CLINICAL STUDY PROTOCOL

A Phase 1 Monotherapy Study to Evaluate the Safety Tolerability and Immunogenicity of Vaccination with Candidate Chimpanzee Adenovirus-vectored Hepatitis B Virus Vaccine (ChAdOx1-HBV) in Healthy Participants and Participants with Chronic Hepatitis B infection
Study Code: HBV001

EudraCT Number: 2019-003420-20

Version: 9.0
24 Nov 2021

Cover page containing NCT Number for the purpose of ClinicalTrials.gov

CLINICAL STUDY PROTOCOL

A Phase 1 Monotherapy Study to Evaluate the Safety Tolerability and Immunogenicity of Vaccination with Candidate Chimpanzee Adenovirus-vectored Hepatitis B Virus Vaccine (ChAdOx1-HBV) in Healthy Participants and Participants with Chronic Hepatitis B infection

Study Code: HBV001

EudraCT Number: 2019-003420-20

Version: 9.0
24 Nov 2021

Sponsor's Authorised Representative: [redacted]

Chief Scientific Officer

Signature [redacted] Date 29-NOV-2021

Printed Name [redacted]

ADMINISTRATIVE AND CONTACT INFORMATION

Sponsor Medical Oversight

Local Medical Monitor

Chief Investigator

Principal Investigator

Principal Investigator	[redacted]
Immunogenicity Laboratory	[redacted]
	[redacted]
Manufacturing Facility	[redacted]
Study Vaccine Packaging, Labelling & Distribution Centre	[redacted]

Central Laboratory (for pgRNA and HBcAg analysis)	[redacted]
Statistics and Data Management	[redacted]

INVESTIGATOR AGREEMENT

I, the undersigned, agree to conduct this study in accordance with this protocol, International Council on Harmonisation Good Clinical Practice (ICH GCP), the ethical principles set forth in the Declaration of Helsinki, and with local regulatory requirements.

Signature Date

Printed Name

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

PROTOCOL AMENDMENT CHANGE HISTORY

This is Version 9.0 of the protocol. The original protocol (Version 1.0) was issued on 30 Sep 2019. All the amendments are listed in the table below - the Summary of Main Changes.

For detailed protocol amendment changes refer to Appendix 2.

SUMMARY OF MAIN CHANGES

Version 8.2, 24 Nov 2021	
Summary and Justification of Change	**Sections Changed**
Inclusion criterion 16 has been updated to allow the inclusion of participants who have received more than two COVID-19 vaccine administrations as the HBV focused T cell response is not expected to vary based on the number of booster doses received.	Section 1.5.1 Rationale for Investigation of Dosing Interval With Respect to COVID-19 Vaccines Section 3.1.1 Study Design Section 3.1.2 Study Methodology Section 4.1 Number of participants Section 4.2: Inclusion Criteria

Version 8.0, 22 Sep 2021	
Summary and Justification of Change	**Sections Changed**
Healthy participants who have received the Moderna mRNA COVID-19 vaccine will now also be eligible to participate in Cohort 6.As no interference on HBV-focused T cell response is expected from either the Pfizer or the Moderna vaccine the interval from the second COVID-19 vaccine has been adjusted to 6 to 30 weeks.	Section 1.5.1: Rationale for Investigation of Dosing Interval With Respect to COVID-19 Vaccines Section 3.1.1: Study Design Section 3.1.2: Study Methodology Section 4.1: Number of Participants Section 4.2: Inclusion Criteria Section 5.7: Method of Assigning Participants to Treatment Groups

Version 7.0, 08 Jul 2021	
Summary and Justification of Change	**Sections Changed**

To evaluate the effect of prior ChAdOx1 vaccines against COVID-19 on the T cell response to ChAdOx1-HBV, and to support the proposed interval of 3 months between the vaccines, two cohorts of 15 healthy participants each have been added (one including those who have previously received AZD1222 and the other including those who have received Pfizer mRNA COVID-19 vaccine).	Section 1.5.1: Rationale for Investigation of Dosing Interval With Respect to COVID-19 Vaccines Section 2: Study Objectives Section 3.1.1: Study Design Section 3.1.2: Study Methodology Section 3.1.4.1: Duration for Each Participant Section 3.2: Discussion of Study Design Section 4.1: Number of Participants Section 4.2: Inclusion Criteria Section 4.3: Exclusion Criteria Section 5.1: Study Vaccines Administered Section 5.7: Method of Assigning Participants to Treatment Groups Section 6: Study Procedures at each Visit/Schedule of Assessments Section 6.2: Treatment Day Assessments (Day 0) Section 6.3.4: Clinic Visit: Days 28, 56 (Cohorts 1-4) and 168 (End of Study Visit Cohorts 1 4) Section 6.3.5: Clinic Visit: Day 84 (including End of Study Visit Cohorts 5 and 6) Section 7.2.1: Secondary Outcomes in Blood Samples
Dr Thomas Evans has replaced Dr Meg Marshall as Sponsor's Authorised Representative	Front Page
Details of a second laboratory for the analysis of immunogenetic samples and a new central laboratory have been added.	Administrative and Contact Information
Abstinence from heterosexual intercourse has been added as a method of contraception	Section 4.2: Inclusion Criteria
The email address for the Investigator to send the SAE form to has been corrected	Section 8.2.4: Reporting Requirements and Procedures for Serious Adverse Events

Version 6.0, 01 Mar 2021

Summary and Justification of Change	Sections Changed
Dr Kingsley Urakpo has left the company and Dr Thomas Evans has taken over the responsibility of Sponsor Medical Oversight for this study.	Administrative and Contact Information
The exclusion criterion 8 has been amended to include patients who have had adenoviral vaccines 3 months prior to screening and 3 months after receiving ChAdOx1-HBV in the study. There is evidence that prior adenoviral vectors may interfere with T cell responses, when given within the first three months of an adenoviral vaccine, especially if it's the same vector encoding the same foreign protein.	Protocol synopsis: Inclusion and Exclusion Criteria Section 4: Exclusion Criteria
Benefit Risk Assessment section has been updated with the available data	Section 3.3: Benefit Risk Assessment
Minimum data required to report an SAE has been updated to be in line with the ICH-GCP guidelines	Section 8.2.4: 8.2.4 Reporting Requirements and Procedures for Serious Adverse Events

Version 5.0, 29 Oct 2020	
Summary and Justification of Change	**Sections Changed**
The acceptable HBsAg level for the CHB patients has been raised from 4000IU/mL to 10000IU/mL.	Protocol synopsis: Inclusion and Exclusion Criteria Section 4.2: Inclusion Criteria

Version 4.0, 24 Feb 2020	
Summary and Justification of Change	**Sections Changed**
Dr Kingsley Urakpo has joined the company and will replace Dr Mariem Charafeddine as responsible for Sponsor Medical Oversight.	Administrative and Contact Information
A new site has been added to the study to accelerate the recruitment.	Administrative and Contact Information
Clarification given on the HBV disease markers assessed in Healthy Volunteers and the participant with CHB infection on each visit.	Protocol Synopsis: Study Procedures and Frequency Section 3.1.2: Study Methodology Section 6: Table 2: Overall Schedule of Assessments at Each Study Visit

	Section 7.1.4.3: HIV, HBV, HCV and HDV Diagnostic Testing
Clarification given on the allocation of participant number to state that a single participant number will be used per participant throughout the study.	Section 5.7: Method of Assigning Participants to Treatment Groups
Pharmacy Manual is replaced with Investigational Product Handling Manual	Section 5: Study Vaccine Section 5.2: Identity of Study Vaccine Section 5.3: Labelling, Packaging and Shipping Section 5.8.2.2: Study Vaccine Preparation and Administration
Clarification given to say that the dressing applied on the vaccination site will be kept on the vaccination site for at least 10 minutes after vaccination	Section 5.8.2.2: Study Vaccine Preparation and Administration
Clarification given that the HDV antibody assessment will be applicable for the participants with CHB infection only	Section 6: Table 2: Overall Schedule of Assessments at Each Study Visit Section 6.1: Screening and Baseline Pre-Dose Assessments
For practical reasons, LFNA test has been moved from Day 0 to within the screening period.	Protocol Synopsis: Study Procedures and Frequency Section 3.1.2: Study Methodology Section 6: Table 2: Overall Schedule of Assessments at Each Study Visit Section 6.1: Screening and Baseline Pre-Dose Assessments
The units of following tests were changed in line with UK's standard practice – • Total bilirubin: mg/dL to µmol/L • Platelet counts: per mL to $x10^9$/L	Protocol Synopsis: Inclusion and Exclusion Criteria: Section 4.3: Exclusion Criteria
Clarification given to state that hospitalisation for scheduled treatments and elective medical/surgical procedures related to a pre-existing condition should not be considered as an SAE	Section 8.2.1.3: Serious Adverse Event

Version 3.0, 04Dec2019

Summary and Justification of Change	Sections Changed
In response to MHRA assessment, state that the only reason why a participant must be discontinued from the study is withdrawal of consent. In all other circumstances, the participants must be encouraged to continue the safety follow-up procedures described in the protocol	Section 4.3.1.2: Study Discontinuation
In response to MHRA assessment, state that it is the investigator's responsibility to determine the causality relationship to the study vaccine	Section 8.1.3: Local Medical Monitor Section 8.2.3.2: Assessment of Relationship Section 8.2.4.3: Medical Review and Reporting by the Sponsor
In response to MHRA assessment, state the following are the only acceptable hormonal methods of contraception to be used during the study: – Combined (estrogen and progestogen containing) hormonal contraception associated with inhibition of ovulation: – oral – intravaginal – transdermal – Progestogen-only hormonal contraception associated with inhibition of ovulation: – oral – injectable – implantable	Section 4.2: Inclusion Criteria

Version 2.0, 13Nov2019	
Summary and Justification of Change	**Sections Changed**
Update collection of HBV disease markers in serum in order to ensure complete serological history is obtained *prior* to vaccination and is appropriate to the development of the study vaccine and ensure alignment with clinical practice in the secondary care setting.	Administrative and Contact Information Section 2: Study Objectives and Endpoints Section 6: Study Procedures at each Visit/Schedule of Assessments Section 7.1.4.3: HIV, HBV, HCV and HDV Diagnostic Testing Section 7.2.1: Secondary Outcomes in Blood Samples. 7.2.2.1: Exploratory Analysis in Blood Samples
Remove transcriptomics at all timepoints as this does not contribute significantly to the study endpoints. It also saves on blood volume contributed by participants and reduces the total number of study visits.	Section 6.3.2: Clinic Visit: 7 Section 7.2: Immunogenicity Assessments
Change the Day 1 visit from a 'face to face' clinic visit to a telephone to ease the burden on participants (vital signs and symptom directed physical examination were also removed appropriately)	Section 3.1.2: Study Methodology Section 6: Study Procedures at each Visit/Schedule of Assessments Section 6.3.1: Telephone Call: Day 1
Clarify the function of the DMSC which is to review safety and tolerability in HBV001 *only* (not efficacy and immunogenicity)	Section 3.1.1: Study Design 8.1.5: Data Safety Monitoring Committee
Provide further clarity and correct typographical errors	Abbreviation Table Throughout

PROTOCOL SYNOPSIS

Title of Study:	A Phase 1 Monotherapy Study to Evaluate the Safety, Tolerability and Immunogenicity of Vaccination with Candidate Chimpanzee Adenovirus-vectored Hepatitis B Virus Vaccine (ChAdOx1-HBV) in Healthy Participants and Participants with chronic hepatitis B infection
Short Title:	First in human study of ChAdOx1-HBV
Sponsor:	[redacted]
Protocol Number:	HBV001
Chief Investigator:	[redacted]
Study Centres:	This study will be performed in at least 2 study centres

Objectives:

Primary Objective

Determine the safety and tolerability of different doses of a single vaccination of ChAdOx1-HBV in healthy participants and in participants with chronic hepatitis B virus (CHB) infection and virally suppressed with oral antiviral medication.

Secondary Objectives

- Determine the immunogenicity of ChAdOx1-HBV in (A) healthy participants and in (B) participants with CHB, virally suppressed with oral antiviral medication

  Determine the effect of ChAdOx1-HBV on the level of hepatitis B surface antigen (HBsAg) in participants with CHB infection, virally suppressed with oral antiviral medication

- Cohorts 5 and 6 only: Assess whether the receipt of prior ChAdOx1-SARS-CoV-2 vaccine (AZD1222) results in decreased T cell responses to ChAdOx1-HBV, when administered 10-18 weeks prior to ChAdOx1-HBV

Endpoints:

Primary Endpoints

Incidence of safety and reactogenicity events:

- Adverse events and/or adverse events leading to study discontinuation
- Serious adverse events
- Grade ≥3 local and systemic reactions

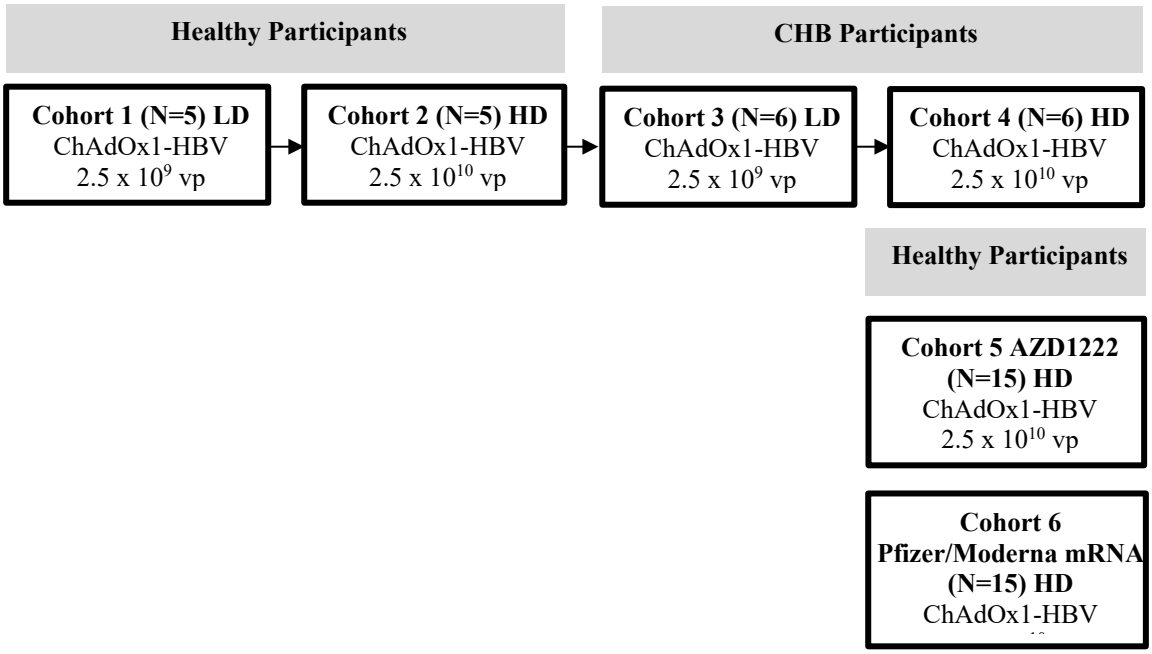
Secondary Endpoints

- A multi-parameter index made of CD4+ magnitude, CD4+ avidity, and CD8+ magnitude
- Mean reduction in HBsAg titre at Day 84 and Day 168 post-vaccination (last visit)
- Proportion of CHB participants with hepatitis B e-antigen (HBeAg) and HBsAg loss
- Proportion of CHB participants with HBeAg and HBsAg seroconversion
- Reduction of hepatitis B deoxyribonucleic acid (DNA) levels
- Total T cell response to the antigens encoded by ChAdOX1-HBV as measured in a peptide

Exploratory objective Determine the effect of ChAdOx1-HBV on virological and immunological systemic and intrahepatic changes in participants with CHB infection and virally suppressed with oral antiviral medication	***Exploratory Endpoints*** • Effect on serum hepatitis B core-related antigen (HBcAg) • Effect on serum hepatitis B circulating pre-genomic ribonucleic acid (pgRNA) • Liver fine needle aspirate (LFNA) assays will be used to quantify and characterise intrahepatic immune and parenchymal cells and/or hepatitis B virus (HBV) DNA and ribonucleic acid (RNA) transcripts • Effect of prior AZD1222 on the CD4+ and CD8+ T cell magnitude and phenotype as measured by multiparameter flow cytometry
Study Design:	This is a Phase 1, first in human study of ChAdOx1-HBV. The study will be conducted in 40 healthy participants and 12 participants with CHB and virally suppressed with oral antiviral medication. This will be an open-label, non-randomised dose escalation study comparing the safety, tolerability and immunogenicity of 2 different doses of ChAdOx1-HBV vaccine. T cell responses in healthy participants who have received a prior two-dose series of AZD1222 will be compared with those who have received at least two doses of the Pfizer mRNA COVID 19 vaccine or the Moderna COVID 19 vaccine. The study design is shown in Figure S1. Participants will be screened in the period Day -42 to Day -1. Informed consent will be obtained before any study specific procedures are performed. Eligible participants will then attend the clinic to receive study vaccine on Day 0. Participants will be enrolled sequentially. The study will investigate the study vaccine as shown in Table S1. On Day 0, an electronic diary (eDiary), tape measure and thermometer will be provided to perform self assessment of local and systemic reactogenicity. All participants will then have a follow-up telephone call on Day 1 and return to the clinic for study assessments on Days 7, 14, 28, 84. Participants in cohorts 1-4 only, will also have follow up visits on Day 56 and Day 168. End of study visit procedures will be performed at the final visit. Five healthy participants will be administered the low dose first (cohort 1). Dose escalation will only be initiated in the next 5 healthy participants (cohort 2) following Safety Monitoring Committee (SMC) review. Six CHB participants will be administered the low dose (cohort 3) before the dose escalation is initiated in the remaining 6 CHB participants (cohort 4). Thirty healthy participants (15 who have received two doses of AZD1222 [cohort 5] and 15 who have received at least two doses of either the Pfizer or Moderna mRNA COVID-19 vaccine [cohort 6]) will be dosed in parallel with the high dose used in cohorts 2 and 4. The first participant in each of cohorts 1-4 will be assessed for 1 hour in the clinic post-vaccination in case of immediate adverse events (timed after the end of study vaccine administration). All other participants will be assessed for 30 minutes. Five reviews of the safety, tolerability and available immunogenicity data will be performed by a SMC: • First review: at least 48 hours after the first healthy participant has received the first low dose of ChAdOx1-HBV vaccine. This review will occur *before* dosing the remaining participants in cohort 1 • Second review: at least 48 hours after the last healthy participant has received the last low dose of ChAdOx1-HBV vaccine. This review will occur *before* dosing high dose healthy participants in cohort 2

	• Third review: at least 48 hours after the last healthy participant has received the last high dose of ChAdOx1-HBV vaccine. This review will occur *before* dosing low dose CHB participants in cohort 3 • Fourth review: at least 48 hours after the first CHB participant has received the first low dose of ChAdOx1-HBV vaccine. This review will occur *before* dosing the remaining low dose CHB participants in cohort 3 • Fifth review: at least 48 hours after the last participant with CHB has received the low dose of ChAdOx1-HBV vaccine cohort 3 *before* dosing high dose CHB participants in cohort 4 A Data Monitoring Committee (DMC) will be appointed to perform unscheduled reviews of the available safety and tolerability study data and make recommendations concerning the continuation, modification or termination of the study if one of the study stopping or holding rules is met.

Figure S1 Overall Study Design



Abbreviations: ChAdOx1-HBV=chimpanzee adenovirus-vectored hepatitis B virus vaccine; CHB=chronic hepatitis B virus; HD=high dose; LD=low dose; N=number of participants; vp=viral particles

Duration of Study:	***Duration for each participant***: Cohorts 1-4: Up to 8 months (up to 1.5 months for screening and 6.5 months on the study with study vaccine given on Day 0). Cohorts 5 and 6: Up to 4.5 months (up to 1.5 months for screening and 3 months on the study with study vaccine given on Day 0). ***Duration of whole study:*** It is planned that the study will take 16 months to implement, enrol and read out.
Participant Numbers:	52 participants: Forty healthy participants and 12 participants with CHB infection and virally suppressed with oral antiviral medication. A total of 10 healthy participants and 12 participants with CHB infection is considered sufficient to confirm the safety, tolerability and immunogenicity of ChAdOx1-HBV and to answer the objectives of the study before progressing to larger studies. An additional 30 healthy participants (15 who have received two doses of AZD1222 and 15 who have received at least two doses of either the Pfizer or Moderna mRNA COVID-19 vaccine) will be recruited to assess whether prior receipt of AZD1222 results in decreased T cell responses to ChAdOx1-HBV.

Inclusion and Exclusion Criteria:	***Inclusion Criteria*** 1. Adult males or females aged ≥18 to ≤65 years at screening 2. Body Mass Index ≤30 kg/m^2 3. Able to provide informed consent indicating they understand the purpose of, and procedures required, for the study and are willing to participate 4. If female, willing not to become pregnant up to 8 weeks after last dose of study vaccine, not breast feeding 5. If female: Not pregnant, and one of the following: • Of non-childbearing potential (i.e. women who have had a hysterectomy or tubal ligation or are post-menopausal, as defined by no menses in ≥1 year) • Sexual abstinence, only if the participant refrains from heterosexual intercourse during the entire study period and it is the usual lifestyle of the participant • Of childbearing potential but agrees to practice highly effective contraception for 4 weeks prior to study vaccine and 8 weeks after study vaccine. Highly effective methods of contraception include one or more of the following: – Male partner who is sterile (medically effective vasectomy) prior to the female participant's entry into the study and is the sole sexual partner for the female participant – Combined (estrogen and progestogen containing) hormonal contraception associated with inhibition of ovulation: – oral – intravaginal – transdermal – Progestogen-only hormonal contraception associated with inhibition of ovulation: – oral – injectable – implantable – An intrauterine device – Bilateral tubal occlusion <u>Healthy participants (cohorts 1 and 2)</u> 6. Considered to be healthy with no current conditions that may significantly impair participant safety or influence study results, in the opinion of the Investigator <u>Participants with well controlled CHB (cohorts 3 and 4):</u> 7. Documented evidence of chronic HBV infection (e.g. HBsAg positive ≥6 months with detectable HBsAg levels at screening) 8. Receipt of only either entecavir or tenofovir for at least 12 months before screening 9. Virally suppressed (HBV DNA <40 IU/mL for ≥6 months) 10. HBsAg <10000 IU/mL <u>Healthy participants (cohort 5):</u> 11. Considered to be healthy with no current conditions that may significantly impair participant safety or influence study results, in the opinion of the Investigator 12. Adult males or females aged ≥40 to ≤60 years at screening

	13. Completed second dose of COVID-19 AZD1222 vaccine 10 to 18 weeks before enrolment Healthy participants (cohort 6): 14. Considered to be healthy with no current conditions that may significantly impair participant safety or influence study results, in the opinion of the Investigator 15. Adult males or females aged ≥40 to ≤60 years at screening 16. Received the latest dose of Pfizer (Comirnaty®) or Moderna (Spikevax®) mRNA COVID 19 vaccine 6 to 30 weeks before enrolment ***Exclusion Criteria*** 1. Presence of any significant acute or chronic, uncontrolled medical/ psychiatric illness 2. Hepatitis C virus antibody positive. 3. Human immunodeficiency virus antibody positive 4. History or evidence of autoimmune disease or known immunodeficiency of any cause 5. Prolonged therapy with immunomodulators (e.g. corticosteroids) or biologics (e.g. monoclonal antibodies, interferon) within 3 months of screening 6. Receipt of immunoglobulin or other blood products within 3 months prior to screening 7. Receipt of any investigational drug or vaccine within 3 months prior to screening 8. Cohorts 1-4: Receipt of any adenoviral vaccine within 3 months prior to administration of ChAdOx1-HBV on Day 0, or plan to receive an adenoviral-based vaccine within 3 months after Day 0 Cohorts 5 and 6: Receipt of any adenoviral vaccine (other than AZD1222 per inclusion criterion 13) within 3 months prior to administration of ChAdOx1-HBV on Day 0, or plan to receive an adenoviral-based vaccine within 3 months after Day 0 9. Receipt of any live vaccines within 30 days prior to screening 10. Receipt of any inactivated vaccines within 14 days prior to screening 11. History of allergic disease or reactions likely to be exacerbated by any component of the vaccine 12. Any history of anaphylaxis in reaction to vaccination 13. Malignancy within 5 years prior to screening with the exception of specific cancers that are cured by surgical resection (e.g. except basal cell skin carcinoma of the skin and cervical carcinoma). Participants under evaluation for possible malignancy are not eligible 14. Current alcohol or substance abuse judged by the Investigator to potentially interfere with participant safety and compliance 15. Significant cardiac disease or unstable uncontrolled cardiac disease 16. Any laboratory test at screening which is abnormal and which is deemed by the Investigator to be clinically significant 17. Any other finding that, in the opinion of the Investigator, deems the participant unsuitable for the study Additionally, for healthy participants (cohorts 1, 2, 5 and 6) 18. HBsAg positive Additionally, for participants with well controlled CHB (cohorts 3 and 4) 19. Co-infection with hepatitis delta 20. Documented cirrhosis or advanced fibrosis indicated by a liver biopsy within 6 months prior to screening.

	In the absence of an appropriate liver biopsy, either 1 of the following: • Screening Fibroscan with a result >9 kPa within ≤6 months of screening or • Screening FibroTest >0.48 and aspartate aminotransferase (AST) to platelet ratio index of >1 In the event of discordant results between non-invasive methods, the Fibroscan result will take precedence. 21. Alanine transaminase (ALT) >3 × upper limit of normal, international normalised ratio (INR) >1.5 unless the participant was stable on an anticoagulant regimen affecting INR, albumin <35 g/L, total bilirubin >34.2 µmol/L, platelet count < 100 x 10^9/L 22. A history of liver decompensation (e.g. ascites, encephalopathy or variceal haemorrhage) 23. Prior or current hepatocellular carcinoma 24. Chronic liver disease of a non-HBV aetiology 25. Any herbal supplements and or other medicines with potential liver toxicity within the previous 3 months prior to enrolment into this study
Study Vaccines:	ChAdOx1-HBV is a non-replicating viral vector encoding HBV consensus sequences from a group C genotype. It will be given by intramuscular injection into the deltoid muscle in the non-dominant arm. The study vaccines to be given in each treatment group are shown in Table S1. All study vaccine will be administered sequentially. Participants will receive study vaccine on Day 0 only. **Table S1 Study Vaccine Treatment Groups in HBV001** Abbreviations: ChAdOx1-HBV=chimpanzee adenovirus-vectored hepatitis B virus vaccine; CHB=chronic hepatitis B virus; HD=high dose; LD=low dose; vp=viral particles
Study Procedures and Frequency:	The safety, tolerability and immunologic assessments to be performed in cohorts 1-4 are: • Haematology (full blood count, haemoglobin, haematocrit, erythrocytes, mean cell volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, leukocytes, neutrophils, lymphocytes, monocytes, eosinophils, basophils, fibrinogen, prothrombin time and INR, activated partial thromboplastin time and platelets) will be analysed at screening, pre-vaccination on Day 0 and on Days 7, 14, 28, 56, 84 and 168 • Biochemistry (sodium, potassium, urea, creatinine and albumin) will be analysed at screening, pre-vaccination on Day 0 and on Days 7, 14, 28, 56, 84 and 168 • Liver function tests (alkaline phosphatase, gamma-glutamyl transpeptidase, ALT, AST and total bilirubin) will be analysed at screening, pre-vaccination on Day 0 and on Days 7, 14, 28, 56, 84 and 168

Treatment Cohort	Vaccine Single Dose	N
Cohort 1 LD Healthy Participants	ChAdOx1-HBV 2.5 x 10^9 vp	5
Cohort 2 HD Healthy Participants	ChAdOx1-HBV 2.5 x 10^{10} vp	5
Cohort 3 LD Participants with CHB	ChAdOx1-HBV 2.5 x 10^9 vp	6
Cohort 4 HD Participants with CHB	ChAdOx1-HBV 2.5 x 10^{10} vp	6
Cohorts 5 and 6 HD Healthy Participants	ChAdOx1-HBV 2.5 x 10^{10} vp	30

	• HBV disease markers in serum:
	  ○ Healthy participants: qualitative HBsAg will be tested at screening, HBsAb and HBcAb will be tested at Day 0 and repeated at the end of the trial if negative at baseline, or at the discretion of the investigator
	  ○ CHB participants: HBV DNA and quantitative HBsAg will be tested at screening and repeated on Days 0, 28, 56, 84 and 168. The following parameters will also be analysed on Days 0, 28, 56, 84 and 168: HBeAg, HBcAg, anti-HBs, anti-HBe, anti-HBc, pgRNA
	• A full physical examination will be performed at screening and pre-vaccination on Day 0. A symptom-directed physical examination will be performed on Days 7, 14, 28, 56, 84 and 168
	• Vital signs will be performed at screening, pre-vaccination and post-vaccination on Day 0 and on Days 7, 14, 28, 56, 84 and 168
	• Local and systemic reactogenicity (pain, induration, warmth, erythema at the vaccination site plus feverishness, chills, myalgia, fatigue, headache, nausea, arthralgia, malaise) will be captured pre-vaccination and post-vaccination on Day 0 and by eDiary for 3 days post-vaccination
	• Unsolicited adverse events will be recorded in the eCRF from the date the informed consent is signed, at all clinic visits to cover the period since the previous visit and during the visit and up to 28 days post-vaccination
	• Serious adverse events and adverse events of special interest will be recorded from the date the informed consent is signed until the end of the study and or resolved or until participant contact discontinues
	• Cellular immune responses will be measured in peripheral blood mononuclear cells samples taken on pre-vaccination on Day 0 and on Days 14, 28, 56, 84, 168. Samples will be analysed by intracellular cytokine staining
	For healthy participants in cohorts 5 and 6, all assessments on Days 0, 7, 14, 28 and 84 above will be performed. In addition, neutralising antibodies to ChadOx1 will be assessed on Days 0 and 84.
	In CHB participants who consent to LFNAs, these will be taken pre-vaccination within screening period and at the clinic visit on Day 84. Liver fine needle aspirate assays may be used to quantify and characterise intrahepatic immune and parenchymal cells and/or HBV DNA and RNA transcripts. These aspirates and subsequent LFNA assessments are optional.

Statistical Methods and Analysis:	All data will be summarised using descriptive statistics. Immunogenicity and efficacy data may be subjected to additional statistical analysis.

ABBREVIATIONS AND DEFINITIONS OF TERMS

ABBREVIATION	DEFINITION
AESI	Adverse Event of Special Interest
ALP	Alkaline Phosphatase
ALT	Alanine Transaminase
anti-HBe	Hepatitis B e-Antibody
aPTT	Activated Partial Thromboplastin Time
AST	Aspartate Transaminase
cccDNA	Closed Circular DNA
ChAdOx1	Chimpanzee Adenovirus Oxford 1
ChAdOx1-HBV	Chimpanzee Adenovirus-vectored Hepatitis B Virus Vaccine
CHB	Chronic Hepatitis B Virus
DNA	Deoxyribonucleic Acid
DMC	Data Monitoring Committee
eCRF	Electronic Case Report Form
eDiary	Electronic Diary
EDTA	Ethylenediaminetetraacetic Acid
ELISpot	Enzyme-linked Immunospot
GCP	Good Clinical Practice
GGT	Gamma-glutamyl Transpeptidase
GMO	Genetically Modified Organism
HBcAb/anti-HBc	Hepatitis B Core Antibody
HBcAg	Hepatitis B Core-related Antigen
HBeAg	Hepatitis B e-Antigen
HBsAb/anti-HBs	Hepatitis B Surface Antibody
HBsAg	Hepatitis B Surface Antigen
HBV	Hepatitis B Virus
HCC	Hepatocellular Carcinoma
HCV	Hepatitis C Virus
HDV Ab	Hepatitis D Virus antibody
HIV	Human Immunodeficiency Virus
HRA	Health Research Authority
ICH	International Council on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
ICS	Intracellular Cytokine Staining
IFN	Interferon
IFN-γ	Interferon-gamma
INR	International Normalised Ratio
IP	Investigational Product
ISF	Investigator's Site File
LFNA	Liver Fine Needle Aspirate
LFTs	Liver Function Tests
MedDRA	Medical Dictionary for Regulatory Activities
MERS	Middle East Respiratory Syndrome
MVA	Modified Vaccinia Ankara
NA	Nucleotide Analogue

ABBREVIATION	DEFINITION
PBMC	Peripheral Blood Mononuclear Cell
PD-1	Programmed Cell Death Protein 1
pgRNA	Pre-genomic RNA
PT	Prothrombin time
REC	Research Ethics Committee
RNA	Ribonucleic Acid
SAE	Serious Adverse Event
SAP	Statistical Analysis Plan
SAS	Statistical Analysis Software
SI	International System of Units
SIi	Shark Class-II invariant Chain
SMC	Safety Monitoring Committee
SOC	System Organ Class
SUSAR	Suspected Unexpected Serious Adverse Reaction
TB	Tuberculosis
TEAE	Treatment Emergent Adverse Event
ULN	Upper Limit of Normal
vp	Viral Particles

1 BACKGROUND

1.1 Disease Review

Hepatitis B virus (HBV) is a complex, small deoxyribonucleic acid (DNA) virus that goes through a ribonucleic acid (RNA) intermediate life cycle requiring reverse transcription. After transmission through infected blood, contaminated body fluids or through perinatal transfer, the virus infects the liver and can then either integrate into the hepatocyte genome or can exist as a stable chromosomal closed circular DNA. Once infection takes place, HBV infection results in chronic liver disease in 5-10% of infected adults, whereas the rate for perinatal transmission is the opposite, with greater than 90% of infected neonates progressing to chronic disease [1]. After a chronic HBV (CHB) infection is established, the rate of natural clearance is minimal. Such CHB infection frequently progresses to necrotic inflammation and ongoing liver damage, which may lead to cirrhosis and hepatocellular carcinoma (HCC) [1].

1.2 Treatment Review

Highly effective prophylactic vaccines were implemented in the early 1980's; however, these vaccines are ineffective once infection is established [2]. Despite the wide use of prophylactic vaccines, there are an estimated 240 million CHB carriers and over 686,000 related deaths per year worldwide [3].

Two classes of antiviral therapies have been approved and recommended for treatment of hepatitis B: interferons (IFNs) and nucleotide analogues (NAs). The use of IFN results in higher rates of hepatitis B e-antigen (HBeAg) and hepatitis B surface antigen (HBsAg) loss compared to NAs.

Pegylated IFN administered for up to 52 weeks results in HBsAg loss in 3-7% of patients compared to 0-3% HBsAg loss after the same duration of NA therapy. Response to IFN is also more durable, and HBsAg loss may occur after cessation of treatment, while virological relapse is frequent after cessation of NA. However, IFN is less effective at suppressing viral replication compared to NAs, requires parenteral administration, is associated with significant side effects, and is contraindicated in patients with decompensated cirrhosis or severe exacerbations of hepatitis and those with autoimmune or psychiatric illnesses.

Nucleotide analogues are administered orally and have negligible adverse effects. The recommended first-line NAs, entecavir and tenofovir, have low risk of drug resistance; but the requirement for indefinite therapy increases the cost and the risk of non-adherence.[4]

Various combinations of IFN and NA have been evaluated, but most studies have not shown an added benefit compared to monotherapy [5,6,7]. A recent study showed that combination of pegylated IFN and tenofovir increased the rate of HBsAg loss to 9.1% at Week 72, but the benefit was mainly observed with those infected with HBV genotype A [8].

Many research programs are ongoing to develop new treatment concepts that focus on the clearance of HBsAg in a significant proportion of patients, with the principle aims of: 1) stopping treatment with no risk of virological relapse and no risk of liver disease progression and, 2) to further decrease the risk of HCC. Several potential target mechanisms for immune modulation to restore HBV specific immune responses in conjunction with profound inhibition of HBV replication and HBsAg production to attain immunological control are being evaluated [3].

The immune clearance of CHB is likely to depend on the use of modalities that induce effective CD8+ T cells [9,10]. For over two decades vaccination strategies to induce these CD8+ T cells

to counter such infections as tuberculosis (TB), human immunodeficiency virus (HIV) and malaria have been investigated [11,12,13]. One platform approach that has induced remarkably high levels of T cells in man has been to use a non-replicating adenovirus "prime" followed by a heterologous viral vector "boost" utilising a non-replicating pox virus (modified vaccinia Ankara [MVA]) [14,15,16]. Non-replicating chimpanzee adenoviruses are often used rather than human adenoviruses, as there is minimal prior immunity to the vector itself. [17,18] The combination of chimpanzee adenoviruses (used in thousands of participants) plus MVA (administered to over 130,000 people) has been shown to induce large immune responses in man in malaria, TB, HIV, influenza and respiratory syncytial virus [13,16,19-22]. The chimpanzee adenovirus vector proposed in this study (chimpanzee adenovirus Oxford 1; ChAdOx1), a serogroup E adenovirus, has been administered to over 200 people in trials of Middle East Respiratory Syndrome (MERS), influenza, TB, chikungunya, and prostate cancer, and has been safe and immunogenic. It has also been modified to enhance immunogenicity through the addition of a short shark invariant chain (SIi) sequence and use of the tissue plasminogen promoter.

Hepatitis B virus circulates in the world as a number of genotypes (A-I), of which the most prevalent (especially in Asia) is genotype C [23]. The proteins expressed by ChAdOx1 in this study include most of the HBV genome from a genotype C consensus, including HBsAg, polymerase and core. In vitro experiments have demonstrated that the HBV polymerase was successfully inactivated through the insertion of point mutations at critical sites. In order to stop aggregation of surface antigen, the protein was split into two separate coding regions in the viral vectors.

The genetic HBV insert was then cloned into both the ChAdOx1 vector (chimpanzee adenovirus vectored hepatitis B virus vaccine; ChAdOx1-HBV), and also into an MVA vector (MVA-HBV) that will be used as a boost in a subsequent study after the evaluation of the ChAdOx1-HBV given alone is completed.

This study is designed to assess the safety and immunogenicity of ChAdOx1-HBV and to assess the breadth of responses to the proteins encoded by the vaccine vector. The cross reactivity to other genotypes will also be assessed. Doses of ChAdOx1 vaccines in previous studies have varied from 10^8 to over 10^{11} viral particles (vp) per dose and the middle two doses will be evaluated in this study to prepare for a further heterologous prime-boost regimen.

1.3 Summary of Non-clinical Studies

Immunogen design: 1,447 HBV genotype C nucleotide sequences (HBV database) were aligned, and an HBV genotype C consensus sequence was generated. Then, a patient's HBV genotype C sequence (accession number: KP017269.1 HBV isolate JP-02) that had maximum similarity to the consensus was chosen for use in the HBV immunogen design. The HBV immunogen encompasses three full length HBV-antigens (pre-core/core, polymerase and preS1/preS2/surface) along with truncated SIi and tissue plasminogen activator genetic adjuvants and it is encoded in the chimpanzee adenoviral vector. Point mutations were introduced within the polymerase protein encoded in the immunogen in order to abolish its function and to ensure no replication-competent HBV genome could arise from the immunogen sequences. An HBV-polymerase assay performed by the laboratory of Prof. Michael Nassal at University Hospital Freiburg, Germany, confirmed the absence of polymerase activity of the polymerase contained in the HBV immunogen. A schematic of the HBV immunogen is shown in Figure 1.

Figure 1 Schematic of Hepatitis B Virus Immunogen

The HBV immunogen was cloned and recovered into the ChAdOx1 vector genome to generate ChAdOx1-HBV. Research stocks of the ChAdOx1-HBV vector were generated at the viral vector core facility of the Jenner Institute, University of Oxford.

Preclinical immunogenicity testing: ChAdOx1-HBV was found to be highly immunogenic when tested in mouse immunogenicity experiments in both inbred (BALB/c, C57BL/6) and outbred (CD-1) mice, measured using interferon-gamma (IFN-γ) enzyme-linked immunospot (ELISpot) and intracellular cytokine staining (ICS) assays.

A Good Laboratory Practice toxicology study is ongoing (see Investigator's Brochure for further study details and results).

1.4 Summary of Clinical Experience

ChAdOx1 has been administered to over 200 participants (MERS, malaria, influenza and prostate cancer) and as the prime for an MVA boost in the malaria, influenza and prostate cancer studies (NCT03399578, NCT03203421, NCT01818362, NCT01623518 and NCT03815942). In all of those studies, extremely high levels of transgene-specific CD4+ and CD8+ T cells have been induced and there have been no vaccine-associated serious adverse events (SAEs). This is the first clinical study for the HBV ChAdOx1 vaccine.

Further information on ChAdOx1-HBV is contained in the Investigator's Brochure; this information should be reviewed prior to study initiation.

1.5 Study Rationale

Chronic hepatitis B virus infection is a global public health challenge on the same scale as TB, HIV and malaria. The current prophylactic vaccine has no effect on established chronic infection. Available treatments suppress viral replication, but they are not curative, largely due to the persistence of the viral covalently closed circular DNA (cccDNA) transcriptional template in infected hepatocytes and the failure of chronically infected patients to mount an immune response that is sufficiently robust, functional, and sustained to clear the infection. Thus, in most cases, treatment must continue for life. However, even successfully virally suppressed patients may still develop liver cancer.

Many research programs are ongoing to develop new treatment concepts that focus on the clearance of HBsAg in a significant proportion of patients, with the principle aims of: 1) stopping treatment with no risk of relapse and no risk of liver disease progression and 2) to further decrease the risk of HCC.

Pre-clinical models still have considerable limitations in this indication, and important gaps in our understanding of the HBV replication cycle and the host immune response must be addressed to expand the exploitable vulnerabilities in the replication cycle that can be targeted therapeutically to cure the infection.

A major effort has been made to compare the efficient integrated response resulting in HBV clearance of acute infection to the dysregulated response observed in patients with chronic hepatitis B. A complex interplay of innate and adaptive immune responses is essential for viral clearance and a failure of these responses can result in liver pathogenesis. CD8+ T cells are the main effector cells that eliminate the virus by cytolytic and non-cytolytic effector functions

[24,25]. Sufficient CD4 T cell activity and the production of neutralising anti-HBV envelope antibodies are required for protective immunity [26].

The combination of chimpanzee adenoviruses (used in thousands of participants) plus MVA (administered to over 130,000 people) has been shown to induce large immune responses in man in malaria, TB, HIV, influenza and respiratory syncytial virus [13,16,19-22].

Doses of ChAdOx1 vaccines in previous studies have varied from 10^8 to over 10^{11} vp per dose and the middle two doses will be evaluated in this study to prepare for a further heterologous prime-boost regimen. The safety of ChAdOx1-HBV and the breadth of systemic and liver-specific immune responses will be explored. A prime-boost strategy using ChAdOx1 vector (ChAdOx1-HBV) and an MVA vector (MVA-HBV), with and without anti-programmed cell death protein 1 (PD-1) will be evaluated in a subsequent study.

1.5.1 Rationale for Investigation of Dosing Interval With Respect to COVID-19 Vaccines

There are concerns that the prior use of a vectored vaccine may result in anti-vector responses, which would decrease the response to a subsequent use of the same vector. Specifically, there have been concerns raised that the use of the AstraZeneca ChAdOx1-SARS-CoV-2 vaccine (AZD1222 or Vaxzevria®) might reduce either the antibody or T cell response to a subsequent vaccine using the same vector. This potential decrease has been attributed to the induction of a neutralising cross-reactive T cell response to the viral vector.

For adenoviral vectors, prior infection with a replicating adenovirus of the same strain (e.g., human adenoviral 5) does appear to have an effect on the subsequent T cell and antibody response. However, this neutralisation is primarily directed against the fibre protein. This interference was a primary motivating factor in the development of replication-incompetent adenoviral vectors based on simian strains (chimpanzee, bonobo, and gorilla). In contrast, the neutralisation resulting from the use of the replication incompetent adenoviral vectors themselves is directed primarily to the abundant hexon capsid protein, and this response may have only minor effect on cell entry and immunogenicity, mainly mediated by the fibre.

In studies of AZD1222, there was no statistical relation between the level of neutralisation response following the first immunisation, and the resultant antibody or T cell response (as measured by IFN-γ ELISpot) following a second immunisation [27]. Likewise prior receipt of a ChAdOx1 vaccine (either ChAdOx1-MERs or ChAdOx1-mening) had no effect on the response to AZD1222.

However, the improvement of antigen-specific immunogenicity of a delayed interval is supported by reports in the literature of clinical trials assessing homologous or heterologous prime-boost regimes with adenoviral vectors [28-33]. In summary, the longer the interval between first and second dose of the adenoviral vector, the better the antigen-specific boosting capacity of the second dose. This has been mostly studied with antibody responses to the same adenoviral vector encoding the same antigen. This has been in part attributed to declining titres of vector-neutralising and vector-binding antibodies with time. Specifically, antigen-specific antibodies were boosted 3-fold when the boosting interval was 4 weeks, but this increased to 10-fold when the interval between the two vaccines was 24 weeks [29]. In another study, antibody titres were boosted 10-fold after a prime-boost interval of 12 weeks, and T cells were also boosted [28]. Nonetheless, it is well-known that increasing interval may boost responses, and the association of the increase in titres with increasing intervals has not been clearly shown in any of the cited studies to be attributed to the level of anti-vector neutralizing antibodies.

It is important to note that all previous clinical experience with second administration of the same adenoviral vector has been in the context of a single antigen, for example, Ad26-gag prime followed by Ad26-gag boost. This is in contrast to the scenario discussed here, where a possible administration of the ChAdOx1-nCoV-19 vaccine is followed by administration of a ChAdOx1 vector encoding HBV antigens. This is not a prime-boost scenario per se but rather the re-use of the same vector platform in a different indication.

Given this uncertainty and the improved responses seen three months after initial immunization compared to one month in studies of homologous prime-boost of the same antigen, and lacking clear data, the Sponsor has advised that participants not receive the ChAdOx1 vaccine in this and other [redacted]-sponsored trials until at least three months after receiving the AZD1222 or J&J hAd26, and that a three-month interval should also be advised if the COVID-19 vaccine follow entry into our study. However, this is not based on firm data, and either a shorter or longer interval may indeed be warranted.

To support this interval, this study aims to produce data of the response in healthy volunteers to our ChAdOx-1 HBV vaccine following the use of the AZD1222 vaccine. T cell responses (the critical immunological factor in chronic HBV infection) will be compared in participants who have received either a prior two-dose series of AZD1222 with those who have received at least two prior doses of either the Pfizer mRNA COVID 19 vaccine (Comirnaty) or the Moderna COVID-19 vaccine (Spikevax). Due to the recommendation to use the AZD1222 in those over the age of 40 years, we will confine our study to 40-60 years of age and keep the interval from the latest COVID-19 vaccine to a narrow window of 10-18 weeks. No interference on HBV -focused T cell responses is expected from either the Pfizer vaccine or the Moderna vaccine, so the interval from the latest COVID-19 vaccine will be between 6 to 30 weeks

2 STUDY OBJECTIVES AND ENDPOINTS

Objectives	Endpoints
Primary Determine the safety and tolerability of different doses of a single vaccination of ChAdOx1-HBV in healthy participants and in participants with CHB infection and virally suppressed with oral antiviral medication.	Primary Incidence of safety and reactogenicity events: • Adverse events and/or adverse events leading to study discontinuation • Serious adverse events • Grade ≥3 local and systemic reactions
Secondary • Determine the immunogenicity of ChAdOx1-HBV in (A) healthy participants and in (B) participants with CHB, virally suppressed with oral antiviral medication Determine the effect of ChAdOx1-HBV on the level of HBsAg in participants with CHB infection, virally suppressed with oral antiviral medication • Cohorts 5 and 6 only: Assess whether the receipt of prior ChAdOx1-SARS-CoV-2 vaccine (AZD1222) results in decreased T cell responses to ChAdOx1-HBV, when administered 10-18 weeks prior to ChAdOx1-HBV	Secondary • A multi-parameter index made of CD4+ magnitude, CD4+ avidity, and CD8+ magnitude • Mean reduction in HbsAg titre at Day 84 and Day 168 post-vaccination (last visit) • Proportion of CHB participants with HbeAg and HbsAg loss • Proportion of CHB participants with HbeAg and HbsAg seroconversion • Reduction of hepatitis B DNA levels • Total T cell response to the antigens encoded by ChAdOx1-HBV as measured in a peptide-stimulated ELISpot assay
Exploratory Determine the effect of ChAdOx1-HBV on virological and immunological systemic and intrahepatic changes in participants with CHB infection and virally suppressed with oral antiviral medication.	Exploratory • Effect on serum hepatitis B core-related antigen (HBcAg) • Effect on serum hepatitis B circulating pre-genomic ribonucleic acid (pgRNA) • Liver fine needle aspirate (LFNA) assays will be used to quantify and characterise intrahepatic immune and parenchymal cells and/or HBV DNA and RNA transcripts • Effect of prior AZD1222 on the CD4+ and CD8+ T cell magnitude and phenotype as measured by multiparameter flow cytometry

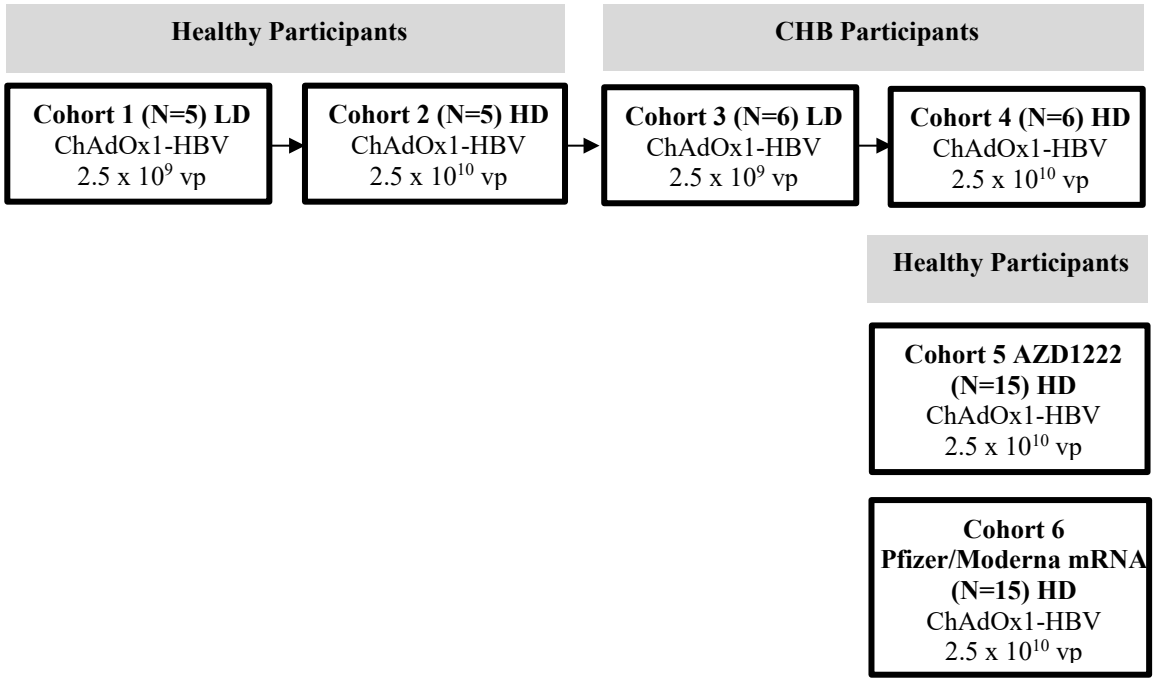
3 STUDY OVERVIEW

3.1 Overall Study Design and Methodology

3.1.1 Study Design

This is a Phase 1, first in human study of ChAdOx1-HBV. The study will be conducted in 40 healthy participants and 12 participants with CHB and virally suppressed with oral antiviral medication. This will be an open-label, non-randomised dose escalation study comparing the safety, tolerability and immunogenicity of 2 different doses of ChAdOx1-HBV vaccine. T cell responses in healthy participants who have received a prior two-dose series of AZD1222 or at least two doses of the Pfizer or Moderna mRNA COVID-19 vaccines will also be analysed. The study design is shown in Figure 2.

Figure 2 Overall Study Design



Abbreviations: ChAdOx1-HBV=chimpanzee adenovirus-vectored hepatitis B virus vaccine; CHB=chronic hepatitis B virus; HD=high dose; LD=low dose; N=number of participants; vp=viral particles

Five reviews of the safety, tolerability and available immunogenicity data will be performed by a Safety Monitoring Committee (SMC):

- First review: at least 48 hours after the first healthy participant has received the first low dose of ChAdOx1-HBV vaccine. This review will occur *before* dosing the remaining participants in cohort 1
- Second review: at least 48 hours after the last healthy participant has received the last low dose of ChAdOx1-HBV vaccine. This review will occur *before* dosing high dose healthy participants in cohort 2
- Third review: at least 48 hours after the last healthy participant has received the last high dose of ChAdOx1-HBV vaccine. This review will occur *before* dosing low dose CHB participants in cohort 3

- Fourth review: at least 48 hours after the first CHB participant has received the first low dose of ChAdOx1-HBV vaccine. This review will occur *before* dosing the remaining participants in cohort 3
- Fifth review: at least 48 hours after the last participant with CHB has received the low dose of ChAdOx1 HBV vaccine cohort 3 *before* dosing high dose CHB participants in cohort 4

No SMC review is required for cohorts 5 and 6 as the dose administered has been assessed in the previous cohorts of healthy participants.

A Data Monitoring Committee (DMC) will be appointed to perform unscheduled reviews of the available data including safety and tolerability study data and make recommendations concerning the continuation, modification or termination of the study if one of the study stopping or holding rules defined in Section 3.1.3 is met.

3.1.2 Study Methodology

Participants will be screened in the period Day -42 to Day -1. Informed consent will be obtained before any study specific procedures are performed. Eligible participants will then attend the clinic to receive study vaccine on Day 0. Participants will be enrolled sequentially.

The study will investigate response to study vaccine as shown in Table 2. On Day 0, an electronic diary (eDiary), tape measure and thermometer will be provided to perform self-assessment of local and systemic reactogenicity. All participants will then have a follow-up telephone call on Day 1 and return to the clinic for study assessments on Days 7, 14, 28, 84. Participants in cohorts 1-4 only, will also have follow up visits on Day 56 and Day 168. End of study visit procedures will be performed at the final visit.

Five healthy participants will be administered the low dose first (cohort 1). Dose escalation will only be initiated in the next 5 healthy participants (cohort 2) following SMC review.

Six CHB participants will be administered the low dose (cohort 3) before the dose escalation is initiated in the remaining 6 CHB participants (cohort 4).

Thirty healthy participants (15 who have received two doses of AZD1222 [cohort 5] and 15 who have received at least two doses of either Pfizer or Moderna mRNA COVID-19 vaccine [cohort 6]) will be dosed in parallel with the high dose used in cohorts 2 and 4.

The first participant in each of cohorts 1-4 will be assessed for 1 hour in the clinic post-vaccination in case of immediate adverse events (timed after the end of study vaccine administration). All other participants will be assessed for 30 minutes.

The safety and immunogenicity assessments to be performed in cohorts 1-4 are:

- Haematology (full blood count, haemoglobin, haematocrit, erythrocytes, mean cell volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, leukocytes, neutrophils, lymphocytes, monocytes, eosinophils, basophils, fibrinogen, prothrombin time [PT] and international normalised ratio [INR], activated partial thromboplastin time [aPTT] and platelets) will be analysed at screening, pre-vaccination on Day 0 and on Days 7, 14, 28, 56, 84 and 168
- Biochemistry (sodium, potassium, urea, creatinine and albumin) will be analysed at screening, pre-vaccination on Day 0 and on Days 7, 14, 28, 56, 84 and 168
- Liver function tests (LFTs; alkaline phosphatase [ALP], gamma-glutamyl transpeptidase [GGT], alanine transaminase [ALT], aspartate transaminase [AST] and

total bilirubin) will be analysed at screening, pre-vaccination on Day 0 and on Days 7, 14, 28, 56, 84 and 168

- HBV disease markers in serum:
    - Healthy participants: qualitative HBsAg will be tested at screening, hepatitis B surface antibody (HBsAb) and HBcAb will be tested at Day 0, and repeated at the end of the trial if negative at baseline, or at discretion of the Investigator
    - CHB participants: HBV DNA and quantitative HBsAg will be tested at screening and repeated on Days 0, 28, 56, 84 and 168. The following parameters will also be analysed on days 0, 28, 56, 84 and 168: HBeAg, HBcAg, anti-HBs, anti-HBe, anti-HBc, pgRNA
- A full physical examination will be performed at screening and pre-vaccination on Day 0. A symptom-directed physical examination will be performed on Days 7, 14, 28, 56, 84 and 168
- Vital signs will be performed at screening, pre-vaccination and post-vaccination at the on Day 0 and on Days 7, 14, 28, 56, 84 and 168
- Local and systemic reactogenicity (pain, induration, warmth, erythema at the vaccination site plus feverishness, chills, myalgia, fatigue, headache, nausea, arthralgia, malaise) will be captured pre-vaccination and post-vaccination on Day 0 and by eDiary for 3 days post-vaccination
- Unsolicited adverse events will be recorded in the eCRF from the date the informed consent is signed, at all clinic visits to cover the period since the previous visit and during the visit and up to 28 days post-vaccination
- Serious adverse events (SAEs) and adverse events of special interest (AESIs) will be recorded from the date the informed consent is signed until the end of the study and or resolved or until participant contact discontinues
- Cellular immune responses will be measured in peripheral blood mononuclear cells (PBMCs) samples taken on pre-vaccination on Day 0 and on Days 14, 28, 56, 84 and 168. Samples will be analysed by ICS

For participants in cohorts 5 and 6, all assessments on Days 0, 7, 14, 28 and 84 above will be performed. In addition, neutralising antibodies to ChadOx1 will be assessed on Days 0 and 84.

In CHB participants who consent to LFNAs, these will be taken pre-vaccination within the screening period and at the clinic visit on Day 84. Liver fine needle aspirate assays may be used to quantify and characterise intrahepatic immune and parenchymal cells and/or HBV DNA and RNA transcripts. These aspirates and subsequent LFNA assessments are optional.

3.1.3 Study Stopping Criteria/Holding Criteria/Dose Escalation Process

There will be a review of the study data by the DMC when there is a reasonable possibility that the investigational vaccine caused any of the listed following adverse events based on the assessment of the Investigator in one or more participants at any dose. Vaccinations will be held pending this review:

- Death
- Life-threatening adverse event or life-threatening suspected reaction
- Serious suspected adverse reaction

- Suspected, unexpected, serious adverse reaction (SUSAR)
- Acute allergic reaction or anaphylactic shock following the administration of vaccine investigational product
- Anaphylaxis or bronchospasm, indicative of an immediate hypersensitivity reaction to study vaccine
- If any participant has any of the following:
  - ALT or AST >8 x upper limit of normal (ULN)
  - ALT or AST >5 x ULN for more than 2 weeks
  - ALT or AST >3 x ULN and (total bilirubin >2 x ULN or INR >1.5)
  - ALT or AST >3 x ULN with the appearance of fatigue, nausea, vomiting, right upper quadrant pain or tenderness, fever, rash, and/or eosinophilia (>5%)

The competent authority and Research Ethics Committee (REC) will be notified of a temporary halt within 15 days according to the local requirements. If, following the review of data, a recommendation is made by the DMC to resume study enrolment and vaccine administration, the DMC will record their judgment in a memorandum to the study file and notify the Sponsor. The DMC memorandum will be forwarded to the local Medical Monitor and Investigators.

3.1.4 Duration of Study

3.1.4.1 *Duration for Each Participant*

Cohorts 1-4: Up to 8 months (up to 1.5 months for screening and 6.5 months on the study with study vaccine given on Day 0).

Cohorts 5 and 6: Up to 4.5 months (up to 1.5 months for screening and 3 months on the study with study vaccine given on Day 0).

3.1.4.2 *Duration of Study*

It is planned that the study will take 16 months to implement, enrol and read out.

The end of the study is defined as the date of database lock.

3.2 Discussion of Study Design

This is a first in human study of the ChAdOx1 vaccine and is designed to assess the safety in both healthy adults, as well as in the target CHB population. As T cell responses to natural infection are blunted or exhausted in participants with CHB, it will be important to determine how responses in CHB compare to healthy participants. Given the uncertainty around prior use of a vectored vaccine resulting in anti-vector responses, this study will also assess the response of healthy participants who have previously received AZD1222 to ChAdOx1-HBV.

In CHB participants, low dose of the vector (selected based on doses given in a large number of other studies (NCT03399578, NCT03203421, NCT01818362, NCT01623518 and NCT03815942), will first be evaluated to minimise the risk that immune responses generated by vaccination do not results in liver toxicity. To further minimise this likelihood, only those participants whose DNA levels are well controlled on antivirals will be included in the study, and those with advanced liver fibrosis or cirrhosis will be excluded. After safety review, a higher dose, but one shown to be safely used in many other ChAdOx1 studies (HIV, malaria, influenza, MERS), will then be given. Dose selection for future studies will be based on safety

and the induction of T cell activity, as measured by both magnitude of response as well as functionality.

Hepatitis B biomarkers will be evaluated as the basis for future comparison and to assess potential efficacy.

The study will be used as a first step prior to the evaluation of a combination vaccine approach using ChAdOx1-HBV combined with both a MVA encoding the same genetic insert, with or without a low dose PD-1 inhibitor.

3.3 Benefit Risk Assessment

There are potential known and unknown risks associated with vaccination. With any vaccine, including those that are licensed, there is a rare risk of anaphylaxis which can be fatal. All participants will be observed in the clinic for at least 30 minutes post-vaccination.

Intramuscular injection of vaccines frequently causes the local and systemic signs and symptoms that are being collected as adverse events. These will be solicited from the participant to ensure they are not occurring more frequently or are more severe than expected based on previous experience with the same or similar viral vectors.

With any new treatment there is always a possibility of an unexpected adverse events.

The administration of ChAdOx1-HBV in this study may interfere with the effectiveness of other vaccines subsequently administered to the participant. The ChAdOx1 vector induces anti-vector immunity in addition to an antigen-specific immune response. Of particular concern are neutralising antibodies directed against the capsid structure of the vector, which may have a negative effect on subsequent immunisations with the same or related adenoviral vector by neutralising the vector before it has a chance to transduce target cells. This effect decreases with longer interval between the vaccines [28,29]. In particular, there is a concern that the ChAdOx1-HBV administered in this study could decrease the response to the AstraZeneca COVID-19 vaccine, which has the same viral vector, especially if it is administered within 3 months of ChAdOx1-HBV. The Janssen (Johnson & Johnson) COVID-19 vaccine is also made with an adenovirus, and some interference cannot be ruled out. Therefore, it is recommended that participants in this study receive an alternative type of COVID-19 vaccine, such as an mRNA or protein vaccine, at least 2 weeks before and after ChAdOx1-HBV in this study.

There are also routinely scheduled reviews of safety and efficacy data throughout the study by the SMC. Stopping and holding rules have been defined for the study (see Section 3.1.3) and a DMC will perform a review of safety data if one of these is met.

Although this is the first time ChAdOx1-HBV has been administered to humans, the ChAdOx1 vector has been widely administered for many pathologies without significant safety concerns (see Section 1.4). Further detail is provided in the Investigator's Brochure.

As this is a first in human study without prior clinical data there may be no benefit to study participants. The participants in the CHB groups will however receive additional monitoring of their HBV status compared to the standard of care.

4 STUDY POPULATION

4.1 Number of Participants

It is planned that 40 healthy participants and 12 participants with CHB infection and virally suppressed with oral antiviral medication will be enrolled in the study. Chronic HBV participants will be recruited/deemed eligible by hepatologists, infectious disease specialists or physicians experienced in treating CHB participants. Centres not meeting the enrolment expectations will be considered for replacement with or addition of a new site. If participants leave the study before all follow-up visits are completed (for any reason), then these may be replaced at the discretion of the study team. A log of all participants enrolled into the study (i.e. having given informed consent) will be maintained in the Investigator's Site File (ISF) at the study centre irrespective of whether they have treated with vaccine or not.

A total of 10 healthy participants and 12 participants with CHB infection is considered sufficient to confirm the safety, tolerability and immunogenicity of ChAdOx1-HBV and to answer the objectives of the study before progressing to larger studies. An additional 30 healthy participants (15 who have received two doses of AZD1222 and 15 who have received at least two doses of either Pfizer or Moderna mRNA COVID-19 vaccine) will be recruited to assess whether prior receipt of AZD1222 results in decreased T cell responses to ChAdOx1-HBV.

The statistical considerations used to determine the number of participants planned and evaluable are presented in Section 10.1.

4.2 Inclusion Criteria

Participants must meet *all* the following criteria to be eligible for the study:

1. Adult males or females aged ≥18 to ≤65 years at screening
2. Body Mass Index ≤30 kg/m^2
3. Able to provide informed consent indicating they understand the purpose of, and procedures required, for the study and are willing to participate
4. If female, willing not to become pregnant up to 8 weeks after last dose of study vaccine, not breast feeding
5. If female: Not pregnant and one of the following:
   - Of non-childbearing potential (i.e. women who have had a hysterectomy or tubal ligation or are post-menopausal, as defined by no menses in ≥1 year)
   - Sexual abstinence, only if the participant refrains from heterosexual intercourse during the entire study period and it is the usual lifestyle of the participant
   - Of childbearing potential but agrees to practice highly effective contraception for 4 weeks prior to study vaccine and 8 weeks after study vaccine. Highly effective methods of contraception include one or more of the following:
     - Male partner who is sterile (medically effective vasectomy) prior to the female participant's entry into the study and is the sole sexual partner for the female participant
     - Combined (estrogen and progestogen containing) hormonal contraception associated with inhibition of ovulation:
       - oral
       - intravaginal

  - transdermal
- Progestogen-only hormonal contraception associated with inhibition of ovulation:
  - oral
  - injectable
  - implantable
- An intrauterine device
- Bilateral tubal occlusion

Healthy participants (cohorts 1 and 2):

6. Considered to be healthy with no current conditions that may significantly impair participant safety or influence study results, in the opinion of the Investigator

Participants with well controlled CHB (cohorts 3 and 4):

7. Documented evidence of chronic HBV infection (e.g. HBsAg positive ≥6 months with detectable HBsAg levels at screening)
8. Receipt of only either entecavir or tenofovir for at least 12 months before screening
9. Virally suppressed (HBV DNA <40 IU/mL for ≥6 months)
10. HBsAg <10000 IU/mL

Healthy participants (cohort 5):

11. Considered to be healthy with no current conditions that may significantly impair participant safety or influence study results, in the opinion of the Investigator
12. Adult males or females aged ≥40 to ≤60 years at screening
13. Completed second dose of COVID-19 AZD1222 vaccine 10 to 18 weeks before enrolment

Healthy participants (cohort 6):

14. Considered to be healthy with no current conditions that may significantly impair participant safety or influence study results, in the opinion of the Investigator
15. Adult males or females aged ≥40 to ≤60 years at screening
16. Received the latest dose of either Pfizer (Comirnaty®) or Moderna (Spikevax) mRNA COVID-19 vaccine 6 to 30 weeks before enrolment

4.3 Exclusion Criteria

1. Presence of any significant acute or chronic, uncontrolled medical/psychiatric illness
2. Hepatitis C virus (HCV) antibody positive.
3. HIV antibody positive
4. History or evidence of autoimmune disease or known immunodeficiency of any cause
5. Prolonged therapy with immunomodulators (e.g. corticosteroids) or biologics (e.g. monoclonal antibodies, IFN) within 3 months of screening
6. Receipt of immunoglobulin or other blood products within 3 months prior to screening
7. Receipt of any investigational drug or vaccine within 3 months prior to screening
8. Cohorts 1-4: Receipt of any adenoviral vaccine within 3 months prior to administration of ChAdOx1-HBV on Day 0, or plan to receive an adenoviral-based vaccine within 3 months after Day 0

Cohorts 5 and 6: Receipt of any adenoviral vaccine (other than AZD1222 per inclusion criterion 13) within 3 months prior to administration of ChAdOx1-HBV on Day 0, or plan to receive an adenoviral-based vaccine within 3 months after Day 0

9. Receipt of any live vaccines within 30 days prior to screening
10. Receipt of any inactivated vaccines within 14 days prior to screening
11. History of allergic disease or reactions likely to be exacerbated by any component of the vaccine
12. Any history of anaphylaxis in reaction to vaccination
13. Malignancy within 5 years prior to screening with the exception of specific cancers that are cured by surgical resection (e.g. except basal cell skin carcinoma of the skin and cervical carcinoma). Participants under evaluation for possible malignancy are not eligible
14. Current alcohol or substance abuse judged by the Investigator to potentially interfere with participant safety and compliance
15. Significant cardiac disease or unstable uncontrolled cardiac disease
16. Any laboratory test at screening which is abnormal and which is deemed by the Investigator to be clinically significant
17. Any other finding that, in the opinion of the Investigator, deems the participant unsuitable for the study

Additionally, for healthy participants (cohorts 1, 2, 5 and 6)

18. HBsAg positive

Additionally, for participants with well controlled CHB (cohorts 3 and 4)

19. Co-infection with hepatitis delta
20. Documented cirrhosis or advanced fibrosis indicated by a liver biopsy within 6 months prior to screening.

    In the absence of an appropriate liver biopsy, either 1 of the following:

    - Screening Fibroscan with a result >9 kPa within ≤6 months of screening or
    - Screening FibroTest >0.48 and AST to platelet ratio index of >1

    In the event of discordant results between non-invasive methods, the Fibroscan result will take precedence.
21. ALT >3 × ULN, INR >1.5 unless the participant was stable on an anticoagulant regimen affecting INR, albumin <35 g/L, total bilirubin >34.2 µmol/L, platelet count <100 x 10^9/L
22. A history of liver decompensation (e.g. ascites, encephalopathy or variceal haemorrhage)
23. Prior or current hepatocellular carcinoma
24. Chronic liver disease of a non-HBV aetiology
25. Any herbal supplements and or other medicines with potential liver toxicity within the previous 3 months prior to enrolment into this study

4.3.1 Removal of Participants from Vaccination or Assessment

4.3.1.1 *Contraindications to Vaccination*

The following events constitute contraindications to administration of study vaccine at that point in time; if any one of these events occurs at the time scheduled for vaccination, the participant may be vaccinated at a later date, or withdrawn from the study at the discretion of the Investigator.

- Acute disease at the time of vaccination. Acute disease is defined as the presence of a moderate or severe illness with or without fever. All study vaccines can be administered to participants with a minor illness such as diarrhoea, mild upper respiratory infection with or without low-grade febrile illness
- Temperature of >38°C (100.4°F) at the time of vaccination

4.3.1.2 *Study Discontinuation*

Participants may choose to discontinue study assessments by withdrawal of consent. If the participant discontinues from taking the study vaccine but does not withdraw consent, the Investigator or research worker should attempt to continue safety follow-up assessments.

Participants **may** be withdrawn from the study vaccine, but followed up for safety, in the event of:

- A severe adverse event or SAE
- Difficulties in obtaining blood or other samples
- Failure of the participant to comply with the protocol requirements or to cooperate with the Investigator
- For safety reasons, it being in the best interest of the participant that he/she be withdrawn, in the Investigator's opinion
- A positive pregnancy test or if the participant is non-compliant with the contraception requirements (see Section 4.2)
- Development of a medical condition that requires concomitant treatment with a potentially toxic therapy

Participants **must** be withdrawn from the study in the event of:

- Withdrawal of consent

4.3.2 Study Termination

The study may be terminated, either at one centre or all centres for the following reasons:

- The discovery of an unexpected, serious or unacceptable risk to participants enrolled in the study
- The decision on the part of the Sponsor to suspend or discontinue testing, evaluation or development of ChAdOx1-HBV. In the event of the Sponsor's decision to no longer supply study vaccine, ample notification will be provided so that appropriate adjustments to treatment can be made

- Serious failure of the Investigator to comply with International Council on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (ICH) Good Clinical Practice (GCP) [34] or local regulations
- Submission of knowingly false information from the study centre to the Sponsor, the Health Research Authority (HRA)/ REC or Competent Authorities
- Major and repeated, non-adherence to the protocol

The Sponsor must be informed immediately in the event of any major protocol deviation or serious breach of ICH GCP.

5 STUDY VACCINE

The Investigator must ensure that study vaccine is handled only by study team members who have been appropriately trained for the conduct of this clinical study and that dosing is only performed by study team members who fully understand the procedures outlined in this section, the Investigator's Brochure and the Investigational Product (IP) Handling Manual. A Delegation of Authority Log will be maintained by the study centre and will identify the individual(s) authorised to prepare and administer the study vaccine.

5.1 Study Vaccines Administered

ChAdOx1-HBV is a non-replicating viral vector encoding HBV consensus sequences from a group C genotype. It will be given by intramuscular injection into the deltoid muscle in the non-dominant arm.

The study vaccine to be given in each treatment group are shown in Table 1.

Table 1 Study Vaccine Treatment Groups in HBV001

Treatment Cohort	Vaccine Single Dose	N
Cohort 1 LD Healthy Participants	ChAdOx1-HBV 2.5×10^9 vp	5
Cohort 2 HD Healthy Participants	ChAdOx1-HBV 2.5×10^{10} vp	5
Cohort 3 LD Participants with CHB	ChAdOx1-HBV 2.5×10^9 vp	6
Cohort 4 HD Participants with CHB	ChAdOx1-HBV 2.5×10^{10} vp	6
Cohorts 5 and 6 HD Healthy Participants	ChAdOx1-HBV 2.5×10^{10} vp	30

Abbreviations: ChAdOx1-HBV=chimpanzee adenovirus-vectored hepatitis B virus vaccine; CHB=chronic hepatitis B virus; HD=high dose; LD=low dose; vp=viral particles

All study vaccine will be administered in a sequential manner. Participants will receive study vaccine on Day 0 only.

5.2 Identity of Study Vaccine

ChAdOx1-HBV will be manufactured to Good Manufacturing Practice, labelled according to local regulations, including Annex 13 of the Good Manufacturing Practice Directive [35] as detailed in the IP Handling Manual.

ChAdOx1-HBV is formulated in an A438 buffer comprising of 10 mM histidine, 35 mM NaCl, 7.5% sucrose (w/v), 1 mM $MgCl_2$, 0.1% (w/v) PS-80, 0.1 mM ethylenediaminetetraacetic acid (EDTA), 0.5% ethanol (v/v), pH 6.6 to a target concentration of 1×10^{11} vp/mL.

ChAdOx1-HBV will be supplied at a target sterile volume of 0.65 mL in 3 mL Type 1 borosilicate glass vials allowing 0.5mL to be drawn by syringe for dilution into high dose HBV vaccine and low dose HBV vaccine. Dilution to both low and high doses will be detailed in the IP Handling Manual. The vials are stoppered with sterilised European Pharmacopoeia/United

States Pharmacopoeia Type 1 chlorobutyl, rubber stopper and sealed with aluminium/copolymer caps. The vaccine is clear to slightly opaque in appearance

The study vaccines will be released by a qualified person.

5.3 Labelling, Packaging and Shipping

Study vaccine will be shipped by Catalent Pharma Solutions to the study centres on dry ice with a continuous temperature monitoring device.

Upon receipt, the pharmacist or designee must immediately inspect all vials for damage. Any damage or discrepancies from the packing list must be documented and promptly discussed with the Sponsor and the Study Monitor to determine the appropriate action. The temperature monitors should be downloaded according to the accompanying instructions in the shipment. Temperature records must be sent to the Sponsor and the centre will receive written confirmation that the vials are clinically released before they are used. If the temperature monitor has been out of range, then the shipment should be placed into quarantine and the Sponsor and Study Monitor contacted immediately to determine whether the study vaccine may be used.

At the start of the study, a sufficient number of study vaccine vials will be shipped to each study centre based on projected recruitment at each centre with approximately 20% overage in case of spillage or breakage. Further study vaccine supplies may be requested following the re-supply process in the IP Handling Manual.

5.4 Storage

Vials of study vaccine in the outer carton (to protect from light) must be stored in a continuously monitored freezer at ≤-65°C.

All study vaccine must be kept in a secured location with no access for unauthorised personnel.

5.5 Accountability

The pharmacist or designee is required to maintain accurate accountability records for the study vaccine. Instructions and forms to be completed and kept for accountability will be provided to the pharmacist or designee. If the pharmacist or designee wishes to use study centre-specific accountability forms, these must be reviewed and approved in advance by the Sponsor. Upon completion of the study, all accountability records will be copied and the copies returned to the Sponsor or designee. The originals must be maintained at the study centre with the rest of the study records.

The number of vials of study vaccine received and dispensed must be recorded on a participant by participant basis, including the applicable batch number.

5.6 Destruction or Disposal

The Sponsor will provide instructions for the return of unused ChAdOx1-HBV. Genetically modified organism (GMO) waste, including empty vials after full study vaccine accountability has been conducted, will be destroyed by appropriately licensed vendors. This destruction will be recorded with an original copy kept at site and a copy sent to the Trial Master File.

5.7 Method of Assigning Participants to Treatment Groups

At the screening visit, participants will be sequentially allocated a participant number once written, informed consent has been obtained. They will be identified by this number throughout the study.

In cohorts 1-4, the healthy and CHB participants will be allocated to low dose or high dose treatment depending on the treatment schedule prepared by statistician.

As cohorts 5 and 6 were introduced following completion of enrolment of the healthy participants in cohorts 1 and 2, all further healthy participants enrolled under protocol version 7.0 and 8.0 will receive high dose treatment.

Participants fulfilling entry criteria will be allocated to treatment based on a treatment schedule.

Once participant numbers have been assigned, no attempt will be made to use those numbers again. If a participant number is allocated incorrectly, no attempt will be made to remedy the error once the study vaccine has been dispensed. Any participants that are withdrawn prior to vaccination will be replaced on a case by case basis following discussion with the Sponsor. Discontinued participants following vaccination who do not complete all follow-up visits will be replaced at the determination of the Sponsor.

Any replacement participants will be given the next sequential participant number.

The treatment schedule list will be held by the statistician during the study.

5.8 Selection of Doses, Dosing Schedule and Administration

5.8.1 Selection of Doses in the Study

The doses of adenoviruses have been taken from historical context of innumerable previous adenovirus vaccine studies. A review of the adenoviral pre-clinical and clinical literature reveals that the overall T cell response may vary for CD4 and CD8+ T cells, and a number of studies have shown that the highest dose of T cell inducing vaccines is not necessarily the most protective dose [36,37]. It is hypothesised that the most therapeutic vaccines will need to induce both CD4+ and CD8+ T cells, as well as highly functional T cell responses. A scoring system will be used to choose the dose to move to further development, which is a combined score for CD4+ magnitude of a pooled cytokine response, CD4+ functionality (as measured by CD4+ IFN-γ mean fluorescence intensity from ICS flow cytometry) and CD8+ magnitude.

5.8.2 Selection and Timing of Dose for each Participant

5.8.2.1 *Dosing Schedule*

The healthy and CHB participants will be allocated to low dose or high dose treatment depending on the assignment. Vaccination will be performed on Day 0 after eligibility has been re-confirmed.

There are no restrictions in the time of day that the vaccination should be administered or in timing of vaccination in relation to meals.

5.8.2.2 *Study Vaccine Preparation and Administration*

ChAdOx1-HBV vials will be allowed to reach room temperature before use.

Doses of ChAdOx1-HBV will be prepared by serial dilution according to the procedure in the Investigational Product (IP) Handling Manual. Dilution kits of commercially available equipment will be provided by the Sponsor.

All vaccinations will be made by a suitably qualified health professional. A second study team member will be present to account for each vaccination given.

The suitably qualified healthcare professional will wear gloves, eye protection and an apron or laboratory coat/gown during the procedure. The vaccination site will be covered with a sterile dressing to minimise dissemination of the recombinant virus into the environment. This should absorb any virus that may leak out through the needle track. The sterile dressing will be kept at least 10 minutes after vaccination. The dressing will be discarded as GMO waste.

Allergic reactions to vaccination are possible, therefore, appropriate drugs and medical equipment to treat acute anaphylactic reactions must be immediately available and a medically qualified study team member trained to recognise and treat anaphylaxis must be present in the clinic during the entire vaccination procedure and post-vaccination observation period.

5.8.2.3 *Dose and Schedule Modifications*

Every effort will be made to administer the planned doses of study vaccine.

5.9 Prior and Concomitant Vaccines and Medications

Prior (within the previous 28 days) and concomitant medications include prescription and non-prescription drugs or other treatments, and any vaccines other than the study vaccine.

Recently used and ongoing medications will be reviewed and recorded during screening.

Concomitant medications used by participants post-vaccination will coincide with the collection period of adverse events. All medications taken will be recorded in the eDiary and will be discussed with the participant at the scheduled clinic visits during the study. All medications taken in the first 28 days will be recorded in the electronic Case Report Form (eCRF). Thereafter, only medications taken to treat SAEs will be recorded.

The name of the medication, treatment start and stop dates (or ‘ongoing’), and indication must be recorded on the concomitant medication eCRF. The indication recorded on the concomitant medication eCRF must correspond to a medical term/diagnosis recorded on the adverse event eCRF, or to a pre-existing condition noted in the participant’s medical history, or be noted as prophylaxis, e.g. dietary supplement.

5.10 Treatment Compliance

Vaccine administration will take place at the study centre and will be performed by a suitably qualified healthcare professional. The precise date and time of vaccination shall be documented in the source documents and eCRF. The study will be monitored by a Study Monitor approved by the Sponsor. During monitoring visits, all procedures will be monitored for compliance with the protocol. Source documents will be reviewed and compared with the data entries in the eCRF to ensure consistency.

5.11 Post-study Vaccine

The Sponsor does not intend to provide ChAdOx1-HBV after the end of the study or after any early participant withdrawal, as there is currently no clinical data supporting their efficacy. These participants will remain under the care of their healthcare professionals and treated according to standard care.

6 STUDY PROCEDURES AT EACH VISIT/SCHEDULE OF ASSESSMENTS

The study consists of the following:

- A 42-day screening period before the start of the study period
- A study period of 168 days, consisting of a vaccination day on Day 0, a follow-up telephone call on Day 1 and follow-up visits on Days 7, 14, 28, 56. Participants in cohorts 1-4 only, will also have follow up visits on Day 56 and Day 168. End of study visit procedures will be performed at the final visit.

The schedule of assessments at each visit is shown in Table 2 and listed in Section 6.1 to Section 6.4. The study assessments are described in Section 7.

Note: any additional visits or assessments performed during the study e.g. to assess adverse events must also be recorded on the unscheduled procedures or unscheduled visits in the eCRF.

Table 2 Overall Schedule of Assessments at Each Study Visit

Visit/Call	Screen	Vaccination			Follow-up						End of Study
Timepoint	Day-42 to -1	Day 0			Day 1	Day 7±1	Day 14±1	Day 28±2	Day 56±3 (cohorts 1-4)	Day 84±7[a]	Day 168±7 (cohorts 1-4)
		Pre-	0	Post-							
Informed consent	X										
Baseline/eligibility variables											
Demographics	X										
Inclusion and exclusion criteria	X	X									
Height and Weight	X										
Medical and disease history	X	X									
HIV Ab, HCV Ab, HBsAg, HDV Ab[b]	X										
Urinalysis	X										
Urine pregnancy test (β-hCG) [c]	X	X						X	X	X	X
Laboratory eligibility and safety tests											
Haematology [d]	X	X				X	X	X	X	X	X
Biochemistry[d]	X	X				X	X	X	X	X	X
Liver function tests[e]	X	X				X	X	X	X	X	X
Study vaccination											
Vaccination			X								
Post-vaccination observation[f]				X							
Other Safety assessments											
Full physical examination	X	X									
Directed physical examination if required						X	X	X	X	X	X
Vital signs[g]	X	X		X		X	X	X	X	X	X
Local/systemic reactogenicity [h]		X		X	X						
Unsolicited adverse events [i]	X	X	X	X	X	X	X	X			
Serious adverse events and adverse events of special interest	X	X	X	X	X	X	X	X	X	X	X
Prior and concomitant medications	X	X	X	X	X	X	X	X	X	X	X
Blood for HBV disease markers											
Healthy participants (HBsAb, HBcAb)		X									X[j]
CHB (HBV DNA, HBsAg quantitative)	X	X						X	X	X	X

Visit/Call	Screen	Vaccination			Follow-up						End of Study
Timepoint	Day-42 to -1	Day 0			Day 1	Day 7±1	Day 14±1	Day 28±2	Day 56±3 (cohorts 1-4)	Day 84±7[a]	Day 168±7 (cohorts 1-4)
		Pre-	0	Post-							
CHB (HBeAg, anti-HBe, anti-HBs, pgRNA, HBcAg, anti-HBc)		X						X	X	X	X
Immunogenicity assessments											
Blood for cellular immunogenicity [k]		X					X	X	X	X	X
Blood for neutralising antibodies (cohorts 5 and 6 only)			X							X	
Liver fine needle aspirates[l]†	X									X	

Abbreviations: ALP=alkaline phosphatase; ALT=alanine transaminase; aPTT=activated partial thromboplastin time; AST=aspartate transaminase; CHB=chronic hepatitis B virus; DNA=desoxyribonucleic acid; GGT=gamma-glutamyl transpeptidase; HBcAb=hepatitis B core antibody; HBsAb=hepatitis B surface antibody; β-hCG=beta human chorionic gonadotrophin; HBcAg=hepatitis B core-related antigen; HBV=hepatitis B virus; HBeAg=hepatitis B e antigen; HBsAg=hepatitis B surface antigen; HCV=Hepatitis C virus; HDV=Hepatitis D virus; HIV=human immunodeficiency virus; ICS=intracellular cytokine staining; INR= international normalised ratio; LFNA=liver fine needle aspirate; pgRNA=pre-genomic RNA; PBMCs=peripheral blood mononuclear cells; PT=prothrombin time
†Optional assessments

[a] End of Study Visit for cohorts 5 and 6

[b] HDV Ab serology and HBsAg quantitative test will be done in CHB participants only; HBsAg qualitative test will be done in healthy participants; all participants will be tested for HIV and HCV serology

[c] Female participants only

[d] Full haematology (including PT/INR and aPTT) and biochemistry panel

[e] Measurement of ALP, GGT, ALT, AST and total bilirubin

[f] The first participant in each cohort will be assessed for 1 hour in case of immediate adverse events (timed after the end of study vaccine administration). All other participants will be assessed for 30 minutes

[g] Pulse, blood pressure and temperature

[h] Captured during clinic visits and then via eDiary for 3 days post-vaccination

[i] Recorded in the eCRF from the date the informed consent is signed, at all clinic visits to cover the period since the previous visit and during the visit and up to 28 days post-vaccination

[j] If negative at baseline, at discretion of the investigator

[k] To be processed into PBMCs for analysis by ICS

[l] Only in CHB participants who consent to LFNAs after confirming eligibility. Coagulation profile must be assessed prior to repeat LFNA on Day 84. For those consenting to LFNAs, blood for PT/INR and aPTT testing must be collected

6.1 Screening and Baseline Pre-Dose Assessments

Written informed consent for participation in the study must be obtained before performing any study specific screening tests or evaluations according to the process in Section 13.4.

Unsolicited adverse events will be recorded from the date the informed consent is signed and at all clinic visits up to 28 days post-vaccination, by questioning the participant at each visit whether they had any adverse events since the previous visit and by observing the participant during clinic visits.

Serious adverse events and AESIs will be recorded from the date the informed consent is signed until the end of the study and/or resolved or until participant contact discontinues.

The following will be performed at screening:

- Demographic data will be collected
- Height and weight will be measured
- A medical and disease history will be recorded, including any medications being taken
- A blood sample will be taken for HIV, HCV, HBV, and in CHB participants only, hepatitis D Virus (HDV) diagnostic testing
- Blood samples will be taken for haematology (including PT/INR and aPTT), biochemistry and LFTs
- A urinalysis dipstick test will be performed
- A urine pregnancy test will be performed, childbearing status recorded and if of childbearing potential, assessment of contraceptive measures performed
- A full physical examination will be performed
- Vital signs (pulse rate, blood pressure and temperature) will be measured

All screening evaluations must be completed and reviewed to confirm that participants meet all eligibility criteria before the first dose of study vaccine. The Investigator will maintain a screening log to record details of all participants screened and to confirm eligibility or record reasons for screening failure, as applicable.

Concomitant medications will be recorded throughout the study by questioning the participant at each visit whether they took any concomitant medications since the previous visit.

In CHB participants who consent to LFNAs: a LFNA will be performed within the screening period, after screening tests have shown eligibility, as directed by the Principal Investigator or delegated physician. These aspirates and subsequent assessments are optional

NB. Coagulation profile must be assessed prior to repeat LFNA

6.2 Treatment Day Assessments (Day 0)

The following will be performed pre-vaccination:

- Re-check of eligibility criteria
- A urine pregnancy test will be performed
- Blood samples will be taken for haematology (including PT/INR and aPTT), biochemistry and LFTs

- A full physical examination will be performed
- Vital signs (pulse rate, blood pressure and temperature) will be measured
- A baseline assessment of local and systemic reactogenicity will be performed
- Blood samples will be taken for immunogenicity assessments (including neutralising antibodies for cohorts 5 and 6 only)

Participants will then receive study vaccine by intramuscular injection according to Table 1. All participants will be assessed for at least 30 minutes in the clinic after receiving the vaccine injection in case of immediate adverse events. The first participant in each cohort will be observed for 1 hour.

The following will be performed post-vaccination:

- The vaccination site will be covered with a sterile dressing during the observation period; it will then be removed and disposed of as GMO waste
- Vital signs (pulse rate, blood pressure and temperature) will be measured at the end of the observation period
- Local and systemic reactogenicity will be assessed at the end of the observation period
- On Day 0, an eDiary, tape measure and thermometer will be provided to perform self-assessment of local and systemic reactogenicity for 3 days after the vaccination. On Day 0 participants will be reminded to do the following daily for the next 3 days:

– Take and record their temperature
– Measure and record the size of vaccination site redness
– Assess the other solicited adverse events listed in the eDiary

6.3 Follow-up Period Assessments

6.3.1 Telephone Call: Day 1

- eDiary to collect local and systemic reactions during 3 consecutive days post-vaccination

6.3.2 Clinic Visit: 7

- A symptom-directed physical examination will be performed if required
- Vital signs (pulse rate, blood pressure and temperature) will be measured
- Blood samples will be taken for haematology (including PT/INR and aPTT), biochemistry and LFTs

6.3.3 Clinic Visit: Day 14

- A symptom-directed physical examination will be performed if required
- Vital signs (pulse rate, blood pressure and temperature) will be measured
- Blood samples will be taken for haematology (including PT/INR and aPTT), biochemistry and LFTs
- Blood samples will be taken for immunogenicity assessments

6.3.4 Clinic Visit: Days 28, 56 (Cohorts 1-4) and 168 (End of Study Visit Cohorts 1-4)

- A urine pregnancy test will be performed
- A symptom-directed physical examination will be performed if required
- Vital signs (pulse rate, blood pressure and temperature) will be measured
- Blood samples will be taken for haematology (including PT/INR and aPTT), biochemistry and LFTs
- Blood samples will be taken for immunogenicity assessments

6.3.5 Clinic Visit: Day 84 (including End of Study Visit Cohorts 5 and 6)

- A symptom-directed physical examination will be performed if required
- Vital signs (pulse rate, blood pressure and temperature) will be measured
- Blood samples will be taken for haematology (including PT/INR and aPTT), biochemistry and LFTs
- Blood samples will be taken for immunogenicity assessments (including neutralising antibodies for cohorts 5 and 6 only)
- In CHB participants who consent to LFNAs: an LFNA will be performed with additional PT/INR and aPTT testing, as directed by the Principal Investigator or delegated physician. These aspirates and subsequent assessments are optional

6.4 Assessments at Unscheduled Visits

Unscheduled visits may be required according to the participant's condition; assessments performed will be recorded in the eCRF.

6.5 Participant Discontinuation

Participants have the right to withdraw from the study at any time for any reason, including their future care. The Investigator should however try to find out why a participant withdraws from the study and document the reason for withdrawal in the source documents and eCRF.

If there is a medical reason for withdrawal, the participant will remain under the supervision of the Investigator until satisfactory health has returned.

Participants who are withdrawn from the study prior to completion of the scheduled study procedures for any reason should complete the end of study visit assessments as far as possible.

7 DETAIL OF STUDY ASSESSMENTS

7.1 Demographic and Baseline Assessments

The following will be collected at screening to determine eligibility and baseline status of the participant. Baseline safety assessments will also be performed as detailed in Section 7.3.

7.1.1 Demographic Data and Baseline Variables

Demographic data will include age, gender and race/ethnicity, smoking history, use of alcohol and drugs of abuse. Height and weight will also be measured.

7.1.2 Medical and Disease History

Disease history (including date of diagnosis) and any allergies or other clinically significant diseases and surgeries will be recorded.

7.1.3 Prior Medications and Therapies

All prior therapies and procedures will be recorded.

All other prior medications (e.g. prescription drugs, over the counter drugs, herbal/homeopathic remedies, nutritional supplements) used by the participant in the 28 days before the screening visit will also be recorded (see Section 5.9).

7.1.4 Laboratory Eligibility Tests

Haematology, biochemistry, LFTs and viral serology blood tests and urinalysis will be performed at screening for all participants. All of these samples will be analysed at the local laboratory in the hospital. Results from tests must be reviewed by the Investigator (or a designee who is a medically qualified study team member) and managed in accordance with the study centre procedures. The clinical significance of all results outside of the normal range will be determined by the Investigator to determine eligibility.

Abnormal results and findings will be discussed as applicable with the participant and the participant will be referred for follow-up with their healthcare provider if necessary.

7.1.4.1 *Haematology*

A sample of venous blood will be collected according to the local laboratory procedures.

The following will be measured: full blood count, haemoglobin, haematocrit, erythrocytes, mean cell volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, leukocytes, neutrophils, lymphocytes, monocytes, eosinophils, basophils, fibrinogen, PT/INR, APTT and platelets.

7.1.4.2 *Biochemistry and Liver Function Tests*

A sample of venous blood will be collected according to the local laboratory procedures.

The following biochemistry parameters will be measured: sodium, potassium, urea, creatinine and albumin and LFTs (ALP, GGT, ALT, AST and total bilirubin).

7.1.4.3 *HIV, HBV, HCV and HDV Diagnostic Testing*

Blood samples will be tested for:

- In all participants:
  - HIV antibodies
  - HCV antibodies
- In Healthy Volunteers: qualitative HBsAg, HBsAb, HBcAb
- In CHB participants: quantitative HBsAg, HBeAg, HBcAg, anti-HBs, anti-HBe, anti-HBc, HBV DNA, pgRNA, HDV serology

7.1.4.4 *Urinalysis*

A urine sample will be collected in a sterile container for dipstick urinalysis. The following will be tested: blood, bilirubin, glucose, ketones, leucocytes, nitrite, pH, protein, specific gravity and urobilinogen. The Investigator may order additional urine tests to be performed.

7.1.5 Urine Pregnancy Test

A urine pregnancy test will be performed in female participants of childbearing potential at screening and confirmed to be negative pre-vaccination on Day 0, as per local standard procedures and at each of the timepoints according to Table 2.

7.2 Immunogenicity Assessments

Immunogenicity samples (PBMCs) will be taken for planned and potential exploratory analyses at each of the timepoints according to Table 2.

Immunogenicity studies will include (but are not limited to) analysis of samples ex vivo by multiparameter flow cytometry (including Mean Fluorescence Intensity measurements) and ex vivo IFN-γ ELISpot assays. The latter will be used for epitope mapping. Innate immune responses will be measured primarily by RNA sequencing of blood and may be assessed in other assays if cell numbers permit. Additional immunogenicity assessments may be performed.

7.2.1 Secondary Outcomes in Blood Samples

Peripheral blood mononuclear cells will be isolated from blood samples taken at each of the timepoints according to Table 2, and tested for recognition of overlapping HBV peptide pools using ICS for multiple cytokines including, and not limited to IFN-γ, and will be analysed by multiparameter flow cytometry to obtain frequencies of HBV-specific T cells within CD4+ and CD8+ T cell subsets before and after vaccination (magnitude of vaccine-induced response). The mean/median fluorescence intensity of cytokine signals will be determined to provide a composite assessment of HBV-specific T cell avidity.

Serum blood samples in CHB participants will be used for analysis of HBV DNA, HBeAg, HBeAb, HBsAg and HBsAb.

Blood samples of healthy participants in cohorts 5 and 6 will be used for analysis of total T cell response to ChAdOx1-HBV antigens using a peptide-stimulated ELISpot assay.

7.2.2 Exploratory Analysis

7.2.2.1 *Exploratory Analysis in Blood Samples*

HBV-specific CD4+ and T cell subsets within PBMC will be further defined by phenotypic markers of activation, differentiation and memory. Peripheral blood mononuclear cells will also be used in IFN-γ ELISpot assays to determine the breadth of HBV-specific T cell responses (defined by number of peptide pools or individual peptides recognised). These data may be used to build immunologic models of the immune system.

Blood samples will be used for extraction of RNA and subsequent unbiased analysis of innate immune responses before and after vaccination by RNA sequencing.

Serum blood samples will be used for analysis of pgRNA and HBcAg.

7.2.2.2 *Exploratory Analysis in Liver Fine Needle Aspirates*

Liver fine needle aspirate assays will be used to quantify and characterise intrahepatic immune and parenchymal cells and/or HBV DNA and RNA transcripts. The local immune landscape at Day 84 will be compared with baseline. Additional exploratory assessments may be performed. This is an optional procedure for CHB participants only. The Principal Investigator will provide a separate consent for this procedure which will explain the procedure in full and all associated risks.

7.3 Safety Assessments

7.3.1 Adverse Events

Recording and reporting of adverse events is described in detail in Section 8.2.

7.3.1.1 *Solicited Adverse Events*

Solicited adverse events are events the participant is specifically asked about. These adverse events are commonly observed soon after receipt of vaccines and relate to local and systemic signs and symptoms. Solicited adverse events will be collected for 3 days post-vaccination[1]. These will be recorded daily in the eDiary for all participants.

For this study, solicited adverse events to be collected include:

- Vaccination site reactions: pain, induration, warmth, erythema (redness)
- Systemic adverse events: feverishness, chills, myalgia, fatigue, headache, nausea, arthralgia, malaise

Participants will be asked to record the presence of these symptoms and grade the severity as described in Section 8.2.3.1. Oral temperature will be measured using the thermometer provided and the diameter of induration will be measured with the tape measure provided.

7.3.1.2 *Unsolicited Adverse Events*

Unsolicited adverse events are other events meeting the criteria for adverse events (see Section 8.2.1) apart from those the participant is specifically asked about. Unsolicited

[1] In the event that they persist longer than 3 days then an adverse event will be recorded

non-serious adverse events will be collected from the date the informed consent is signed to 28 days post-vaccination. Hospitalisations, other SAEs and AESIs will be collected for the duration of the study.

Participants will collect unsolicited adverse events in the eDiary and these will be reviewed during clinic visits with the participant and relevant information (event term, start and end date, severity, causality, outcome and seriousness) collected and entered into the eCRF as described in Section 8.2.3.

7.3.2 Laboratory Safety Tests

Laboratory safety tests will be performed at each of the timepoints according to Table 2, as per Sections 7.1.4.1 and 7.1.4.2. Extra laboratory safety tests may be performed if the Investigator deems them to be necessary to fully evaluate an adverse event. In the event that the Investigator elects to order non-protocol-specified laboratory tests, the Investigator must record the rationale for the tests and a determination of clinical significance of the result in the source documents. The Investigator must keep the local Medical Monitor informed of adverse events of clinical significance.

Abnormal results and findings will be discussed as applicable with the participant, and the participant will be referred for follow-up with their healthcare provider if necessary.

Results from the LFTs obtained on the study must be reviewed by the Investigator (or a designee who is a medically qualified study team member) and managed in accordance with the study centre procedures. The clinical significance of all results outside of the normal range will be determined by the Investigator and may be reported as an adverse event at the discretion of the Investigator.

7.3.3 Physical Examination

A skin, respiratory, cardiovascular, abdominal and lymphatic system examination will be performed at screening and pre-vaccination on Day 0. A symptom-directed physical examination will be performed at all other clinic visits if required. Results will be recorded as normal, abnormal and not clinically significant or abnormal and clinically significant.

7.3.4 Vital Signs

Vital signs will be performed at screening, pre-vaccination and post-vaccination (at the end of the observation period) at the vaccination visit, at all visits during the follow-up and the end of study visit. At each timepoint blood pressure, pulse rate and oral temperature, will be measured after the participant has been in a sitting position for 5 minutes.

8 SAFETY MANAGEMENT

8.1 Responsibilities for Ensuring the Safety of Study Participants

The national Competent Authority, the study Sponsor, the Institution through which the research is performed, and all members of the Investigator's study team share responsibility for ensuring that participants in this study are exposed to the least possible risk of adverse events that may result from participation in this protocol.

8.1.1 Investigator

The Investigator has a personal responsibility to closely monitor study participants and an inherent authority to take whatever measures necessary to ensure their safety. The Investigator may delay a participant's study vaccine administration or pause study vaccine administration in the whole study if they have some suspicion that the study vaccine might place a participant at significant risk. The Investigator determines severity and causality for each adverse event.

Responsibilities of the Investigator may be assigned to a designee who is a medically qualified study team member, however the accountability for the specific task remains with the Investigator.

8.1.2 Study Sponsor

The Sponsor also has an institutional responsibility to ensure participant safety. This responsibility is vested in the local Medical Monitor and an SMC.

8.1.3 Local Medical Monitor

The local Medical Monitor (LMM) is the Sponsor's medical representative. The LMM reviews safety information collected throughout the study. The LMM can add another causality to the Investigator's causality assessment, however the causality assessment given by the Investigator must not be downgraded by the LMM. If the LMM disagrees with the Investigator's causality assessment, the opinion of both the Investigator and the Sponsor should be provided with the report.

8.1.4 Safety Monitoring Committee

The SMC will constitute the Sponsor Representative, the Principal Investigator at each centre, the local Medical Monitor and the Chief Investigator. The SMC will operate in accordance with the study-specific charter, which will be agreed prior to the start of enrolment.

An SMC will be appointed to review the study data and make recommendations concerning the continuation, modification, or termination of the study. The SMC will perform the following:

- Scheduled evaluation of study conduct, progress and review of the safety data. Scheduled meetings will take place as follows:
  - Kick off meeting before the first participant is enrolled
  - At least 48 hours after the first healthy participant is dosed in cohort 1
  - At least 48 hours after cohort 1 dosing is complete
  - At least 48 hours after cohort 2 dosing is complete
  - At least 48 hours after the first CHB participant is dosed in cohort 3
  - At least 48 hours after cohort 3 dosing is complete

- The SMC will be notified within 24 hours of the Investigators' being aware of any severe local or systemic reaction or vaccine-related SAE. If a severe local or systemic reaction or vaccine-related SAE occurs at any time point during the study, the SMC will review the case immediately.
- The SMC will be convened if any of the stopping or holding rules are met (see Section 3.1.3)

The SMC has the power to place the study on hold if deemed necessary

8.1.5 Data Monitoring Committee

A DMC will be appointed to perform unscheduled reviews of the available data including safety and tolerability study data and make recommendations concerning the continuation, modification, or termination of the study if one of the study stopping or holding rules defined in Section 3.1.3 is met.

There will be a minimum of three appropriately qualified independent committee members of whom one will be the designated chair. The DMC will operate in accordance with the study-specific charter, which will be agreed prior to the start of enrolment.

The DMC will be notified within 24 hours of the Sponsor being aware of any study stopping or holding rules.

The chair of the DMC may be contacted for advice and independent review by the Investigator or Sponsor in any other situation where the Investigator or Sponsor feels independent advice or review is important.

8.1.6 Research Ethics Committee

The HRA/REC has institutional responsibility for the safety of participants in clinical studies. The HRA/REC has the authority to terminate, suspend or require changes to a clinical study.

8.1.7 Competent Authority

Since the Competent Authority receives all expedited safety reports for the study it also has the authority to terminate, suspend or require changes to a clinical study.

8.2 Adverse Events

8.2.1 Definitions

8.2.1.1 *Adverse Event*

An adverse event is:

- Any untoward medical occurrence in a patient or clinical investigation participant administered a pharmaceutical product and which does not necessarily have to have a causal relationship with this treatment
- An adverse event can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom or disease temporally associated with the use of study vaccine, whether or not considered related to the investigational product

An adverse event includes but is not limited to:

- Any clinically significant worsening of a pre-existing condition

- An adverse event occurring from overdose (i.e. a dosage higher than that prescribed by a healthcare professional for clinical reasons, or a dosage higher than that described in the Investigator's Brochure or on the marketed product label) of an investigational or marketed product, whether accidental or intentional
- An adverse event occurring from abuse (e.g. use for non-clinical reasons) of an investigational or marketed product
- An event related to a medical procedure or associated with the discontinuation of the previous use of an investigational or marketed product required by protocol (protocol-related adverse event)
- Not all vital sign or laboratory abnormalities will be considered an adverse event. Usually these will be considered as an adverse event:
  - If Grade 3 (severe) or greater in severity
  - If judged to be clinically significant by the Investigator
  - If accompanied by clinical symptoms
  - If meeting the definition of a SAE
  - If resulting in dose modification, interruption or discontinuation of study vaccine
  - If requiring specific treatment

8.2.1.2 *Adverse Reaction*

An adverse reaction is defined as any untoward and unintended response to study vaccine related to any dose administered.

An unexpected adverse reaction is an adverse reaction in which the nature or severity of which is not consistent with the Investigator's Brochure or Prescribing Information.

8.2.1.3 *Serious Adverse Event*

SAEs are adverse events meeting at least one of the following criteria:

- It results in **death** (i.e. the adverse event caused or led to the fatality). Serious does not describe an event which hypothetically might have caused death if it were more severe
- It was immediately **life-threatening** (i.e. the adverse event placed the participant at immediate risk of dying. It does not refer to an event which hypothetically may have led to death if it were more severe)
- It required inpatient **hospitalisation** or prolonged hospitalisation beyond the expected length of stay. Hospitalisations for scheduled treatments and elective medical/surgical procedures related to a pre-existing condition that did not increase in severity or frequency following receipt of study vaccine, are **not** serious by this criterion. Hospitalisation is defined as a hospital admission or an emergency room visit for a period greater than 24 hours
- It resulted in a persistent or significant **disability/incapacity** (i.e. substantial reduction of the participant's ability to carry out activities of daily living)
- It resulted in a **congenital anomaly or birth defect** (i.e. an adverse finding in a child or foetus of a participant exposed to the study vaccine prior to conception or during pregnancy)

- Other **medically important conditions** that may not result in death, threaten life or require hospitalisation (i.e. the adverse event does not meet any of the above serious criteria) may be considered an SAE when, based on appropriate medical judgment, they may jeopardise the participant and require medical or surgical intervention to prevent one of the serious outcomes listed in these criteria (e.g. allergic bronchospasm requiring intensive treatment in an emergency room or at home; blood dyscrasias or convulsions that do not result in hospitalisation, or the development of drug dependency or drug abuse)

An **SAE** is an adverse event meeting the outcome criteria for seriousness regardless of relationship to the study vaccine. The following are not (and should not be reported as) SAEs: hospitalisation for scheduled treatments and elective medical/surgical procedures related to a pre-existing condition that did not increase in severity or frequency following receipt of study vaccine, and admissions for social reasons.

8.2.1.4 *Suspected Unexpected Serious Adverse Reaction*

A SUSAR is any suspected adverse reaction related to the study vaccine that is both unexpected and serious.

8.2.2 Study Reporting Period for Adverse Events

The reporting period for all adverse events begins on the date the informed consent is signed. Solicited adverse events are recorded in the eDiary up to 3 days post-vaccination. All other events are recorded in the eCRF up until Day 28. All SAEs and or Adverse events of special interest are recorded until at least 168 days after the last study vaccination dose and for all serious or study vaccine-related adverse events until these are resolved or until participant contact discontinues.

Adverse event information will be collected at study visits and participant will be instructed to call study team members to report any abnormalities during the intervals between study visits and to come to the study centre if medical evaluation is needed and the urgency of the situation permits.

8.2.3 Recording and Assessment of Adverse Events by the Investigator

Solicited adverse events are recorded in the eDiary up to 3 days post-vaccination. All other adverse events are recorded in the eCRF up to until Day 28. All adverse events, both those observed by study team members and those spontaneously reported by the participant, will be recorded in the eCRF. Solicited adverse events will be reported using pre-defined terms. Unsolicited adverse events will be reported using a recognised medical term or diagnosis that accurately reflects the event.

Adverse events will be assessed by the Investigator, or a medically qualified study team member, for severity, relationship to study vaccine, action taken, outcome and whether the event meets criteria as an SAE according to the following guidelines:

8.2.3.1 *Assessment of Severity*

The severity of adverse events will be graded according to the toxicity table in Appendix 1. The scale shown in Table 3 will be used to assess severity of adverse events not listed in Appendix 1.

Table 3 Severity Grading of Adverse Events not listed in the Toxicity Table for Clinical and Laboratory Abnormalities

Grade	Severity	
1	Mild	Asymptomatic or mild symptoms; clinical or diagnostic observations only; or intervention not indicated; no disruption of normal daily activity.
2	Moderate	Minimal, local, or non-invasive intervention indicated; discomfort sufficient to reduce or affect daily activity.
3	Severe	Severe or medically significant, but not immediately life-threatening; hospitalisation or prolongation of hospitalisation indicated; inability to work or perform normal daily activity.
4	Life-threatening	Represents an immediate threat to life.
5	Fatal	Death as a result of this adverse event

A change in severity of an adverse event will not be recorded as a new adverse event. Only the highest severity level that occurs during the entire period of the adverse event will be recorded on the eCRF with the onset and resolution dates encompassing the entire duration of the event.

Note: It is important to distinguish between serious and severe adverse events. Severity is a measure of intensity whereas seriousness is defined by the criteria in Section 8.2.1.3.

8.2.3.2 *Assessment of Relationship*

Solicited adverse events of vaccination site reactions will be considered causally related to study vaccine.

For all other adverse events, the Investigator will determine a **causal relationship** to the study vaccine. A number of factors will be considered in making this assessment, including: 1) the temporal relationship of the event to the administration of the study vaccine 2) whether an alternative aetiology has been identified and 3) biological plausibility.

Causality of all adverse events should be assessed by the Investigator using the following question:

"Is there a reasonable possibility that the adverse event may have been caused by the study vaccine?"

- YES (related): There is a reasonable possibility that the study vaccine contributed to the adverse event
- NO (not related): There is no reasonable possibility that the adverse event is causally related to the administration of the study vaccine. There are other, more likely causes and administration of the study vaccine is not suspected to have contributed to the adverse event

The Investigator determines causality. The Sponsor and LMM can add another causality assessment to the Investigator's causality assessment. The causality assessment given by the Investigator must not be downgraded by the Sponsor. If the Sponsor disagrees with the

Investigator's causality assessment, the opinion of both the Investigator and the Sponsor should be provided with the report.

Every effort should be made by the Investigator to determine the existence of any pre-existing conditions (e.g. headache in adults or rashes in infants on study Day 0 with onset prior to study vaccination) that must be taken into consideration when assessing causal relationship of an adverse event. Pre-existing conditions should be recorded in the eCRF as baseline history and substantiated by appropriate source documentation. Intermittent conditions such as headaches in adults or irritability in infants may not be present on study Day 0 but may represent an adverse event if the intensity or duration of the event is worse than usual following study vaccine.

8.2.3.3 *Action Taken with Study Vaccine*

The action taken with the study vaccine will be recorded as None, as only one dose is given.

8.2.3.4 *Assessment of Outcome*

The outcome of the adverse event will be assessed as:

- Recovered/resolved
- Recovered/resolved with sequelae
- Not recovered/resolved
- Unknown
- Fatal

8.2.4 Reporting Requirements and Procedures for Serious Adverse Events

All SAEs are reported to the Sponsor for the entire study period. Suspected, unexpected, serious adverse reactions are reported even after the study is over, if the Sponsor, local Medical Monitor or Principal Investigator becomes aware of them. The study centre will be provided with specific reporting procedures including the SAE paper form and any supplemental reporting forms to be used. SAEs will be reported on the SAE paper form and the adverse event eCRF using a recognised medical term or diagnosis that accurately reflects the event.

SAEs will be assessed for severity, causal relationship to the study vaccine or alternative aetiology by the Sponsor and Investigator. The onset and resolution dates of the event and medical care taken in response to the event will be documented. If the event has not resolved by the end of the study is enrolled into, it will be documented as "ongoing" on the eCRF, however, follow-up of the SAE must continue until resolved or the condition has stabilised. Information recorded on the eCRF must be substantiated in the source documents.

The SAE form for that event must be completed by the Investigator, within 24 hours of the study centre becoming aware of the event. The SAE form should be completed with all information known at the time and scanned and emailed to the local Medical Monitor and to [redacted]

Fatal or life-threatening SAEs that the Investigator suspects are related to the study vaccine should be telephoned to the local Medical Monitor immediately upon the Investigator's awareness of the event. If the local Medical Monitor is required by the protocol or chooses to suspend enrolment s/he shall immediately create a written memorandum for record to the study file and telephonically notify the Sponsor of this act.

Contact information for all safety personnel are contained in the Team Contact List, which will be stored at the study centre in the ISF and maintained by the study Sponsor.

Investigators must not wait to collect additional information to fully document the event before notifying the local Medical Monitor of an SAE. The initial notification should include the following (at minimum):

- Protocol number and name and contact number of the Investigator
- Participant identification number (and initials and date of birth, if available)
- Investigational product
- SAE(s)

The Sponsor will notify the DMC of all SUSARs within 24 hours of becoming aware of an event and will provide all follow-up information in a timely manner.

8.2.4.1 *Follow-up Information on an SAE*

Appropriate diagnostic tests should be performed and therapeutic measures, as medically indicated, should be instituted. Appropriate consultation and follow-up evaluations should be carried out until the event has resolved or is otherwise explained by the Investigator. For all SAEs, the Investigator is obligated to pursue and provide information to the Sponsor. In addition, an Investigator may be requested by the Sponsor to obtain specific information in an expedited manner. This information may be more detailed than that captured on the SAE form. In general, this will include a description of the adverse event in sufficient detail to allow for a complete medical assessment of the case and independent determination of possible causality. Information on other possible causes such as concomitant medication and illnesses must be provided.

After the initial SAE report, the Investigator is required to follow each participant proactively and to report new significant follow-up by submitting an updated SAE report form to the Sponsor (or designee). New significant information includes, but is not limited to, the following:

- New signs or symptoms or a change in the diagnosis
- Significant new diagnostic test results and hospital records if applicable
- Change in causality based on new information
- Change in the event's outcome, including recovery
- Additional narrative information on the clinical course of the event

8.2.4.2 *Required Follow-up for SAEs*

There should be attempted follow-up until the end of the study in all participants. If an SAE continues after this period, then the participant must be followed up until the event resolves or stabilises. The local Medical Monitor may specify a longer period of time, if required to assure the safety of the participant.

8.2.4.3 *Medical Review and Reporting by the Sponsor*

The Sponsor (or designee) will determine expedited reporting requirements for each reported SAE according to local requirements based upon:

- Investigator's assessment of causality and seriousness
- Expectedness

The expectedness of an SAE is assessed by the Sponsor in the overall classification of SAEs for expedited reportability. As no clinical data are available with ChAdOx1-HBV, all serious adverse reactions will be considered unexpected for the purposes of expedited reporting.

8.2.4.4 *Sponsor Responsibility for Expedited Safety Reports*

The Sponsor (or designee) will notify Investigators of all reportable SAEs. This notification will be in the form of an expedited safety report. Upon receiving such notices, the Investigator must review and retain the notice with other study related documentation.

The Investigator should also comply with the HRA/REC procedures for reporting any other safety information.

The Sponsor will ensure that SAEs are reported to the HRA/REC and Competent Authority according to local requirements.

Suspected, unexpected, serious adverse reactions and other significant safety issues reported from the development programme shall be reported to the relevant Competent Authorities according to local regulations (either as expedited safety reports and/or in aggregate reports), by the Sponsor (or designee).

8.2.5 Follow-up of Participants after Adverse Events

8.2.5.1 *Investigator Follow-up*

Treatment of any adverse events will be determined by the Investigator using his/her best medical judgment and according to current clinical practice guidelines. All applied measures as well as follow-up will be recorded in the appropriate eCRF.

The Investigator will continue follow-up on adverse events, including laboratory abnormalities and solicited adverse events, until the event has resolved, is otherwise satisfactorily explained, or the participant completes the study. The resolution date will be recorded on the eCRF as the last date on which the participant experienced the adverse event. If an adverse event resolution date is uncertain the Investigator should estimate the completion date based on medical judgment and interview of the participant. Approximate dates of resolution from phone or other communications may be taken as adverse event resolution dates. Some examples of estimation of adverse event resolution are: 1) an asymptomatic laboratory abnormality on one visit that has not been followed-up between visits but has resolved by the next visit may be assumed to have resolved by the midpoint of the inter-visit interval; 2) A resolved adverse event that was treated may be assumed to have been resolved by the end of treatment.

Adverse events that are still present at the end of the study should be recorded as ongoing.

Information recorded on the eCRF must be substantiated in the source documents. If an adverse event evolves into a condition that becomes “serious,” it will be designated as serious on the adverse event eCRF and a supplemental SAE report form will be completed.

Follow-up for SAEs must continue until resolution and the outcome reported to the Sponsor, even if this extends beyond the SAE reporting period.

8.2.6 Post-study Adverse Events

At the final study visit, the Investigator should instruct each participant to report to the Investigator any subsequent adverse events that the participant's personal physician believes could be related to study vaccine or study procedures.

The Investigator should notify the Sponsor (or designee) of any death, SAE or other adverse event of concern occurring at any time after a participant has discontinued study vaccine if the event is believed to be related to study vaccine or study procedures. The Sponsor (or designee) should also be notified if the Investigator becomes aware of the development of cancer or a congenital anomaly/birth defect in a subsequently conceived offspring of a participant that participated in this study.

The Investigator should record the event in the eCRF; if the eCRF is no longer available, the Investigator should report the event directly to the Sponsor (or designee).

8.3 Other Events of Special Interest

Adverse events of special interest represent a subset of adverse events that include autoimmune diseases and other systemic disorders of interest which could potentially have an autoimmune aetiology. The Investigator should use clinical and scientific judgment in deciding whether other adverse events (i.e. events not listed here) could have an autoimmune origin and should therefore be reported as AESIs (see Section 8.2.4).

8.4 Overdoses or Incorrect Administration

Study vaccine overdose is the accidental or intentional use of the study vaccine in an amount higher than the dose being studied. An overdose or incorrect administration of study vaccine is not an adverse event unless it results in untoward medical effects:

- Any study vaccine overdose or incorrect administration of study vaccine should be recorded in the eCRF as part of the study vaccine administration information
- Any untoward medical effects associated with an overdose or incorrect administration of study vaccine should be recorded in the eCRF as an adverse event.

If the associated adverse event fulfils serious criteria, the event should be reported to the Sponsor (or designee) within 24 hours of knowledge of the event

8.5 Management of Pregnancy

8.5.1 Notification and Follow-up

Any pregnancies occurring during the study or within 6 months after the last dose of study vaccine must be reported to the Sponsor (or designee) within 24 hours of knowledge of the event. The paper pregnancy form should be completed with all information known at the time and scanned and emailed to [redacted]

Female participants of childbearing potential will be instructed to immediately inform the Investigator if they become pregnant during the reporting window and must be immediately discontinued from study vaccine. The Investigator should counsel the participant, discussing

the risks of the pregnancy and the unknown effects on the foetus. Monitoring of the participant should continue until conclusion of the pregnancy.

Male participants will be instructed to immediately inform the Investigator if their partner becomes pregnant during the reporting window. Attempts should be made to collect and report details of the course and outcome of any pregnancy in the partner of a male participant exposed to study vaccine. The pregnant partner will need to sign an authorisation for use and disclosure of pregnancy health information to allow for follow-up on her pregnancy. The Investigator may provide information on the risks of the pregnancy and the unknown effects on the foetus, to support an informed decision in cooperation with the treating physician and/or obstetrician.

8.5.2 Outcome

Additional information on the course and outcome of the pregnancy should be provided to the Sponsor (or designee) when available using the pregnancy report form.

The following pregnancy outcomes will be considered to be SAEs and should be reported according to the procedure in Section 8.2.4. Pregnancy is not considered an adverse event unless one of these criteria is met:

- Spontaneous abortion (as the Sponsor considers spontaneous abortions to be medically significant events)
- Any congenital anomaly/birth defect in a child born to a female participant or female partner of a male participant

9 DATA QUALITY ASSURANCE AND QUALITY CONTROL

9.1 Protocol Violations and Deviations

Major protocol deviations are defined as those that result in harm to the study participants or significantly affect the scientific value of the reported results of the study. Other deviations will be considered minor.

Protocol deviations will be recorded on the source documents and with an explanation for the deviation. All protocol deviations will also be recorded as specified in the monitoring plan and statistical analysis plan (SAP).

Major protocol deviations that meet the criteria for a serious breach of ICH GCP [34] should be reported to the Sponsor immediately. No deviation from the inclusion/exclusion criteria will be permitted.

9.2 Monitoring and Source Document Verification

The Sponsor will arrange for the study to be monitored in accordance with the principles of ICH GCP [34]. The frequency of monitoring visits will be determined by the rate of participant recruitment.

The following are examples of items that will be reviewed at these visits:

- Compliance with the protocol
- Consent procedure
- Source documents
- Adverse event procedures
- Storage and accountability of study vaccine

The monitoring visits also provide the Sponsor with the opportunity to ensure that timely participant accrual and the other Investigator's obligations and all applicable requirements are being fulfilled.

The Investigator must permit the Study Monitor, the HRA/REC, the Sponsor's auditors and representatives from Competent Authorities direct access to all source documents (see Section 11.1) for confirmation of the accuracy and reliability of data contained within the eCRF (source document verification). Participant confidentiality will be protected at all times. The Study Monitor will carry out source document verification at regular intervals. This is an essential element of quality control, as it allows the rectification of transcription errors and omissions.

9.3 Data Management and Coding

Data for each participant will be recorded on an eCRF. Data collection must be completed for each participant who signs an informed consent form and receives at least one dose of study vaccine.

Electronic CRFs will be designed and produced by the Sponsor (or designee) and should be completed in accordance with instructions. The Investigator is responsible for maintaining adequate and accurate medical records from which accurate information will be transcribed directly into the eCRFs using a secure internet connection. The eCRFs should be filled out completely by the Investigator (or designee) as stated on the delegation of responsibilities form.

The eCRF system will be Food and Drug Administration (FDA) Code of Federal Regulations (CFR) 21 Part 11 compliant.

The eCRFs must be reviewed and approved by the Investigator.

Data entered into the eCRF will be validated as defined in the data validation specifications. Validation includes, but is not limited to, validity checks (e.g. range checks), consistency checks and customised checks (logical checks between variables to ensure that study data are accurately reported) for eCRF data and external data (e.g. laboratory data). A majority of edit checks will be triggered during data entry and will therefore facilitate efficient 'point of entry' data cleaning.

Data management personnel will perform both manual eCRF review and review of electronic edit checks to ensure that the data are complete, consistent and reasonable. The electronic edit checks will run continually throughout the course of the study and the issues will be reviewed manually online to determine what action needs to be taken.

Manual queries may be added to the system by clinical data management, local Medical Monitor or Study Monitor. Clinical Data Managers and Study Monitors are able to remotely and proactively monitor the eCRFs to improve data quality.

External data will be transferred electronically into the study database. Discrepancies will be queried to the study centre and/or the laboratory until the electronic data and the database are reconciled.

All updates to queried data will be made by authorised study team members only and all modifications to the database will be recorded in an audit trail. Once all the queries have been resolved, eCRFs will be locked. Any changes to locked eCRFs will be approved by the Investigator.

Once the full set of eCRFs has been completed and locked, and SAE reconciliation has been completed, the Sponsor will authorise database lock and all electronic data will be sent to the designated statistician for analysis. Subsequent changes to the database will then only be made only by written agreement of the Sponsor. The Investigator will be provided with a copy of the eCRFs in a non-editable format.

Adverse events and medical/cancer history terms will be coded from the verbatim description (Investigator term) using the Medical Dictionary for Regulatory Activities (MedDRA) (Version 22.0 or later). Prior and concomitant medications and therapies will be coded according to the World Health Organisation drug dictionary (Version Drug B3 Global September 2019). Coding review will be performed by the Sponsor (or designee) prior to database lock.

The clinical data (in SAS® dataset format) will be transferred to the Sponsor at the end of the study.

10 STATISTICAL CONSIDERATIONS AND ANALYSIS PLAN

10.1 Sample Size Determination

No formal sample size calculation for cohorts 1 to 4 was performed and sample size is based upon feasibility considerations. The chosen number of participants is considered sufficient to meet the objectives for the study.

The sample size for cohorts 5 and 6 is based on the ELISpot results of ChAdOx1-HBV administered to healthy adults at a dose of 2.5 x 10^{10} which resulted in a mean of 1,000 +/-500 spot forming units in three adults who had samples examined by same day analysis at Oxford University. Assuming the same response in 15 volunteers in each cohort, the sample size is powered to detect a 45% decrease in the T cell response in the participants receiving prior AZD1222 compared to the Pfizer/Moderna mRNA COVID-19 vaccine.

10.2 Statistical and Analytical Plans

This section presents a summary of the planned statistical analyses. Full details of the analysis will be described in the SAP that will be approved before database lock. Any analysis that deviates from the SAP will be documented and justified in the clinical study report.

The computer program package SAS System for Windows® (Version 9.4 or later) will be used for statistical analysis.

10.2.1 Analysis Sets

- The intent-to-treat/safety analysis set will consist of all participants who received at least one vaccination (data will be summarised according to the vaccination actually received)
- The per-protocol analysis set will consist of all participants in the safety analysis set who received the correct study vaccine and who had no major protocol deviations
- The immunogenicity analysis set will consist of all participants in the per-protocol set and have available immunogenicity data to evaluate the immunogenicity endpoints and did not have any major protocol deviations that would impact on the results of the immunological analysis

Allocation of participant to the analysis sets (and whether any participant or specific data from a participant will be excluded) will be determined at the pre-database lock Data Review meeting.

10.2.2 Study Analyses

10.2.2.1 *General Principles*

The majority of the analysis will be descriptive in nature. Unless stated otherwise, continuous variables will be summarised using descriptive statistics (number of participants, mean, standard deviation, median, minimum and maximum values) and the number and percentage of participants will be used for categorical variables.

Data will be summarised by study vaccine, participant group and where appropriate, overall.

Immunogenicity and efficacy data may be subjected to additional statistical analysis, which will be detailed in the SAP.

10.2.2.2 *Disposition*

Participant disposition, including reasons for study withdrawal if applicable, will be summarised descriptively.

10.2.2.3 *Protocol Deviations*

Protocol deviations will be listed including: nature (missed procedure(s), early or late procedure(s), missed visits, visit performed out of protocol defined window, prohibited medication taken, study vaccine dosing deviation, inclusion/exclusion criteria violation, participants not withdrawn but meeting withdrawal criteria, biological sample handling error, other) overall and categorised by major and minor.

10.2.2.4 *Demographics and Baseline Data*

The safety analysis set will be used for the analysis of demographic and baseline data.

Demographics and baseline characteristics will be listed and summarised descriptively. Medications and therapies will be summarised by anatomic therapeutic chemical classification.

10.2.2.5 *Immunogenicity Analyses*

The following endpoints will be presented by study vaccine and participant group:

- A multi-parameter index made of CD4+ magnitude, CD4+ avidity, and CD8+ magnitude
- Reduction in HBsAg titre at Week 12 and Week 24 post-vaccination (last visit)
- Proportion of subjects with HBeAg and HBsAg loss
- Proportion of HBeAg and HBsAg seroconversion
- Reduction of hepatitis B DNA level
- Effect on hepatitis core-related antigen (HBcAg)
- Effect of hepatitis B circulation pre-genomic RNA (pgRNA)
- Liver fine needle aspirate assays will include measures of immune and parenchymal cell frequency, function and phenotype, HBV cccDNA and RNA transcripts
- Additional exploratory analysis can be performed on blood samples and LFNAs, including comparing the local immune landscape at Day 84 with baseline

10.2.2.6 *Safety Data*

Safety data will be summarised descriptively.

10.2.2.6.1 Adverse Events

All adverse events will be listed, including the verbatim description and MedDRA preferred terms and system organ class (SOC).

Treatment emergent adverse events (TEAEs) are defined as those occurring after the study vaccine administration.

TEAEs will be summarised by SOC and by preferred term. The incidence of TEAEs will be based on the numbers and percentages of participants with events and number of events. TEAEs will be further summarised by severity and relationship to study vaccine.

An overall summary of adverse event incidence will also be presented by study vaccine dose and overall to include the number and percentage of participants with at least one: TEAE, vaccine-related TEAE, Grade 3 to Grade 5 TEAE, death, SAE, vaccine-related SAE, AESI, vaccine-related AESI, TEAE leading to study vaccine discontinuation and TEAE leading to study discontinuation.

Narratives will be written for any deaths, other SAEs, AESIs, TEAEs leading to study vaccine discontinuation and TEAEs leading to study discontinuation.

10.2.2.6.2 Laboratory Safety Tests

Severity grading using the toxicity table in Appendix 1 will be assigned to laboratory safety values where applicable. Laboratory results in reported units and standard international (SI) units will be listed, including high and low flags, severity grades where applicable, the corresponding normal range and clinical significance.

Laboratory safety tests summaries will be based on results in SI units. Absolute and change from baseline results, including the worst change for each variable for each participant will be summarised using descriptive statistics.

Treatment emergent out of range results with the corresponding severity grade, normal ranges, baseline results and clinical significance will be separately listed. Shift tables will be used to show changes from baseline at each timepoint and the worst change in each participant and scatterplots of the worst change will be produced. The number of participants showing shifts of at least two severity grades will be summarised.

The number of participants with treatment emergent clinically significant laboratory safety test results and laboratory safety test results of Grade 3 or 4 will be summarised.

10.2.2.6.3 Vital Signs

Absolute and change from baseline vital sign results, including the worst change for each variable for each participant will be summarised using descriptive statistics. Treatment emergent out of range results with the corresponding normal ranges, baseline results and clinical significance will be separately listed. Shift tables will be used to show changes from baseline at each timepoint and the worst change in each participant and scatterplots of the worst change will be produced. The number of participants with treatment emergent clinically significant results will be summarised.

10.2.2.6.4 Physical Examination

Treatment emergent abnormal physical examination with the corresponding clinical significance will be listed. Shift tables will be used to show changes in physical examination status from baseline at each timepoint and the worst shift in each participant will be included.

10.2.3 Interim Analyses

No interim analyses are planned.

10.2.4 Handling Missing, Unused or Spurious Data

The SAP will describe and account for the occurrence of and extent of missing data, and its possible impact on the study analysis.

11 STUDY DOCUMENTATION, INSPECTIONS AND RECORD KEEPING

11.1 Study Documentation

The Investigator must maintain adequate and accurate records to enable the conduct of the study to be fully documented and the study data to be subsequently verified. These documents should contain all essential documents required by ICH GCP [34] and include:

1. ISF (e.g. protocol and amendments, HRA/REC and Competent Authority approval with correspondence, sample informed consent, study vaccine accountability records, staff curriculum vitae and authorisation forms, correspondence)
2. Participant source documents

   Source documents are defined as the results of original observations and activities of a clinical investigation, including medical notes. All source documents produced in this study will be maintained by the Investigator and made available for inspection. Source data include, but is not limited to, the following and will be identified in a source data location log:

   - Screening/enrolment log
   - Medical notes - which should be updated after each visit to include visit dates, medical history and cancer history, concomitant medication, any clinically relevant findings of clinical examinations or clinically relevant adverse events/medication changes, SAEs and information on participant withdrawal
   - Informed consent form
   - Safety laboratory reports
   - Visit dates
   - Study vaccine accountability and inventory forms
3. Participant eCRF data (which includes an audit trail containing a complete record of all changes to data) will be sent to the Investigator at the end of the study

11.2 Audits and Study Centre Inspections

Authorised personnel from Competent Authorities and the Sponsor quality assurance function may carry out inspections and audits respectively. The purpose of an audit or inspection is to ensure that ethical, regulatory and quality requirements are fulfilled in Sponsor studies.

If an audit or inspection occurs, the Investigator and institution agree to allow the auditor/inspector direct access to all relevant documents and to allocate his time and the time of his staff to the auditor or inspector to discuss findings and any relevant issues.

11.3 Retention of Records

Records and documents pertaining to the conduct of this study and the distribution of study vaccine, including eCRFs, informed consent forms, laboratory safety test results and study vaccine inventory and accountability records, must be retained by the Investigator for at least 15 years after completion or discontinuation of the study, or according to local requirements, whichever is longer. After that period of time, the documents may be destroyed, subject to local regulations.

No records may be disposed of without the written approval of the Sponsor. Written notification should be provided to the Sponsor prior to transferring any records to another party or moving them to another location.

12 FINANCE AND INSURANCE

Financial arrangements are detailed in the Investigator Agreement between the Sponsor and Investigator.

Details of the Sponsor's arrangement for clinical study insurance to provide for compensation to participant for any claim for bodily injury or death arising from participation in the clinical study are provided in the informed consent form.

13 ETHICAL AND REGULATORY CONSIDERATIONS

13.1 Local Regulations/Declaration of Helsinki

The Investigator will ensure that this study is conducted in full conformance with the protocol and the principles of the "Declaration of Helsinki" [38] or with the laws and regulations of the country in which the research is conducted, whichever affords the greater protection to the individual.

The study must fully adhere to ICH GCP [34] or with local regulations if they afford greater protection to the participant.

13.2 Competent Authority Approval

The study will not commence before approval from the Competent Authority been granted according to local requirements. The Sponsor (or designee) will be responsible for the preparation, submission and confirmation of receipt of any Competent Authority approvals required prior to release of study vaccine for shipment to the study centre.

During the study, the Sponsor (or designee) is also responsible for submitting subsequent amendments and notifications to the Competent Authority according to local requirements.

13.3 Research Ethics Committee Approval

The Investigator will be responsible for submitting submit all documents required by the HRA/REC e.g. this protocol, the informed consent form relevant supporting information and all types of study specific participant recruitment information (including advertisements) and any other written information to be provided to participants, to the HRA/REC for review. The Investigator or Sponsor should also provide the HRA/REC with a copy of the Investigator's Brochure or product labelling, information to be provided to participants and any updates. If the HRA/REC requires modification of the submitted information, the documentation supporting this requirement must be provided to the Sponsor (or designee).

The study must have the initial and at least annual (when required) approval of an HRA/REC. The signed approval letter must identify the exact protocol title and number, documents approved and the date of approval of the protocol and the informed consent document. The Sponsor will not ship clinical supplies until a signed approval letter has been received and a Clinical Trial Agreement has been signed by the Sponsor and the study centre.

A list of HRA/REC members must be provided to the Sponsor (or designee).

The Investigator or Sponsor should provide the HRA/REC with reports, updates and other information (e.g. expedited safety reports, amendments and administrative letters) according to regulatory requirements or study centre procedures.

The Sponsor (or designee) will ensure the HRA/REC is notified of any adverse events meeting the criteria for expedited reporting, annual updates and when the study has been completed according to local requirements. The Sponsor (or designee) will provide information to the Investigator who will be responsible for forwarding this to the HRA/REC.

13.4 Informed Consent

It is the responsibility of the Investigator to obtain written informed consent from participants prior to conducting any study related procedures. All consent documentation must be in accordance with applicable regulations and ICH GCP [34]. Each participant is requested to

sign and date the informed consent form after (s)he has received and read the participant information sheet and received an explanation of what the study involves, including but not limited to: the objectives, potential benefits and risk, inconveniences and the participant's rights and responsibilities. participants will be given adequate time to evaluate the information given to them before signing and dating the informed consent form. The informed consent form also be signed and dated by the person obtaining consent. The original signed informed consent form for each participant will be retained on file by the Investigator and the second signed original given to the participant.

Informed consent forms must be retained for enrolled participants and for participants who are not subsequently enrolled and must be available for verification by the Study Monitor at any time.

In the event of changes to the informed consent form during the study, the Investigator must always use the most current HRA/REC approved form for documenting written informed consent.

CHB participants will also be asked whether they would like to undergo a liver fine needle aspirate procedure. This is an optional test and the PI will provide a separate consent form for this procedure which will explain the procedure in full and all associated risks.

13.5 Protocol Amendments

The protocol (and other supporting documents that have received approval before the start of the study e.g. informed consent form) may not be modified without written approval from the Sponsor. In the event that an amendment to the protocol (and/or supporting documents) is required, it will be classified into one of the following categories by the Sponsor:

- Substantial amendments are those considered 'substantial' to the conduct of the clinical study and are likely to have a significant impact on e.g. the safety or physical or mental integrity of the participants, the scientific value of the study, the conduct or management of the study or the quality or safety of the study vaccine used in the study
- Non-substantial amendments only involve administrative or logistical changes, typographical errors

The Sponsor will determine if Competent Authority and/or HRA/REC review is required prior to the implementation of the amendment according to local regulations. In general, substantial amendments may not be initiated without approval except when necessary to eliminate immediate hazards to the participants or when the change(s) involves. Non-substantial amendments do not require approval.

The Sponsor (or designee) is responsible for obtaining approval for substantial amendments from the Competent Authority. The Investigator is responsible for promptly informing the HRA/REC of any substantial amendments and providing documentation of favourable opinion to the Sponsor (or designee).

13.6 Confidentiality of Study Documents and Participant Records

The Investigator must ensure that participant's anonymity will be maintained and that their identities are protected from unauthorised parties. The Sponsor (or designee) will maintain confidentiality standards by assigning a unique coded identification number to each participant included in the study. Participant names will never be included in data sets that are transmitted

to the Sponsor or their representatives or to third parties as permitted by the informed consent form.

Participants should be identified by an identification code rather than by their names on eCRFs or other documents submitted to the Sponsor. The Investigator should keep a participant enrolment log relating codes to the names of participants. The Investigator should maintain documents not for submission to the Sponsor e.g. informed consent forms, in strict confidence. Records will not be destroyed without giving the Sponsor prior written notice and the opportunity to further store such records, at the Sponsor's cost and expense.

Participant medical information obtained by this study is confidential and may only be disclosed to third parties as permitted by the informed consent form signed by the participant, unless permitted or required by law.

14 STUDY COMPLETION

All materials or supplies provided by the Sponsor will be returned to the Sponsor upon study completion. The Investigator will notify the HRA/REC when the study has been completed. The study will be considered complete when the last participant completes their final follow-up visit and all the data clarification forms have been resolved. This report should be made within 3 months of the completion or termination of the study. The final report sent to the HRA/REC should also be sent to the Sponsor and, along with the completed eCRFs, constitutes the final summary to the Sponsor, thereby fulfilling the Investigator's regulatory responsibility.

15 PUBLICATION OF DATA

The final study report will be made available to the Investigator for purposes of publications. The Investigator and study team must send all manuscripts, abstracts, and presentations using data from this study to the Sponsor for review prior to their submission. The Sponsor reserves the right to delete any part or parts of such materials deemed to be confidential or proprietary.

16 REFERENCES

1. World Health Organization. Guidelines for the prevention, care and treatment of persons with chronic hepatitis B infection. March 2015. Available at: https://www.who.int/hepatitis/topics/hepatitis-b/en/. Last accessed on 11 July 2019.
2. Cesar Aguilar J, Lobiana Y. Immunotherapy for chronic hepatitis B using HBsAg-based vaccine formulations: from preventive commercial vaccines to therapeutic approach. Euroasian J Hepato-Gastroenterol. 2014;4(2):92-97.
3. European Association for the Study of the Liver. EASL 2017 Clinical Practice Guidelines on the management of hepatitis B virus infection. J Hepatol. 2017;67:370-398.
4. Viganò M, Grossi G, Loglio A, Lampertico P. Treatment of hepatitis B: Is there still a role for interferon? Liver Int. 2018;38(Suppl. 1):79–83.
5. Lau GK, Piratvisuth T, Luo KX, et al. Peginterferon Alfa-2a, lamivudine, and the combination for HBeAg-positive chronic hepatitis B. N Engl J Med. 2005;352:2682-2695.
6. Marcellin P, Lau GK, Bonino F, et al. Peginterferon alfa-2a alone, lamivudine alone, and the two in combination in patients with HBeAg-negative chronic hepatitis B. N Engl J Med. 2004;351:1206-1217.
7. Xie Q, Zhou H, Bai X, et al. A randomized, open-label clinical study of combined pegylated interferon alfa-2a (40kD) and entecavir treatment for hepatitis B “e” antigen-positive chronic Hepatitis B. Clin Infect Dis. 2014;59:1714-1723.
8. Marcellin P, Ahn SH, Ma X, et al. Combination of tenofovir disoproxil fumarate and peginterferon α-2a increases loss of hepatitis B surface antigen in patients with chronic hepatitis B. Gastroenterol. 2016;150:134-144.
9. Zhang Y, Wu Y, Deng M, et al. CD8+ T-cell response-associated evolution of hepatitis B virus core protein and disease progress. J Virol. 2018;92(17):e02120-17.
10. Knolle PA, Thimme R. Hepatic immune regulation and its involvement in viral hepatitis infection. Gastroenterol. 2014;146:1193–1207.
11. Seder RA, Darah PA, Roederer M. T-cell quality in memory and protection: implications for vaccine design. Nature Rev Immunol. 2008;8:247-258.
12. Moyo N, Borthwick N, Wee EG et al. Long-term follow up of human T-cell responses to conserved HIV-1 regions elicited by DNA/simian adenovirus/MVA vaccine regimens. PLoS One 2017;12(7): e0181382.
13. Rampling T, Ewer KJ, Bowyer G et al. Safety and high level efficacy of the combination malaria vaccine regimen of RTS,S/AS01$_B$ with chimpanzee adenovirus 63 and modified vaccinia Ankara vectored vaccines expressing ME-TRAP. J Infect Dis. 2016;214:772-781.
14. Lu S. Heterologous prime-boost vaccination. Curr Opin Immunol. 2009;21(3):346-351.
15. Schneider J, Gilbert SC, Hannan CM et al. Induction of CD8+ T cells using heterologous prime-boost immunisation strategies. Immunol Rev. 1999;170:29-38.
16. Coughlan L, Sridhar S, Payne R et al. Heterologous two-dose vaccination with simian adenovirus and poxvirus vectors elicits long-lasting cellular immunity to influenza virus A in healthy adults. EBioMedicine. 2018;29:146-154.

17. Ewer K, Sebastian S, Spencer AJ, Gilbert S, Hill AVS, Lambe T. Chimpanzee adenoviral vectors as vaccines for outbreak pathogens. Hum Vaccin Immunother. 2017;13(12):3020–3032.
18. Morris SJ, Sebastian S, Spencer AJ, Gilbert SC. Simian adenoviruses as vaccine vectors. Future Virol. 2016;11(9):649–659.
19. Sheehan S, Harris SA, Satti I, et al. A Phase I, open-label trial, evaluating the safety and immunogenicity of candidate tuberculosis vaccines AERAS-402 and MVA85A, administered by prime-boost regime in BCG-vaccinated healthy adults. PLoS One. 2015;10(11):e0141687.
20. Hodgson SH, Ewer KJ, Bliss CM, et al. Evaluation of the efficacy of ChAd63-MVA vectored vaccines expressing circumsporozoite protein and ME-TRAP against controlled human malaria infection in malaria-naive individuals. J Infect Dis. 2015;211:1076-1086.
21. Mothe B, Manzardo C, Sanchez-Bernabeuet A, et al. Therapeutic vaccination refocuses T-cell responses towards conserved regions of HIV-1 in early treated individuals (BCN 01 study). EClinicalMedicine 2019;11:65–80.
22. Green CA, Sande CJ, Scarselli E, et al. Novel genetically-modified chimpanzee adenovirus and MVA-vectored respiratory syncytial virus vaccine safely boosts humoral and cellular immunity in healthy older adults. J Infect. 2019;78(5):382-392.
23. Velkov S, Ott JJ, Protzer U, Michler T. The global hepatitis B virus genotype distribution approximated from available genotyping data. Genes (Basel) 2018;9(10):495.
24. Thimme R, Wieland S, Steiger C, et al. $CD8^+$ T cells mediate viral clearance and disease pathogenesis during acute hepatitis B virus infection. J Virol. 2003;77(1):68–76.
25. Guidotti LG, Rochford R, Chung J, Shapiro M, Purcell R, Chisari FV. Viral clearance without destruction of infected cells during acute HBV infection. Science 1999;284:825-829.
26. Asabe S, Wieland SF, Chattopadhyay PK, et al. The size of the viral inoculum contributes to the outcome of hepatitis B virus infection. J Virol. 2009;83:9652-9662.
27. Voysey M, Clemend SAC, Madhi SA, et al. Safety and efficacy of the ChAdOx1 nCoV-19 vaccine (AZD1222) against SARS-CoV-2: an interim analysis of four randomised controlled trials in Brazil, South Africa, and the UK. Lancet 2021;397:99-111.
28. Baden LR, Karita E, Mutua G, et al. Assessment of the safety and immunogenicity of 2 novel vaccine platforms for HIV-1 prevention: a randomized trial. Ann Intern Med 2016;164:313-22.
29. Baden LR, Walsh SR, Seaman MS, et al. First-in-human evaluation of the safety and immunogenicity of a recombinant adenovirus serotype 26 HIV-1 Env vaccine (IPCAVD 001). J Infect Dis 2013;207:240-7.
30. Capone S, Brown A, Hartnell F, et al. Optimising T cell (re)boosting strategies for adenoviral and modified vaccinia Ankara vaccine regimens in humans. npj Vaccines 2020;5:94.
31. Barouch DH, Tomaka FL, Wegmann F, et al. Evaluation of a mosaic HIV-1 vaccine in a multicentre, randomised, double-blinded, placebo-controlled, phase 1/2a clinical trial (APPROACH) and in rhesus monkeys. Lancet 2018;392:232-243.
32. Goldstein N, Bockstal V, Bart S, et al. Safety and immunogenicity of heterologous and homologous two dose regimens of Ad26- and MVA-vectored Ebola vaccines: a randomized, controlled phase 1 Study. J Infect Dis. 2020; jiaa586.

33. Wilkie M, Satti I, Minhinnick A, et al. A phase I trial evaluating the safety and immunogenicity of a candidate tuberculosis vaccination regimen, ChAdOx1 85A prime – MVA85A boost in healthy UK adults. Vaccine 2020;38:779-789.
34. International Conference on Harmonisation. ICH E6(R2). Integrated addendum to ICH E6(R1) Guideline for Good Clinical Practice. 11 June 2015.
35. EU Guidelines to Good Manufacturing Practice. Medicinal Products for Human and Veterinary Use Annex 13.
36. Ewer KJ, Lambe T, Rollier CS, Spencer AJ, Hill AV, Dorrell L. Viral vectors as vaccine platforms: from immunogenicity to impact. Curr Opin Immunol. 2016;41:47-54.
37. de Barra E, Hodgson SH, Ewer KJ, et al. A Phase Ia study to assess the safety and immunogenicity of new malaria vaccine candidates ChAd63 CS administered alone and with MVA CS. PLoS One. 2014;9(12):e115161.
38. World Medical Association, Declaration of Helsinki. Ethical Principles for Medical Research Involving Human Subjects as amended by the 59th World Medical Association General Assembly, Seoul, Korea, October 2008.

APPENDICES

Appendix 1 Toxicity Table for Clinical and Laboratory Abnormalities

Guidance for Industry

Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials

Additional copies of this guidance are available from the Office of Communication, Training and Manufacturers Assistance (HFM-40), 1401 Rockville Pike, Suite 200N, Rockville, MD 20852-1448, or by calling 1-800-835-4709 or 301-827-1800, or from the Internet at http://www.fda.gov/cber/guidelines.htm.

For questions on the content of this guidance, contact the Division of Vaccines and Related Products Applications, Office of Vaccines Research and Review at 301-827-3070.

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Biologics Evaluation and Research
September 2007

Contains Nonbinding Recommendations

Table of Contents

Contains Nonbinding Recommendations

Guidance for Industry

Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials

This guidance represents the Food and Drug Administration's (FDA's) current thinking on this topic. It does not create or confer any rights for or on any person and does not operate to bind FDA or the public. You can use an alternative approach if the approach satisfies the requirements of the applicable statutes and regulations. If you want to discuss an alternative approach, contact the appropriate FDA staff. If you cannot identify the appropriate FDA staff, call the appropriate number listed on the title page of this guidance.

I. INTRODUCTION

Preventive vaccines are usually developed to prevent disease in a healthy population. The Office of Vaccines Research and Review, Center for Biologics Evaluation and Research, regulates preventive vaccines under authority of section 351 of the Public Health Service Act (42 U.S.C. 262), as well as specific sections of the Federal Food, Drug, and Cosmetic Act, and reviews investigational new drug applications (INDs) and biologics license applications (BLAs). (See, for example, Title 21 Code of Federal Regulations (CFR) Parts 312, 600, and 601). Most of the clinical trials of preventive vaccines conducted to support INDs and BLAs enroll healthy volunteers in all phases of vaccine testing. The enrollment of healthy volunteers warrants a very low tolerance for risk in those clinical trials.

This guidance provides you, sponsors, monitors, and investigators of vaccine trials, with recommendations on assessing the severity of clinical and laboratory abnormalities in healthy adult and adolescent volunteers enrolled in clinical trials. The grading system described in the table can also be useful in defining a particular study's stopping rules (e.g., a certain number of adverse events, as defined in the table, may call for stopping the study). Less extreme observations (e.g., mild) may not require discontinuing the study vaccine but can still contribute to evaluating safety by identifying parameters to focus upon in subsequent product development. Uniform criteria for categorizing toxicities in healthy volunteers can improve comparisons of safety data among groups within the same study and also between different studies. We, FDA, recommend using toxicity grading scale tables, provided below, as a guideline for selecting the assessment criteria to be used in a clinical trial of a preventive vaccine. We recommend incorporation of such appropriate, uniform, criteria into the investigational plan, case report forms, and study reports and correspondence with FDA, sponsors, monitors, investigators, and IRBs.

This guidance finalizes the draft guidance of the same title dated April 2005 (70 FR 22664, May 2, 2005).

1

Contains Nonbinding Recommendations

FDA's guidance documents, including this guidance, do not establish legally enforceable responsibilities. Instead, guidances describe FDA's current thinking on a topic and should be viewed only as recommendations, unless specific regulatory or statutory requirements are cited. The use of the word *should* in FDA's guidances means that something is suggested or recommended, but not required.

II. BACKGROUND

Standardized toxicity assessment scales have been widely used to evaluate products treating specific diseases. For example, the National Cancer Institute's Common Toxicity Criteria Scale and the Division of AIDS' Toxicity Grading Scale standardize the evaluation of adverse events among patients with cancer and HIV/AIDS, respectively (Refs. 1, 2). The defined toxicity parameters in those scales are designed for patients who may already experience mild, moderate, or severe adverse clinical or laboratory events due to the disease process, and may not be appropriate for healthy volunteers.

In the development of the toxicity grading scales for healthy volunteers, we chose parameter limit values based on published information, when such values were available (Refs. 1-6). For example, the Brighton Collaboration has developed case definitions and guidelines to evaluate some adverse events associated with administering vaccines (Ref. 3). In some cases, parameter limit values were based on clinical experience and experience reviewing vaccine clinical trials that enroll normal healthy subjects.

Toxicity grading scales for laboratory abnormalities should consider the local laboratory reference values when the parameter limit values are defined. The characterization of laboratory parameters among some populations of healthy adults and adolescents may require the exercise of clinical judgment, for example, consideration of the potential for ethnic differences in white blood cell (WBC) counts or gender differences in creatine phosphokinase (CPK) values.

III. TOXICITY GRADING SCALE TABLES

Adverse events in a clinical trial of an investigational vaccine must be recorded and monitored and, when appropriate, reported to FDA and others involved in an investigation (sponsors, IRBs, and investigators). (See, for example, 21 CFR 312.32, 312.33, 312.50, 312.55, 312.56, 312.60, 312.62, 312.64, 312.66). Although the use of a toxicity grading scale for adverse events would not replace these regulatory requirements, using a scale to categorize adverse events observed during a clinical trial may assist you in monitoring safety and making required reports. Nonetheless, we believe that categorization or grading of data as outlined in this document is supplementary to and should not replace full and complete data analysis.

These guidelines for toxicity grading scales are primarily intended for healthy adult and adolescent volunteers. The parameters in the tables below are not necessarily applicable to every clinical trial of healthy volunteers. The parameters monitored should be appropriate for the specific study vaccine. For some preventive vaccines under development, it may be appropriate

Contains Nonbinding Recommendations

to include additional parameters to be monitored during a clinical trial or to alter the choice of values in the toxicity table. For example, additional parameters might be added based on one or more of the following: safety signals observed in pre-clinical toxicology studies, the biological plausibility of the occurrence of certain adverse events, or previous experience with a similar licensed product.

As discussed above, the tables do not represent a recommendation to monitor all the listed parameters in all clinical trials of healthy volunteers, nor do the tables represent all possible parameters to be monitored. In addition, these tables do not represent study inclusion or exclusion criteria. We recommend that the parameters monitored be appropriate for the study vaccine administered to healthy volunteers participating in the clinical trial.

A. Tables for Clinical Abnormalities

Local Reaction to Injectable Product	Mild (Grade 1)	Moderate(Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)
Pain	Does not interfere with activity	Repeated use of non-narcotic pain reliever > 24 hours or interferes with activity	Any use of narcotic pain reliever or prevents daily activity	Emergency room (ER) visit or hospitalization
Tenderness	Mild discomfort to touch	Discomfort with movement	Significant discomfort at rest	ER visit or hospitalization
Erythema/Redness *	2.5 – 5 cm	5.1 – 10 cm	> 10 cm	Necrosis or exfoliative dermatitis
Induration/Swelling **	2.5 – 5 cm and does not interfere with activity	5.1 – 10 cm or interferes with activity	> 10 cm or prevents daily activity	Necrosis

* In addition to grading the measured local reaction at the greatest single diameter, the measurement should be recorded as a continuous variable.
** Induration/Swelling should be evaluated and graded using the functional scale as well as the actual measurement.

Contains Nonbinding Recommendations

Vital Signs *	Mild (Grade 1)	Moderate(Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)
Fever (°C) ** (°F) **	38.0 – 38.4 100.4 – 101.1	38.5 – 38.9 101.2 – 102.0	39.0 – 40 102.1 – 104	> 40 > 104
Tachycardia - beats per minute	101 – 115	116 – 130	> 130	ER visit or hospitalization for arrhythmia
Bradycardia - beats per minute***	50 – 54	45 – 49	< 45	ER visit or hospitalization for arrhythmia
Hypertension (systolic) - mm Hg	141 – 150	151 – 155	> 155	ER visit or hospitalization for malignant hypertension
Hypertension (diastolic) - mm Hg	91 – 95	96 – 100	> 100	ER visit or hospitalization for malignant hypertension
Hypotension (systolic) – mm Hg	85 – 89	80 – 84	< 80	ER visit or hospitalization for hypotensive shock
Respiratory Rate – breaths per minute	17 – 20	21 – 25	> 25	Intubation

* Subject should be at rest for all vital sign measurements.
** Oral temperature; no recent hot or cold beverages or smoking.
*** When resting heart rate is between 60 – 100 beats per minute. Use clinical judgement when characterizing bradycardia among some healthy subject populations, for example, conditioned athletes.

Systemic (General)	Mild (Grade 1)	Moderate(Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)
Nausea/vomiting	No interference with activity or 1 – 2 episodes/24 hours	Some interference with activity or > 2 episodes/24 hours	Prevents daily activity, requires outpatient IV hydration	ER visit or hospitalization for hypotensive shock
Diarrhea	2 – 3 loose stools or < 400 gms/24 hours	4 – 5 stools or 400 – 800 gms/24 hours	6 or more watery stools or > 800gms/24 hours or requires outpatient IV hydration	ER visit or hospitalization
Headache	No interference with activity	Repeated use of non-narcotic pain reliever > 24 hours or some interference with activity	Significant; any use of narcotic pain reliever or prevents daily activity	ER visit or hospitalization
Fatigue	No interference with activity	Some interference with activity	Significant; prevents daily activity	ER visit or hospitalization
Myalgia	No interference with activity	Some interference with activity	Significant; prevents daily activity	ER visit or hospitalization

Contains Nonbinding Recommendations

Systemic Illnes	Mild (Grade 1)	(Moderate(Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)
Illness or clinical adverse event (as defined according to applicable regulations)	No interference with activity	Some interference with activity not requiring medical intervention	Prevents daily activity and requires medical intervention	ER visit or hospitalization

5

Contains Nonbinding Recommendations

B. Tables for Laboratory Abnormalities

The laboratory values provided in the tables below serve as guidelines and are dependent upon institutional normal parameters. Institutional normal reference ranges should be provided to demonstrate that they are appropriate.

Serum *	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)**
Sodium – Hyponatremia mEq/L	132 – 134	130 – 131	125 – 129	< 125
Sodium – Hypernatremia mEq/L	144 – 145	146 – 147	148 – 150	> 150
Potassium – Hyperkalemia mEq/L	5.1 – 5.2	5.3 – 5.4	5.5 – 5.6	> 5.6
Potassium – Hypokalemia mEq/L	3.5 – 3.6	3.3 – 3.4	3.1 – 3.2	< 3.1
Glucose – Hypoglycemia mg/dL	65 – 69	55 – 64	45 – 54	< 45
Glucose – Hyperglycemia Fasting – mg/dL Random – mg/dL	 100 – 110 110 – 125	 111 – 125 126 – 200	 >125 >200	Insulin requirements or hyperosmolar coma
Blood Urea Nitrogen BUN mg/dL	23 – 26	27 – 31	> 31	Requires dialysis
Creatinine – mg/dL	1.5 – 1.7	1.8 – 2.0	2.1 – 2.5	> 2.5 or requires dialysis
Calcium – hypocalcemia mg/dL	8.0 – 8.4	7.5 – 7.9	7.0 – 7.4	< 7.0
Calcium – hypercalcemia mg/dL	10.5 – 11.0	11.1 – 11.5	11.6 – 12.0	> 12.0
Magnesium – hypomagnesemia mg/dL	1.3 – 1.5	1.1 – 1.2	0.9 – 1.0	< 0.9
Phosphorous – hypophosphatemia mg/dL	2.3 – 2.5	2.0 – 2.2	1.6 – 1.9	< 1.6
CPK – mg/dL	1.25 – 1.5 x ULN***	1.6 – 3.0 x ULN	3.1 –10 x ULN	> 10 x ULN
Albumin – Hypoalbuminemia g/dL	2.8 – 3.1	2.5 – 2.7	< 2.5	–
Total Protein – Hypoproteinemia g/dL	5.5 – 6.0	5.0 – 5.4	< 5.0	--
Alkaline phosphate – increase by factor	1.1 – 2.0 x ULN	2.1 – 3.0 x ULN	3.1 – 10 x ULN	> 10 x ULN
Liver Function Tests –ALT, AST increase by factor	1.1 – 2.5 x ULN	2.6 – 5.0 x ULN	5.1 – 10 x ULN	> 10 x ULN
Bilirubin – when accompanied by any increase in Liver Function Test increase by factor	1.1 – 1.25 x ULN	1.26 – 1.5 x ULN	1.51 – 1.75 x ULN	> 1.75 x ULN
Bilirubin – when Liver Function Test is normal, increase by factor	1.1 – 1.5 x ULN	1.6 – 2.0 x ULN	2.0 – 3.0 x ULN	> 3.0 x ULN
Cholesterol	201 – 210	211 – 225	> 226	---
Pancreatic enzymes – amylase, lipase	1.1 – 1.5 x ULN	1.6 – 2.0 x ULN	2.1 – 5.0 x ULN	> 5.0 x ULN

* The laboratory values provided in the tables serve as guidelines and are dependent upon institutional normal parameters. Institutional normal reference ranges should be provided to demonstrate that they are appropriate.

** The clinical signs or symptoms associated with laboratory abnormalities might result in characterization of the laboratory abnormalities as Potentially Life Threatening (Grade 4). For example, a low sodium value that falls within a grade 3 parameter (125-129 mE/L) should be recorded as a grade 4 hyponatremia event if the subject had a new seizure associated with the low sodium value.

***"ULN" is the upper limit of the normal range.

Contains Nonbinding Recommendations

Hematology *	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)
Hemoglobin (Female) - gm/dL	11.0 – 12.0	9.5 – 10.9	8.0 – 9.4	< 8.0
Hemoglobin (Female) change from baseline value - gm/dL	Any decrease – 1.5	1.6 – 2.0	2.1 – 5.0	> 5.0
Hemoglobin (Male) - gm/dL	12.5 – 13.5	10.5 – 12.4	8.5 – 10.4	< 8.5
Hemoglobin (Male) change from baseline value – gm/dL	Any decrease – 1.5	1.6 – 2.0	2.1 – 5.0	> 5.0
WBC Increase - cell/mm^3	10,800 – 15,000	15,001 – 20,000	20,001 – 25, 000	> 25,000
WBC Decrease - cell/mm^3	2,500 – 3,500	1,500 – 2,499	1,000 – 1,499	< 1,000
Lymphocytes Decrease - cell/mm^3	750 – 1,000	500 – 749	250 – 499	< 250
Neutrophils Decrease - cell/mm^3	1,500 – 2,000	1,000 – 1,499	500 – 999	< 500
Eosinophils - cell/mm^3	650 – 1500	1501 - 5000	> 5000	Hypereosinophilic
Platelets Decreased - cell/mm^3	125,000 – 140,000	100,000 – 124,000	25,000 – 99,000	< 25,000
PT – increase by factor (prothrombin time)	1.0 – 1.10 x ULN**	1.11 – 1.20 x ULN	1.21 – 1.25 x ULN	> 1.25 ULN
PTT – increase by factor (partial thromboplastin time)	1.0 – 1.2 x ULN	1.21 – 1.4 x ULN	1.41 – 1.5 x ULN	> 1.5 x ULN
Fibrinogen increase - mg/dL	400 – 500	501 – 600	> 600	--
Fibrinogen decrease - mg/dL	150 – 200	125 – 149	100 – 124	< 100 or associated with gross bleeding or disseminated intravascular coagulation (DIC)

* The laboratory values provided in the tables serve as guidelines and are dependent upon institutional normal parameters. Institutional normal reference ranges should be provided to demonstrate that they are appropriate.
** "ULN" is the upper limit of the normal range.

Urine *	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)
Protein	Trace	1+	2+	Hospitalization or dialysis
Glucose	Trace	1+	2+	Hospitalization for hyperglycemia
Blood (microscopic) – red blood cells per high power field (rbc/hpf)	1 - 10	11 – 50	> 50 and/or gross blood	Hospitalization or packed red blood cells (PRBC) transfusion

* The laboratory values provided in the tables serve as guidelines and are dependent upon institutional normal parameters. Institutional normal reference ranges should be provided to demonstrate that they are appropriate.

Contains Nonbinding Recommendations

IV. REFERENCES

1. National Cancer Institute Common Toxicity Criteria, April 30, 1999. (http://ctep.cancer.gov/reporting/CTC-3.html)

2. Division of AIDS Table for Grading Severity of Adult Adverse Experiences; August 1992. (http://rcc.tech-res-intl.com/tox_tables.htm)

3. The Brighton Collaboration. Finalized Case Definitions and Guidelines. (http://brightoncollaboration.org/internet/en/index/definition___guidelines.html)

4. HIV Vaccine Trials Network Table for Grading Severity of Adverse Experiences; September 18, 2002. (http://rcc.tech-res-intl.com/tox_tables.htm)

5. Division of AIDS Table for Grading the Severity of Adult and Pediatric Adverse Events, December 2004. (http://www3.niaid.nih.gov/research/resources/DAIDSClinRsrch/PDF/Safety/DAIDSAEGradingTable.pdf)

6. Kratz A, Ferraro M, Sluss PM, Lewandrowski KB. Laboratory Reference Values. New England Journal of Medicine. 2004;351:1548-1563.

Appendix 2 Details of Changes Made in Protocol Amendments

Details of Changes Made in Protocol Amendment 8 (Protocol Version 9.0, 24 Nov 21)

1.5.1 Rationale for Investigation of Dosing Interval With Respect to COVID-19 Vaccines

To support this interval, this study aims to produce data of the response in healthy volunteers to our ChAdOx-1 HBV vaccine following the use of the AZD1222 vaccine. T cell responses (the critical immunological factor in chronic HBV infection) will be compared in participants who have received-who have received either a prior two-dose series of AZD1222 with those who have received **at least two prior doses of** either the Pfizer mRNA COVID 19 vaccine (Comirnaty®) or the Moderna COVID-19 vaccine (Spikevax). Due to the recommendation to use the AZD1222 in those over the age of 40 years, we will confine our study to 40-60 years of age and keep the interval from the ~~second~~ **latest** COVID-19 vaccine ~~in~~ **to** a narrow window of 10-18 weeks. No interference on HBV-focused T cell responses is expected from either the Pfizer vaccine or the Moderna vaccine, so the interval from the ~~second~~ **latest** COVID-19 vaccine will be between 6 to 30 weeks

3.1.1 Study Design

This is a Phase 1, first in human study of ChAdOx1-HBV. The study will be conducted in 40 healthy participants and 12 participants with CHB and virally suppressed with oral antiviral medication. This will be an open-label, non-randomised dose escalation study comparing the safety, tolerability and immunogenicity of 2 different doses of ChAdOx1-HBV vaccine. T cell responses in healthy participants who have received a prior two dose series of AZD1222 or **at least two doses of** Pfizer or Moderna mRNA COVID-19 vaccines will also be analysed. The study design is shown in Figure 2.

3.1.2 Study Methodology

….

Thirty healthy participants (15 who have received two doses of AZD1222 [cohort 5] and 15 who have received **at least** two doses of either Pfizer or Moderna mRNA COVID-19 vaccine [cohort 6]) will be dosed in parallel with the high dose used in cohorts 2 and 4.

4.1 Number of participants

….

A total of 10 healthy participants and 12 participants with CHB infection is considered sufficient to confirm the safety, tolerability and immunogenicity of ChAdOx1-HBV and to answer the objectives of the study before progressing to larger studies. An additional 30 healthy participants (15 who have received two doses dose of AZD1222 and 15 who have received **at least** two dose of either Pfizer or Moderna mRNA COVID-19 vaccine) will be recruited to assess whether prior receipt of AZD1222 results in decreased T cell responses to ChAdOx1-HBV.

4.2 Inclusion Criteria

Healthy participants (cohort 6):

16. ~~Completed second~~ **Received the latest** dose of Pfizer (Comirnaty®) or Moderna (Spikevax) mRNA COVID 19 vaccine 6 to 30 weeks before enrolment

Details of Changes Made in Protocol Amendment 7 (Protocol Version 8.0, 22 Sep 2021)

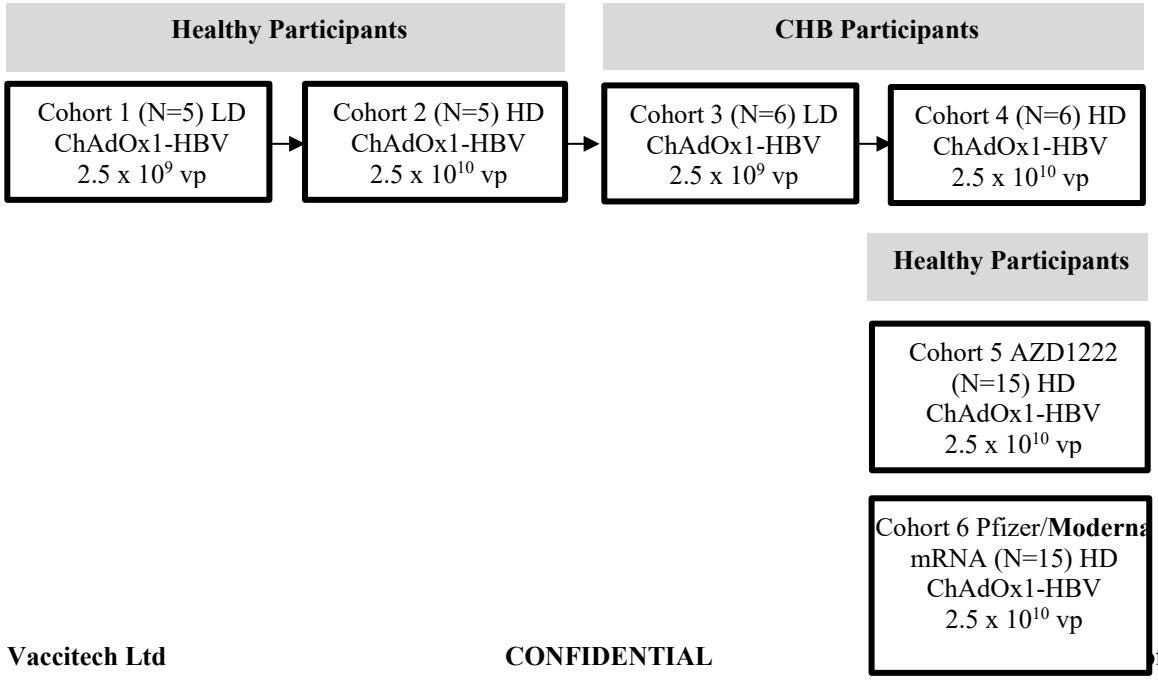
1.5.1 Rationale for Investigation of Dosing Interval With Respect to COVID-19 Vaccines

…..

To support this interval, this study aims to produce data of the response in healthy volunteers to our ChAdOx-1 HBV vaccine following the use of the AZD1222 vaccine. T cell responses (the critical immunological factor in chronic HBV infection) will be compared in participants who have received a prior two-dose series of AZD1222 with those who have received either the Pfizer mRNA COVID-19 vaccine (Comirnaty®) or Moderna COVID-19 vaccine (Spikevax). Due to the recommendation to use the AZD1222 in those over the age of 40 years, we will confine our study to 40-60 years of age and keep the interval from the second COVID 19 vaccine in a narrow window of 10-18 weeks. No interference on HBV-focused T cell responses is expected from the Pfizer **or Moderna** vaccine, **so the interval from the second COVID-19 vaccine will be between 6 to 30 weeks.**

3.1.1 Study Design

This is a Phase 1, first in human study of ChAdOx1-HBV. The study will be conducted in ~~10~~**40** healthy participants and 12 participants with CHB and virally suppressed with oral antiviral medication. This will be an open-label, non-randomised dose escalation study comparing the safety, tolerability and immunogenicity of 2 different doses of ChAdOx1-HBV vaccine. **T cell responses in healthy participants who have received a prior two-dose series of either AZD1222 or the Pfizer or Moderna mRNA COVID-19 vaccines will also be analysed.** The study design is shown in Figure 2.



3.1.2 Study Methodology

….

Thirty healthy participants (15 who have received two doses of AZD1222 [cohort 5] and 15 who have received two doses of Pfizer/**Moderna** mRNA COVID-19 vaccine [cohort 6]) will be dosed in parallel with the high dose used in cohorts 2 and 4.

4.1 Number of Participants

….

A total of 10 healthy participants and 12 participants with CHB infection is considered sufficient to confirm the safety, tolerability and immunogenicity of ChAdOx1-HBV and to answer the objectives of the study before progressing to larger studies. An additional 30 healthy participants (15 who have received two doses of AZD1222 and 15 who have received two doses of Pfizer/**Moderna** mRNA COVID-19 vaccine) will be recruited to assess whether prior receipt of AZD1222 results in decreased T cell responses to ChAdOx1-HBV.

4.2 Inclusion criteria

Healthy participants (cohort 6):

14. Considered to be healthy with no current conditions that may significantly impair participant safety or influence study results, in the opinion of the Investigator
15. Adult males or females aged ≥40 to ≤60 years at screening
16. Completed second dose of Pfizer (Comirnaty®) or **Moderna (Spikevax)** mRNA COVID-19 vaccine **6 to 30 weeks** before enrolment

5.7 Method of Assigning Participants to Treatment Groups

In cohorts 1-4**, t**he healthy and CHB participants will be allocated to low dose or high dose treatment depending on the treatment schedule prepared by statistician.

As cohorts 5 and 6 were introduced following completion of enrolment of the healthy participants in cohorts 1 and 2, all further healthy participants enrolled under protocol version 7.0 **and 8.0** will receive high dose treatment.

The Synopsis is updated to reflect the changes above.

Details of Changes Made in Protocol Amendment 6 (Protocol Version 7.0, 08 Jul 2021)

Front Page

Sponsor's Authorised Representative: [redacted]

Chief Scientific Officer

ADMINISTRATIVE AND CONTACT INFORMATION

Immunogenicity Laboratory	
Central Laboratory (for pgRNA and HBcAg analysis)	

1.5.1 Rationale for Investigation of Dosing Interval With Respect to COVID-19 Vaccines

There are concerns that the prior use of a vectored vaccine may result in anti-vector responses, which would decrease the response to a subsequent use of the same vector. Specifically, there have been concerns raised that the use of the AstraZeneca ChAdOx1-SARS-CoV-2 vaccine (AZD1222 or Vaxzevria®) might reduce either the antibody or T cell response to a subsequent vaccine using the same vector. This potential decrease has been attributed to the induction of a neutralising cross-reactive T cell response to the viral vector.

For adenoviral vectors, prior infection with a replicating adenovirus of the same strain (e.g., human adenoviral 5) does appear to have an effect on the subsequent T cell and antibody response. However, this neutralisation is primarily directed against the fibre protein. This interference was a primary motivating factor in the development of replication-incompetent adenoviral vectors based on simian strains (chimpanzee, bonobo, and gorilla). In contrast, the neutralisation resulting from the use of the replication incompetent adenoviral vectors themselves is directed primarily to the abundant hexon capsid protein, and this response may have only minor effect on cell entry and immunogenicity, mainly mediated by the fibre.

In studies of AZD1222, there was no statistical relation between the level of neutralisation response following the first immunisation, and the resultant antibody or T cell response (as measured by IFN-γ ELISpot) following a second immunisation [27]. Likewise prior receipt of a ChAdOx1 vaccine (either ChAdOx1-MERs or ChAdOx1-mening) had no effect on the response to AZD1222.

However, the improvement of antigen-specific immunogenicity of a delayed interval is supported by reports in the literature of clinical trials assessing homologous or heterologous prime-boost regimes with adenoviral vectors [28-33]. In summary, the longer the interval between first and second dose of the adenoviral vector, the better the antigen-specific boosting capacity of the second dose. This has been mostly studied with antibody responses to the same adenoviral vector encoding the same antigen. This has been in part attributed to declining titres of vector-neutralising and vector-binding antibodies with time. Specifically, antigen-specific antibodies were boosted 3-fold when the boosting interval was 4 weeks, but this increased to 10-fold when the interval between the two vaccines was 24 weeks [29]. In another study, antibody titres were boosted 10-fold after a prime-boost interval of 12 weeks, and T cells were also boosted [28]. Nonetheless, it is well-known that increasing interval may boost responses, and the association of the increase in titres with increasing intervals has not been clearly shown in any of the cited studies to be attributed to the level of anti-vector neutralizing antibodies.

It is important to note that all previous clinical experience with second administration of the same adenoviral vector has been in the context of a single antigen, for example, Ad26-gag prime followed by Ad26-gag boost. This is in contrast to the scenario discussed here, where a possible administration of the ChAdOx1-nCoV-19 vaccine is followed by administration of a ChAdOx1 vector encoding HBV antigens. This is not a prime-boost scenario per se but rather the re-use of the same vector platform in a different indication.

Given this uncertainty and the improved responses seen three months after initial immunization compared to one month in studies of homologous prime-boost of the same antigen, and lacking clear data, the Sponsor has advised that participants not receive the ChAdOX1 vaccine in this and other [redacted]-sponsored trials until at least three months after receiving the AZD1222 or J&J hAd26, and that a three-month interval should also be advised if the COVID-19 vaccine follow entry into our study. However, this is not based on firm data, and either a shorter or longer interval may indeed be warranted.

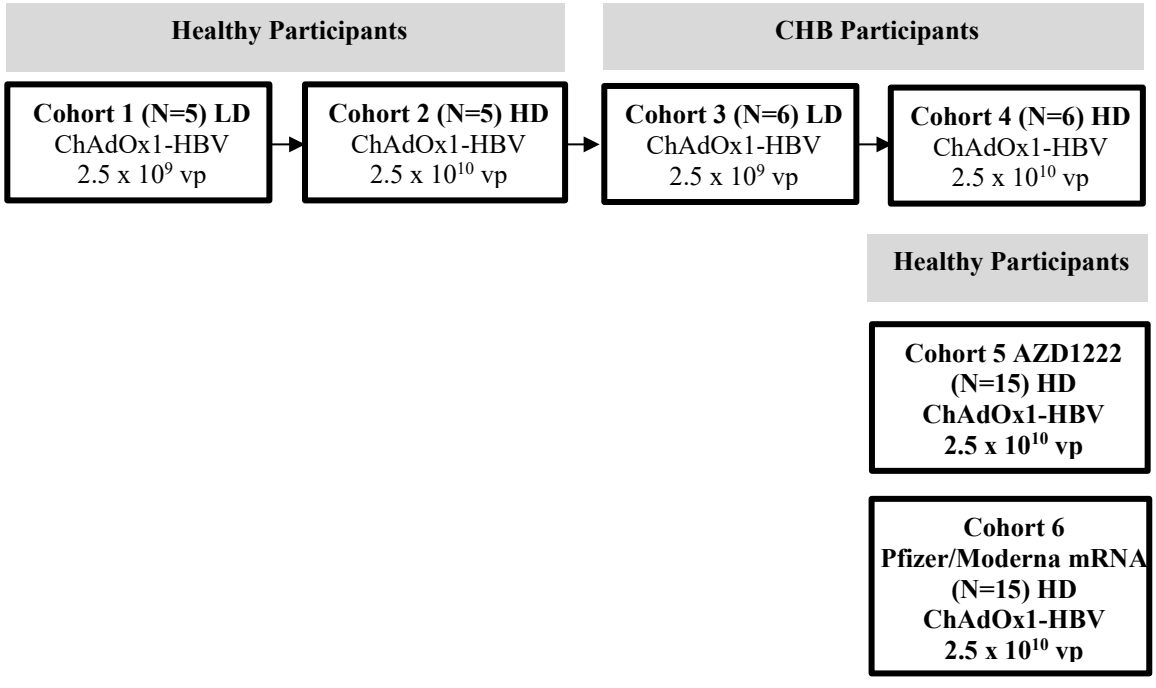
To support this interval, this study aims to produce data of the response in healthy volunteers to our ChAdOx-1 HBV vaccine following the use of the AZD1222 vaccine. T cell responses (the critical immunological factor in chronic HBV infection) will be compared in participants who have received a prior two-dose series of AZD1222 with those who have received either the Pfizer mRNA COVID-19 vaccine (Comirnaty®) or Moderna COVID-19 vaccine (Spikevax). Due to the recommendation to use the AZD1222 in those over the age of 40 years, we will confine our study to 40-60 years of age and keep the interval from the second COVID 19 vaccine in a narrow window of 10-18 weeks. No interference on HBV-focused T cell responses is expected from the Pfizer or Moderna vaccine, so the interval from the second COVID-19 vaccine will be between 6 to 30 weeks.

2 STUDY OBJECTIVES

Objectives	Endpoints
Secondary	Secondary
• **Cohorts 5 and 6 only: Assess whether the receipt of prior ChAdOx1 SARS-CoV-2 vaccine (AZD1222) results in decreased T cell responses to ChAdOx1-HBV, when administered 10-18 weeks prior to ChAdOx1-HBV**	• **Total T cell response to the antigens encoded by ChAdOX1-HBV as measured in a peptide-stimulated ELISpot assay**
Exploratory	
Determine the effect of ChAdOx1-HBV on virological and immunological systemic and intrahepatic changes in participants with CHB infection and virally suppressed with oral antiviral medication.	•
	• **Effect of prior AZD1222 on the CD4+ and CD8+ T cell magnitude and phenotype as measured by multiparameter flow cytometry**

3.1.1 Study Design

This is a Phase 1, first in human study of ChAdOx1-HBV. The study will be conducted in ~~10~~**40** healthy participants and 12 participants with CHB and virally suppressed with oral antiviral medication. This will be an open-label, non-randomised dose escalation study comparing the safety, tolerability and immunogenicity of 2 different doses of ChAdOx1-HBV vaccine. **T cell responses in healthy participants who have received a prior two-dose series of either AZD1222 or the Pfizer or ModernamRNA COVID-19 vaccines will also be analysed.** The study design is shown in Figure 2.



Five reviews of the safety, tolerability and available immunogenicity data will be performed by a Safety Monitoring Committee (SMC):

-

No SMC review is required for cohorts 5 and 6 as the dose administered has been assessed in the previous cohorts of healthy participants.

3.1.2 Study Methodology

The study will investigate response to study vaccine as shown in Table 2. On Day 0, an electronic diary (eDiary), tape measure and thermometer will be provided to perform self-assessment of local and systemic reactogenicity. **All participants will then have a follow-up telephone call on Day 1 and return to the clinic for study assessments on Days 7, 14, 28, 84. Participants in cohorts 1-4 only, will also have follow up visits on Day 56 and Day 168. End of study visit procedures will be performed at the final visit**~~Participants will then have a follow-up telephone call on Day 1 and return to the clinic on Days 7, 14, 28, 56, 84 and 168 for study assessments, with end of study visit procedures performed at the final visit~~.

Five healthy participants will be administered the low dose first (cohort 1). Dose escalation will only be initiated in the ~~remaining~~**next** 5 healthy participants (cohort 2) following SMC review.

Six CHB participants will be administered the low dose (cohort 3) before the dose escalation is initiated in the remaining 6 CHB participants (cohort 4).

Thirty healthy participants (15 who have received two doses of AZD1222 [cohort 5] and 15 who have received two doses of Pfizer/Moderna mRNA COVID-19 vaccine [cohort 6]) will be dosed in parallel with the high dose used in cohorts 2 and 4.

The first participant in each **of** cohort**s 1-4** will be assessed for 1 hour in the clinic post-vaccination in case of immediate adverse events (timed after the end of study vaccine administration). ~~The~~**All** other participants will be assessed for 30 minutes.

The safety and immunogenicity assessments to be performed in **cohorts 1-4** are:

-

For participants in cohorts 5 and 6, all assessments on Days 0, 7, 14, 28 and 84 above will be performed. In addition, neutralising antibodies to ChadOx1 will be assessed on Days 0 and 84.

3.1.4.1 Duration for Each Participant

Cohorts 1-4: Up to 8 months (up to 1.5 months for screening and 6.5 months on the study with study vaccine given on Day 0).

Cohorts 5 and 6: Up to 4.5 months (up to 1.5 months for screening and 3 months on the study with study vaccine given on Day 0).

3.2 Discussion of Study Design

This is a first in human study of the ChAdOx1 vaccine and is designed to assess the safety in both healthy adults, as well as in the target CHB population. As T cell responses to natural infection are blunted or exhausted in participants with CHB, it will be important to determine how responses in CHB compare to healthy participants. **Given the uncertainty around prior use of a vectored vaccine resulting in anti-vector responses, this study will also assess the response of healthy participants who have previously received AZD1222 to ChAdOx1-HBV.**

4.1 Number of Participants

It is planned that ~~10~~**40** healthy participants and 12 participants with CHB infection and virally suppressed with oral antiviral medication will be enrolled in the study. Chronic HBV participants will be recruited/deemed eligible by hepatologists, infectious disease specialists or physicians experienced in treating CHB participants. Centres not meeting the enrolment expectations will be considered for replacement with or addition of a new site. If participants leave the study before all follow-up visits are completed (for any reason), then these may be replaced at the discretion of the study team. A log of all participants enrolled into the study (i.e. having given informed consent) will be maintained in the Investigator's Site File (ISF) at the study centre irrespective of whether they have treated with vaccine or not.

~~The number of participants is based on feasibility considerations rather than formal sample size calculations.~~ A total of 10 healthy participants and 12 participants with CHB infection is considered sufficient to confirm the safety, tolerability and immunogenicity of ChAdOx1-HBV and to answer the objectives of the study before progressing to larger studies. **An additional 30 healthy participants (15 who have received two doses of AZD1222 and 15 who have received two doses of Pfizer/Moderna mRNA COVID-19 vaccine) will be recruited to assess whether prior receipt of AZD1222 results in decreased T cell responses to ChAdOx1-HBV.**

4.2 Inclusion criteria

Participants must meet *all* the following criteria to be eligible for the study:

5. If female: Not pregnant and one of the following:
   -
   - **Sexual abstinence, only if the participant refrains from heterosexual intercourse during the entire study period and it is the usual lifestyle of the participant**

<u>Healthy participants (cohort 5):</u>

11. **Considered to be healthy with no current conditions that may significantly impair participant safety or influence study results, in the opinion of the Investigator**
12. **Adult males or females aged ≥40 to ≤60 years at screening**
13. **Completed second dose of COVID-19 AZD1222 vaccine 10 to 18 weeks before enrolment**

Healthy participants (cohort 6):

14. **Considered to be healthy with no current conditions that may significantly impair participant safety or influence study results, in the opinion of the Investigator**
15. **Adult males or females aged ≥40 to ≤60 years at screening**
16. **Completed second dose of Pfizer/Moderna mRNA COVID-19 vaccine 6 to 30 weeks before enrolment**

4.3 Exclusion Criteria

8. **Cohorts 1-4:** Receipt of any adenoviral vaccine within 3 months prior to administration of ChAdOx1-HBV on Day 0, or plan to receive an adenoviral-based vaccine within 3 months after Day 0

   Cohorts 5 and 6: Receipt of any adenoviral vaccine (other than AZD1222 per inclusion criterion 13) within 3 months prior to administration of ChAdOx1-HBV on Day 0, or plan to receive an adenoviral-based vaccine within 3 months after Day 0

Additionally, for healthy participants (cohorts 1, **2, 5 and 6**)

18. HBsAg positive

5.1 Study Vaccines Administered

The study vaccine to be given in each treatment group are shown in Table 1.

Table 1 Study Vaccine Treatment Groups in HBV001

Treatment Cohort	Vaccine Single Dose	N
Cohort 1 LD Healthy Participants	ChAdOx1-HBV 2.5 x 10^9 vp	5
Cohort 2 HD Healthy Participants	ChAdOx1-HBV 2.5 x 10^{10} vp	5
Cohort 3 LD Participants with CHB	ChAdOx1-HBV 2.5 x 10^9 vp	6
Cohort 4 HD Participants with CHB	ChAdOx1-HBV 2.5 x 10^{10} vp	6
Cohorts 5 and 6 HD Healthy Participants	**ChAdOx1-HBV 2.5 x 10^{10} vp**	**30**

Abbreviations: ChAdOx1-HBV=chimpanzee adenovirus-vectored hepatitis B virus vaccine; CHB=chronic hepatitis B virus; HD=high dose; LD=low dose; vp=viral particles

5.7 Method of Assigning Participants to Treatment Groups

In cohorts 1-4, ~~T~~the healthy and CHB participants will be allocated to low dose or high dose treatment depending on the treatment schedule prepared by statistician.

As cohorts 5 and 6 were introduced following completion of enrolment of the healthy participants in cohorts 1 and 2, all further healthy participants enrolled under protocol version 7.0 will receive high dose treatment.

6 STUDY PROCEDURES AT EACH VISIT/SCHEDULE OF ASSESSMENTS

The study consists of the following:

- A 42-day screening period before the start of the study period
- A study period of 168 days, consisting of a vaccination day on Day 0, a follow-up telephone call on Day 1 and follow-up visits on Days 7, 14, 28, 56 ~~and end of study visit on Day 168~~**. Participants in cohorts 1-4 only, will also have follow up visits on Day 56 and Day 168. End of study visit procedures will be performed at the final visit**.

Table 2 Overall Schedule of Assessments at Each Study Visit

Visit/Call	Screen	Vaccination			Follow-up						End of Study
Timepoint	Day-42 to -1	Day 0			Day 1	Day 7±1	Day 14±1	Day 28±2	Day 56±3 (cohorts 1-4)	Day 84±7[a]	Day 168±7 (cohorts 1-4)
		Pre-	0	Post-							
Informed consent	X										
Baseline/eligibility variables											
Demographics	X										
Inclusion and exclusion criteria	X	X									
Height and Weight	X										
Medical and disease history	X	X									
HIV **Ab**, HCV **Ab**, **HBsAg, HDV Ab**[(a)][b]	X										
Urinalysis	X										
Urine pregnancy test (β-hCG) [c]	X	X						X	X	X	X
Laboratory eligibility and safety tests											
Haematology [d]	X	X				X	X	X	X	X	X
Biochemistry[d]	X	X				X	X	X	X	X	X
Liver function tests[e]	X	X				X	X	X	X	X	X
Study vaccination											
Vaccination			X								
Post-vaccination observation[f]				X							
Other Safety assessments											
Full physical examination	X	X									
Directed physical examination if required						X	X	X	X	X	X
Vital signs[g]	X	X		X		X	X	X	X	X	X
Local/systemic reactogenicity [h]		X		X	X	~~X~~					
Unsolicited adverse events [i]	X	X	X	X	X	X	X	X			
Serious adverse events and adverse events of special interest	X	X	X	X	X	X	X	X	X	X	X
Prior and concomitant medications	X	X	X	X	X	X	X	X	X	X	X
Blood for HBV disease markers											
Healthy participants (HBsAb, HbcAb)		**X**									**X[j]**
CHB (HBV DNA, HBsAg quantitative)	X	**X**						**X**	**X**	**X**	**X**

Visit/Call	Screen	Vaccination			Follow-up						End of Study
Timepoint	Day-42 to -1	Day 0			Day 1	Day 7±1	Day 14±1	Day 28±2	Day 56±3 (cohorts 1-4)	Day 84±7[a]	Day 168±7 (cohorts 1-4)
		Pre-	0	Post-							
CHB (HBeAg, anti-HBe, anti-HBs, pgRNA, HBcAg, anti-HBc)		**X**						**X**	**X**	**X**	**X**
Immunogenicity assessments											
Blood for cellular immunogenicity [k]		X					X	X	X	X	X
Blood for neutralising antibodies (cohorts 5 and 6 only)			**X**							**X**	
Liver fine needle aspirates[l]†	X	~~X~~								X	

Abbreviations: ALP=alkaline phosphatase; ALT=alanine transaminase; aPTT=activated partial thromboplastin time; AST=aspartate transaminase; CHB=chronic hepatitis B virus; DNA=desoxyribonucleic acid; GGT=gamma-glutamyl transpeptidase; HBcAb=hepatitis B core antibody; HBsAb=hepatitis B surface antibody; β-hCG=beta human chorionic gonadotrophin; HBcAg=hepatitis B core-related antigen; HBV=hepatitis B virus; HBeAg=hepatitis B e antigen; HBsAg=hepatitis B surface antigen; HCV=Hepatitis C virus; HDV=Hepatitis D virus; HIV=human immunodeficiency virus; ICS=intracellular cytokine staining; INR= international normalised ratio; LFNA=liver fine needle aspirate; pgRNA=pre-genomic RNA; PBMCs=peripheral blood mononuclear cells; PT=prothrombin time
†Optional assessments

[a] End of Study Visit for cohorts 5 and 6

[b] HDV Ab serology and HBsAg quantitative test will be done in CHB participants only; HBsAg qualitative test will be done in healthy participants; all participants will be tested for HIV and HCV serology

[c] Female participants only

[d] Full haematology (including PT/INR and aPTT) and biochemistry panel

[e] Measurement of ALP, GGT, ALT, AST and total bilirubin

[f] The first participant in each cohort will be assessed for 1 hour in case of immediate adverse events (timed after the end of study vaccine administration). The other participants will be assessed for 30 minutes

[g] Pulse, blood pressure and temperature

[h] Captured during clinic visits and then via eDiary for 3 days post-vaccination

[i] Recorded in the eCRF from the date the informed consent is signed, at all clinic visits to cover the period since the previous visit and during the visit and up to 28 days post-vaccination

[j] If negative at baseline, at discretion of the investigator

[k] To be processed into PBMCs for analysis by ICS

[l] Only in CHB participants who consent to LFNAs **after confirming eligibility. Coagulation profile must be assessed prior to repeat LFNA on Day 84**. For those consenting to LFNAs, blood for PT/INR and aPTT testing must be collected

6.2 Treatment Day Assessments (Day 0)

The following will be performed pre-vaccination:

-
- Blood samples will be taken for immunogenicity assessments **(including neutralising antibodies for cohorts 5 and 6 only)**

6.3.4 Clinic Visit: Days 28, 56 (Cohorts 1-4) and 168 (End of Study Visit Cohorts 1-4)

6.3.5 Clinic Visit: Day 84 (including End of Study Visit Cohorts 5 and 6)

-
- Blood samples will be taken for immunogenicity assessments **(including neutralising antibodies for cohorts 5 and 6 only)**

7.2.1 Secondary Outcomes in Blood Samples

Blood samples of healthy participants in cohorts 5 and 6 will be used for analysis of total T cell response to ChAdOx1-HBV antigens using a peptide-stimulated ELISpot assay.

8.2.4 Reporting Requirements and Procedures for Serious Adverse Events

The SAE form for that event must be completed by the Investigator, within 24 hours of the study centre becoming aware of the event. The SAE form should be completed with all information known at the time and scanned and emailed to the local Medical Monitor and to [redacted]

The Synopsis is updated to reflect the changes above.

Details of Changes Made in Protocol Amendment 5 (Protocol Version 6.0, 01 Mar 2021)

ADMINISTRATIVE AND CONTACT INFORMATION

Sponsor Medical Oversight	[redacted]

4.3 Exclusion Criteria

1. Presence of any significant acute or chronic, uncontrolled medical/psychiatric illness
2. Hepatitis C virus (HCV) antibody positive…..

……..

8. ~~Any History of~~ receipt of any adenoviral vaccine **within 3 months prior to administration of ChAdOx1-HBV on Day 0, or plan to receive an adenoviral-based vaccine within 3 months after Day 0**

3.3 Benefit Risk Assessment

…….

With any new treatment there is always a possibility of an unexpected adverse events.

The administration of ChAdOx1-HBV in this study may interfere with the effectiveness of other vaccines subsequently administered to the participant. The ChAdOx1 vector induces anti-vector immunity in addition to an antigen-specific immune response. Of particular concern are neutralising antibodies directed against the capsid structure of the vector, which may have a negative effect on subsequent immunisations with the same or related adenoviral vector by neutralising the vector before it has a chance to transduce target cells. This effect decreases with longer interval between the vaccines [Error! Reference source not found.,Error! Reference source not found.**]. In particular, there is a concern that the ChAdOx1-HBV administered in this study could decrease the response to the AstraZeneca Covid19 vaccine, which has the same viral vector, especially if it is administered within 3 months of ChAdOx1-HBV. The Janssen (Johnson & Johnson) Covid19 vaccine is also made with an adenovirus, and some interference cannot be ruled out. Therefore it is recommended that participants in this study receive an alternative type of Covid19 vaccine, such as an mRNA or protein vaccine, at least 2 weeks before and after ChAdOx1-HBV in this study.**

There are also routinely scheduled reviews of safety and efficacy data throughout the study by the SMC……

8.2.4 Reporting Requirements and Procedures for Serious Adverse Events

…….

Investigators must not wait to collect additional information to fully document the event before notifying the local Medical Monitor of an SAE. The initial notification should include the following (at minimum):

- Protocol number and name and contact number of the Investigator
- Participant identification number (and initials and date of birth, if available)
- ~~Date participant received~~ iInvestigational product
- SAE(s) ~~and date of event onset~~
- ~~Current status of participant~~

Details of Changes Made in Protocol Amendment 4 (Protocol Version 5.0, 29 Oct 2020)

4.2 Inclusion Criteria

Participants must meet *all* the following criteria to be eligible for the study:

1. Adult males or females aged ≥18 to ≤65 years at screening
2. Body Mass Index ≤30kg/m^2……..

…….

10. HBsAg <~~4000~~**10000**IU/mL

Details of Changes Made in Protocol Amendment 3 (Protocol Version 4.0, 24 Feb 2020)

ADMINISTRATIVE AND CONTACT INFORMATION

Sponsor Medical Oversight	[redacted]
Principal Investigator	[redacted]

[redacted]

3.1.2 **Study Methodology**

Participants will be screened in the period Day -42 to Day -1. Informed consent will be obtained before any study specific procedures are performed. Eligible participants will then attend the clinic to receive study vaccine on Day 0. Participants will be enrolled sequentially.

….

- HBV disease markers in serum:
    - Healthy participants: HBsAg **will be tested at screening**, hepatitis B surface antibody (HBsAb) **and** HBcAb **will be tested at Day 0, and repeated at the end of the trial if negative at baseline, or at discretion of the Investigator**
    - CHB participants: **HBV DNA and quantitative HBsAg will be tested at screening and repeated on Days 0 28, 56, 84 and 168. The following parameters will be analysed on Days 0, 28, 56, 84 and 168: HBeAg, HBcAg, anti-HBs, anti-HBe, anti-HBc, pgRNA** ~~HBsAg, HBeAg, HBcAg, anti-HBs, anti-HBe, anti-HBc, HBV DNA, pgRNA will be analysed at screening, pre vaccination on Day 0 and on Days 14, 56, 84 and 168~~
    - …

In CHB participants who consent to LFNAs, these will be taken pre-vaccination **within the screening period** ~~on Day 0~~ and at the clinic visit on Day 84. Liver fine needle aspirate assays may be used to quantify and characterise intrahepatic immune and parenchymal cells and/or HBV DNA and RNA transcripts. These aspirates and subsequent LFNA assessments are optional.

4.3 Exclusion criteria

Additionally, for participants with well controlled CHB (cohorts 3 and 4)

21. ALT >3 × ULN, INR >1.5 unless the participant was stable on an anticoagulant regimen affecting INR, albumin <35 g/L, total bilirubin >**34.2 µmol/L** ~~2mg/dL~~, platelet count <**100 x 10^9/L** ~~100,000/mL~~

5 STUDY VACCINE

The Investigator must ensure that study vaccine is handled only by study team members who have been appropriately trained for the conduct of this clinical study and that dosing is only performed by study team members who fully understand the procedures outlined in this section, the Investigator's Brochure and the ~~Pharmacy~~ **Investigational Product (IP) Handling** Manual.

A Delegation of Authority Log will be maintained by the study centre and will identify the individual(s) authorised to prepare and administer the study vaccine.

5.2 Identity of Study Vaccine

ChAdOx1-HBV will be manufactured to Good Manufacturing Practice, labelled according to local regulations, including Annex 13 of the Good Manufacturing Practice Directive [28] as detailed in the ~~Pharmacy~~ **IP Handling** Manual.

ChAdOx1-HBV is formulated in an A438 buffer comprising of 10 mM histidine, 35 mM NaCl, 7.5% sucrose (w/v), 1 mM MgCl2, 0.1% (w/v) PS-80, 0.1 mM ethylenediaminetetraacetic acid (EDTA), 0.5% ethanol (v/v), pH 6.6 to a target concentration of 1 x 1011 vp/mL.

ChAdOx1-HBV will be supplied at a target sterile volume of 0.65 mL in 3 mL Type 1 borosilicate glass vials allowing 0.5mL to be drawn by syringe for dilution into high dose HBV vaccine and low dose HBV vaccine. Dilution to both low and high doses will be detailed in the ~~Pharmacy~~ **IP Handling** Manual. The vials are stoppered with sterilised European Pharmacopoeia/United States Pharmacopoeia Type 1 chlorobutyl, rubber stopper and sealed with aluminium/copolymer caps. The vaccine is clear to slightly opaque in appearance

The study vaccines will be released by a qualified person.

5.3 Labelling, Packaging and Shipping

At the start of the study, a sufficient number of study vaccine vials will be shipped to each study centre based on projected recruitment at each centre with approximately 20% overage in case of spillage or breakage. Further study vaccine supplies may be requested following the re supply process in the ~~Pharmacy~~ **IP Handling** Manual.

5.7 Method of Assigning Participants to Treatment Groups

At the screening visit, participants will be sequentially allocated a ~~screening~~ **participant** number once written, informed consent has been obtained. They will be identified by this number ~~until entry into~~**throughout** the study.

The healthy and CHB participants will be allocated to low dose or high dose treatment depending on the treatment schedule prepared by statistician.

Participants fulfilling entry criteria will be ~~allocated an enrolment number and~~ allocated to treatment based on a treatment schedule.

Once ~~screening and enrolment~~**participant** numbers have been assigned, no attempt will be made to use those numbers again. If a~~n enrolment~~ **participant** number is allocated incorrectly, no attempt will be made to remedy the error once the study vaccine has been dispensed. Any participants that are withdrawn prior to vaccination will be replaced on a case by case basis following discussion with the Sponsor. Discontinued participants following vaccination who do not complete all follow up visits will be replaced at the determination of the Sponsor.

Any replacement participants will be given the next sequential participant number.

The treatment schedule list will be held by the statistician during the study.

5.8.2.2 Study Vaccine Preparation and Administration

ChAdOx1-HBV vials will be allowed to reach room temperature before use.

Doses of ChAdOx1 HBV will be prepared by serial dilution according to the procedure in the ~~Pharmacy~~ **IP Handling** Manual. Dilution kits of commercially available equipment will be provided by the Sponsor.

All vaccinations will be made by a suitably qualified health professional. A second study team member will be present to account for each vaccination given.

The suitably qualified healthcare professional will wear gloves, eye protection and an apron or laboratory coat/gown during the procedure. The vaccination site will be covered with a sterile dressing to minimise dissemination of the recombinant virus into the environment. This should absorb any virus that may leak out through the needle track. The sterile dressing will be removed ~~approximately~~**kept on the vaccination site at least** 10 minutes after vaccination. The dressing will be discarded as GMO waste.

6 STUDY PROCEDURES AT EACH VISIT/SCHEDULE OF ASSESSMENTS

Table 2 Overall Schedule of Assessments at Each Study Visit

Visit/Call	Screen	Vaccination			Follow-up						End of Study
Timepoint	Day-42 to -1	Day 0			Day 1	Day 7±1	Day 14±1	Day 28±2	Day 56±3	Day 84±7	Day 168±7
		Pre-	0	Post-							
Informed consent	X										
Baseline/eligibility variables											
Demographics	X										
Inclusion and exclusion criteria	X	X									
Height and Weight	X										
Medical and disease history	X	X									
HIV **Ab**, HCV **Ab**, **HBsAg, HDV Ab**[(a)], ~~HBV, HDV serology~~	X										
Urinalysis	X										
Urine pregnancy test (β-hCG) [c]	X	X						X	X	X	X
Laboratory eligibility and safety tests											
Haematology [d]	X	X				X	X	X	X	X	X
Biochemistry[d]	X	X				X	X	X	X	X	X
Liver function tests[e]	X	X				X	X	X	X	X	X
Study vaccination											
Vaccination			X								
Post-vaccination observation[f]				X							
Other Safety assessments											
Full physical examination	X	X									
Directed physical examination if required						X	X	X	X	X	X
Vital signs[g]	X	X		X		X	X	X	X	X	X
Local/systemic reactogenicity [h]		X		X	X	~~X~~					
Unsolicited adverse events [i]	X	X	X	X	X	X	X	X			
Serious adverse events and adverse events of special interest	X	X	X	X	X	X	X	X	X	X	X
Prior and concomitant medications	X	X	X	X	X	X	X	X	X	X	X
Blood for HBV disease markers											
Healthy participants (HBsAb, HbcAb)		**X**									**X**[i]
CHB (HBV DNA, HBsAg quantitative)	X	**X**						**X**	**X**	**X**	**X**

CHB (HBeAg, anti-HBe, anti-HBs, pgRNA, HBcAg, anti-HBc)		**X**						**X**	**X**	**X**	**X**
Immunogenicity assessments											
Blood for cellular immunogenicity [k]		X					X	X	X	X	X
Liver fine needle aspirates[l]†	X	~~X~~								X	

Abbreviations: ALP=alkaline phosphatase; ALT=alanine transaminase; aPTT=activated partial thromboplastin time; AST=aspartate transaminase; CHB=chronic hepatitis B virus; DNA=desoxyribonucleic acid; GGT=gamma-glutamyl transpeptidase; HBcAb=hepatitis B core antibody; HBsAb=hepatitis B surface antibody; β-hCG=beta human chorionic gonadotrophin; HBcAg=hepatitis B core-related antigen; HBV=hepatitis B virus; HBeAg=hepatitis B e antigen; HBsAg=hepatitis B surface antigen; HCV=Hepatitis C virus; HDV=Hepatitis D virus; HIV=human immunodeficiency virus; ICS=intracellular cytokine staining; INR= international normalised ratio; LFNA=liver fine needle aspirate; pgRNA=pre-genomic RNA; PBMCs=peripheral blood mononuclear cells; PT=prothrombin time
†Optional assessments

[m] HDV Ab serology and HBsAg quantitative test will be done in CHB participants only; HBsAg qualitative test will be done in healthy participants; all participants will be tested for HIV and HCV serology

[n] Female participants only

[o] Full haematology (including PT/INR and aPTT) and biochemistry panel

[p] Measurement of ALP, GGT, ALT, AST and total bilirubin

[q] The first participant in each cohort will be assessed for 1 hour in case of immediate adverse events (timed after the end of study vaccine administration). The other participants will be assessed for 30 minutes

[r] Pulse, blood pressure and temperature

[s] Captured during clinic visits and then via eDiary for 3 days post-vaccination

[t] Recorded in the eCRF from the date the informed consent is signed, at all clinic visits to cover the period since the previous visit and during the visit and up to 28 days post-vaccination

[u] **If negative at baseline, at discretion of the** ~~investigator healthy participants: HBsAg, HBsAb, HBeAb. In CHB participants: HBsAg, HBeAg, HBeAg, anti-HBs, anti-HBe, anti-HBc, HBV DNA, pgRNA~~

[v] To be processed into PBMCs for analysis by ICS

[w] Only in CHB participants who consent to LFNAs **after confirming eligibility. Coagulation profile must be assessed prior to repeat LFNA on Day 84**. For those consenting to LFNAs, blood for PT/INR and aPTT testing must be collected

6.1 Screening and Baseline Pre-Dose Assessments

Written informed consent for participation in the study must be obtained before performing any study specific screening tests or evaluations according to the process in Section 13.4.

…..

- A blood sample will be taken for HIV, HCV, HBV, and **in CHB participants only**, hepatitis D Virus (HDV) diagnostic testing

…

Concomitant medications will be recorded throughout the study by questioning the participant at each visit whether they took any concomitant medications since the previous visit.

In CHB participants who consent to LFNAs: an LFNA will be performed within screening period, after screening tests have shown eligibility, as directed by the Principal Investigator or delegated physician. These aspirates and subsequent assessments are optional

NB. Coagulation profile must be assessed prior to repeat LFNA

6.2 Treatment Day Assessments (Day 0)

The following will be performed pre-vaccination:

- ~~In CHB participants who consent to LFNAs: an LFNA will be performed with additional PT/INR and aPTT testing, as directed by the Principal Investigator or delegated physician. These aspirates and subsequent assessments are optional~~

7.1.4.3 **HIV, HBV, HCV and HDV Diagnostic Testing**

Blood samples will be tested for:

- In all participants:
  - HIV antibodies
  - HCV antibodies
- In Healthy Volunteers: **qualitative** HBsAg, HBsAb, HBcAb
- In CHB participants: **quantitative** HBsAg, HBeAg, HBcAg, anti-HBs, anti-HBe, anti-HBc, HBV DNA, pgRNA, HDV serology

8.2.1.3 Serious Adverse Event

An **SAE** is an adverse event meeting the outcome criteria for seriousness regardless of relationship to the study vaccine. **The following are not (and should not be reported as) SAEs: hospitalisation for scheduled treatments and elective medical/surgical procedures related to a pre-existing condition that did not increase in severity or frequency following receipt of study vaccine, and admissions for social reasons.**

Details of Changes Made in Protocol Amendment 2 (Protocol Version 3.0, 04Dec2019)

4.2 Inclusion Criteria

The following text in bold has been added:

5. If female: Not pregnant and one of the following:
   - ~~Hormonal (oral, intravaginal, transdermal, implantable or injectable)~~:
   - **Combined (estrogen and progestogen containing) hormonal contraception associated with inhibition of ovulation:**
     - **oral**
     - **intravaginal**
     - **transdermal**
   - **Progestogen-only hormonal contraception associated with inhibition of ovulation:**
     - **oral**
     - **injectable**
     - **implantable**
   - ~~An intrauterine hormone-releasing system~~

4.3.1.2 *Study Discontinuation*

The following text in bold has been clarified:

Participants may choose to discontinue study assessments by withdrawal of consent. If the participant discontinues from taking the study vaccine but does not withdraw consent, the Investigator or research worker should attempt to continue safety follow-up assessments.

Participants **may** be withdrawn from taking the study vaccine**, but followed up for safety,** in the event of:

- A severe adverse event or SAE
- Difficulties in obtaining blood or other samples
- Failure of the participant to comply with the protocol requirements or to cooperate with the Investigator
- **For safety reasons, it being in the best interest of the participant that he/she be withdrawn, in the Investigator's opinion**
- **A positive pregnancy test or if the participant is non-compliant with the contraception requirements (see Section 4.2)**
- **Development of a medical condition that requires concomitant treatment with a potentially toxic therapy**

Participants **must** be withdrawn from the study in the event of:

- Withdrawal of consent
- ~~For safety reasons, it being in the best interest of the participant that he/she be withdrawn, in the Investigator's opinion~~
- ~~A positive pregnancy test or if the participant is non-compliant with the contraception requirements (see Section 4.2)~~

- ~~Development of a medical condition that requires concomitant treatment with a potentially toxic therapy~~

8.1.3 Local Medical Monitor

The following text in bold has been added:

The local Medical Monitor **(LMM)** is the Sponsor's **medical** representative ~~and is a credentialed physician or surgeon in their country of residence with the necessary expertise to act in such capacity~~. The **LMM** ~~local Medical Monitor reviews the safety of the product for protocols in a specific region and, in conjunction with the Sponsor, determines expectedness of study vaccine related SAEs. The local Medical Monitor, in consultation with the Sponsor, may assess the causality for adverse events and may upgrade the causality determined by the Investigator~~**can add another causality to the investigator's causality assessment, however the causality assessment given by the investigator must not be downgraded by the LMM. If the LMM disagrees with the investigator's causality assessment, the opinion of both the investigator and the sponsor should be provided with the report**.

8.2.3.2 Assessment of Relationship

The following text in bold has been added:

For all other adverse events, the Investigator ~~and the Sponsor (the local Medical Monitor)~~ will determine a **causal relationship** to the study vaccine.

The Investigator ~~and the Sponsor both~~ determine**s** causality. ~~It is expected that communication and consultation may occur in the assessment of the causality of adverse **events**~~**The Sponsor and LMM can add another causality assessment to the Investigator's causality assessment. The causality assessment given by the investigator must not be downgraded by the Sponsor. If the Sponsor disagrees with the Investigator's causality assessment, the opinion of both the Investigator and the Sponsor should be provided with the report**.

8.2.4.3 Medical Review and Reporting by the Sponsor

The following text has been clarified:

The Sponsor (or designee) will determine expedited reporting requirements for each reported SAE according to local requirements based upon:

- Investigator's assessment of causality and seriousness~~, with allowance for upgrading following independent review by the Sponsor (or designee) as needed~~

The Synopsis is updated to reflect the changes above.

Details of Changes Made in Protocol Amendment 1 (Protocol Version 2.0, 13Nov2019)

ADMINISTRATIVE AND CONTACT INFORMATION
The following text in bold has been added:

Central Laboratory (for pgRNA and ~~HBcrAg~~ **HBcAg** analysis)	[redacted]

2 STUDY OBJECTIVES AND ENDPOINTS
The following text in bold has been added:

Exploratory Determine the effect of ChAdOx1-HBV on virological and immunological systemic and intrahepatic changes in participants with CHB infection and virally suppressed with oral antiviral medication.	Exploratory • Effect on serum hepatitis B core-related antigen (~~HBcrAg~~**HBcAg**)

3.1.1 Study Design

The following text in bold has been added:

A Data ~~Safety~~ Monitoring Committee (D~~S~~MC) will be appointed to perform unscheduled reviews of the available safety~~,~~ **and** tolerability~~, efficacy and immunogenicity~~ study data and make recommendations concerning the continuation, modification or termination of the study if one of the study stopping or holding rules defined in Section 3.1.3 is met.

3.1.2 Study Methodology

The following text in bold has been added:

The study will investigate response to study vaccine as shown in Table 2. On Day 0, an electronic diary (eDiary), tape measure and thermometer will be provided to perform self-assessment of local and systemic reactogenicity. Participants will then **have a follow-up telephone call on Day 1 and** return to the clinic on Days ~~1,~~ 7, 14, 28, 56, 84 and 168 for study assessments, with end of study visit procedures performed at the final visit.

- HBV disease markers in serum (~~HBV DNA, HBeAg,~~ **in healthy participants: HBsAg, hepatitis B surface antibody (**~~anti-HBs~~**HBsAb), HBcAb; in CHB participants:** HBsAg, HBeAg, ~~HBcrAg~~ **HBcAg,** anti-HBs~~),~~ **anti-HBe, anti-HBc,** HBV DNA, pgRNA) will be analysed at screening, pre-vaccination on Day 0 and on Days 14, 56, 84 and 168 in the CHB cohorts

6 STUDY PROCEDURES AT EACH VISIT/SCHEDULE OF ASSESSMENTS

The following text in bold has been added:

A study period of 168 days, consisting of a vaccination day on Day 0**, a follow-up telephone call on Day 1** and follow-up visits on Days ~~1,~~ 7, 14, 28, 56 and end of study visit on Day 168

Table 1 Overall Schedule of Assessments at Each Study Visit

Visit/Call	Screen	Vaccination			Follow-up						End of Study
Timepoint	Day-42 to -1	Day 0			Day 1	Day 7±1	Day 14±1	Day 28±2	Day 56±3	Day 84±7	Day 168±7
		Pre-	0	Post-							
Other Safety assessments											
Full physical examination	X	X									
Directed physical examination if required					~~X~~	X	X	X	X	X	X
Vital signs [g]	X	X		X	~~X~~	X	X	X	X	X	X
Immunogenicity assessments											
~~Transcriptomics~~		~~X~~			~~X~~	~~X~~	~~X~~	~~X~~	~~X~~	~~X~~	~~X~~

Abbreviations: ALP=alkaline phosphatase; ALT=alanine transaminase; aPTT=activated partial thromboplastin time; AST=aspartate transaminase; CHB=chronic hepatitis B virus; DNA=desoxyribonucleic acid; GGT=gamma-glutamyl transpeptidase; **HBcAb=hepatitis B core antibody;** ~~anti-HBs~~**HBsAb**=hepatitis B surface antibody; β-hCG=beta human chorionic gonadotrophin; ~~HBcrAg~~**HBcAg**=hepatitis B core-related antigen; HBV=hepatitis B virus; HBeAg=hepatitis B e antigen; HBsAg=hepatitis B surface antigen; HCV=Hepatitis C virus; HDV=Hepatitis D virus; HIV=human immunodeficiency virus; ICS=intracellular cytokine staining; INR= international normalised ratio; LFNA=liver fine needle aspirate; pgRNA=pre-genomic RNA; PBMCs=peripheral blood mononuclear cells; PT=prothrombin time

[h] In ~~all~~**healthy** participants: HBsAg, **HBsAb, HBcAb**. In CHB participants ~~only HBV DNA,~~**:** HBsAg, HBeAg, **HBcAg, anti-HBs,** anti-HBe, anti-~~HBs~~**HBc, HBV DNA**, pgRNA~~, HBcrAg~~

6.3.1 ~~Clinic Visit~~Telephone Call: Day 1

The following text has been clarified:

- ~~A symptom-directed physical examination will be performed if required~~
- ~~Vital signs (pulse rate, blood pressure and temperature) will be measured~~
- ~~Blood samples will be taken for immunogenicity assessment [transcriptomics only]~~
- eDiary to collect local and systemic reactions during 3 consecutive days post-vaccination

6.3.2 Clinic Visit: 7

The following text has been clarified:

- A symptom-directed physical examination will be performed if required
- Vital signs (pulse rate, blood pressure and temperature) will be measured
- Blood samples will be taken for haematology (including PT/INR and aPTT), biochemistry and LFTs
- ~~Blood samples will be taken for immunogenicity assessment [transcriptomics only]~~

7.1.4.3 HIV, HBV, HCV and HDV Diagnostic Testing

Blood samples will be tested for:

- In all participants:
  - HIV antibodies
  - HCV antibodies
  - ~~HBsAg~~

- **In Healthy Volunteers: HBsAg, HBsAb, HBcAb**
- In CHB participants only~~: HDV serology, HBV DNA,~~**: HBsAg,** HBeAg, **HBcAg,** anti~~-HBe antibody~~**-HBs**, anti~~-HBs antibody~~**-HBe, anti-HBc, HBV DNA, pgRNA, HDV serology**

7.2 Immunogenicity Assessments

The following text has been clarified:

Immunogenicity samples (PBMCs) will be taken for planned and potential exploratory analyses ~~and transcriptomics~~ at each of the timepoints according to Table 2.

7.2.2.1 Exploratory Analysis in Blood Samples

The following text in bold has been added:

Serum blood samples will be used for analysis of pgRNA and ~~HBcrAg~~**HBcAg**.

7.3 Secondary Outcomes in Blood Samples

The following text in bold has been added:

Serum blood samples in CHB participants will be used for analysis of HBV DNA, HBeAg, anti-HBe antibody, HBsAg and ~~anti-HBs antibody~~**HBsAb**.

8.1.5 Data Safety Monitoring Committee

The following text in bold has been added:

A ~~DSMC~~ will be appointed to perform unscheduled reviews of the available safety~~,~~ **and** tolerability~~, efficacy and immunogenicity~~ study data and make recommendations concerning the continuation, modification, or termination of the study if one of the study stopping or holding rules defined in Section 3.1.3 is met.