INVESTIGATOR'S BROCHURE

Chimpanzee Adenovirus Oxford 1-vectored Hepatitis B Virus Vaccine (ChAdOx1-HBV)

A non-replicating viral vector encoding consensus sequences from a group C genotype.

Version: 4.0
27 Apr 2021

Sponsor's Authorised Representative: [redacted]

Chief Medical Officer

Signature [redacted] Date 27-APR-2021

Margaret Marshall

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

VERSION HISTORY

This is the fourth version of the Investigator's Brochure.

Summary of Modifications since Version 3.0		
Date	**Section**	**Modification**
27/04/2021	6.4	Addition of warnings and precautions on thrombotic events and thrombocytopenia following ChAdOx1-HBV.

Summary of Modifications since Version 2.0		
Date	**Section**	**Modification**
01/04/2021	Front Page	[redacted] added as Sponsor's Authorised Representative.
01/04/21	1.2 4.2	Results of biodistribution and shedding study of ChAdOx1-HBV included. Information on related simian adenoviruses deleted.
01/04/21	1.3 2.2 5.1 6.1.2	Updated the summary of clinical experience to reflect use of the ChAdOx1-vectored COVID-19 vaccine and Vaccitech access to study data. Safety information amended.
01/04/21	1.3 5.1 6.1.2	Information of doses of ChAdOx1-HBV updated.
01/04/21	4.1	Information on lack of oncogenic potential of ChAdOx1-HBV included.
01/04/21	4.4	Results of biodistribution and shedding study of ChAdOx1-HBV included.
01/04/21	5.2	Status of influenza clinical programme updated to "completed".
01/04/21	5.3	Section including specific information on clinical studies in chikungunya, Middle East respiratory syndrome, prostate cancer, malaria, tuberculosis, meningitis B and MERS deleted.
01/04/21	6.1.1	Results of biodistribution and shedding study of ChAdOx1-HBV included.
01/04/21	6.4	The following section deleted: "Mild tenderness, bruising, light-headedness, or rarely, infection or bleeding at the injection site, or vasovagal syncope, may result from venepuncture. The volume of

		blood drawn over the study period should not compromise the populations being studied."
01/04/21	6.5	The following section deleted: "With any new treatment there is always a possibility of an unexpected adverse events. Stopping and holding rules have been defined for study HBV002, and a Data Monitoring Committee will perform a review of safety data if one of these is met. There will also perform unscheduled reviews of safety during the study-." And replaced with: Vaccines may interfere with the response to each other if administered too closely together. • Live vaccines should not be administered within 30 days before or after study vaccine. • Inactivated vaccines should not be administered within 14 days of study vaccine. • Adenoviral-vectored vaccines should not be administered within 3 months of the ChAdOx1-HBV component of VTP-300, or as described in the protocol.
01/04/21	6.7	Addition of: "There is no rescue medication."
01/04/21	6.8	Statement on RSI updated and corrected to read: "All serious adverse reactions will be considered unexpected for the purposes of expedited reporting."
01/04/21	Throughout	General editorial amendments

Summary of Modifications since Version 1.0		
Date	**Section**	**Modification**
01/04/20	1.1	Chimpanzee adenovirus Oxford 1 (ChAdOx1)-vectored hepatitis B virus (HBV) vaccine (ChAdOx1-HBV) is a chimpanzee adenoviral-vectored vaccine encoding **HBV** consensus sequences from a group C genotype. The ChAdOx1 virus has been engineered to be replication-~~deficient~~**incompetent** and can be manufactured in well-established HEK293 cell lines containing the adenoviral E1 gene.
01/04/20	1.3	Based on these results, doses of 2 .5 x 10^9 vp and 2.5 x 10^{10} vp ~~will be~~**are being** investigated in the **First-in-Human (FIH)** HBV001 clinical study in healthy volunteers ~~as a first step, then~~**and** subjects with chronic HBV infection. **Depending on the results of HBV001,** ~~T~~the ~~optimal~~ **higher** dose of **2.5 x 10^{10} vp** will be further evaluated in ~~a~~

Summary of Modifications since Version 1.0		
Date	**Section**	**Modification**
		~~subsequent~~ study ~~(~~HBV-002~~)~~, with MVA and with or without programmed cell death protein 1 (PD-1) inhibitors.
01/04/20	2.2	ChAdOx1), a serogroup E adenovirus, has been administered to over 200 people in studies of MERS, influenza, TB, chikungunya, and prostate cancer, and **found to be well tolerated** ~~has been safe~~ and immunogenic. Immunogenicity of the vector encoded antigens can be enhanced through the addition of molecular adjuvants such as a short shark invariant chain (SIi) sequence or use of the tissue plasminogen **signal sequence**~~promoter~~ fused to the N-terminus of the antigen.
01/04/20	2.2	The genetic HBV insert was then cloned into both the ChAdOx1 vector (chimpanzee adenovirus-vectored HBV vaccine; ChAdOx1-HBV), and also into an MVA vector (MVA-HBV), **which will be** ~~that will be~~ used as a boost in ~~a subsequent~~ study **HBV-002.** ~~after the evaluation of the ChAdOx1-HBV given alone is completed.~~
01/04/20	2.3	Doses of ChAdOx1 vaccines in previous studies have varied from 10^8 to over 10^{10}~~11~~ vp per dose **and this study will test the dose of 2.5 x 10^{10} vp.**~~the middle two doses will be evaluated in this study to prepare for a further heterologous prime boost regimen.~~
01/04/20	2.3	This approach has been used in a prior non-published study without major toxicity occurring. For this reason, a prime-boost strategy using ChAdOx1 vector (ChAdOx1-HBV) and an MVA vector (MVA-HBV), with and without PD-1 inhibitors will be evaluated.~~in a subsequent study.~~
01/04/20	3.1	The HBV immunogen encompasses three full length HBV-antigens (precore,core, polymerase and preS1~~/~~, preS2/, surface) along with truncated SIi and ~~T~~tPA **molecular**~~genetic~~ adjuvants and it is encoded in the chimpanzee adenoviral vector.
01/04/20	3.1	The HBV immunogen was cloned and **inserted** ~~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~~**by** [redacted] The vector is derived from chimpanzee adenovirus isolate Y25 and rendered replication-~~deficient~~**incompetent** by deletion of the essential E1 genes. ChAdOx1 cannot replicate to produce infectious virus in any primary human

Summary of Modifications since Version 1.0		
Date	**Section**	**Modification**
		cell type. The vector is used to deliver an **HBV** immunogen comprising sequences from a group C genotype.
01/04/20	3.3	Specific tests are performed on the bulk harvest, bulk drug substance **and safety testing is performed on the control cells.**~~vaccine preparation and harvested media derived from the tested Master Cell Bank.~~
01/04/20	3.3	ChAdOx1-HBV will be supplied in stoppered and sealed vials at a target sterile volume of 0.65 mL in **43** mL vials for a 0.5 mL injection volume.
01/04/20	3.4	A confirmatory in-use study of the potency (vp/mL and infectious units [ifu]/mL) of the ChAdOx1-HBV held in the vial at room temperature and mimicking the clinic processes ~~will be conducted prior to any clinical study~~**has been conducted** to show there is no detectable decline in viral titre over this period of time.
01/04/20	3.4	ChAdOx1-HBV will be administered by intramuscular injection at doses of 2.5×10^9 vp and 2.5×10^{10} vp **in Studies HBV001 and 002**. The appropriate doses will be prepared by aseptic serial dilution of individual vials on a per participant basis by unblinded study staff according to the procedure in the Pharmacy Manual. Dilution kits of commercially available equipment will be provided by the Sponsor.
01/04/20	Figure 2	**(a)** LP based mammalian expression cassettes, encoding SIi-CPmutS and HBV-CP_{mut}S immunogens, were inserted into E1 locus of replication-~~deficient~~**incompetent** ChAdOx2 vector and recombinant ChAdOx2-SIi-CPmutS and ChAdOx2-HBV-CP_{mut}S viruses were generated in T-REx™-293 cells.
01/04/20	Figure 3	**(a)** LP or SP based mammalian expression cassettes, encoding SIi-CP_{mut}TPA-$S_{(sh)}$ immunogen were inserted into E1 locus of replication-**incompetent**~~deficient~~ ChAdOx1 or ChAdOx2 vector and four recombinant ChAdOx1-LP-SIi-CP_{mut}TPA-$S_{(sh)}$, ChAdOx1-SP-SIi-CP_{mut}TPA-$S_{(sh)}$, ChAdOx2-LP-SIi-CP_{mut}TPA-$S_{(sh)}$ and ChAdOx2-SP-SIi-CP_{mut}TPA-$S_{(sh)}$ viruses were generated in T-REx™-293 cells.
01/04/20	5.1	The~~first~~ **FIH** study ~~(~~HBV001~~)~~ is a non-randomised, open-label, dose escalation study to evaluate the safety, tolerability and immunogenicity of two different doses of ChAdOx1-HBV vaccine (2.5×10^9 vp and 2.5×10^{10} vp) in 10 healthy participants and 12 participants with CHB and virally suppressed with oral antiviral medication. **Depending on the results of HBV001, the higher dose of 2.5×10^{10} vp** ~~The optimal dose~~will be further evaluated in

Summary of Modifications since Version 1.0		
Date	**Section**	**Modification**
		study HBV-002 with MVA, with or without PD-1 inhibitors.
01/04/20	6	ChAdOx1-HBV vaccine is a chimpanzee adenoviral-vectored vaccine encoding consensus sequences from a group C genotype. The ChAdOx1 virus has been engineered to be replication-**incompetent**~~deficient~~ and can be manufactured in well-established HEK293 cell lines containing the adenoviral E1 gene.
01/04/20	6.1	Based on these results, doses of 2.5 x 10^9 vp and 2.5 x 10^{10}vp ~~will be~~**are being** investigated in the **FIH** HBV001 clinical study, in healthy volunteers~~as a first step, then~~ **and** subjects with CHB infection. **Depending on the results of HBV001, the higher dose of 2.5 x 10^{10} vp**~~The optimal dose~~ will be further evaluated in~~a subsequent~~ study ~~(~~HBV-002~~)~~ with MVA, with or without PD-1 inhibitors.
01/04/20	6.2	**In study HBV002,** ~~I~~it will be administered by intramuscular injection ~~at doses 2.5 x 10^9 vp and~~**a dose of** 2.5 x 10^{10} vp.
01/04/20	6.5	With any new treatment there is always a possibility of an unexpected adverse events. Stopping and holding rules have been defined for ~~the~~study **HBV002**, and a ~~Safety Review~~**Data Monitoring** Committee will perform a review of safety data if one of these is met. There ~~are also~~**will also perform** ~~routinely~~**unscheduled**~~scheduled~~ reviews of safety during the study. ~~A Data Safety Monitoring Committee will meet upon request from the SMC and will perform a review of safety data if stopping criteria are met.~~

ABBREVIATIONS AND DEFINITIONS OF TERMS

ABBREVIATION	DEFINITION
PD-1	Programmed Cell Death Protein 1
ChAdOx1	Chimpanzee Adenovirus Oxford 1
ChAdOx2	Chimpanzee Adenovirus Oxford 2
CHB	Chronic Hepatitis B
CMV	Cytomegalovirus
$CP_{mut}S$	Core, Mutated Polymerase, Surface Antigen
DNA	Deoxyribonucleic Acid
ELISA	Enzyme-linked Immunosorbent Assay
ELISPOT	Enzyme Linked Immunospot
GLP	Good Laboratory Practice
HBsAb	Hepatitis B Surface Antibody
HBsAg	Hepatitis B Surface Antigen
HBV	Hepatitis B Virus
HCC	Hepatocellular Carcinoma
HIT	Heparin-induced Thrombocytopenia
HIV	Human Immunodeficiency Virus
ICS	Intracellular Cytokine Staining
IFN	Interferon
IFN-γ	Interferon Gamma
ifu	Infectious Units
IL-2	Interleukin-2
IM	Intramuscular
MERS	Middle East Respiratory Syndrome
MVA	Modified Vaccinia Ankara
NA	Nucleotide Analogue
pfu	Plaque Forming Units
SAE	Serious Adverse Event
SFU	Spot Forming Units
SIi	Shark Invariant Chain
TB	Tuberculosis
TNF-α	Tumour Necrosis Factor Alpha
TPA	Tissue Plasminogen Activator
vp	Viral Particles

1 SUMMARY

1.1 Physical, Chemical and Pharmaceutical Properties and Formulation

Chimpanzee adenovirus Oxford 1 (ChAdOx1)-vectored hepatitis B virus (HBV) vaccine (ChAdOx1--HBV) is a chimpanzee adenoviral-vectored vaccine encoding HBV consensus sequences from a group C genotype. The ChAdOx1 virus has been engineered to be replication-incompetent and can be manufactured in well-established HEK293 cell lines containing the adenoviral E1 gene.

1.2 Nonclinical Studies

The HBV antigen was found to be highly immunogenic in both inbred (BALB/c and C57BL/6) and outbred CD-1 mice when delivered using the ChAdOx1 viral vector platform, measured using interferon gamma (IFN-γ) enzyme linked immunospot (ELISPOT), intracellular cytokine staining (ICS) and enzyme-linked immunosorbent assay (ELISA). Insert-specific responses could be detected in the liver as well as in the periphery following intramuscular (IM) vaccination and could be increased by a booster vaccination with a Modified vaccinia Ankara (MVA) vector encoding the same HBV antigens.

In a Good Laboratory Practice (GLP) compliant toxicology study (number NP08QM), BALB/c mice were dosed with ChAdOx1-HBV at 2.5×10^{10} viral particles (vp), a dose level approximating to the maximum anticipated clinical dose. Animals were dosed on Day 1 and Day 15 and toxicity was assessed 2 days after cessation of dosing and 14 days after cessation of dosing (the latter to evaluate recovery of effects and persistence of an immune response). Additional animals were dosed once and killed 2 days later to assess acute toxicity. Dosing resulted in an immune response that was sustained for 2 weeks after cessation of dosing. There were no toxicologically significant findings.

The biodistribution and shedding of ChAdOx1A-HBV was assessed in BALB/c mice in a GLP-compliant study. In this study, groups of mice were dosed with ChAdOx1-HBV at 2.4×10^{10} vp on Day 1, or on Days 1 and 28. Viral genomic DNA levels were evaluated in whole blood, skeletal muscle (at the site of injection), brain, heart, draining inguinal lymph node, kidney, liver, lung, gonads and spleen, and additionally in urine and faeces to evaluate viral shedding. No DNA was detected in the blood at any timepoint in the study; it was also not detectable in any urine or faecal samples, indicating that no viral shedding had occurred. The biodistribution of the vaccine was as expected, with low levels/results below the limit of quantification noted in most tissues. As evidenced by data from samples taken 24 hours after a single dose, high levels were apparent in skeletal muscle, at the site of administration, and some distribution to draining lymph nodes was noted.

1.3 Clinical Experience

Vaccitech is conducting a programme of studies with a ChAdOx1-vectored vaccine containing an HBV antigen.

The ChAdOx1-vectored COVID-19 vaccine has recently been authorised throughout the world and has now been administered to millions of people at a dose of 5×10^{10} vp [1]. Prior to the COVID-19 pandemic, ChAdOx1 has also been the vector for different vaccine trials conducted by the University of Oxford for a range of diseases including influenza (FLU004 & 005), MERS [2] and prostate cancer [3]. The doses administered to participants ranged from 5×10^9 vp, to 5×10^{10} vp.

In the FLU004 study, ChAdOx1 prime followed by an MVA boost significantly increased the influenza-specific cellular immune response and maintained high responses up to 8 weeks post-boost in healthy participants naturally primed by influenza infection in the past. This study found that at a dose of 5×10^{10} vp, the reactogenicity profile of the prime-boost regimen was less well tolerated. Three of the six participants developed fever (38.2 to 38.5°C) and two of these three participants also developed severe local and systemic adverse reactions. Laboratory adverse events (lymphopenia and neutropoenia) also occurred in three participants.

In the FLU005 study heterologous prime-boost vaccination with a combination of MVA-NP+M1 and ChAdOx1 NP+M1 at a dose of 2.5×10^{10} vp generated high frequencies of influenza-specific T-cells that lasted up to 18 months following the first vaccination. The majority of adverse events were mild to moderate in nature and lasted for 1-2 days. The most common local adverse event was arm pain at the site of injection and the most common systemic adverse event was mild fatigue and headache. No participant had a documented fever.

Results from the two influenza studies are provided in more detail in Section 5.2 and show that the ChAdOx1-NP + M1 vaccine generated positive immunogenicity data and had an acceptable safety profile.

Published results from the MERS and prostate cancer studies also show positive immunogenicity and an acceptable safety profile for the ChAdOx1-vectored vaccines [2,3].

Based on these results, doses of 2.5×10^{9} vp and 2.5×10^{10} vp are being investigated in the First-in-Human (FIH) HBV001 clinical study in healthy volunteers and subjects with chronic HBV infection. Based on the interim results of HBV001, the higher dose of 2.5×10^{10} vp will be further evaluated in study HBV-002, with MVA and with or without programmed cell death protein 1 (PD-1) inhibitors.

2 INTRODUCTION

2.1 Disease Review

Hepatitis B virus is a complex, small deoxyribonucleic acid (DNA) virus that goes through a ribonucleic acid 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 [4]. 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) [4].

2.2 Treatment Review

Highly effective prophylactic vaccines were implemented in the early 1980's; however, these vaccines are ineffective once infection is established [5]. Despite the wide use of prophylactic vaccines, there are an estimated 240 million CHB carriers and over 686,000 related deaths per year worldwide [6].

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 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.[7]

Various combinations of IFN and NA have been evaluated, but most studies have not shown an added benefit compared to monotherapy [8,9,10]. 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 [11].

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 [6].

The immune clearance of CHB is likely to depend on the use of modalities that induce effective CD8+ T cells [12,13]. 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 [14,15,16]. 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 (MVA) [17,18,19]. Non-replicating chimpanzee adenoviruses are often used rather than human adenoviruses, as there is minimal prior immunity to the vector itself [20,21]. The combination of chimpanzee adenoviruses (used in millions of people) 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 [16,19,22-25]. The ChAdOx1-vectored COVID-19 vaccine has recently been authorised throughout the world and has now been administered to millions of people at a dose of 5×1010 vp [1]. Prior to the COVID-19 pandemic, ChAdOx1 has also been the vector for different vaccine trials conducted by the University of Oxford for a range of diseases including influenza (FLU004 & 005), MERS [2] and prostate cancer [3]. The doses administered to participants ranged from 5 x 10^9 vp, to 5 x 10^{10} vp. Immunogenicity of the vector encoded antigens can be enhanced through the addition of molecular adjuvants such as a short shark invariant chain (SIi) sequence or use of the tissue plasminogen signal sequence fused to the N-terminus of the antigen.

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 [26]. 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 HBV vaccine; ChAdOx1-HBV), and also into an MVA vector (MVA-HBV), which will be used as a boost in study HBV-002.

2.3 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 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. 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.

Nonclinical 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 CHB. 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 [27,28]. Sufficient CD4 T cell activity and the production of neutralising anti-HBV envelope antibodies are required for protective immunity [29].

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 [16,19,22-25]. Furthermore, millions of people have been immunised with an authorised ChAdOx1 COVID-19 vaccine at a dose of 5×10^{10} vp.

Doses of ChAdOx1 vaccines in previous studies have varied from 10^8 to over 10^{10} vp per dose and this study will test the dose of 2.5×10^{10} vp. The safety of ChAdOx1-HBV and the breadth of systemic and liver-specific immune responses are currently being explored in studies HBV001 and HBV002.

T cell exhaustion has been a feature of chronic hepatitis B, and methods to overcome this immunosuppression have included the use of low dose checkpoint inhibitors. This approach has been used in a prior non-published study without major toxicity occurring. For this reason, a prime-boost strategy using ChAdOx1 vector (ChAdOx1-HBV) and an MVA vector (MVA-HBV), with and without PD-1 inhibitors will be evaluated.

3 PHYSICAL, CHEMICAL AND PHARMACEUTICAL PROPERTIES AND FORMULATION

3.1 Description of Investigational Medicinal Product

The Investigational Medicinal Product, ChAdOx1-HBV is a chimpanzee adenoviral-vectored vaccine encoding consensus sequences from an HBV group C genotype.

In early nonclinical studies (Section 4) this vaccine was named ChAdOx1-SIi-CP_{mut}TPA-$S_{(sh)}$.

Immunogen design:

Figure 1 Schematic of Hepatitis B Virus Immunogen

3.2 Description of Formulation

3.3 Specification and Testing

Each lot of ChAdOx1-HBV drug product is tested to confirm that its purity, identity, potency and safety meets specifications. Specific tests are performed on the bulk harvest, bulk drug substance and safety testing is performed on the control cells.

3.4 ChAdOx1-HBV Vaccine Storage

ChAdOx1-HBV will be supplied in stoppered and sealed vials at a target sterile volume of 0.65 mL in 3 mL vials for a 0.5 mL injection volume. The vaccine is shipped on dry ice at ≤-65°C. Specific storage information including lot number, dose strength, fill volume and storage temperature is detailed on the vaccine product labels. The storage unit should be monitored and any excursions outside the specified range should be reported to the study centre pharmacist and the study team as soon as possible.

3.5 Preparation and Administration

To prepare, the ChAdOx1-HBV vial is thawed at room temperature (15 to 25°C). The vial is mixed thoroughly prior to administration according to the instructions in the Pharmacy Manual. If the vaccine is not administered immediately, the thawed vial may be stored at room temperature for up to 2 hours before being used (based upon available stability data with other ChAdOx1-vectored vaccines and feedback from pharmacies), after which time the thawed material must be disposed of. A confirmatory in-use study of the potency (vp/mL and infectious units [ifu]/mL) of the ChAdOx1-HBV held in the vial at room temperature and mimicking the clinic processes has been conducted to show there is no detectable decline in viral titre over this period of time.

ChAdOx1-HBV will be administered by IM injection at doses of 2.5×10^9 vp and 2.5×10^{10} vp in Studies HBV001 and 002. The appropriate doses will be prepared by aseptic serial dilution of individual vials on a per participant basis by unblinded study staff according to the procedure in the Pharmacy Manual. Dilution kits of commercially available equipment will be provided by the Sponsor.

4 NONCLINICAL STUDIES

4.1 Nonclinical Immunogenicity

The immunogenicity of the HBV insert, delivered by ChAdOx1 and MVA, as a single dose and heterologous prime-boost regimens have been evaluated in inbred (BALB/c) and outbred (CD-1) mice using *ex vivo* IFN-γ ELISPOT, ICS and ELISA.

Nonclinical studies were conducted to evaluate the magnitude of antigen-specific T cell and antibody response to vaccination with the HBV insert. The addition of a molecular adjuvant was investigated, both peripheral and tissue-specific responses were assessed, prime-boost administration was assessed, and the immunogenicity using peptides representing discrete areas of the transgene product was established.

Six-week-old female BALB/c and CD-1 mice were vaccinated with a dose of 2×10^6 plaque forming units (pfu) of MVA and/or 4-5 $\times 10^7$ ifu of ChAdOx1. All viruses were resuspended in endotoxin-free phosphate buffered saline for vaccination. Vaccinations were performed by IM injection in a final volume of 50 µL and injected into the tibialis anterior muscle of each animal.

Toxicity was assessed in BALB/c mice, dosed with 2.5×10^{10} vp by IM injection. Animals received one or two administrations 14 days apart. Acute toxicity and recovery were assessed. The persistence of an immune response was assessed as was in-life tolerability, clinical observations, body temperature, clinical and anatomic pathology.

The transforming potential of the ChAdOx1-HBV vector was not evaluated as the risk of oncogenicity was assessed to be negligible. Hepatitis B virus itself is an oncogenic agent, and integration of the viral genome into the host genome is an important mechanism responsible for HCC development. In addition, the HBx protein has been identified as an essential driver of HBV-induced tumorigenesis. The ChAdOx1-HBV vector neither integrates into the host genome nor encodes the HBx protein, and it is, therefore, considered that the risk of ChAdOx1-HBV induced oncogenicity to be negligible.

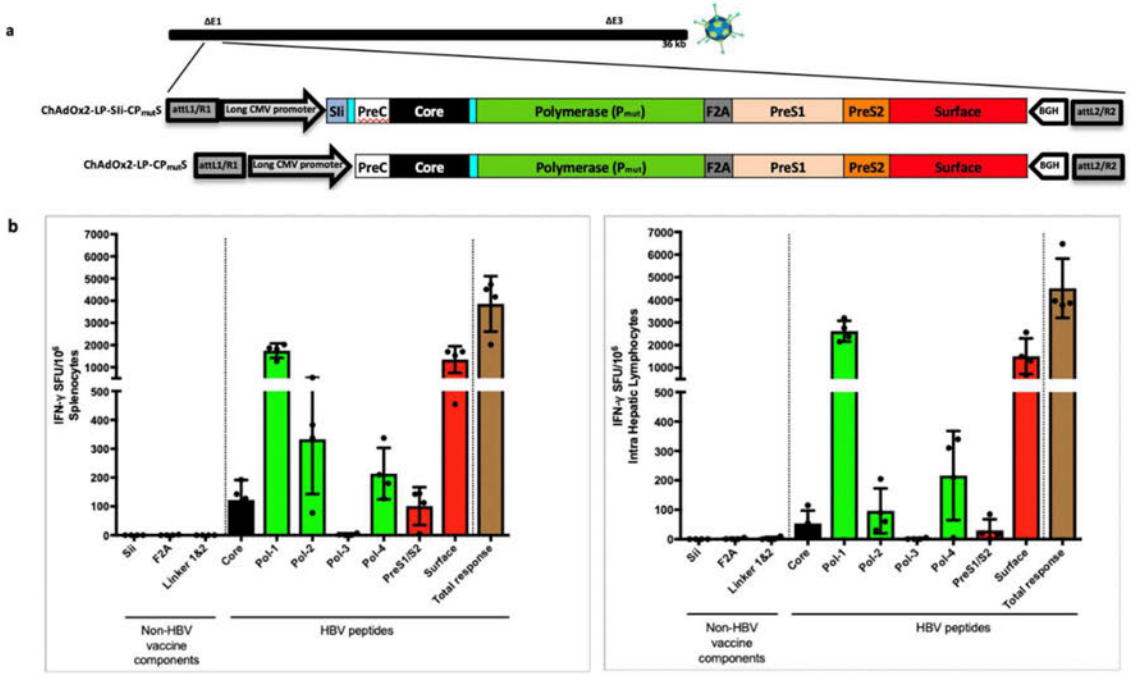
4.1.1 Effect of the SIi to the Amino-terminus of CPmutS on the Immune Response

The first-generation HBV immunogen tested was core, mutated polymerase, surface antigen ($CP_{mut}S$), encoding HBV core, polymerase (with inactivating mutations), and HBsAg. It was previously shown that full-length or truncated versions of the major histocompatibility complex class II-associated invariant chain can act as molecular adjuvants and can enhance antigen-specific immunogenicity when fused to the N-terminus of the antigen. This has been observed nonclinically in mice and in rhesus macaques [30,31,32] and has also recently been assessed in the clinic (NCT03688061 and NCT03203421).

To test immunogenicity of $CP_{mut}S$ and the influence of tethering SIi transmembrane sequence as a molecular adjuvant to the amino-terminus of $CP_{mut}S$, chimpanzee adenoviral vectors (chimpanzee adenovirus Oxford 2 [ChAdOx2]) encoding $CP_{mut}S$ with and without SIi, were generated (Figure 2a) and tested in mouse immunogenicity experiments. Vaccination generated very high magnitude HBV specific T cell responses to all HBV peptides. The mean magnitude of total HBV-specific T cell responses in inbred BALB/c (Figure 2b) and outbred CD-1 (Figure 2c) mice were 3858 and 3155 spot forming units [SFU]/10^6 splenocytes and 4514 and 2979 SFU/10^6 intrahepatic lymphocytes respectively. Intracellular cytokine staining showed that HBV specific CD8+ T cells were polyfunctional producing IFN-γ, tumour necrosis

factor-alpha (TNF-α) and interleukin-2 (IL-2). The inclusion of SIi significantly enhances the T cell magnitude for both splenocytes (3155 mean total SFU/10^6 with SIi versus 386 mean total SFU/10^6 without SIi, p<0.0001) and intrahepatic lymphocytes (2979 mean total SFU/10^6 with SIi versus 461 mean total SFU/10^6 without SIi, p=0.0002; Figure 2c). Importantly, T cells to the non-HBV SIi, F2A and the inter-gene regions were not generated.

Figure 2 Assessing the Effect of a Molecular Adjuvant (SIi) to the HBV Immunogen

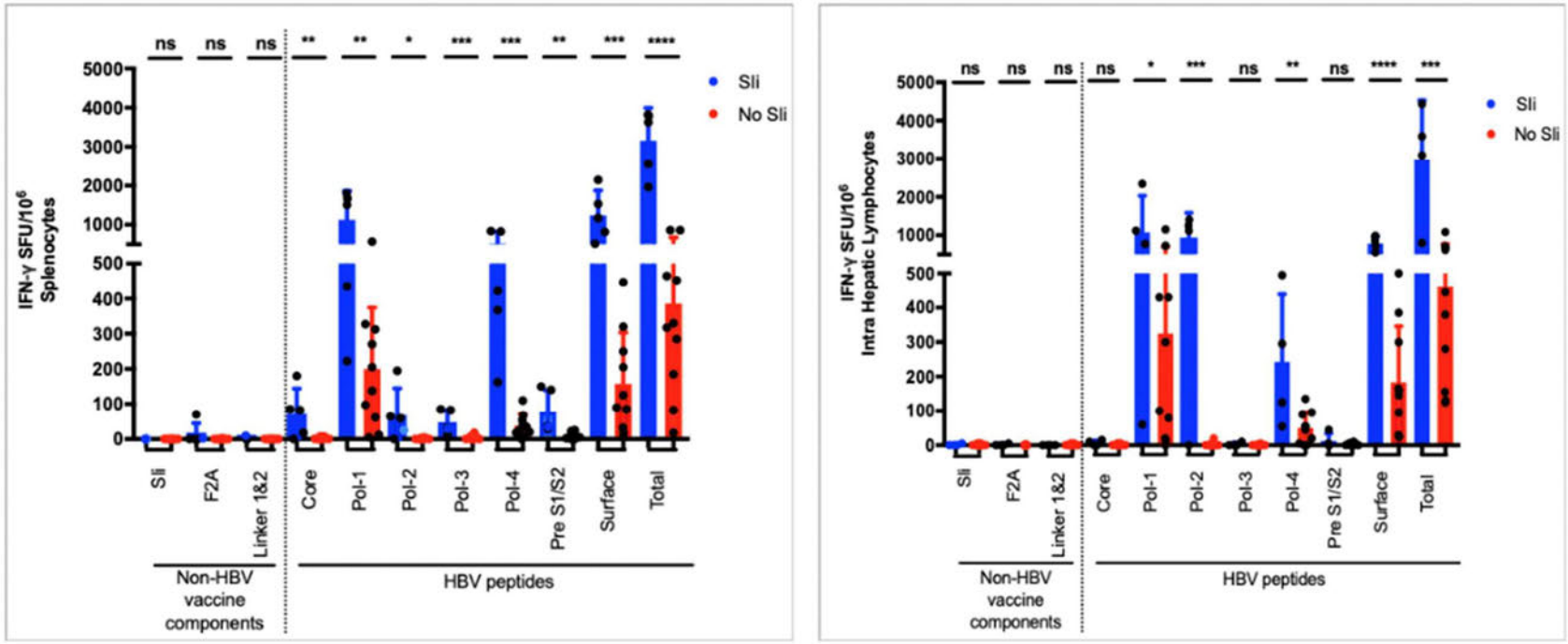


Abbreviations: ANOVA=analysis of variance; ChAdOx2=Chimpanzee Adenovirus Oxford 2; CMV=cytomegalovirus ELISPOT=enzyme linked immunospot; HBV=hepatitis B virus; IFN-γ=interferon gamma; ifu=infectious units; IHL=intrahepatic lymphocytes; LP=long-CMV promoter; SFU=spot forming units

Tethering truncated SIi to the HBV immunogen ($CP_{mut}S$) enhances the magnitude of vaccine induced T cell response in naive inbred BALB/c and outbred CD-1 mice: (a) LP based mammalian expression cassettes, encoding SIi-CPmutS and HBV-$CP_{mut}S$ immunogens, were inserted into E1 locus of replication-incompetent ChAdOx2 vector and recombinant ChAdOx2-SIi-CPmutS and ChAdOx2-HBV-$CP_{mut}S$ viruses were generated in T-REx™-293 cells. **(b)** BALB/c mice (n=4) were vaccinated intramuscularly with 4 x 10^7 ifu per mice of ChAdOx1-SIi-CPmutS vaccine. 14 days post-vaccination, splenocyte and IHLs were harvested and stimulated overnight with three non-HBV peptide pools (SIi, F2A and linker 1&2) and seven HBV-specific peptide pools (core, pol-1, pol-2, pol-3, pol-4, PreS1/S2 and surface, with approximately 50 peptides per pool) and the IFN-γ response for each peptide pool was analysed using IFN-γ ELISPOT assay. The mean magnitude of IFN-γ ELISPOT response, in SFU, per 10^6 splenocyte (left image) or IHL (right image) to three the non-HBV peptide pools, seven HBV-specific peptide pools and cumulative total response to seven HBV-peptide pools are shown.

Figure 2 Assessing the Effect of a Molecular Adjuvant (SIi) to the HBV Immunogen *continued*

c



Tethering truncated SIi to the HBV immunogen ($CP_{mut}S$) enhances the magnitude of vaccine induced T cell response in naive inbred BALB/c and outbred CD-1 mice: (c) 14 days post-vaccination splenocyte and IHL IFN-γ ELISPOT response from five and 10 CD1 mice, vaccinated with $5x10^7$ ifu per mice of ChAdOx2-HBV-SIi-CPmutS and ChAdOx2-HBV-CPmutS, respectively, were analysed using Mann Whitney and ANOVA with multiple comparisons, p values expressed as p<0.0001 ****, p=0.0001-0.0008 ***, p=0.0019-0.0096 **, p=0.0108-0.0486 * ns=not significant. Statistical significance of the IFN-γ ELISPOT response for each peptide pool and total response for both vaccines are shown on top of the image.

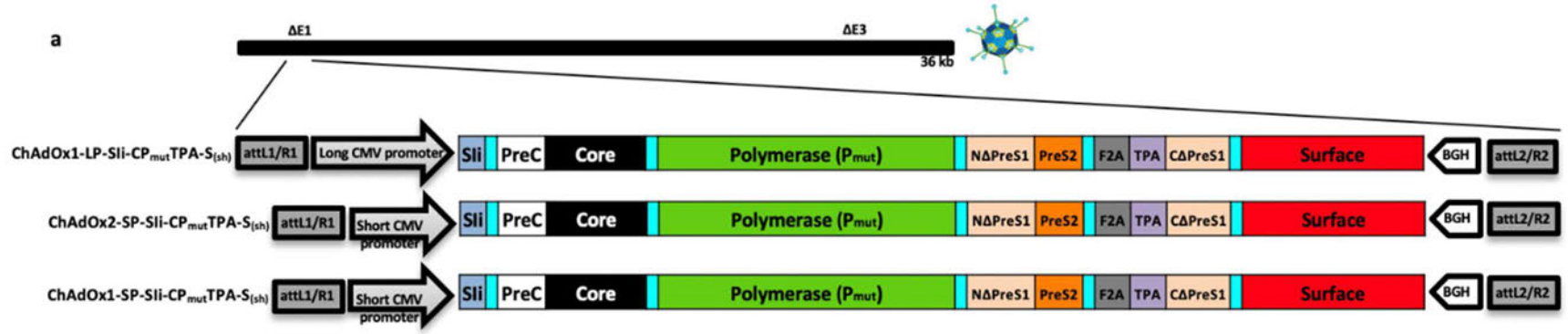
4.1.2 *Immunogenicity of ChAdOx and MVA Viral Vectors Encoding the Optimised HBV-immunogen SIi-CP$_{mut}$TPA-S$_{(sh)}$*

Since the large surface protein encoded by the SIi-CP$_{mut}$S immunogen failed to induce hepatitis B surface antibodies (HBsAbs), the surface protein region of SIi-CP$_{mut}$S was re-engineered and generated a second-generation HBV immunogen SIi-CP$_{mut}$TPA-S$_{(sh)}$ (Figure 3a). Two modifications were made to SIi-CP$_{mut}$S (1) regions non-essential for antibody induction, the carboxy-terminus of PreS1 and entire PreS2, were re-located to the amino-terminus of F2A and (2) TPA, a molecular adjuvant, was inserted to the amino-terminus of the remaining modified surface protein S$_{(sh)}$. During chimpanzee adenoviral vector generation, it was noticed that the SIi adjuvanted ChAdOx2 long-cytomegalovirus (CMV) promoter-based SIi-CP$_{mut}$S to be unstable, however the non-adjuvanted ChAdOx2 long-CMV promoter-based HBV-CP$_{mut}$S did not show any stability issues. It was hypothesised that the instability could be related to exceeding the gene-insert size limit of ChAdOx2. To overcome the instability, the possibility of generating stable adeno-viral vectors by (1) reducing the size of the transgene cassette, using a short-CMV promoter instead of the long-CMV promoter and (2) utilising another viral vector, ChAdOx1 was analysed. To test this, ChAdOx1 and ChAdOx2 vectors with either short-CMV or long-CMV promoter based SIi-CP$_{mut}$TPA-S$_{(sh)}$ immunogen were generated. During chimpanzee adenoviral vector production it was observed that except ChAdOx2 long-CMV promoter based SIi-CP$_{mut}$TPA-S$_{(sh)}$, all other ChAdOx viral vectors in three different combinations were stable during multiple passages in cell culture. Immunogenicity studies showed that ChAdOx1 short-CMV promoter based CP$_{mut}$S$_{(sh)}$ induced higher mean magnitude of total HBV-specific T cell response (4231 SPF/10^6 splenocytes) compared to the other two stable chimpanzee adenoviral vectors (2980 and 2024 SPF/10^6 splenocytes for ChAdOx2 short-CMV promoter and ChAdOx1 long-CMV promoter, respectively) (Figure 3b).

Intracellular cytokine staining showed that all second-generation HBV immunogen encoding vaccines were able to induce polyfunctional HBV specific CD8+ T and CD4+ T cells (Figure 3c). Based on these results, an MVA viral vector encoding the second-generation HBV immunogen SIi-CP$_{mut}$TPA-S$_{(sh)}$ was generated (Figure 3d). TPA-S$_{(sh)}$ region of SIi-CP$_{mut}$TPA-S$_{(sh)}$ was encoded using mH5 promoter and SIi-CP$_{mut}$ region of SIi-CP$_{mut}$TPA-S$_{(sh)}$ was encoded under the F11 promoter. The order of PreCore-Core, P$_{mut}$, PreS1 and PreS2 of CP$_{mut}$ encoded by the MVA was rearranged to prevent the generation of immune responses to inter-gene regions when used in prime boost strategies. MVA-SIi-CP$_{mut}$TPA-S$_{(sh)}$ vaccination studies showed induction of a mean magnitude of total HBV-specific INF-γ ELISPOT response of 878 SPF/10^6 splenocytes. Intracellular cytokine staining showed that MVA vaccination induced polyfunctional HBV-specific CD8+ T cells. Overall, prime-only vaccination studies showed that chimpanzee adenoviral-vectored HBV vaccine induces higher magnitude of IFN-γ ELISPOT responses compared to MVA-vectored HBV vaccines.

The optimised, second-generation vaccine constructs containing the SIi-CP$_{mut}$TPA-S$_{(sh)}$ immunogen were re-named ChAdOx1-HBV and MVA-HBV.

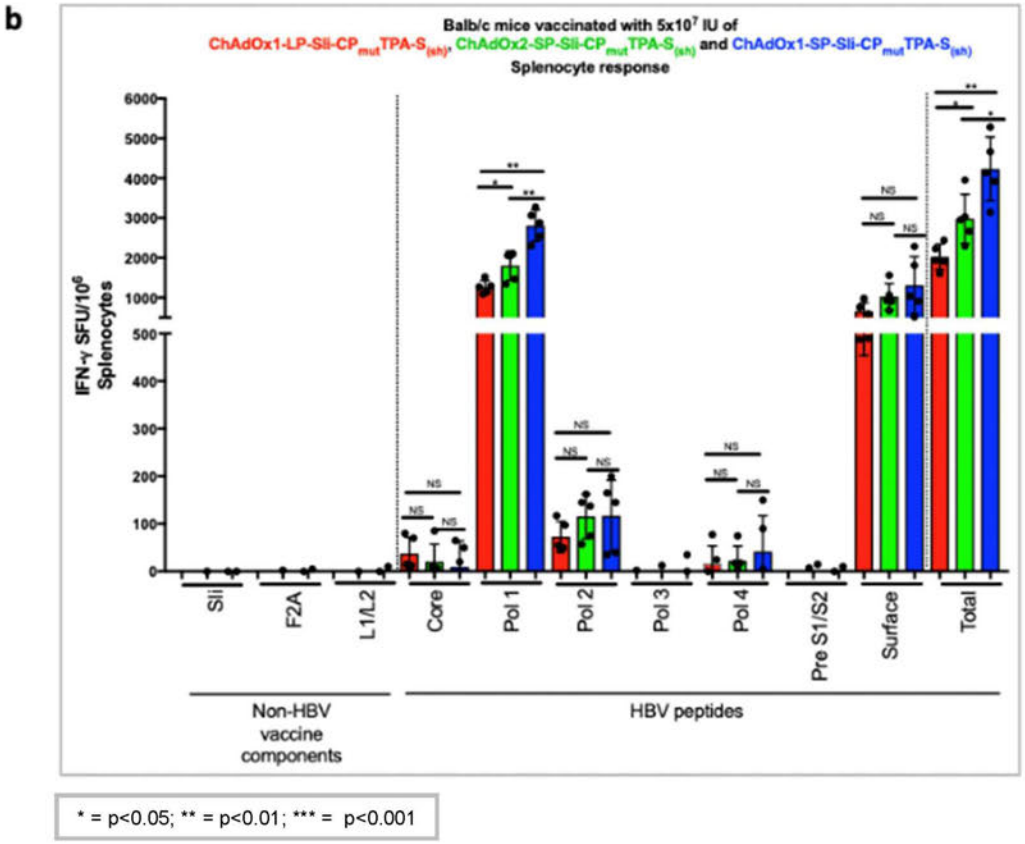
Figure 3 Immunogenicity of Optimised, Second-generation HBV Immunogens



Abbreviations: ChAdOx1=Chimpanzee Adenovirus Oxford 1; ChAdOx2=Chimpanzee Adenovirus Oxford 2; ELISPOT=enzyme linked immunospot; HBV=hepatitis B virus; IFN-γ=interferon gamma; LP=long-CMV promoter; MVA=Modified vaccinia Ankara; SP=Short-CMV promoter; TNF=tumour necrosis factor

Immune response to ChAdOx and MVA viral vectors encoding modified HBV immunogen (SIi-CP_{mut}TPA-$S_{(sh)}$) in naive inbred BALB/c mice: (a) LP or SP based mammalian expression cassettes, encoding SIi-CP_{mut}TPA-$S_{(sh)}$ immunogen were inserted into E1 locus of replication-incompetent ChAdOx1 or ChAdOx2 vector and four recombinant ChAdOx1-LP-SIi-CP_{mut}TPA-$S_{(sh)}$, ChAdOx1-SP-SIi-CP_{mut}TPA-$S_{(sh)}$, ChAdOx2-LP-SIi-CP_{mut}TPA-$S_{(sh)}$ and ChAdOx2-SP-SIi-CP_{mut}TPA-$S_{(sh)}$ viruses were generated in T-REx™-293 cells. Subsequently, all four recombinant viruses were screened by PCR, for stability analysis. Except ChAdOx2-LP-SIi-CP_{mut}TPA-$S_{(sh)}$, other three viruses showed positive PCR result for the presence of an intact HBV-immunogen cassette. The stability was also confirmed by Sanger sequencing of the entire HBV-immunogen cassette.

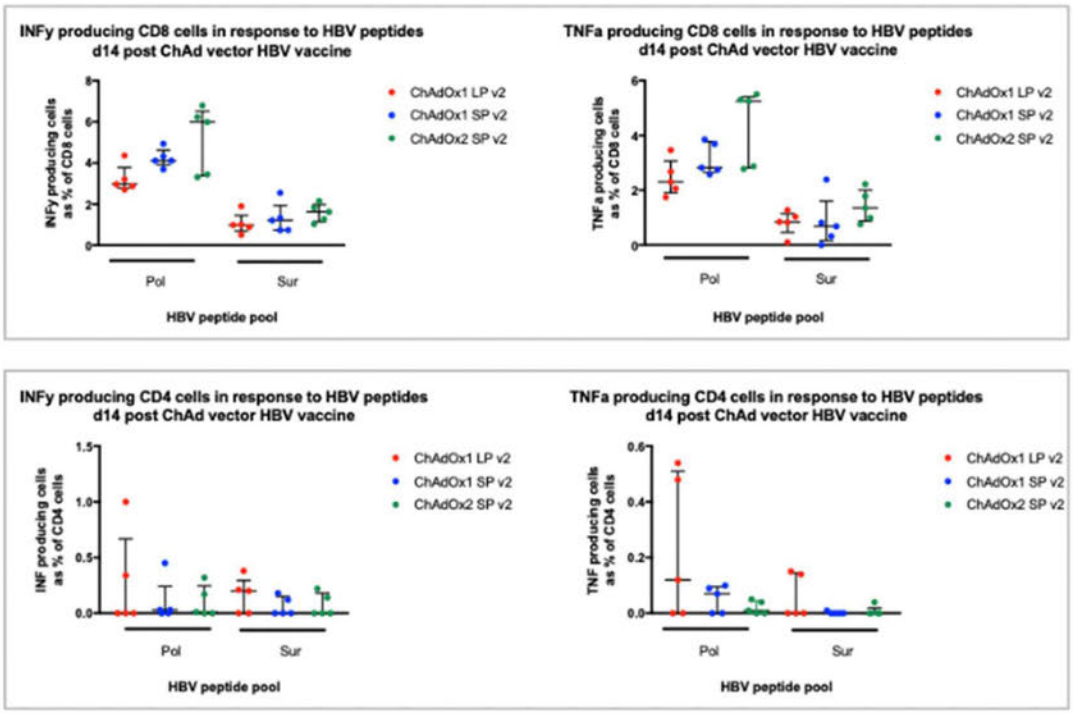
Figure 3 Immunogenicity of Optimised, Second-generation HBV Immunogens ***continued***



Immune response to ChAdOx and MVA viral vectors encoding modified HBV immunogen (SIi-CP$_{mut}$TPA-S$_{(sh)}$) in naive inbred BALB/c mice: (b) BALB/c mice (n=5) were vaccinated intramuscularly with 5x10^7 ifu per mice of ChAdOx1-LP-SIi-CP$_{mut}$TPA-S$_{(sh)}$, or ChAdOx1-SP-SIi-CP$_{mut}$TPA-S$_{(sh)}$ or ChAdOx2-SP-SIi-CP$_{mut}$TPA-S$_{(sh)}$ vaccine. 14 days post-vaccination splenocyte were harvested and stimulated overnight with 10 different peptide pools and the IFN-γ ELISPOT response was measured. The mean magnitude of IFN-γ ELISPOT response generated by three different vaccines for each peptide pool were analysed using Mann-Whitney-U test and the statistical significance are shown.

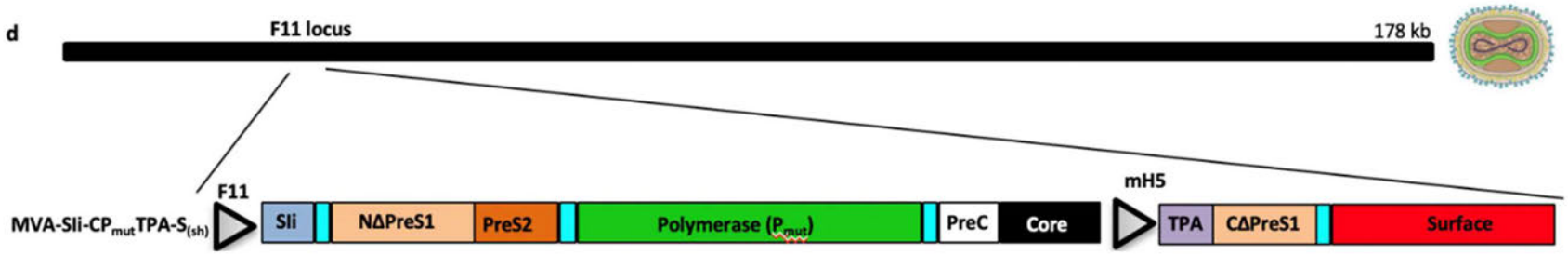
Figure 3 Immunogenicity of Optimised, Second-generation HBV Immunogens *continued*

c



Immune response to ChAdOx and MVA viral vectors encoding modified HBV immunogen (SIi-CP$_{mut}$TPA-S$_{(sh)}$) in naive inbred BALB/c mice: (c) Splenocytes harvested 14-days post vaccination were stimulated with combined peptide pools for polymerase (Pol1 + Pol2 + Pol3 + Pol4) and surface (PreS1/S2 + Surface) and stained for IFN-γ and TNF production. The percentage of CD8+ T and CD4+ T cells producing IFN-γ and TNF-α in response to three different vaccines are shown.

Figure 3 Immunogenicity of Optimised, Second-generation HBV Immunogens *continued*

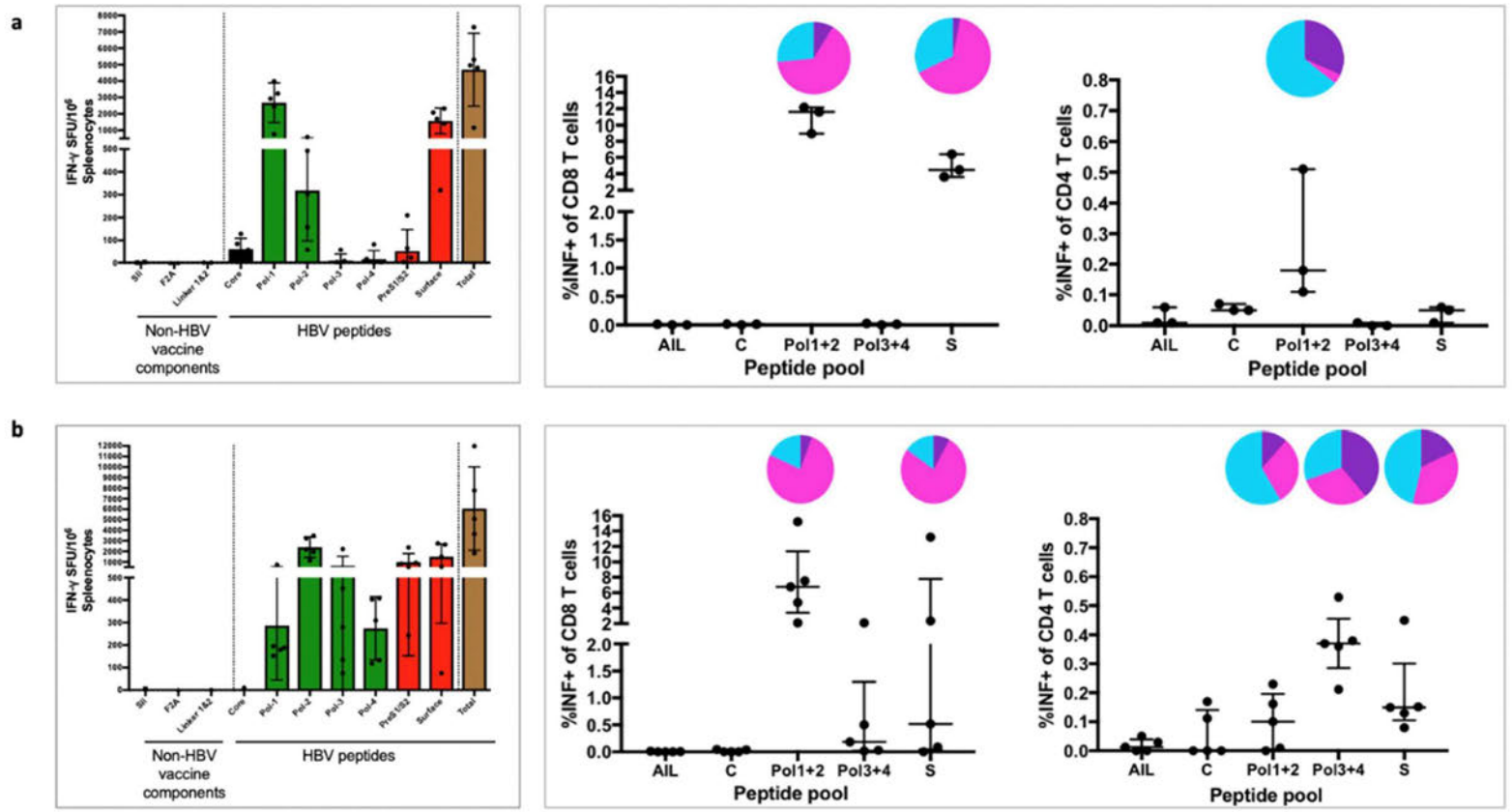


Immune response to ChAdOx and MVA viral vectors encoding modified HBV immunogen (SIi-CP_{mut}TPA-$S_{(sh)}$) in naive inbred BALB/c mice: (d) Recombinant MVA-SIi-CP_{mut}TPA-$S_{(sh)}$ was generated in chicken embryo fibroblast cells by recombining a plasmid shuttle vector encoding SIi-HBV-$CP_{mut}S_{(sh)}$ with wild-type MVA in chicken embryo fibroblast cells.

4.1.3 Effect of ChAdOx1-HBV Prime-MVA-HBV Boost Compared to Prime Only on Immune Response

Previous studies from multiple vaccination programmes employing chimpanzee adenoviral and MVA viral-vectored vaccines have shown that priming with chimpanzee adenovirus and boosting with MVA viral-vectored vaccine induces a greater magnitude of T cell immune responses compared to prime-only vaccination with either of the vaccines. To analyse the immune-response induction potential of combining ChAd and MVA HBV vaccines, prime-boost vaccination studies were performed, by giving 100-fold lower dose of ChAdOx1-HBV priming vaccine followed by a standard-dose of MVA-HBV boosting vaccine. Prime-boost vaccination showed an enhancement in the magnitude of T cells with a mean total HBV-specific T-cell response of 4703 and 6063 SFU/10^6 splenocytes in BALB/c and C57BL6, respectively (Figure 4a and Figure 4b). Intracellular cytokine staining showed that HBV specific CD8+ and CD4+ T cells were highly polyfunctional producing INF-γ, TNF-α, and IL-2. Furthermore, prime-boost vaccination regime also showed induction of anti-hepatitis B surface antibodies in a lower proportion of vaccinated mice (Figure 4c). An HBsAg secretion assay, performed with ChAdOx and MVA viral vectors encoding the second-generation HBV immunogen, showed that this immunogen is capable of secreting HBsAg (Figure 5).

Figure 4 **Prime-boost Vaccination with ChAdOx1 and MVA Viral-vectored HBV Vaccines Encoding the Optimised HBV Immunogen**

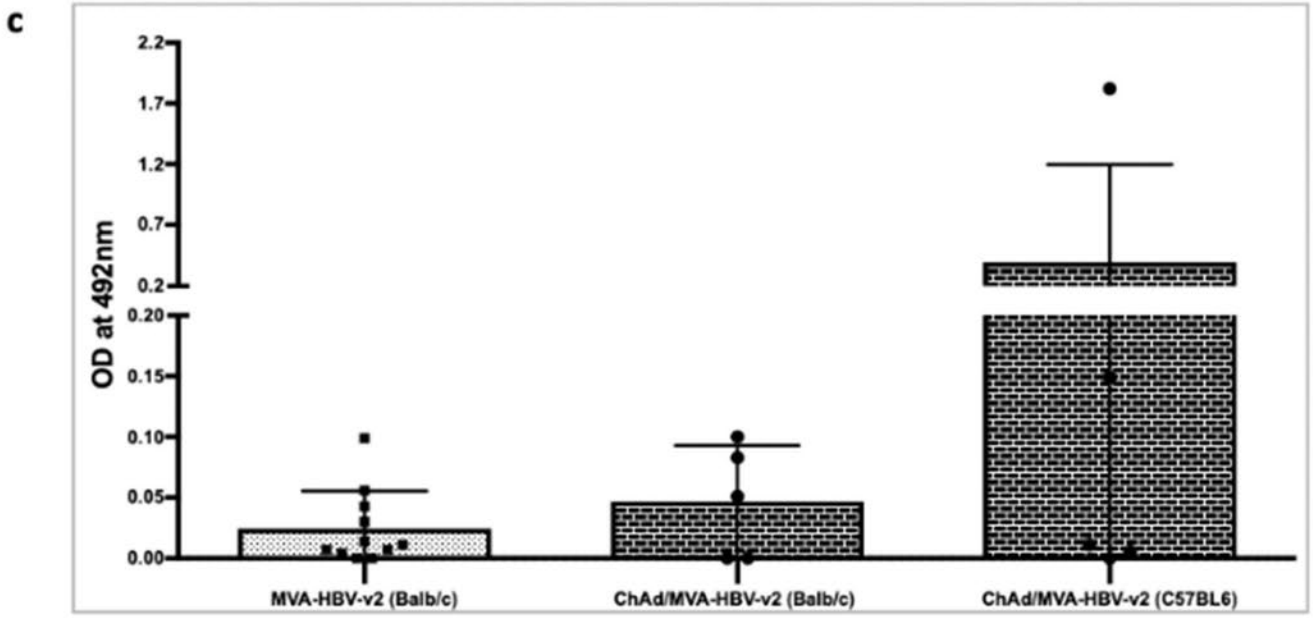


Abbreviations: ChAdOx1=chimpanzee adenovirus Oxford 1; ELISPOT=enzyme linked immunospot; HBV=hepatitis B virus; ICS=intracellular cytokine staining; IFN-γ=interferon gamma; ifu=infectious units; IL-2=interleukin-2; pfu=plaque forming units; MVA=Modified vaccinia Ankara; OD=optical density; SPICE=Simplified presentation of incredibly complex evaluations; TNF-α= Tumour Necrosis Factor alpha

Footnote overleaf

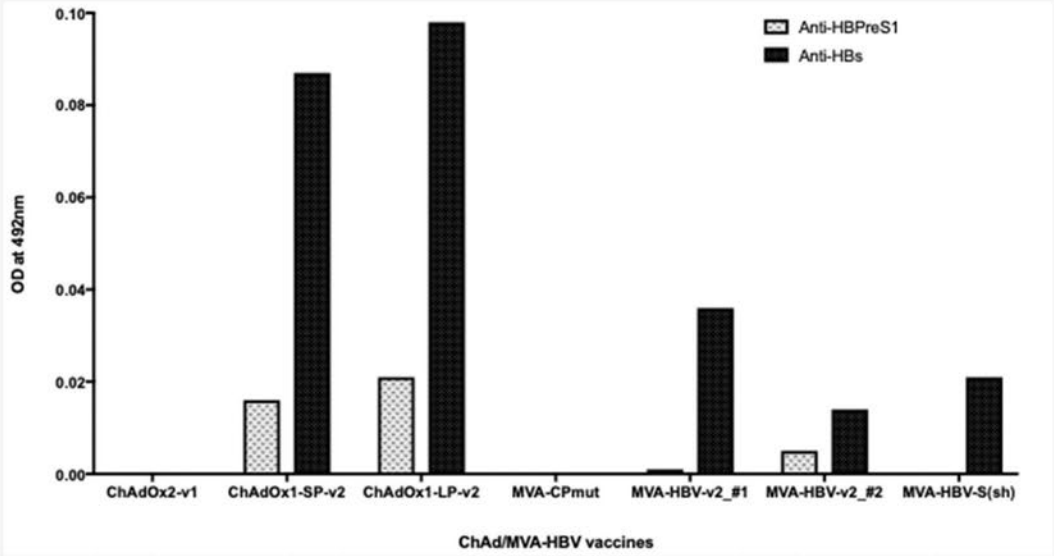
Prime-boost vaccination with ChAdOx and MVA viral-vectored HBV vaccines encoding optimised HBV immunogen (SIi-CP$_{mut}$TPA-S$_{(sh)}$) induces robust T-cell response and anti-HBsAb response in naive inbred BALB/c and C57BL/6 mice: (a) BALB/c and **(b)** C57BL/6 mice, n=5 per group, were given intramuscular injections with 5 x 10^5 ifu per mouse of ChAdOx1-SIi-CP$_{mut}$-TPA-S$_{sh}$ at Week 0 followed by 2 x 10^6 pfu per mouse of MVA-SIi-HBV-CP$_{mut}$TPA-S$_{sh}$ at Week 7. 14 days post-MVA-vaccination (Week 9) splenocytes were harvested and non-HBV and HBV-specific INF-γ ELISPOT response (left panel), and intracellular cytokine levels of INF-γ, TNF-α and IL-2 of CD8+ T cells and CD4+ T cells (right panel) in ICS were analysed. SPICE analysis was performed and where IFN-γ producing cells are present pie chart above shows single (blue), double (pink) and triple (purple) cytokine producing cells. Mean and interquartile range shown for all plots.

Figure 4 Prime-boost Vaccination with ChAdOx1 and MVA Viral-Vectored HBV Vaccines Encoding the Optimised HBV Immunogen *continued*



Prime-boost vaccination with ChAdOx and MVA viral-vectored HBV vaccines encoding optimised HBV immunogen (SIi-CP$_{mut}$TPA-S$_{(sh)}$) induces robust T-cell response and anti-HBsAb response in naive inbred BALB/c and C57BL/6 mice: (c) Level of anti-HBs induction, in response to vaccination, was quantified in ELISA using sera collected 14 days post-MVA vaccination or ChAdOx-MVA prime-boost vaccination. ELISA values above background value of naïve un-vaccinated sera are shown.

Figure 5 Secretion of HBs Antigen from Vector-infected Cells



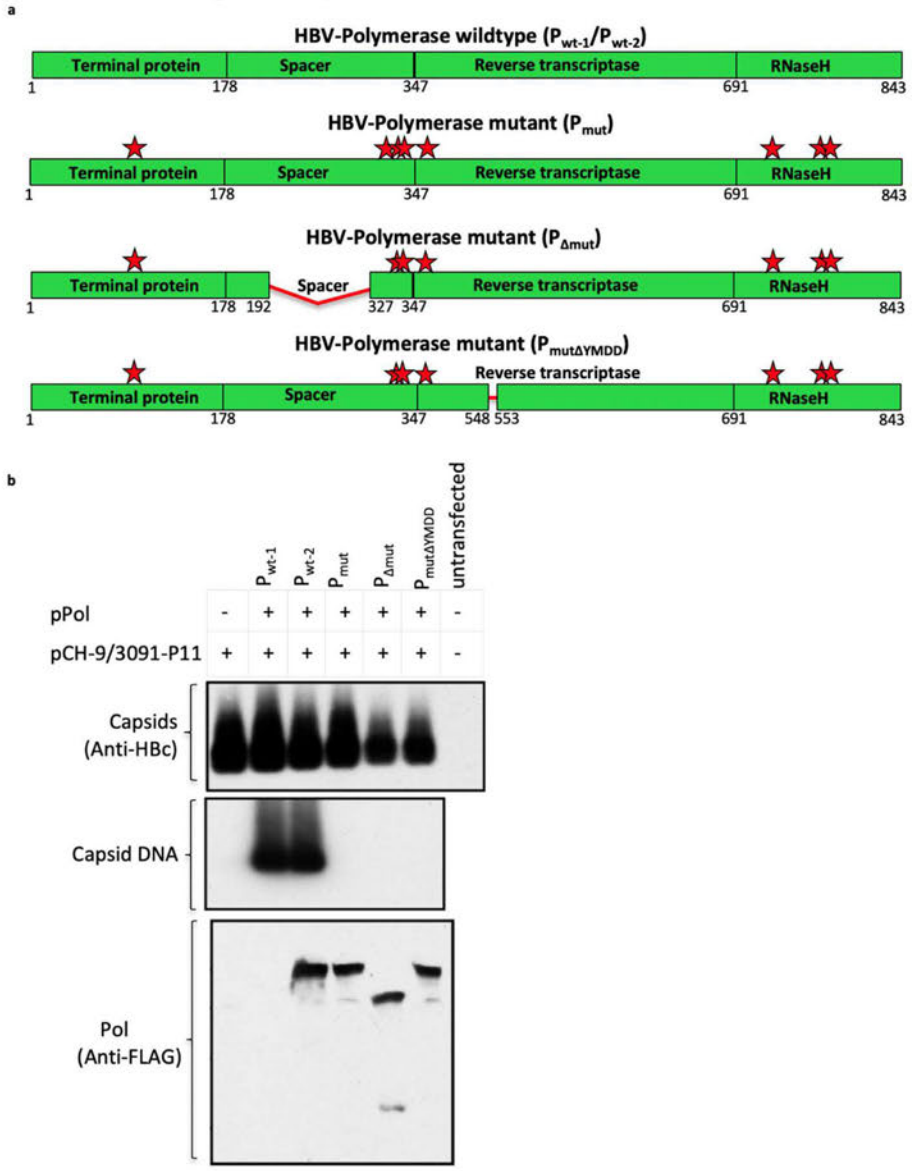
Abbreviations: ChAdOx1=chimpanzee adenovirus Oxford 1; ChAdOx2=chimpanzee adenovirus Oxford 2; ELISA=enzyme-linked immunosorbent assay; HBV=hepatitis B virus; MVA=Modified vaccinia Ankara; OD=optical density

The optimised HBV immunogen encoded in the ChAdOx and MVA viral-vectored HBV vaccines have secretory potential: Supernatants from ChAdOx and MVA-vectored HBV vaccine infected HEK293A cells were harvested 24 hours post-infection and quantified in PreS1 and HBsAg capture ELISA. ELISA values obtained from different ChAdOx and MVA-vectored HBV vaccines, above background value of ChAdOx1-GFP and MVA-GFP, respectively, are shown.

4.1.4 Functionality Assessment of the Mutant polymerase (P_{mut}) encoded within the HBV-immunogen

Encoding a functional polymerase in the vaccine could enable its participation in HBV replication. To avoid this, eight-amino acids (Figure 6a) that were previously shown to be essential for the functionality of HBV-polymerase were mutated in the polymerase codons of SIi-CP$_{mut}$S and SIi-CP$_{mut}$TPA-S$_{(sh)}$ immunogens [33-37]. To verify if these eight-point mutations were able to abolish the functionality of the vaccine encoded polymerase, plasmids encoding mutant polymerase (P_{mut}) and wild-type polymerase (P_{wt}) were generated and tested in an assay that requires trans-complementation of a functional polymerase to rescue the replication of polymerase-null HBV replicon. As expected, the wild-type polymerase was able to support replication of the polymerase-null HBV replicon, however, the mutant-polymerase did not show any evidence of rescue of replication of polymerase-null HBV replicon (Figure 6b). These results demonstrated that the mutant-polymerase encoded in the HBV-vaccine to be non-functional.

Figure 6 Confirmation that the HBV Immunogen Encodes a Non-functional Polymerase, P_{mut}



Abbreviations: Anti-HBc=Anti hepatitis B virus core; DNA=deoxyribonucleic acid; HBV=hepatitis B virus; mut=mutant; Pol=polymerase; wt=wild type

Mutant HBV-polymerase (P_{mut}) encoded by the ChAdOx and MVA viral-vectored HBV vaccines is non-functional: (a) Schematic representation of wild-type (P_{wt-1}/P_{wt-2}) and mutant (P_{mut}, $P_{\Delta mut}$, $P_{mut\Delta YMDD}$) HBV polymerase used to evaluate the possibility of abolishing the functionality of the HBV polymerase encoded in the vaccine using eight-point mutations (alanine substitutions at positions Y63, C323, C334, C338, C352, R714, D777, R781, represented in red stars) on its own, or in combination with either 193-326 spacer region deletion or 549-552 YMDD motif deletion. **(b)** Mammalian expression plasmids encoding two wild-type (P_{wt-1} and FLAG-P_{wt-2}) and three mutant HBV polymerase (FLAG-P_{mut}, FLAG-$P_{\Delta mut}$, FLAG-$P_{mut\Delta YMDD}$) were generated and tested in a trans-complementation assay that requires co-transfection of plasmid encoding functional HBV polymerase, to rescue the replication of polymerase deficient HBV-genome (encoded via plasmid, pCH-9/3091-P11). Plasmid constructs, as indicated in the top of the figure, were co-transfected into Huh7 cells. 4 days post-transfection cells were lysed, capsids were isolated and the levels of capsid protein and capsid DNA from each sample were analysed in western blot and Southern blot, using anti-capsid antibody and ^{32}P-labelled

HBV specific probe, respectively. Western blot probed with anti-FLAG antibody confirmed equivalent level of expression HBV polymerase in samples receiving FLAG-P_{wt-2}, FLAG-P_{mut}, FLAG-$P_{\Delta mut}$ and FLAG-$P_{mut\Delta YMDD}$.

4.2 Biodistribution Studies

The biodistribution and shedding of ChAdOx1-HBV was assessed in BALB/c mice following IM administration of the vector (Study 0841MV38.001). In this GLP-compliant study conducted at Calvert Laboratories Inc. (Philadelphia, USA), ChAdOx1-HBV (0.1 mL IM to the right-side muscle mass over the posterior femur) was administered as a prime on Day 1 or Days 1 and 28, followed by a boost with MVA-HBV (0.1 mL IM to the left-side muscle mass), as summarised in Table 1.

Table 1: Dosing of Biodistribution Study 0841MV38.001

Group	# of Animals/ Sex	Dose Route	Dose Vol (mL/dose)	Dose Concentration		Dose Level	
				ChAdOx1-HBV (vp/mL)	MVA-HBV (pfu/mL)	ChAdOx-1 HBV (vp/dose)	MVA-HBV (pfu/dose)
1	2M/2F	IM	0.1 (saline)	0	0	0	0
2	5M/5F	IM	0.1	$2.4\ 10^{11}$	0	$2.4\ 10^{10}$	0
3	4M/5F	IM	0.1	$2.4\ 10^{11}$	6.1×10^{8}	$2.4\ 10^{10}$	6.1×10^{7}
4	6M/5F	IM	0.1	$2.4\ 10^{11}$	6.1×10^{8}	$2.4\ 10^{10}$	6.1×10^{7}

Abbreviations: F=female, M=male, pfu=plaque forming units; vp=viral particles.

Groups 2 to 4 were dosed with ChAdOx1-HBV on Day 1, and Groups 3 and 4 were dosed with both ChAdOx1-HBV and MVA-HBV on Day 28. Groups 1 and 2 were killed on Day 2, Group 3 on Day 29 and Group 4 on Day 56 and blood and tissue samples were taken.

Biodistribution was measured using a validated quantitative polymerase chain reaction method on samples of whole blood, skeletal muscle (at the site of injection), brain, heart, draining inguinal lymph node, kidney, liver, lung, gonads and spleen. Shedding into biological matrices (urine and faeces; collection from Group 3 on Days 2, 4 and 8 and from Groups 4 on Days 29, 31 and 35) was also measured by quantitative polymerase chain reaction.

There were incidences of decreased activity (Days 5-8) in individual animals in Group 4. In this group one male was found dead on each of Days 5 and 6 and 1 male and 1 female on Day 9. Additionally, 1 male from Group 3 was found dead on Day 28. There were no visible lesions at necropsy and a cause of death was not established. The dose of ChAdOx1-HBV used in Study 0841MV38.001 was well tolerated in a GLP toxicology study (Study NP08QM), as described in Section 4.3, with no instances of morbidity or premature mortality.

Quantitative polymerase chain reaction data indicated that ChAdOx1-HBV DNA was not present in whole blood samples at any timepoint. Distribution to at least some samples of all tissues was noted on Days 2 and 29. The highest levels were noted at the site of administration into the skeletal muscle; the copy number on Day 2 ranged from 3×10^{8} to 9.97×10^{9} copies/mg sample. In the majority of samples of other tissues taken on Day 56 i.e. brain, kidney, lung and spleen, the levels were below the limit of quantification, indicating elimination. Low levels were noted in 1/6 samples of heart and liver, 1/3 samples of ovary and testes and 3/6 samples of lymph nodes. No shedding into urine or faeces was observed.

4.3 Nonclinical Toxicology

In a GLP compliant study (study number NP08QM), the toxicity of ChAdOx1-HBV has been assessed following IM administration to BALB/c mice. Main test animals (10 per sex) were

dosed on Days 1 and 15 and terminated on Day 17. Additional groups (six per sex) were included to assess effects i) 48 hours after a single administration (Day 3) and ii) at Day 29, the end of a recovery period of 14 days following the second dose administration. Dose levels were 0 (vehicle) or 2.5 x 10^{10} vp ChAdOX1-HBV.

The following were assessed: mortality, clinical observations, body weight, food consumption, body temperature, haematology, clinical chemistry, immune response in spleocytes (IFN-γ secretion), organ weight and gross and microscopic pathology (Day 17 only).

There was no mortality or morbidity and dosing was well tolerated with no noteworthy effects on food intake, body temperature or clinical condition. A slight reduction in body weight gain was observed in males following the second dose, which resulted in overall lower gains at Day 29, and in females during the first 3 days following the first dose.

ChAdOx1-HBV caused an increase in the antigen-specific IFN-γ response in splenocytes incubated with P4 peptide pool. An equivalent antigen specific IFN-γ response was measured in main test animals on Day 17 and recovery animals 2 weeks after cessation of dosing.

On Day 3, a decrease (~60%) in reticulocytes was noted and there was a decrease in white blood cell count (~60%) due primarily to a decrease in lymphocytes. On Day 17, white blood cell count decreased in females and not males, whilst on Day 29, the decrease was in males only. Changes in red cell indices had recovered by Day 29.

Minor changes in clinical chemistry parameters were observed, including reversible decreases in glucose and increases in cholesterol, were observed.

Histopathologically, on Day 17, increased extramedullary hemopoiesis was noted in the spleen which correlated with increased spleen enlargement macroscopically and increased spleen weight. Unilateral generalised increased cellularity (minimal or slight) was seen in the inguinal lymph node (the draining lymph node), which was considered to be a local stimulation due to the presence of ChAdOx1-HBV. There was an increased incidence and severity of inflammatory infiltrate at the injection site in the thigh muscle, suggesting a heightened local reaction to ChAdOx1-HBV compared to the vehicle control. Overall, the limited histopathological findings were consistent with vaccine administration.

In conclusion, dosing ChAdOx1-HBV on Days 1 and 15 at 2.5 x 10^{10} vp was well tolerated in BALB/c mice. There was evidence of an immune response and minor changes associated with the presence of a vaccine, which were not considered adverse.

4.4 Conclusions from Nonclinical Studies and Relevance to the Clinical Studies

Studies in both inbred (BALB/c and C57BL/6) and outbred (CD-1) mice illustrate the HBV antigen is highly immunogenic when delivered using the ChAdOx1 viral vector platform. This immune response was measured using IFN-γ ELISPOT, ICS and ELISA. Insert-specific responses could be detected in the liver as well as in the periphery following IM vaccination and could be increased by a boost vaccination with an MVA vector encoding the same HBV antigens. These data illustrate ChAdOx1-HBV has the potential to elicit the immune response required for efficacy.

In a GLP compliant toxicology study in BALB/c mice, a dose of ChAdOx1-HBV of 2.5 x 10^{10} vp, a dose level approximating to the maximum anticipated clinical dose, was well tolerated, produced an immune response that was sustained for 2 weeks following dosing, and was not associated with adverse effects. Thus, the nonclinical safety profile is consistent with the planned clinical investigation.

A biodistribution and shedding study conducted in BALB/c mice, showed that following IM injection of ChAdOx1-HBV, high levels of viral DNA were localised to the site of injection, with low levels in other tissues (many samples below the limit of quantification) at all timepoints. No DNA was detected in the blood at any timepoint in the study; it was also not detectable in any urine or faecal samples, indicating that no viral shedding had occurred..

5 EFFECTS IN HUMANS

5.1 Summary of Clinical Studies

Vaccitech is conducting a programme of studies with vaccines containing an HBV antigen. The FIH study HBV001 is a non-randomised, open-label, dose escalation study to evaluate the safety, tolerability and immunogenicity of two different doses of ChAdOx1-HBV vaccine (2.5×10^9 vp and 2.5×10^{10} vp) in 10 healthy participants and 12 participants with CHB and virally suppressed with oral antiviral medication. Based on the interim results of HBV001, the higher dose of 2.5×10^{10} vp will be further evaluated in study HBV-002 with MVA, with or without PD-1 inhibitors.

The ChAdOx1-vectored COVID-19 vaccine has recently been authorised throughout the world and has now been administered to millions of people at a dose of 5×10^{10} vp [1]. Prior to the COVID-19 pandemic, ChAdOx1 has also been the vector for different vaccine trials conducted by the University of Oxford for a range of diseases including influenza (FLU004 & 005), MERS [2] and prostate cancer [3]. The doses administered to participants ranged from 5×10^9 vp, to 5×10^{10} vp.

Results from the two influenza studies are provided in more detail in Section 5.2 and show that the ChAdOx1-NP + M1 vaccine generated positive immunogenicity data and had an acceptable safety profile.

5.2 Influenza Studies with ChAdOx1

Vaccitech previously completed a clinical programme for influenza, which has included ChAdOx1 and MVA-vectored vaccines encoding the influenza nucleoprotein and matrix 1 antigens (ChAdOx-1-NP+M1 and MVA-NP+M1, respectively). The ChAdOx1-vectored vaccine was administered in two of the completed studies (FLU004 and FLU005). Immunogenicity data from FLU004 is provided in Section 5.2.1 and safety data from both studies in Section 5.2.2. The influenza programme no longer utilises ChAdOx-1-NP+M1 as studies showed that MVA-NP+M1 alone produced a sufficient immune response.

FLU004 (NCT01623518) [38] was a Phase I open-label non-randomised dose escalation study to determine the safety and immunogenicity of the ChAdOx1 NP+M1candidate influenza vaccine in 15 healthy participants aged 18 to 50 years. The first three groups (three participants each) received single ascending doses of 5×10^8 vp, 5×10^8 vp and 2.5×10^{10} vp ChAdOx1-NP+M1. Six participants in the final group received 5×10^{10} vp ChAdOx1-NP+M1, of which three also received a boost of 1.5×10^8 pfu MVA-NP+M1 7 to 14 weeks after adenovirus prime. All vaccinations were given by IM injection.

This study demonstrated that a prime-boost regime of ChAdOx1-NP+M1 followed by MVA-NP+M1 significantly increased the influenza-specific cellular immune response and maintained high responses up to 8 weeks post-boost. This study found that the reactogenicity profile of the prime-boost regime was found to be unacceptable at the highest dose of ChAdOx1-NP+M1 used (5×10^{10} vp) and therefore a lower dose 2.5×10^{10} vp was chosen for further studies with ChAdOx1-NP+M1.

FLU005 (NCT01818362) [19] was a Phase I randomised observational study which assessed the safety and immunogenicity of ChAdOx1-NP+M1 at a dose of 2.5×10^{10} vp given in a prime-boost regimen with MVA-NP+M1 1.5×10^{8} pfu:

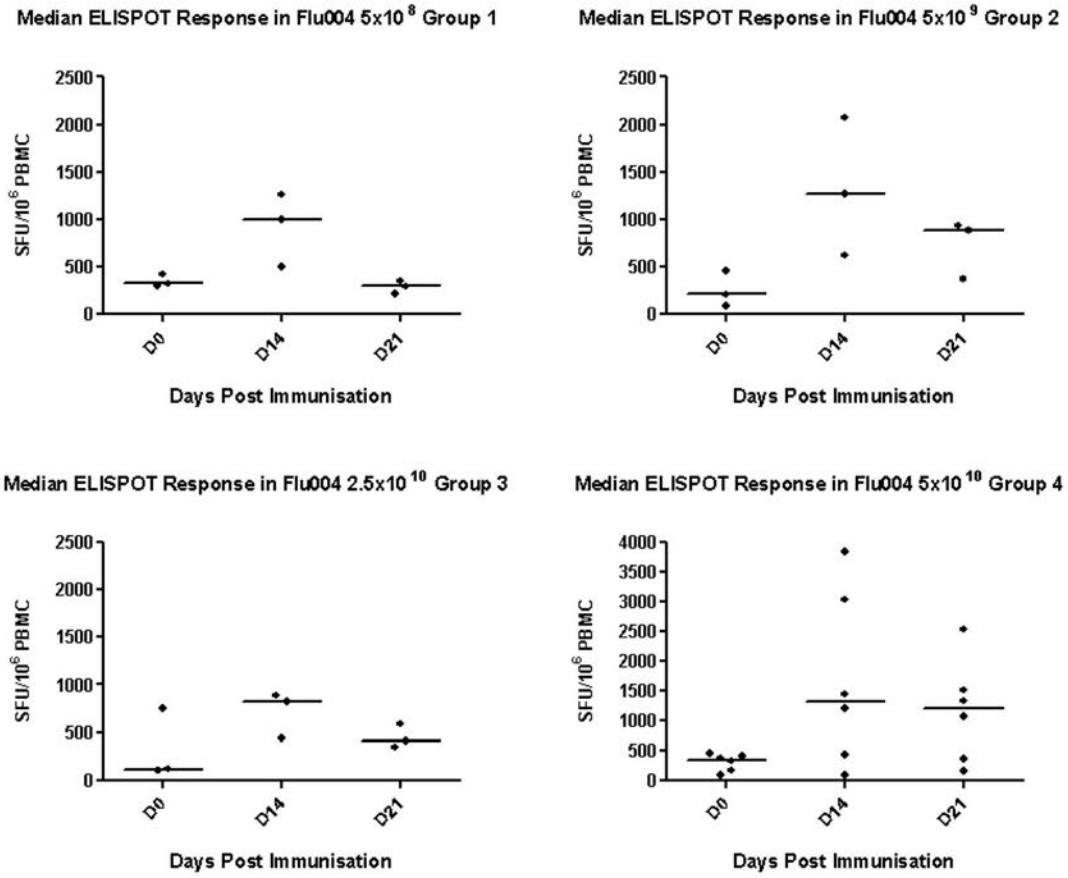
- 48 healthy adult participants (required to be aged 18 to 50 years) were randomised to receive either ChAdOx1-NP+M1 or MVA-NP+M1 on Day 0, with a boost at 8 weeks or 52 weeks with the alternative vaccine
- 24 elderly participants (>50 years) were randomised to receive either ChAdOx1-NP+M1 alone or ChAdOx1 NP+M1 followed by a boost at 8 weeks with MVA-NP+M1

5.2.1 Immunogenicity

The median ELISPOT responses achieved post-vaccination with increasing doses of the ChAdOx1-NP+M1 influenza vaccine on Day 0 in FLU004 are shown in Figure 7. Responses increased with dose but were not statistically significant due to variability within a small sample size.

The peak ELISPOT response was seen at Day 14. Responses to vaccine were maintained at Day 21; with median values of 298.3, 885, 411.7 and 1197 SFU/10^6 peripheral blood mononuclear cells for the 5×10^8 vp, 5×10^9 vp, 2.5×10^{10} vp and 5×10^{10} vp doses of ChAdOx1-NP+M1, respectively.

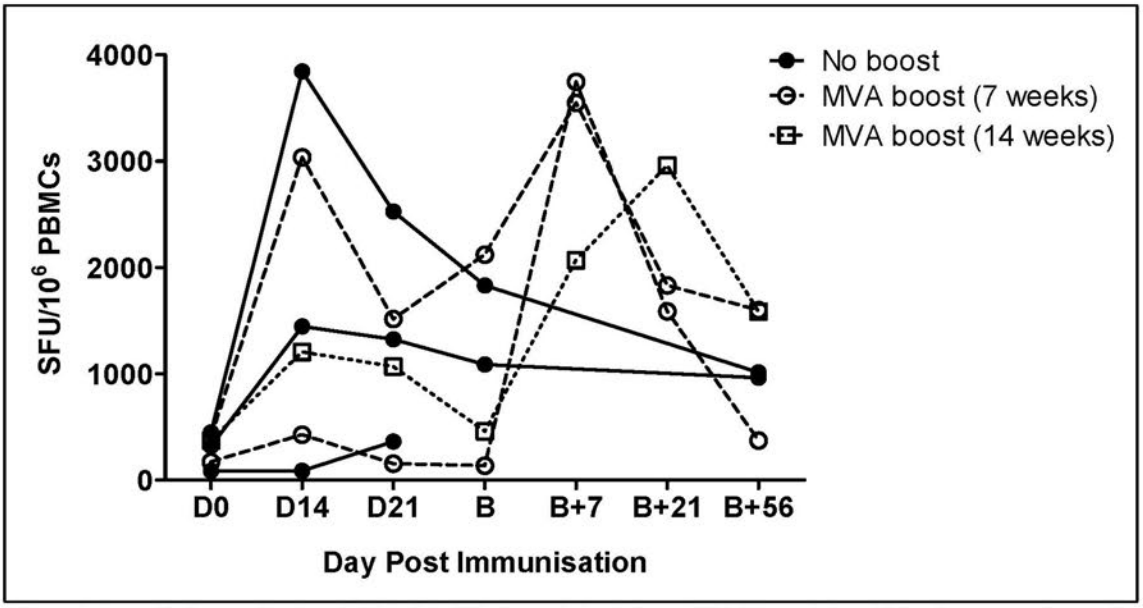
Figure 7 Median ELISPOT Responses after Vaccination with the ChAdOx1-NP+M1 Influenza Vaccine at Increasing Doses in FLU004



Abbreviations: D=day; PBMC=peripheral blood mononuclear cells; SFU=spot forming units
Note: The y-axis for Group 4 is different to Groups 1 to 3

Figure 8 shows the effect on the response to the highest dose of ChAdOx1-NP+M1 (5×10^{10} vp), with an MVA-NP+M1 boost at 7 days or 14 days. Median ELISPOT responses 7 days post-MVA-NP+M1 boost were increased approximately threefold and remained at high levels up to 56 days post-boost.

Figure 8 ***Ex Vivo*** **ELISPOT Responses for Three Participants Boosted with MVA-NP+M1 Following ChAdOx1-NP+M1 Prime Compared to Two Participants who did not receive a Boost in FLU004**



Abbreviations: B=boost; D=day; MVA=Modified vaccinia Ankara; PBMC=peripheral blood mononuclear cell; SFU=spot forming units

5.2.2 Safety

In FLU004, the ChAdOx1-NP+M1 influenza vaccine was well tolerated in healthy participants naturally primed by influenza infection in the past at doses below 5×10^{10} vp with the majority (83%) of adverse events being mild (12% moderate and 5% severe). The reactogenicity profile at 5×10^{10} vp was considered unacceptable; the difference between the 2.5×10^{10} vp and 5×10^{10} vp doses is shown in Figure 9 for local reactions and Figure 10 for systemic reactions. Three of the six participants developed fevers (38.2 to 38.5°C) and two of these three participants also developed severe local and systemic adverse reactions at the highest dose. Laboratory adverse events (lymphopenia and neutropoenia) also occurred in three participants at the highest dose.

Boosting with MVA-NP+M1 7 to 14 days after the highest dose of ChAdOx1-NP+M1 appeared to result in more severe local reactions, with two of three participants reporting severe local pain. Severe systemic adverse events of feverishness, fatigue, malaise and myalgia were reported by one participant; the other two reported mild systemic events.

Figure 9 Percentage of Participants and Severity of Local Adverse Events after 2.5 x 10^{10} vp and 5 x 10^{10} vp ChAdOx1 Prime in Participants aged 18 to 50 years in FLU004

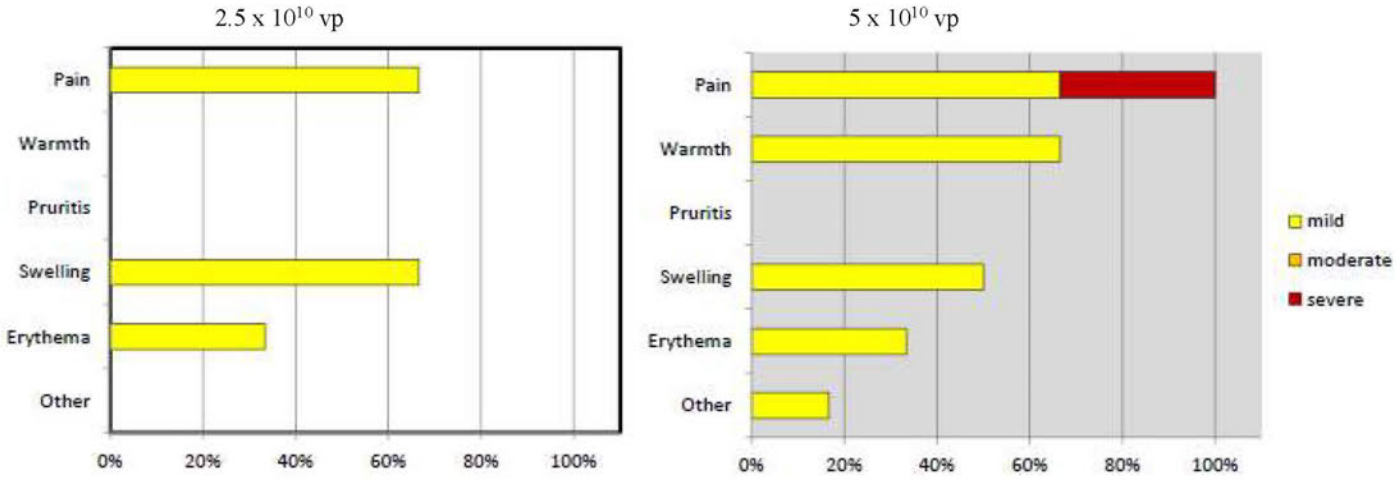
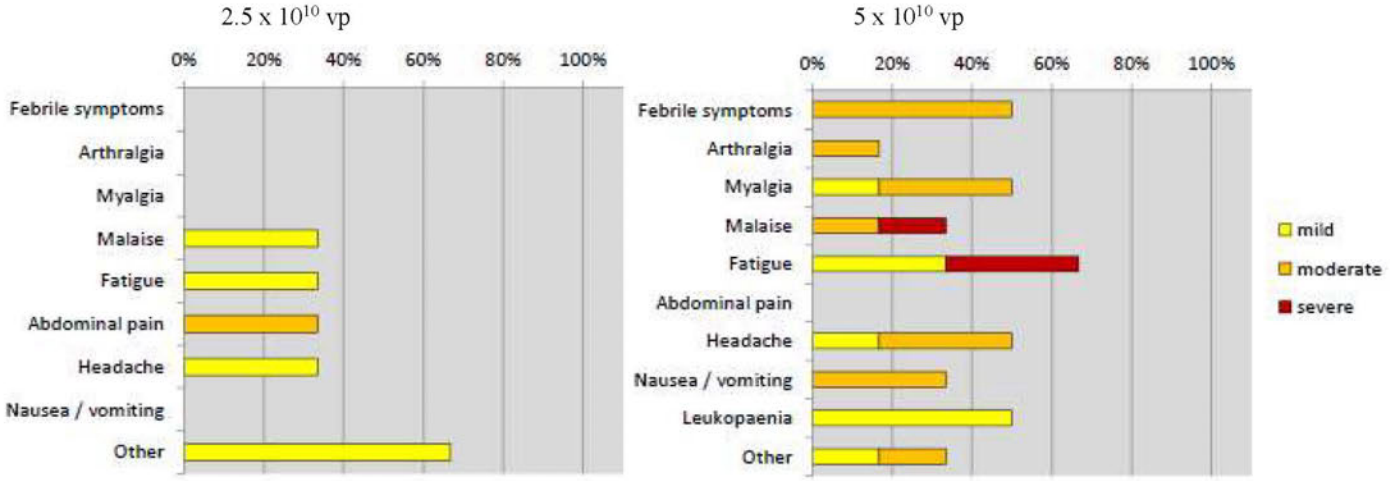


Figure 10 Percentage of Participants and Severity of Systemic Adverse Events after 2.5 x 10^{10} vp and 5 x 10^{10} vp ChAdOx1 Prime in Participants aged 18 to 50 years in FLU004



A dose of 2.5 x 10^{10} vp dose was therefore selected for use in FLU005.

The local and systemic adverse event profile following ChAdOx1-NP+M1 prime in participants aged 18 to 50 years in FLU005 are shown Figure 11. Local and systemic adverse events following ChAdOx1-NP+M1 prime in participants aged >50 years are shown in Figure 12A and B for MVA-NP+M1 boost at 8 weeks in Figure 12C and D at 52 weeks.

The majority of adverse events were mild to moderate. The most common local adverse event was pain at the vaccination site and the most common systemic adverse events were fatigue and headache. The proportion of participants experiencing local and systemic adverse events of any severity after ChAdOx1-NP+M1 and MVA-NP+M1 vaccination was lower in the participants aged >50 years than those aged 18 to 50 years. The majority of laboratory adverse events were mild (leucocytosis, thrombocytopenia, eosinophilia, alkaline phosphatase elevation, hypoalbuminaemia, increase in serum urea, hypokalaemia). Hyperkalaemia was

observed in three participants in the older age group in the first week after ChAdOx1 vaccination, including one Grade 3 resolving within 1 week.

There were no serious adverse reactions in FLU005.

Doses of ChAdOx-1-HBV 2.5 10^9 vp and 2.5 x 10^{10} vp will be investigated in the HBV clinical programme.

Figure 11 Percentage of Participants and Severity of Local and Systemic Adverse Events after Prime Vaccination with ChAdOx1-MVA by Intramuscular Injection in 32 Participants aged 18 to 50 years in FLU005

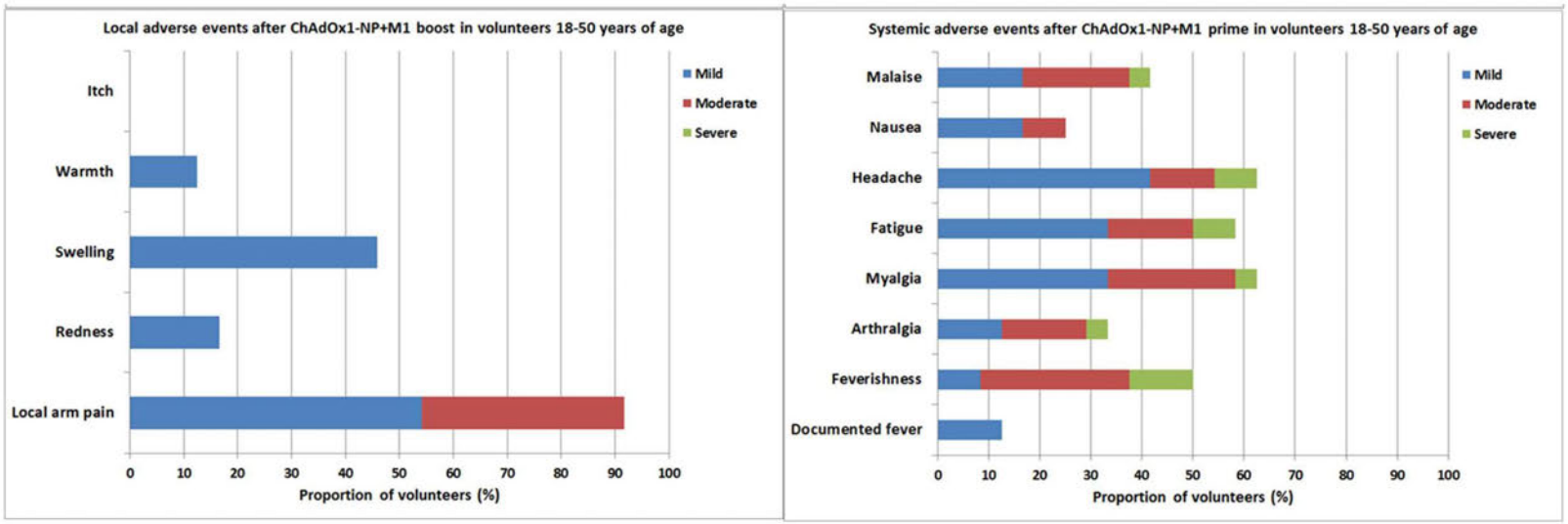
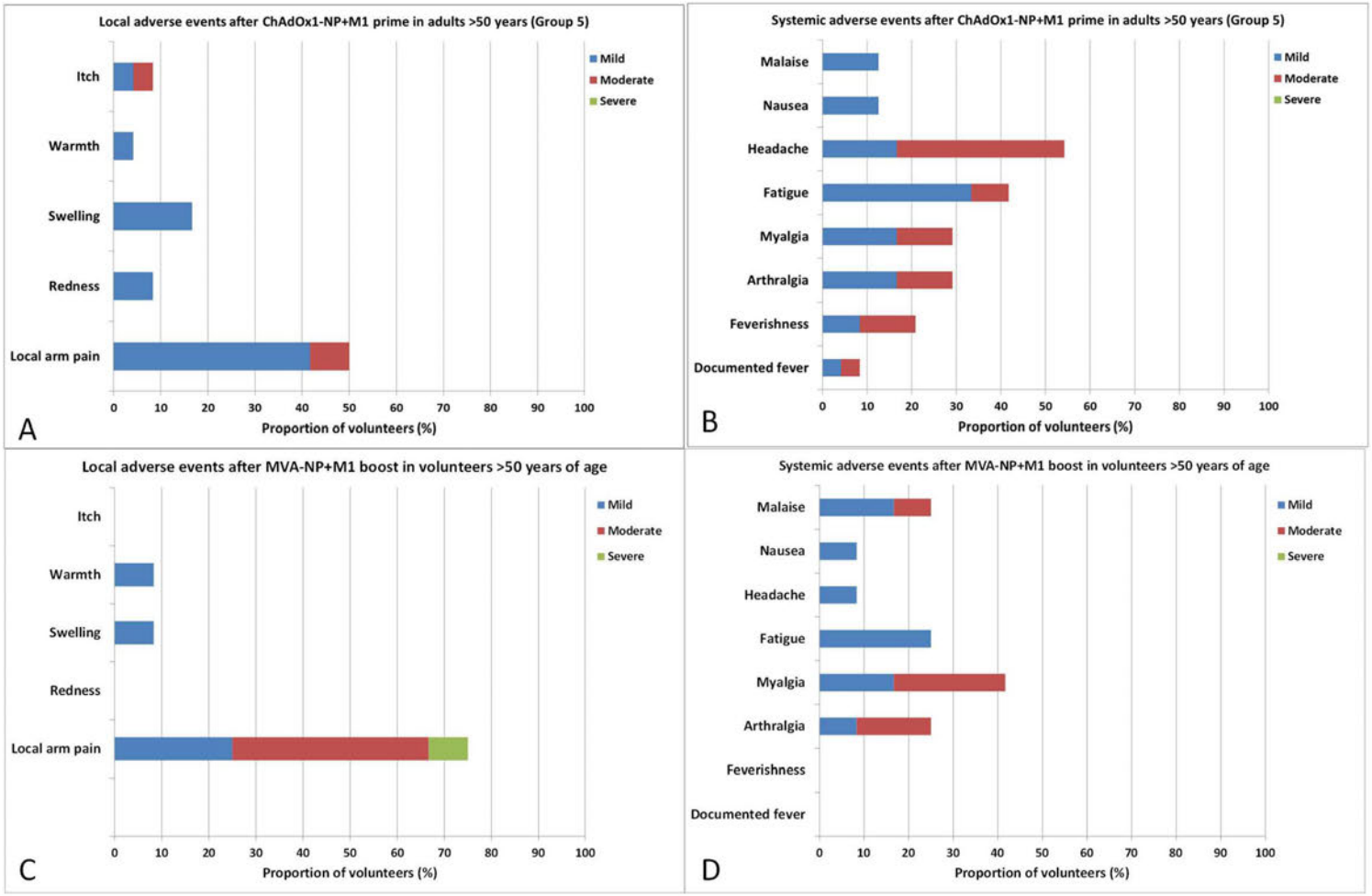


Figure 12 **Percentage of Participants and Severity of Local and Systemic Adverse Events after Prime Vaccination with ChAdOx1-NP+M1 followed by MVA-NP+M1 Boost at 8 and 52 weeks in 12 Participants aged >50 years in FLU005**



5.3 Marketing Experience

ChAdOx1-HBV has not been commercialised and there is therefore no current marketing experience.

6 SUMMARY OF DATA, REFERENCE SAFETY INFORMATION AND GUIDANCE FOR THE INVESTIGATOR

ChAdOx1-HBV vaccine is being investigated for its potential in treating CHB infection in a prime-boost regimen including MVA-HBV, in patients with prior viral suppression.

ChAdOx1-HBV vaccine is a chimpanzee adenoviral-vectored vaccine encoding consensus sequences from a group C genotype. The ChAdOx1 virus has been engineered to be replication-incompetent and can be manufactured in well-established HEK293 cell lines containing the adenoviral E1 gene.

The data provided in this document comprises a summary of the known data of ChAdOx1-HBV in animal models and in clinical studies with other ChAdOx1-vectored vaccines to date.

6.1 Summary of Data

6.1.1 Nonclinical Studies

A nonclinical immunogenicity study was conducted in which 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 IFN-γ ELISPOT and ICS assays as well as ELISA.

A GLP toxicology study has been conducted in BALB/c mice. In this study, animals were dosed on Day 1 and Day 15 and terminated on Day 17 or after a 2-week recovery period, on Day 29, while an additional group was dosed once on Day 1 and terminated on Day 3 to assess acute effects. The dose level of ChAdOx1-HBV administered was 2.5×10^{10} vp, which is the maximum anticipated human clinical dose level. The dose was well tolerated in all animals, with no morbidity or mortality, no changes in body temperature or adverse clinical observations. There was evidence of an increase in the antigen-specific IFN-γ response in splenocytes (ELISPOT data) and the response measured was equivalent at 2 days and at 2 weeks after cessation of dosing. Anatomic pathology changes were limited to the spleen (increased extramedullary hemopoiesis which correlated with increased organ size/weight), inguinal lymph node (the draining lymph node, in which there was a generalised increase in cellularity) and the injection site (thigh muscle in which the incidence and severity of inflammatory cell infiltration was noted which was above that observed in animals administered vehicle alone). These changes were consistent with the administration of vaccine.

A GLP nonclinical biodistribution and shedding study of the ChAdOx1-HBV, conducted in BALB/c mice, showed there to be insignificant levels of viral DNA detected in the majority of organs and tissues, with the majority of the IM dose remaining localised to the site of injection and lymphoid organs (Study No. 0841MV038.001); this is to be anticipated as the vaccines are processed by the immune system. Furthermore, no DNA was detected in the blood at any timepoint in the study; it was also not detectable in any urine or faecal samples, indicating that no viral shedding had occurred.; this is to be expected, as the viral vector is unable to replicate.

Overall, the data from the nonclinical safety assessment support the proposed clinical plan.

6.1.2 Clinical Studies

Vaccitech is planning to conduct a programme of studies with vaccines containing an HBV antigen. The ChAdOx1-vectored COVID-19 vaccine has recently been authorised throughout the world and has now been administered to millions of people at a dose of 5×10^{10} vp [1]. Prior to the COVID-19 pandemic, ChAdOx1 has also been the vector for different vaccine trials conducted by the University of Oxford for a range of diseases including influenza (FLU004 & 005), MERS [2] and prostate cancer [3]. The doses administered to participants ranged from 5×10^9 vp, to 5×10^{10} vp.

Results from the two influenza studies show that the ChAdOx1-NP + M1 vaccine generated positive immunogenicity data and had an acceptable safety profile.

Based on these results, doses of 2.5×10^9 vp and 2.5×10^{10} vp are being investigated in the FIH HBV001 clinical study, in healthy volunteers and subjects with CHB infection. Based on the interim results of HBV001, the higher dose of 2.5×10^{10} vp is being further evaluated in study HBV002 with MVA, with or without PD-1 inhibitors.

6.2 Posology and Method of Administration

ChAdOx1-HBV will be supplied in stoppered and sealed vials at a target sterile volume of 0.65 mL in 4 mL vials for a 0.5mL injection volume. In study HBV002, it will be administered by IM injection at a dose of 2.5×10^{10} vp. The appropriate doses will be prepared by aseptic serial dilution according to the procedure in the Pharmacy Manual. Dilution kits of commercially available equipment will be provided by the Sponsor.

ChAdOx1-HBV is classed as a genetically modified organism. It must be administered by suitably qualified healthcare professionals wearing 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 10 minutes after vaccination. The dressing and any unused vaccine at the end of the study must both be discarded as genetically modified organism waste.

6.3 Contraindications

Treatment with ChAdOx1-HBV is contraindicated in patients with clinically significant autoimmune or immunosuppressive disease or any history of anaphylaxis in reaction to vaccination or history of allergic reactions likely to be exacerbated by any component of the vaccine.

6.4 Special Warnings and Special Precautions for Use

The potential risks to participants in clinical studies of ChAdOx1-HBV are associated with vaccination and phlebotomy.

There are potential known and unknown risks associated with vaccination, although severe systemic adverse reactions with any vaccine are rare. With any vaccine, including licensed ones, there is a rare risk of anaphylaxis which can be fatal. The incidence of this is unknown but is estimated at one per 10^5 to 10^6 vaccinations. Participants should be vaccinated in a clinical area where appropriate drugs and medical equipment to treat acute anaphylactic reactions are immediately available for the management of SAEs. Participants should also be observed in the clinic for at least 30 minutes post-vaccination.

ChAdOx1-HBV is made with the same viral vector as the AstraZeneca vaccine for COVID-19. There have been reports of a very rare type of blood clot in the brain, known as cerebral venous sinus thrombosis, and clots in other organs, associated with thrombocytopenia in people who received this AstraZeneca vaccine, or another vaccine for COVID-19 made with a different adenoviral vector and manufactured by Johnson and Johnson. These events have typically started 6-14 days after vaccination and have been reported as very rare events - up to 1 in 250,000 in the general population. It is not known whether these very rare clotting problems are related to the ChAdOx1 vector, and not known if they might also occur with ChAdOx1-HBV. Cerebral venous sinus thrombosis should be considered in participants who have recently received ChAdOx1-HBV and who present with symptoms that might represent serious thrombotic events or thrombocytopenia, including severe headache, backache, new neurologic symptoms, severe abdominal pain, shortness of breath, leg swelling, petechiae, or new or easy bruising. Obtain platelet counts and screen for evidence of immune thrombotic thrombocytopenia. Participants with a thrombotic event and thrombocytopenia after ChAdOx1-HBV, should be initially evaluated with a screening PF4 ELISA as would be performed for autoimmune heparin-induced thrombocytopenia (HIT). Consultation with a haematologist is strongly recommended. Do not treat participants with thrombotic events and thrombocytopenia following receipt of ChAdOx1-HBV with heparin, unless HIT testing is negative. If HIT testing is positive or unable to be performed in participants with thrombotic events and thrombocytopenia following receipt of ChAdOx1-HBV, non-heparin anticoagulants and high-dose intravenous immune globulin should be strongly considered.

Intramuscular injection of vaccines frequently causes the following local and systemic signs and symptoms that will be solicited from the participant to ensure they are nor occurring more frequently or are more severe than expected:

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

6.5 Interactions

Vaccines may interfere with the response to each other if administered too closely together.

- Live vaccines should not be administered within 30 days before or after study vaccine.
- Inactivated vaccines should not be administered within 14 days of study vaccine.
- Adenoviral-vectored vaccines should not be administered within 3 months of the ChAdOx1-HBV component of VTP-300, or as described in the protocol.

6.6 Pregnancy, Lactation and Fertility

There are no data on the use of ChAdOx1-HBV in pregnant or breast-feeding women. ChAdOx1-HBV is therefore not to be administered in clinical studies to women of childbearing potential who are not using highly effective contraception or to women who are breastfeeding

Studies to evaluate the effect on ChAdOx1-HBV on fertility have not been performed. The effect of ChAdOx1-HBV on female fertility is therefore unknown.

Female participants of childbearing potential will be required to undertake a pregnancy test at screening which would exclude them from the clinical studies, and at pre-vaccination that would preclude them from further treatment. All participants agree to maintain highly effective contraception throughout treatment.

6.7 Overdose

In the case of overdose the participant should be monitored for evidence of toxicity and standard supportive treatment provided based on any signs or symptoms experienced. There is no rescue medication.

6.8 Reference Safety Information

All serious adverse reactions will be considered unexpected for the purposes of expedited reporting.

7 REFERENCES AND REPORTS

1. World Health Organisation Summary of Product Characteristics COVID-19 Vaccine AstraZeneca, solution for injection in multidose container COVID-19 Vaccine (ChAdOx1-S [recombinant]). 15 February 2021. Available at: https://extranet.who.int/pqweb/sites/default/files/documents/WHO_SMPC_azd1222.pdf. Last accessed on 30 March 2021.
2. Folegatti PM, Bittaye M, Flaxman A, et al. Safety and immunogenicity of a candidate Middle East respiratory syndrome coronavirus viral-vectored vaccine: a dose-escalation, open-label, non-randomised, uncontrolled, phase 1 trial. Lancet Infect Dis. 2020;20(7):816-826.
3. Cappuccini F, Bryant R, Pollock E, et al. Safety and immunogenicity of novel 5T4 viral vectored vaccination regimens in early stage prostate cancer: a phase I clinical trial. JITC. 2020;8:e000928.
4. 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.
5. 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.
6. 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.
7. 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.
8. 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.
9. 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.
10. 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.
11. 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.
12. 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.
13. Knolle PA, Thimme R. Hepatic immune regulation and its involvement in viral hepatitis infection. Gastroenterol. 2014;146:1193–1207.
14. Seder RA, Darah PA, Roederer M. T-cell quality in memory and protection: implications for vaccine design. Nature Rev Immunol. 2008;8:247-258.
15. 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.

16. 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.
17. Lu S. Heterologous prime-boost vaccination. Curr Opin Immunol. 2009;21(3):346-351.
18. 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.
19. 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.
20. 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.
21. Morris SJ, Sebastian S, Spencer AJ, Gilbert SC. Simian adenoviruses as vaccine vectors. Future Virol. 2016;11(9):649–659.
22. 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.
23. 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.
24. 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.
25. 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.
26. 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.
27. 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.
28. 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.
29. 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.
30. Halbroth BR, Sebastian S, Poyntz HC, et al. Development of a molecular adjuvant to enhance antigen-specific CD8+ T cell responses. Sci Rep. 2018;8(1):15020.
31. Capone S, Naddeo M, D'Alise AM, et al. Fusion of HCV nonstructural antigen to MHC class II-associated invariant chain enhances T-cell responses induced by vectored vaccines in nonhuman primates. Mol Ther. 2014;22(5):1039-1047.

32. Spencer AJ, Cottingham MG, Jenks JA, et al. Enhanced vaccine-induced CD8+ T cell responses to malaria antigen ME-TRAP by fusion to MHC Class II invariant chain. PLoS One. 2014; 9(6): e100538.

33. Nassal M. Hepatitis B viruses: reverse transcription a different way. Virus Res 2008;134(1-2):235-249.

34. Jones SA, Hu J. Protein-primed terminal transferase activity of hepatitis B virus polymerase. J Virol 2013;87(5):2563-2576.

35. Jones SA, Clark DN, Cao F, Tavis JE, Hu J. Comparative analysis of hepatitis B virus polymerase sequences required for viral RNA binding, RNA packaging, and protein priming. J Virol 2014;88(3):1564–1572.

36. Ko C, Shin YC, Park WJ, Kim S, Kim J, Ryu WS. Residues Arg703, Asp777, and Arg781 of the RNase H domain of hepatitis B virus polymerase are critical for viral DNA synthesis. J Virol 2014;88(1):154-63.

37. Nassal M. HBV cccDNA: viral persistence reservoir and key obstacle for a cure of chronic hepatitis B. Gut 2015;64(12):1972-1984.

38. Antrobus RD, Couglan L, Berthoud TK et al. 2014. Clinical assessment of a novel recombinant simian adenovirus ChAdOx1 as a vectored vaccine expressing conserved Influenza A antigens. Mol Ther. 2014; 22(3): 668-674.