately placed to absorb the spilled GMO, and then the contaminated surface will be decontaminated with a standard disinfectant according to appropriate measures in place.

5 plans for protecting human health and the environment in case of the occurrence of an undesirable effect.

The proposed study is a FTIH for the proposed ChAd155-hIi-HBV candidate vaccine. The target recruitment is approximately 148 patients who will receive different dose regimens.

To treat an immediate systemic allergic reaction to vaccination, all study subjects will remain under observation (visibly followed, no specific procedure) at the study site for at least 60 minutes after vaccination. First aid kit for anaphylactic reaction will be available at sites.

In addition to the planned iSRC evaluations, ad hoc safety evaluations can take place if a safety concern is identified by an investigator.

The safety holding rules which will be assessed by the iSRC are defined in Table 5.

- 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 5 Holding rules

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

Holding Rule	Event	Number of patients
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.

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