Trial Title: A phase I study to assess the safety and immunogenicity of a recombinant adenovirus-based vaccine against plague

Internal Reference Number / Short title: Investigating a Vaccine Against Plague (PlaVac)

Ethics Ref: 20/SC/0405

IRAS Project ID: 279095

EudraCT Number: 2020-000710-15

ISRCTN: 41077863

Date and Version No: 19-Nov-2021 V3.4

Chief Investigator: **Professor Andrew J Pollard**
Oxford Vaccine Group (OVG)
Department of Paediatrics
University of Oxford
Centre for Clinical Vaccinology & Tropical Medicine (CCVTM)
Churchill Hospital
Oxford
OX3 7LE
United Kingdom

Co-Investigators: **Professor Brian Angus**
Dr Maheshi Ramasamy

Sponsor: University of Oxford

Funder: Innovate UK

Investigator Agreement

"I have read this protocol and agree to abide by all provisions set forth therein. I agree to comply with the International Conference on Harmonisation Tripartite Guideline on Good Clinical Practice. We declare no financial conflict of interest in this study."

Chief Investigator Signature:

Co-Investigator Signatures: _____ _____

Statistician Signature: _____

Confidentiality Statement

Contents

1. KEY TRIAL CONTACTS

Chief Investigator	**Prof Andrew Pollard** Professor of Paediatrics & Immunity Oxford Vaccine Group Centre for Clinical Vaccinology & Tropical Medicine (CCVTM) Churchill Hospital Old Road, Headington Oxford, OX3 7LE

	Tel +44 (0)1865 234226/611313 Fax +44 (0)1865 289695 andrew.pollard@paediatrics.ox.ac.uk
Investigators **(Clinical)**	**Prof Brian Angus** Professor of Infectious Diseases / hosptial Physician / Director Oxford Centre for Clinical Tropical Medicine, University of Oxford Nuffield Department of Medicine Research Building (NDRMB) Old Road Campus, Headington Oxford, OX3 7FZ, United Kingdom brian.angus@ndm.ox.ac.uk **Dr Maheshi Ramasamy** Consultant Physician, Oxford University Hospitals NHS Foundation Trust/ Senior Clinical Researcher Oxford Vaccine Group Centre for Clinical Vaccinology and Tropical Medicine (CCVTM) Churchill Hospital, Oxford, OX3 7LE, United Kingdom maheshi.ramasamy@paediatrics.ox.ac.uk
Investigators **(Laboratory)**	**Prof Christine Rollier** Associate Professor in Vaccinology Oxford Vaccine Group Centre for Clinical Vaccinology and Tropical Medicine (CCVTM) Churchill Hospital, Old Road Campus, Headington Oxford, OX3 7LE, United Kingdom Christine.rollier@paediatrics.ox.ac.uk **Dr Christina Dold** Senior Postdoctoral Researcher Oxford Vaccine Group Centre for Clinical Vaccinology and Tropical Medicine (CCVTM) Churchill Hospital, Old Road Campus, Headington Oxford, OX3 7LE, United Kingdom Christina.dold@paediatrics.ox.ac.uk
Sponsor	**University of Oxford** Clinical Trials and Research Governance Joint Research Office Boundary Brook House Churchill Drive Headington Oxford OX3 7GB United Kingdom
Clinical Trials Unit	**Centre for Clinical Vaccinology & Tropical Medicine (CCVTM)** Churchill Hospital

	Old Road Campus Headington Oxford OX3 7LE United Kingdom Tel +44 (0)1865 611400
Statistician	**Xinxue Liu** Oxford Vaccine Group Centre for Clinical Vaccinology and Tropical Medicine (CCVTM) Churchill Hospital, Old Road Campus, Headington Oxford, OX3 7LE, United Kingdom Xinxue.liu@paediatrics.ox.ac.uk
Data Safety Monitoring Committee Chair	**Prof Graham S Cooke** Professor of Infectious Diseases Faculty of Medicine, Department of Infectious Disease Infectious Diseases Section Winston Churchill Wing St Mary's Campus g.cooke@imperial.ac.uk

2. LAY SUMMARY

Plague is a disease caused by infection with *Yersinia pestis,* which is a type of bacteria. In humans, this infection can cause high fevers, swollen lymph nodes, shortness of breath, coughing up blood, bloodstream infection and, if left untreated, death.

It is spread by the bite of an infected flea, the handling of an animal infected with plague or from inhaling respiratory droplets from an infected person. Plague is found across the world, but the biggest burden is in very remote and poor parts of Africa and Asia. During 1998-2016, a total of 13,234 suspected cases of plague were recorded in Madagascar alone (1). Plague can be treated effectively with antibiotics, if treated early, however this is often not possible in rural areas, where a vaccine would be much more effective.

Some effective vaccines against plague are available in some parts of the world, however they are not ideal for various reasons [2]. The University of Oxford has developed a novel plague vaccine, which can be given intramuscularly. The vaccine is made with a modified harmless virus, which has already been given to humans as a vaccine platform, and has been shown to be safe. This novel vaccine needs testing to see if it is safe and produces an effective immune response in healthy participants. All participants will be monitored closely throughout the trial.

This type of vaccine has been given to participants in previous Oxford Vaccine Group and Jenner Laboratories studies, most recently with a vaccine against COVID-19 and Meningitis B, and shown to be safe. Blood and mucosal samples will be taken and used to test the effectiveness of this potential vaccine.

3. SYNOPSIS

Trial Title	**A phase I study to assess the safety and immunogenicity of a recombinant adenovirus-based vaccine against plague**

Internal ref. no. (or short title)	Investigating a Vaccine Against Plague (PlaVac) / (OVG2019/05)	
Clinical Phase	Phase I	
Trial Design	An open label, non-randomized safety and immunogenicity interventional study.	
Trial Participants	Healthy adults aged 18 to 55 years, inclusive	
Planned Sample Size	10 - 45 participants **Group 1 –**15 individuals, 1 dose **Group 2 –**15 individuals, 2 doses (2 month boost interval) **Group 3 –** 15 individual, 2 doses (6 month boost interval)	
Treatment duration	Single dose Prime dose + booster dose at 2 months Prime dose + booster dose at 6 months	
Follow up duration	Up to 12 months (52 weeks) from prime dose	
Planned Trial Period	32 months (completion of the last laboratory assay on the last participant sample)	
	Objectives	Outcome Measures
Primary	To investigate safety and tolerability of 5 x 10^{10} VP of the proposed ChAdOx1 Plague vaccine in healthy adults aged 18 to 55 years, when given one or two dose(s) intramuscularly	The recording and assessment of local and systemic adverse events for 28 days following administration of each vaccine dose
Secondary	To investigate immunogenicity of 5 x 10^{10} VP of the proposed ChAdOx1 Plague vaccine in healthy adults aged 18 to 55 years, when given as one or two dose(s) intramuscularly, with different prime-boost intervals To determine the safety of 5 x 10^{10} VP of the proposed ChAdOx1 Plague vaccine, in healthy adults aged 18 to 55 years when given one or two dose(s) intramuscularly with different prime-boost intervals	ELISA to measure antibody responses to the vaccine antigens 1 months post vaccination The recording and assessment of local and systemic adverse events for 28 days following administration of each vaccine dose via eDiaries Serious adverse events (SAEs) Safety blood tests

Tertiary	To utilize exploratory immunogenicity assays to determine the immunogenicity of 5 x 10^{10} VP of the proposed ChAdOx1 Plague vaccine, in healthy adults aged 18 to 55 years, when given one or two dose(s) intramuscularly, with different prime-boost intervals	Immunological assays from blood and mucosa, to study the immune responses to vaccines potentially including: 1. Antibody concentration against vaccine antigens before and after vaccination. 2. Quantification of circulating vaccine-induced B-cell responses specific for vaccine antigens before and after the dose. 3. Quantification of vaccine-induced, antigen specific T-cell responses and associated cytokine production before and after the dose. 4. Antibody functional capacity in *in vitro* assays (serum bactericidal, phagocytosis, invasion) 5. Antibody functional capacity in *in vivo* challenge assay (by serum transfer) 6. Gene expression profile after immunisation and DNA storage for investigation of the genetic associations with the immune response. 7. Mucosal and Innate immune activation
Investigational Medicinal Product(s)	Vaccine ChAdOx1 Plague	
Formulation	Liquid, The ChAdOx1 Plague vaccine is formulated in 10 mM Histidine, 35 mM NaCl, 1 mM $MgCl_2$, 0.1 mM EDTA, 0.5 % (v/v) ethanol, 7.5 % (w/v) sucrose, 0.1 % (w/v) PS80, in Water for Injection (WFI) at pH 6.6, sterile-filtered. The vaccine is stored frozen (-80°C nominal) in Type 1 glass, particle free (as per USP or Ph. Eur. Method), sterile and depyrogenated vials each containing 0.5 mL.	
Target Dose	5 x 10^{10} VP	

<table>
<tr><td>Route of administration</td><td>Intramuscular (IM)</td></tr>
<tr><td>Vaccine Schedule</td><td>The schedule for the 3 arms receiving the IMP:

<table>
<tr><th>Group</th><th>Vaccine Dose</th><th>Schedule</th><th>Route</th><th>Day</th></tr>
<tr><td>1</td><td>5 x 10^{10} VP</td><td>Single</td><td>IM</td><td>0</td></tr>
<tr><td>2</td><td>5 x 10^{10} VP</td><td>Two dose, 2 month boost</td><td>IM</td><td>0, 56</td></tr>
<tr><td>3</td><td>5 x 10^{10} VP,</td><td>Two dose, 6 month boost</td><td>IM</td><td>0, 182</td></tr>
</table>

See Fig. 7</td></tr>
</table>

4. ABBREVIATIONS

Ad	Adenovirus
AE	Adverse event
AR	Adverse reaction
ARD	Acute respiratory disease
ASC	Antibody Secreting Cell
AST	Aspartate Transaminase
ALT	Alanine Aminotransferase
CBF	Clinical BioManufacturing Facility
CCVTM	Centre for Clinical Vaccinology & Tropical Medicine
CFU	Colony Forming Units
ChAd	Chimpanzee adenovirus
CI	Chief Investigator
CMV	Cytomegalovirus
CRF	Case Report Form

CSM	Centre for Statistics in Medicine, University of Oxford
DC	Dendritic cell
DSUR	Development Safety Update Reports
DSMC	Data Safety Monitoring Committee
eCRF	Electronic Case Report Form
ELISA	Enzyme Linked Immunosorbent Assay
EMEA	European Medicines Agency
EPR	Electronic Patient Records
F1	Fraction 1
FG	Functional Genomics
GCP	Good Clinical Practice
GLP	Good Laboratory Practice
GMO	Genetically Modified Organism
GMP	Good Manufacturing Practice
GP	General Practitioner
HRA	Health Research Authority
IB	Investigators Brochure
ICF	Informed Consent Form
ICH	International Conference of Harmonisation
IFN-?	Interferon gamma
IM	Intramuscular
IMP	Investigational Medicinal Product
IMPD	Investigational Medicinal Product Dossier
LLN	Lower Limit of Normal
MHRA	Medicines and Healthcare products Regulatory Agency
MLST	Multi-Locus Sequence Typing
MVA	Modified Vaccinia Ankara Virus

NAAT	Nucleic acid amplification test
NHAIS	National Health Applications and Infrastructure Services
OVC	Oxford Vaccine Centre
OVG	Oxford Vaccine Group
PBMC	Peripheral Blood Mononuclear Cell
PI	Principal Investigator
PRN	Pro Re Nata (as required)
QC	Quality Control
QP	Qualified person
R&D	NHS Trust R&D Department
RCA	Replication Competent Adenovirus
REC	Research Ethics Committee
RGEA	Research Governance, Ethics and Assurance
RRT	Renal Replacement Therapy
RSV	Respiratory syncytial virus
SAE	Serious Adverse Event
SAM	Synthetic Absorptive Matrix
SAR	Serious Adverse Reaction
SOP	Standard Operating Procedure
ST	Sequence Type
SUSAR	Suspected Unexpected Serious Adverse Reactions
TB	Tuberculosis
TOPS	The Over volunteering Prevention System (http://www.tops.org.uk)
TMF	Trial Master File
ULN	Upper Limit of Normal
VP	Viral Particle
WFI	Water For Injection

5. BACKGROUND AND RATIONALE

5.1. Plague disease

Plague is a highly contagious and virulent infectious disease caused by the intracellular bacterium *Yersinia pestis.* Since the 1990s, the number of human plague cases has increased in 25 countries (3). Plague is now considered a re-emerging infectious disease due to large reservoirs in Africa, Asia and the Americas[2] and the emergence of antibiotic-resistance strains[4]. Its endemicity throughout the world results in sporadic infections and outbreaks (including recent epidemics in the 21st century, with surprisingly large epidemics in Madagascar in 2017-2018). In 2020, there have been fatal cases of bubonic plague in Mongolia and septicaemic plague in the USA.

Depending on the mode of infection, there are three clinicopathological forms of human plague: bubonic, septicaemic, and pneumonic (3). Bubonic plague is characterized by painful and swollen lymph nodes, which develop near the port of bacterial entry through the skin. Primary pneumonic plague in humans begins as a bronchopneumonia characterized by numerous bacteria and proteinaceous effusion in the alveoli. There is near 100% mortality unless antibiotic treatment of pneumonic plague is commenced within 24 hours after the onset of symptoms; fever, shortness of breath and haemoptysis[5]. Both bubonic and pneumonic plague can progress to the septicaemic form, which is a life-threatening infection of the blood.

5.2. Transmission

Plague is a zoonotic disease; it mainly infects rodents, while humans are only accidental hosts. Plague infections can result from a flea bite, direct handling of infected animal tissues, ingestion of infective materials, or inhalation of aerosolized bacteria. Pneumonic plague, which can be primary from aerosol exposure or secondary from haematological spread, is the form of plague that results in human-to-human transmission and epidemic transmission.

5.3. Vaccine prevention, and the need for a new vaccine

Currently, the only method for controlling plague endemicity and outbreaks is the reactive use of antibiotics. An effective vaccine to prevent pneumonic plague would therefore be preferable to attempting to reduce mortality using treatment alone. In addition, the extreme virulence of *Yersinia pestis* and its ease of spread in aerosolized form, raise the risk of its potential use as a bioweapon.

Vaccine development efforts against plague have been dominated by the use of live attenuated and sub-unit protein vaccines. Live attenuated vaccines pose significant safety concerns and there is disagreement over the level of protection achieved, it is highly reactogenic and the protection does not extent readily to a second year post vaccination, making it unsuitable for the poorest affected countries. Sub-unit proteins in adjuvant have demonstrated a certain degree of protection in animal models, but the immune response seems limited to antibodies, whilst a cellular response correlates with increased efficacy in animal models. This highlights the need for another vaccine strategy that improves over the existing platforms. Moreover, plague disease affects mainly poor populations in hard-to-reach areas within developing countries, where a single dose vaccine eliciting persistent protection for more than a year would be preferable. Vaccines based on conventional delivery platforms (purified or recombinant proteins, in adjuvant) require 2 to 3

doses to induce some form of protection and are expensive, again highlighting the need for improvement over these vaccine candidates[2].

5.4. Plague F1 and LcrV as vaccine antigens

Antigens that have a role in virulence and have a proven track-record of inducing protection against plague in animal models have been incorporated in the ChAdOx1 Plague vaccine [6]: The fraction 1 antigen (F1), and the V antigen involved in the secretion system of the bacteria. Both proteins are virulence factors that generate protective antibodies during natural infection and have been investigated as subunit vaccines in humans (phase I and II). There is availability of serologic markers of efficacy (antibodies against F1 and V), and efficacy testing in animal models has demonstrated that immunization with these antigens, formulated as recombinant proteins in adjuvant, is protective. Moreover, these antigens formulated in Alum adjuvant are safe when administered as recombinant vaccines to humans (phase I and II trials). Studies using these two protein antigens, confirmed that two plague antigens can induce a protective immune response.

5.5. Adenovirus based vaccine platform and use for a vaccine against plague

Adenoviruses have been investigated as vaccine delivery platforms since the 1970s. Vaccination of 2 million US military personnel, using orally administered live human adenovirus serotype 4 and 7, have shown good safety and efficacy [7]. Adenoviruses can infect several cell types, but no evidence of insertional mutagenesis has been observed. The adenoviral genome is well characterized and easy to manipulate. Adenoviruses cause mild disease but deletion of key genes (E1, which is required for viral replication) renders them replication-defective. Replication-deficient adenoviruses can be propagated in cell lines approved by regulatory agencies for human product development (human embryonic kidney cells 293) and following Good Manufacturing Practice. Recombinant Adenoviral vectors expressing antigens from SARS-CoV-2, Ebola, HIV-1, TB, malaria, influenza, RSV and hepatitis C virus, are in phase I/II and III clinical trials and elicit strong antibody responses in humans with a first-rate safety record. Excellent safety and immunogenicity was observed in trials, including in 10 week-old Gambian infants (8), with the Adenoviral-based EBOLA vaccines being used in the field (2019), and with the SARS-CoV-2 vaccine.

Pre-existing immunity against highly prevalent human serotypes, present in up to 80% of individuals, may render certain serotypes, such as human serotype 5 vectors, partially ineffective. Simian adenovirus serotypes can be rendered replication deficient, do not cause human disease, produce high yields during manufacture and are highly immunogenic, making them attractive vaccine vectors for human use (9, 10). Such vectors have hexon structures homologous to human serotype 4, and thus can infect human cells, but do not circulate at detectable levels in human populations; neutralizing antibody prevalence is very low in humans on all continents [11]. These vectors are highly immunogenic: the most developed, based on ChAd63 and ChAdOx1 serotypes, have shown very good safety and immunogenicity in thousands of participants.

Chimpanzee adenovirus Oxford 1 (ChAdOx1) was developed, at Oxford University, from the group E chimpanzee adenovirus Y258 (12). In a UK cohort of 100 people, no individual possessed a neutralisation titre above 200 (the threshold for a positive titre during routine pre-vaccination screening). The low seroprevalence of ChAdOx1 in humans suggests that this relatively new vector could be particularly efficacious in a clinical setting. Therefore, ChAdOx1 now offers an attractive option for vaccine development against *Yersinia pestis*. We elected to employ this novel, replication-deficient viral vector, to develop an adenovirus-vectored vaccine, expressing known immunogenic antigens, F1 and LcrV, fused by a flexible linker to induce protective antibody responses to *Yersinia pestis*.

Chimpanzee adenovirus vaccine vectors have been safely administered to thousands of people, targeting a wide range of pathogens including SARS-CoV-2, another bacterial disease (meningococcus group B) and also cancer. ChAdOx1 has been administered to over 7,000 healthy participants taking part in clinical trials in the UK led by the University of Oxford. The vaccine studies, doses, and number of participants are outlined in Table 1. The ChAdOx1 vaccine platform therefore benefits from a large amount of safety data relevant to the consideration of ChAdOx1 Plague.

Table 1. University of Oxford clinical experience with ChAdOx1 viral vectored vaccines.

Country	Trial	Vaccine	Age	Route	Dose	Number of Volunteers Received ChAdOx1
UK	FLU004	ChAdOx1 NP+M1	18-50	IM	5×10^8 vp	3
					5×10^9 vp	3
					2.5×10^{10} vp	3
					5×10^{10} vp	6
UK	FLU005	ChAdOx1 NP+M1 MVA NP+M1 (week 8)	18-50	IM	2.5×10^{10} vp	12
		ChAdOx1 NP+M1 MVA NP+M1 (week 52)	18-50	IM	2.5×10^{10} vp	12
		MVA NP+M1 ChAdOx1 NP+M1 (week 8)	18-50	IM	2.5×10^{10} vp	12
		MVA NP+M1 ChAdOx1 NP+M1 (week 52)	18-50	IM	2.5×10^{10} vp	9
		ChAdOx1 NP+M1	>50	IM	2.5×10^{10} vp	12
		ChAdOx1 NP+M1 MVA NP+M1 (week 8)	>50	IM	2.5×10^{10} vp	12
UK	TB034	ChAdOx1 85A	18-50	IM	5×10^9 vp	6
					2.5×10^{10} vp	12
		ChAdOx1 85A MVA85A (week 8)	18-50	IM	2.5×10^{10} vp	12
UK	VANCE01	ChAdOx1.5T4 MVA.5T4	18 – 75	IM	2.5×10^{10} vp	34
UK	ADVANCE	ChAdOx1.5T4	>18	IM	2.5×10^{10} vp	23 (as of Feb 20)

	(on-going)	MVA.5T4				
UK	VAC067	ChAdOx1 LS2	18-45	IM	5×10^9 vp	3
					2.5×10^{10} vp	10
UK	MERS001	ChAdOx1 MERS	18-50	IM	5×10^9 vp	6
					2.5×10^{10} vp	9
					5×10^{10} vp	9
UK	CHIK001	ChAdOx1 Chik	18-50	IM	5×10^9 vp	6
					2.5×10^{10} vp	9
					5×10^{10} vp	9
UK	VAMBOX	ChAdOx1 MenB.1	18-50	IM	$2.5x10^{10}$ vp	3
					$5x10^{10}$ vp	24
UK	COV001 (on-going)	ChAdOx1 nCov-19	18-55	IM	$5x10^{10}$vp	1077
UK	COV002 (on-going)	ChAdOx1 nCov-19	18-70+	IM	$2.5x10^{10}$vp $5x10^{10}$vp	10812
Brazil	COV003 (ongoing)	ChAdOx1 nCoV-19	>18	IM	$2.5x10^{10}$vp $5x10^{10}$vp	10416
South Africa	COV005 (ongoing)	ChAdOx1 nCoV-19	18-65	IM	$5x10^{10}$vp	2130
USA Argentina Chile Colombia France Peru	AstraZeneca	AZD1222 (ChAdOx1 nCoV-19)	>18	IM	$5x10^{10}$vp	32459

5.6. The investigational product ChAdOx1 Plague

The ChAdOx1 backbone has been demonstrated previously to be safe in several clinical trials with different inserts (Table 1). All participants in Groups 1, 2 and 3 will receive at least one intramuscular vaccination with ChAdOx1 Plague at the dose of 5×10^{10} VP.

5.6.1 Description of ChAdOx1 Plague

The vaccine consists of the replication-deficient (E1 and E3 deleted) simian adenovirus vector ChAdOx1, containing a genetic cassette encoding for the plague antigens F1 and V, fused with a flexible linker, codon optimized for mammalian expression, under the control of the strong cytomegalovirus (CMV) immediate early promoter.

5.6.2 Generation of ChAdOx1 Plague adenovirus vector

The vaccine construct was generated at the Oxford Vaccine Group, University of Oxford. Manufacture of the vaccine was carried out in accordance with the requirements of GMP by:

Clinical BioManufacturing Facility (CBF)

Old Road, Headington

Oxford OX3 7JT, UK

MIA (IMP) Number: MIA(IMP) 21584

ChAdOx1 Plague encodes the *Yersinia pestis* antigens F1 and LcrV, fused by a flexible linker. A genomic clone of ChAdOx1 Plague was prepared by Gateway® recombination between an entry plasmid containing the native coding sequence for F1, a flexible linker and LcrV under the transcriptional control of an Intron-A containing human cytomegalovirus immediate-early promoter, and the E1-and E3-deleted ChAdOx1 destination vector.

The manufactured vaccine is currently being stored at the CBF. Full technical details of the assembly process can be found in the Investigator's Brochure (IB).

5.6.3 ChAdOx1 vector

The ChAdOx1 vector is replication-deficient as the E1 gene region, essential for viral replication, has been deleted. The virus will not replicate in cells within the human body. In addition**,** the E3 locus, which promotes viral particle release and inhibits the host's antiviral response, is also deleted. ChAdOx1 propagates only in cells expressing E1, such as HEK293 cells and their derivatives or similar cell lines such as Per.C6 (Crucell).

Vaccines constructed using the same method (adenovirus vector) have been generated for SARS-CoV-2, Ebola, malaria, HIV, TB, cancer antigens, HCV, chikungunya, Zika, HPV and meningococcus. They have been, or are currently being, tested in clinical trials (EudraCT 2017-000965-61, 2011-003589-34, 2011-005477-24, 2014-004714-28, 2010-018341-56, 2009-012591-27, 2017-001992-22, 2019-000075-16, 2017-004483-35 add COV are a few examples), including large field studies (EudraCT 2019-000691-42), with excellent safety profiles, including in babies (ClinicalTrials.gov NCT01635647. Pactr.org PACTR201208000404131.).

5.6.4 Rationale for ChAdOx1 Plague as a trial intervention

The ChAdOx1 vector has been used in over 14,000 participants from phase 1 to 3 clinical trials in Oxford, UK, and other countries to date. The experience with these adenovirus vaccine vectors demonstrates that they are well tolerated with minimal side effects, even in comparison to licensed vaccine such as 4CMenB (Bexsero®) or other viral vectored vaccines such as Modified Vaccinia Ankara (MVA). The adenoviral vector technology has shown to induce potent cellular immunity (required for protection against pneumonic plague) in addition to excellent antibody responses, combining the advantage of live attenuated vaccines with the safety of sub-unit vaccine (no risk of reversion). A single shot regimen induces remarkably rapid and persistent responses as seen with a simian viral vector expressing Ebola antigens administered to

humans (13). Furthermore, a single dose of a viral vectored meningococcal vaccine developed by OVG induces a stronger and longer lasting protection as compared to the licensed vaccines based on sub-unit proteins and alum-based adjuvant in mice. The single dose regimen is a realistic path for use in low-income countries (shown by their development against Ebola, Malaria and TB). Moreover, vaccine development of the ChAdOx1 nCoV-19 vaccine recently showed that a higher antibody response was obtained when using a 2 dose schedule..

ChAdOx1 Plague incorporates known immunogenic antigens, which have been shown to provide protection in animal models when delivered as sub-unit proteins. The results from pre-clinical studies (see below) suggest that an adenoviral-based Plague vaccine induces plague-specific immune responses, able to induce 100% protection after only one dose; an unprecedented achievement in the field of sub-unit vaccines against plague. Therefore, this new vaccine candidate has potentially more protective immune response from fewer doses than the protein in adjuvant –based candidates currently in development. We are therefore testing it in a first-in-man Phase I clinical setting, primarily for safety, but also to obtain descriptive immunogenicity data.

The target dose chosen (5×10^{10} viral particles (VP) is based on the large experience of this vector in human as described above (Table 1).

The rationale for the differing boost groups relates to recent evidence from COVID-19 vaccine trials using the ChAdOx1 platform. There was evidence of both 1) improved immunogenicity with a second dose (compared to single dose), and 2) improved immunogenicity and clinical efficacy with a delayed boost from at least twelve weeks post-prime.[14,15] There may also be a logistical justification in preferring a longer six month interval when considering the deployment of a vaccine during a plague outbreak or epidemic. A delayed boost at six months may be operationally preferable in terms of mobilising health teams; in the most recent plague outbreak in Madagascar in 2017 the peak of cases was seen around eight weeks.[3]

This Phase I study will provide valuable data on safety and immunogenicity against *Yersinia pestis* antigens following vaccination with ChAdOx1 Plague. The data from this first-in-human trial will be used to support further trials in the target population in endemic settings.

5.7 Findings from non-clinical studies with the ChAdOx1 Plague vaccine

Two types of non-clinical studies were performed with ChAdOx1 Plague: Immunogenicity and biological activity in mouse models, and a GLP toxicology study.

5.7.1 Preclinical studies

In order to design an optimal transgene to incorporate in the adenoviral vaccine platform, several constructs were screened in mice using a serotype 5 adenovirus (AdHu5) platform, because of its ease of production, while ChAdOx1 is relevant for human use. Various versions of the F1 and LcrV fusion were incorporated into the AdHu5 serotype vector and immunogenicity studies were conducted in mice. Groups of mice were immunized once with a vaccine candidate, by the intramuscular route, and the immune response assessed by serum ELISA against recombinant F1 and V, to measure the production of antigen-specific antibody responses, and by T-cell ELISPOT assay, using peptide pools covering the full F1 and V antigens, to enumerate the number of antigen-specific interferon gamma (IFN-γ) producing T-cell responses. Full details are available in the current version of the ChAdOx1 Plague Investigator's Brochure (IB). All vaccine candidates were immunogenic, as exemplified below with one of the vaccine candidates, named AdHu5 Plague (expressing the exact same transgene as incorporated in ChAdOx1 Plague, F1, flexible linker and LcrV), inducing antibody responses after a single intramuscular dose in mice (Figure 1).

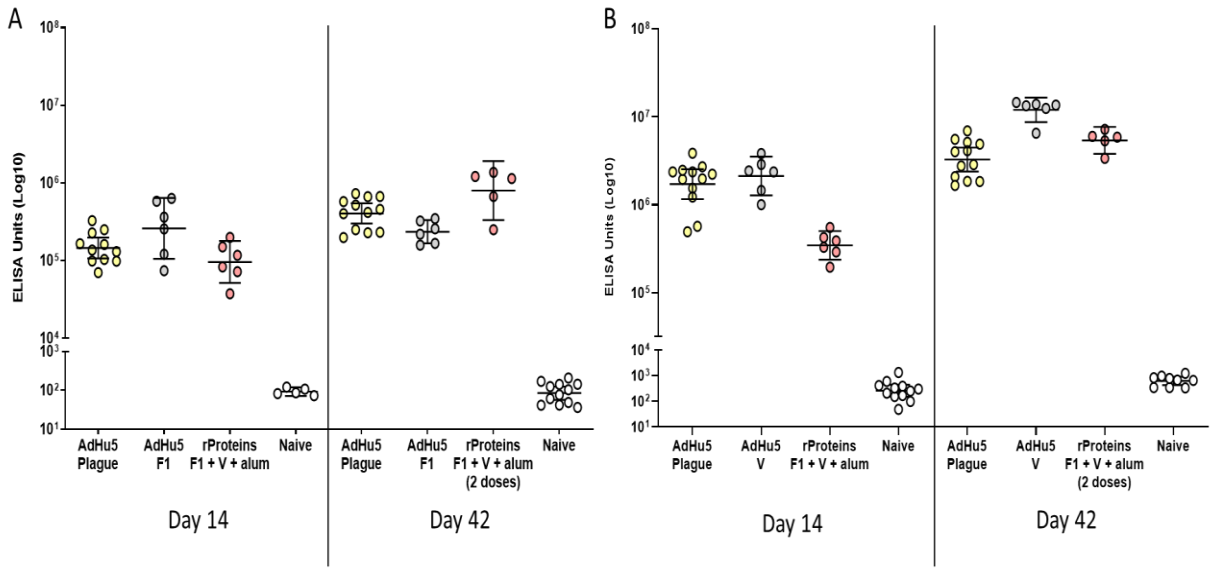

Figure 1. Immunogenicity of AdHu5 viral vectored vaccines, expressing F1 and V antigens in NIH Swiss mice at day 14 and 42 post immunisation. Groups of mice were administered a single dose of AdHu5 Plague, expressing both F1 and V antigens, or a single dose of AdHu5 expressing either F1 or V. The positive control group was immunised with two doses of recombinant F1 and V proteins in alum. The IgG response specific for F1 (A) and V (B) for each mouse is represented by a dot and the geometric mean with 95% confidence intervals of the group are displayed**.** The results show that a single dose of AdHu5 Plague induces a strong antibody response to both antigens.

Furthermore, a single dose of AdHu5 Plague outperformed two doses of twice the human dose of a F1 and LcrV protein-based vaccine adjuvanted in Alum (the most advanced plague vaccine in clinical trials) in a challenge study: a single dose protected 100% of animals from any signs of illness following aerosol challenge with *Y. pestis* 6 weeks post vaccination (Figure 2).

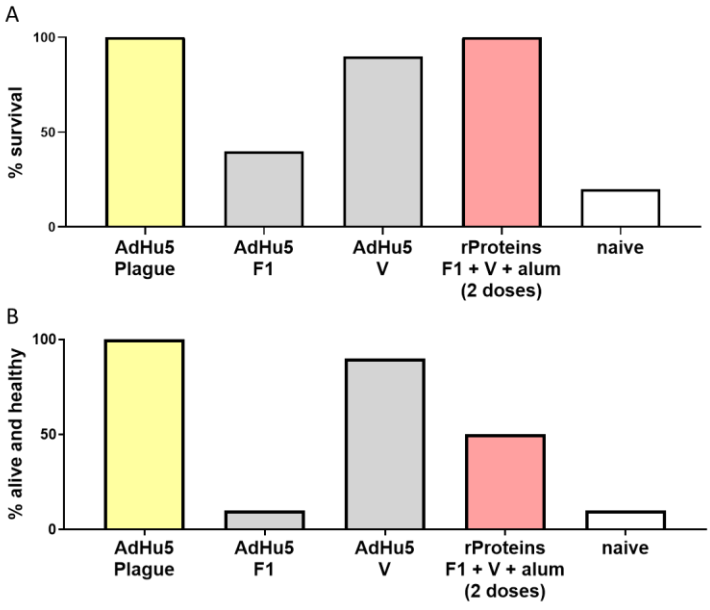

Figure 2. Outcome of aerosol challenge with *Yersinia pestis,* measured by survival (A) and protection against illness (B) in NIH Swiss mice immunised with AdHu5 vaccines or recombinant proteins. **While both AdHu5 Plague and the protein vaccine comparator protected all mice against death (A), Only mice immunised with a single dose of AdHu5 Plague, expressing both F1 and V, fully protected all mice against illness**. **(B). In comparison, 90% of the mice immunized with 2 doses of the protein-based vaccines demonstrated signs of illness.**

Therefore, this transgene was selected for clinical development, and incorporated in the clinically relevant ChAdOx1 backbone, resulting in ChAdOx1 Plague. The biological activity of ChAdOx1 Plague was assessed in mice, using non GMP grade material:

ChAdOx1 Plague is immunogenic in two different strains of mice, including in an outbred strain, after a single intramuscular injection (Figure 3).

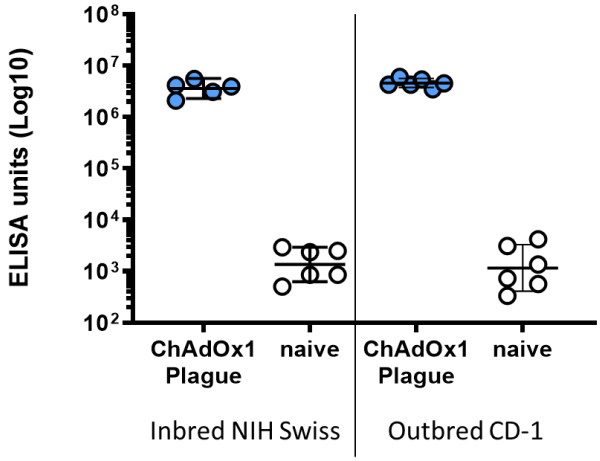

Figure 3. Immunogenicity of ChAdOx1 Plague, expressing F1 and V antigens in inbred NIH Swiss and outbred CD-1 mice at day 28 post immunisation. Groups of mice were administered a single dose of ChAdOx1 Plague and the IgG response specific for the V antigen for each mouse is represented by a dot and the geometric mean with 95% confidence intervals of the group are displayed.

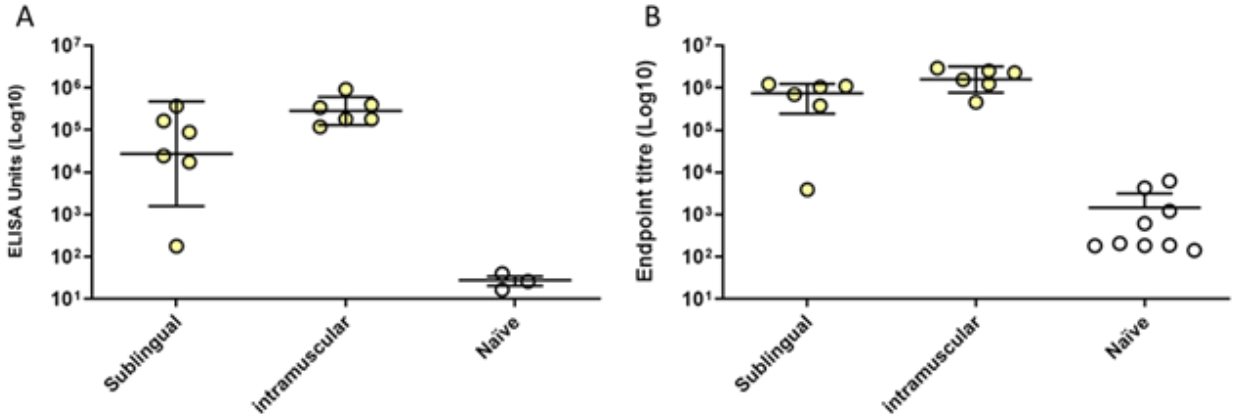

Figure 4. Immunogenicity of AdHu5 Plague in NIH Swiss mice at day 42 post immunisation. Groups of mice were administered a single dose of AdHu5 Plague, expressing both F1 and V antigens, via the sublingual or intramuscular route. The IgG systemic response in sera specific for F1 (A) and V (B) for each

mouse is represented by a dot and the geometric mean with 95% confidence intervals of the group are displayed.

A dose response study was performed in two mouse strains (including an outbred strain (Figure 5).

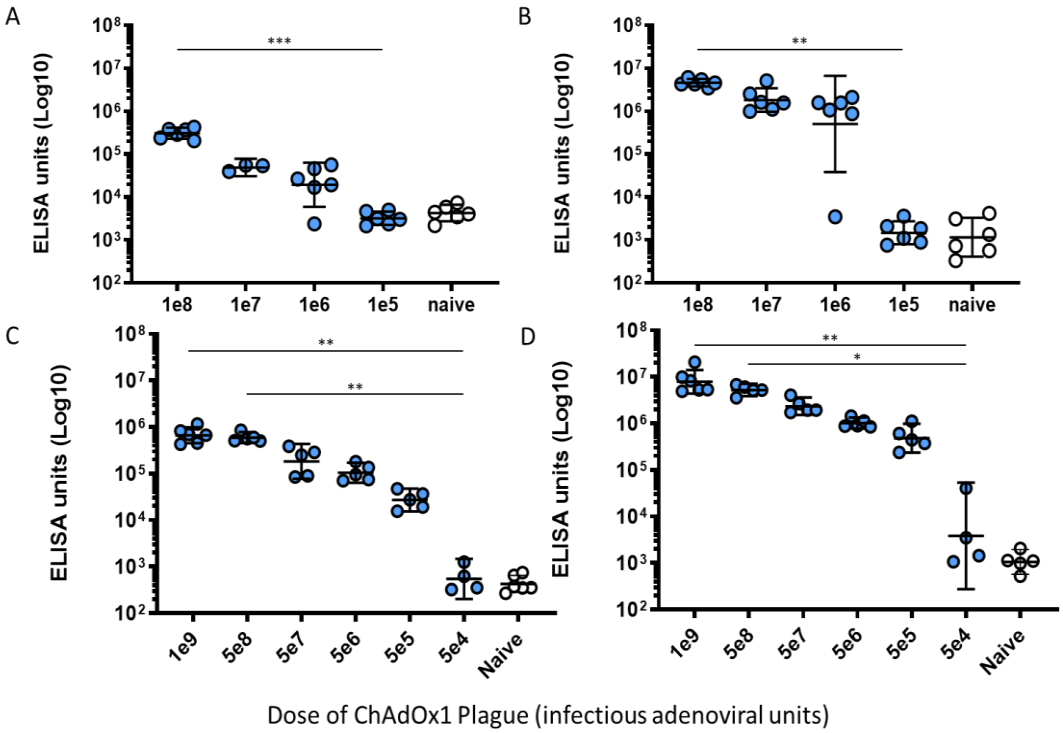

Figure 5. Immunogenicity of varying doses of ChAdOx1 Plague, expressing F1 and V antigens in outbred CD-1 (A-B) and inbred NIH Swiss (C-D) mice at day 28 and 42 post immunisation respectively. Groups of mice were administered a single dose of ChAdOx1 Plague and the IgG response specific for F1 (A, C) and V (B, D) for each mouse is represented by a dot and the geometric mean with 95% confidence intervals of the group are displayed.

A potency assay was also performed on a clinical vial from the GMP production used for this trial, and demonstrated that the clinical grade vaccine induced the expected immunogenicity (Figure 6)

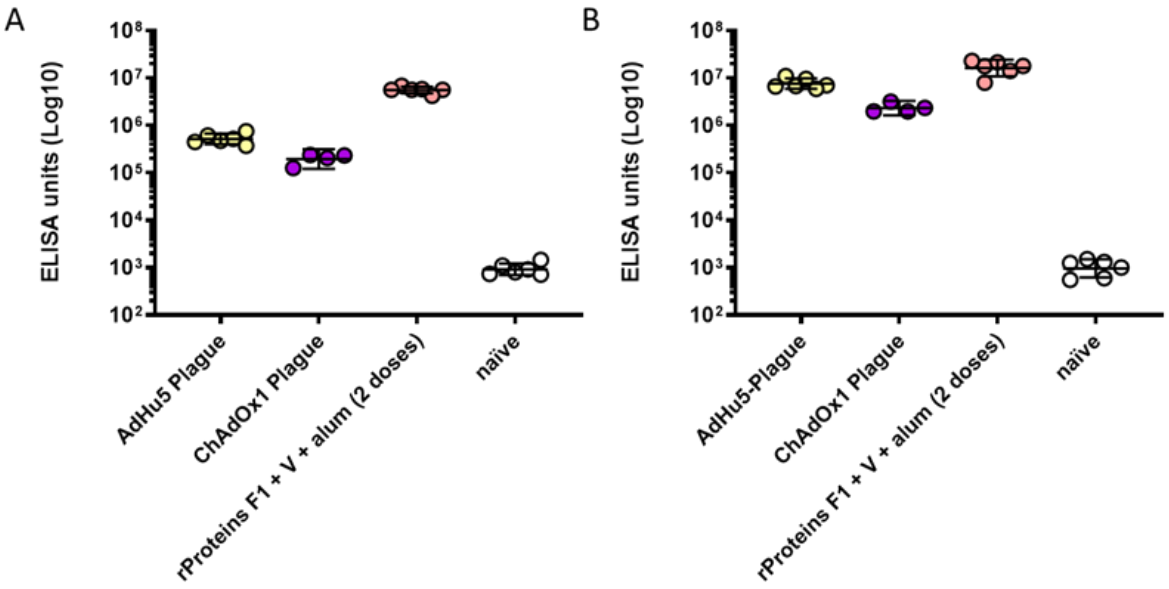

Figure 6. Potency assay of a clinical vial of ChAdOx1 Plague. Groups of mice were administered a single dose of ChAdOx1 Plague clinical vial, or a single dose of the AdHu5 version. The positive control group was immunised with two doses of recombinant F1 and V proteins in alum (human dose). The IgG response specific for F1 (A) and V (B) for each mouse is represented by a dot and the geometric mean with 95% confidence intervals of the group are displayed. The results show that a single dose of ChadOx1 Plague from a clinical vial induces a strong antibody response to both antigens.

5.7.2 Toxicity study of ChAdOx1 Plague administered in mice (Covance)

A toxicology study was performed by injecting mice with 35µl of either phosphate-buffered solution or ChAdOx1 Plague and comparing the two groups.

The aim of this study is to investigate the potential toxicity of ChAdOx1 Plague when administered to Balb/c mice on two occasions with a 14 day interval followed by a 13 day observation period, as described below:

The animal model was mouse (BALB/c strain), accepted by regulatory agencies, background data available and relevant strain for the vaccine construct, and the route intramuscular, to simulate the conditions of clinical administration. The rationale for choosing BALB/c mice is based in the best science available: Mouse is a relevant species to identify and characterize the potential toxic effect of a vaccine based on a non-replicating adenovirus vector. To date, there have been 10 experimental recombinant ChAdOx1 vaccines tested in GLP pre-clinical toxicity studies at ENVIGO, now Covance in this species (Table 2) and all these studies uniformly concluded that the intramuscular delivery of the ChAdOx1-vectored vaccines was well tolerated and was not associated with any adverse effects other than those expected from IM injection and induction of immune responses. For most of these vaccines, there are safety data already available from humans demonstrating no safety concerns so far. In addition, safety data in humans are accumulating from thousands of participants that received either one or two doses of ChAdOx1 nCoV19.

Table 2. *Toxicity studies on ChAdOx1-vectored experimental vaccines and clinical studies.*

Envigo Study Number	Toxicology Study Title	Trial Registration	rChAdOx1 Dose (vp) in clinical trial
GG05TY	ChAdOx1 LS2 and MVA LS2: Toxicity Study by Intramuscular and Intravenous Administration to Mice	NCT03203421	5×10^9 2.5×10^{10}

QS18DL	ChAdOx1 CHIK Vaccine or ChAdOx1 MERS: Toxicity Study by Intramuscular Administration to Mice	NCT03399578	5×10^9 2.5×10^{10} 5×10^{10}
WC71GC	ChAdOx1 ZIKA and ChAdOx1 CHIK: Toxicity Study by Intramuscular Administration to BALB/cJ Mice	NCT03590392 2019-000075-16	5×10^9 2.5×10^{10} 5×10^{10} 1^10^{11}
FP09PP	ChAdOx1 RVF: Toxicity Study by Intramuscular Administration to BALB/cJ Mice	2017-004482-27	5×10^9 2.5×10^{10} 5×10^{10}
TX05CW	ChAdOx1.HTI: Toxicity Study by Intramuscular Administration to BALB/cJ Mice	NCT03204617	5×10^{10}
KH75CL	ChAdOx1 MenB.1: Toxicity Study by Intramuscular Administration to Mice	ISRCTN46336916	2.5×10^{10} 5×10^{10}
XMM0003	ChAd OX1 NP+M1 and MVA NP+M1: Toxicity Study by Intramuscular Administration to Mice	NCT01623518 NCT01818362	5×10^8 5×10^9 2.5×10^{10} 5×10^{10}
XMM0012	ChAdOx1 5T4: Toxicity Study by Intramuscular Administration to Mice	NCT02390063 NCT03815942	2.5×10^{10}
XMM0005	ChAdOx1 85A: Toxicity Study by Intramuscular Administration to Mice	NCT01829490 NCT03681860	5×10^9 2.5×10^{10}

Table 2. Treatment Groups and Doses

Group	**1**	**2**
Compound	PBS	ChAdOx1 Plague
Dose (per occasion)	0	1.25×10^{10} vp*

** Due to a dosing accuracy of 5 µL, the animals will receive $1.32x10^{10}$ vp per occasion (0.035 ml instead of 0.033ml)*

Administration of 35µL ChAdOx1 Plague at a titre of 3.81×10^{11} VP/mL will result in animals receiving a concentration of 1.32×10^{10} vp on each occasion. This will result in a safety margin of 792 fold when using 5×10^{10}vp dose of ChAdOx1 Plague once in the clinical trial.This safety margin is achieved using the following calculation:

$$\frac{\text{dose for mouse/average mouse wt (20 g) x 1000}}{\text{clinical dose/average human wt (60 kg)}} = \text{x fold (safety margin)}$$

Therefore, the volume calculation is:
- Vaccine titre is 3.81×10^{11} VP/mL
- Mouse dose is 1.25×10^{10} VP (0.033 ml)

Therefore mice received 0.035 ml (35µL) of 3.81×10^{11} Vp/mL, at two injections two weeks apart.

There was no unscheduled death on the study. There were no clinical signs considered related to treatment and there was no apparent reaction to treatment at the dose site. There was no difference in

clinical features between the two groups (no difference in body weight, food consumption). Haematology investigations performed on Day 28 did not reveal any clear treatment-related findings.

A post-mortem examination revealed a higher spleen weight for treated females, and enlarged lumbar and axillary lymph nodes, compatible with the administration of a vaccine.

In conclusion, treatment with the vaccine ChAdOx1 Plague was well tolerated and was not associated with any adverse effects.

Full details of these studies can be found in the IB

5.8 Previous clinical experience

There is no clinical experience with ChAdOx1 Plague, but there is ample clinical experience with ChAdOx1 vaccines expressing other antigens, as described in Table 1, and with the antigens formulated as recombinant proteins.

The proposed study is the first-in-human clinical trial of ChAdOx1 Plague. The gene product of the antigenic insert, F1 and LcrV, is present in previously trialled vaccines, and thus has been administered successfully to individuals (NCT00332956, NCT00246467, NCT00097396, NCT01381744, NCT02596308, NCT01122784).

ChAdOx1 vectored vaccines expressing various different antigenic inserts have been administered to over 5000 healthy participants in clinical trials conducted by the University of Oxford. Four clinical trials administered ChAdOx1-based vaccine, containing antigens as varied as the influenza virus, MERS, chikungunya virus and capsular group B meningococcus, at the dose proposed in the current study (5 x 10^{10} VP), and there were no serious adverse events (SAEs) associated with the vaccines reported to date, following one or two injections of the vaccine. The number of participants previously receiving ChAdOx1 at 5 x 10^{10} viral particles (48 over the 4 trials) are outlined in Table 1, and thus there is sufficient evidence that this dose of ChAdOx1-based vaccine would be well tolerated in humans.

In addition, the on-going COV001 and COV002 trials, using the proposed ChAdOx1 nCOV-19 vaccine, has had a good safety profile with thousands of participants so far, highlighting the safety of the prime-boost schedule, and the lower reactogenicity upon second injection (14).

Moreover, previous studies using 1 x 10^{10} VP in mice have been used with chimpanzee adenoviruses vaccines (ChAd63 ME-TRAP, ChAdOx1 MP+N1, ChAdOx1 Chick) with no overt toxicity apparent, and used at doses including 5 x 10^{10} VP in humans (and up to 2 x 10^{11} VP for ChAd63 ME-TRAP).

The dose to be used in this study is not expected to cause undue toxicity. The clinical trial will be using a dose of 5 x 10^{10} VP, as the safety profile was acceptable at this dose with ChAdOx1 vaccines containing other inserts (ChAdOx1 NP+M1, MERS, Chick, MenB.1). Moreover, the inserts included in this vaccine have been used as proteins in adjuvant vaccines in humans with acceptable safety profile.

6. OBJECTIVES AND OUTCOME MEASURES

Objectives	Outcome Measures	Timepoint(s) of evaluation of this outcome measure (if applicable)
Primary To investigate the safety and tolerability of 5 x 10^{10} VP of	Local and systemic adverse events for 28 days following administration of each	eDiary records from Day 0 (day of vaccination) to Day 7 for

the proposed ChAdOx1 Plague vaccine in, , healthy adults aged 18 to 55 years when given one or two dose(s) intramuscularly	vaccine dose as recorded in participant eDiaries	solicited symptoms (plus later if any persisting symptoms). Unsolicited adverse events up until and including 28 days following vaccination. Serious adverse events (SAEs) during the entire study. Safety blood tests at Day D0, and D7 following each vaccination
Secondary To determine the immunogenicity of 5 x 10^{10} VP of the proposed ChAdOx1 Plague vaccine, in healthy adults aged 18 to 55 years when given one or two dose(s) intramuscularly with different prime-boost intervals To determine the safety of 5 x 10^{10} VP of the proposed ChAdOx1 Plague vaccine, in healthy adults aged 18 to 55 years when given one or two dose(s) intramuscularly with different prime-boost intervals	ELISA to measure antibody responses to the vaccine antigens 1 month post vaccination The recording and assessment of local and systemic adverse events for 28 days following administration of each vaccine dose via eDiaries Serious adverse events (SAEs) Safety blood tests	1-month post vaccination eDiary records from Day 0 (day of vaccination) to Day 7 for solicited symptoms (plus later if any persisting symptoms). Unsolicited adverse events up until and including 28 days following vaccination. Throughout the entire study. At Day D0, and D7 following each vaccination
Tertiary To utilize exploratory immunogenicity assays to determine the immunogenicity of 5 x 10^{10} VP of the proposed ChAdOx1 Plague vaccine in healthy adults aged 18 to 55 years when given one or two dose(s) intramuscularly with different prime-boost intervals	Immunological assays to study the immune responses to vaccines potentially including: 1. Antibody concentration against vaccine antigens before and after vaccination 2. Quantification of circulating vaccine-induced B-cell responses specific for vaccine antigens before and after vaccination. 3. Quantification of vaccine-induced, antigen specific T-cell responses and associated cytokine production before and after vaccination. 4. Antibody functional capacity in in vitro assays (serum bactericidal, phagocytosis, invasion) 5. Antibody functional capacity in *in vivo* challenge assay (by serum transfer)	Blood and mucosal samples from study visits at D0, D1, D7, D14, D28, D56, D57, D63, D70, D84, D182, D183, D189, D196, D210 and one visit between D250 and D365

	6. Gene expression profile after immunisation and DNA storage for investigation of the genetic associations with the immune response. 7. Mucosal and Innate immune activation	

7. TRIAL DESIGN

7.1. Overview of Trial Design

The study is a phase I, single centre study to assess the safety and immunogenicity of one or two doses at different prime-boost intervals of the recombinant adenovirus Plague vaccine candidate ChAdOx1 Plague in healthy adults. The participants will be divided into 3 subgroups as described below. The total number of participants required to reach the primary endpoint will be 10.

1. **Group 1 – Single dose arm** – This group will consist of 10 to 15 individuals who will receive a single dose of 5 x 10^{10} VP of ChAdOx1 Plague via the intramuscular route as outlined in section 7.2.
2. **Group 2 – Prime-Boost (two-month boost) arm** – This group will consist of up to 15 participants assigned to receive two doses of 5 x 10^{10} VP of ChAdOx1 Plague IM, two-months apart
3. **Group 3 – Prime-Boost (six-month boost) arm** - This group will consist of up to 15 participants assigned to receive two doses of 5 x 10^{10} VP of ChAdOx1 Plague IM six months apart.

A schematic of the vaccination arms and when they receive the vaccine is seen in Figure 7. Aside from vaccination, these participants are followed up according to the schedules detailed in Table 3, Table 4 and Table 5.

Figure 7. Schematic of trial design.

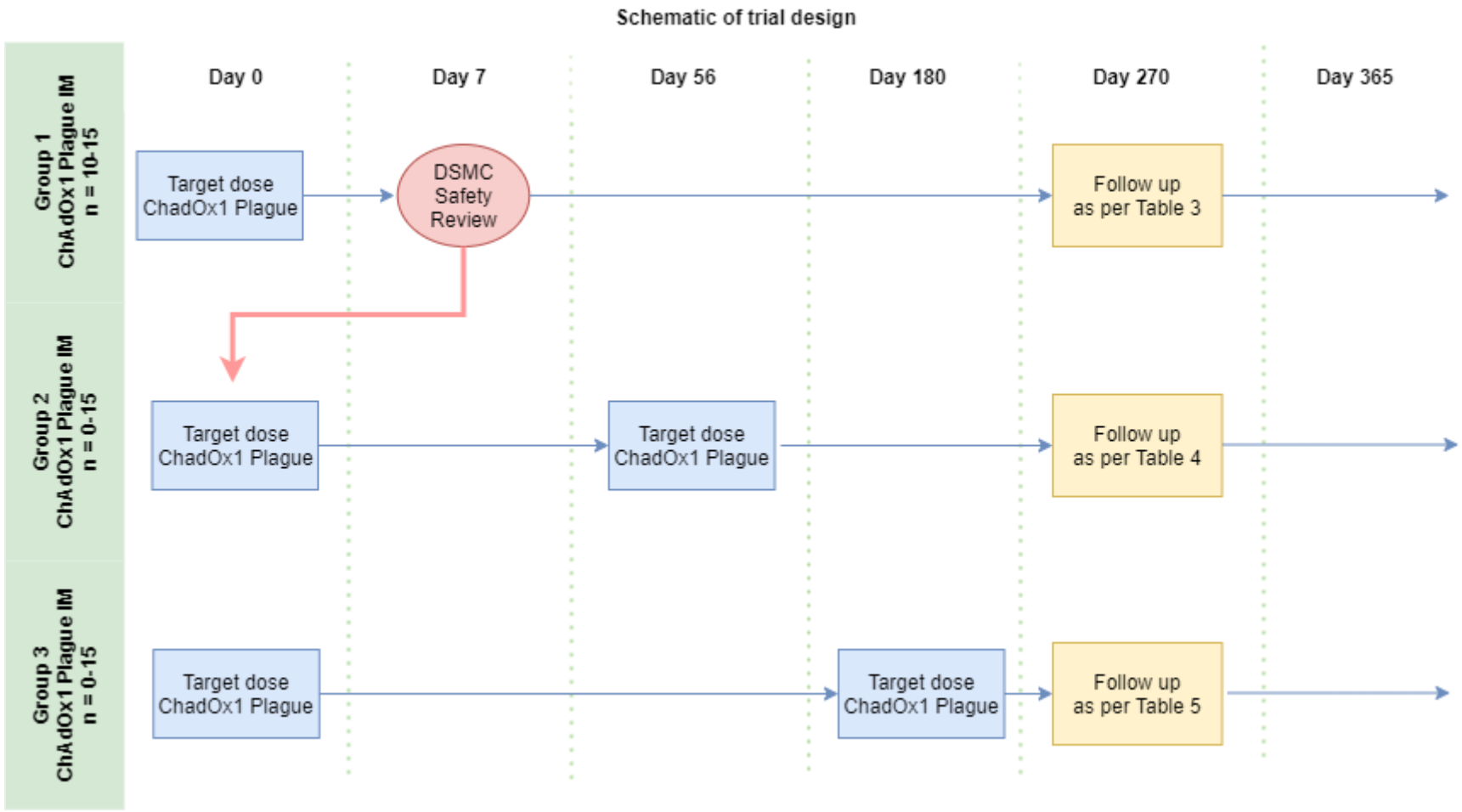

Table 3. Scheduled visits per participant and procedures performed at each visit (Group 1)

Visit Name	Screening (V0)	V1	V2	V3	V4	V5	V6	V7	V8	V9	V10	V11	V12	V13	V14	V15	V16
Indicative Study Day		**1**	**2**	**7**	**14**	**28**	**56**	**57**	**63**	**70**	**84**	**182**	**183**	**189**	**196**	**210**	**250 - 365**
Day post last vaccine		0	1	7	14	28	56	(57)	(63)	(70)	84	182	183	189	196	210	250 - 365
Visit Window (days)		N/A		+/- 1	+/- 2	+/- 4	+/- 4				+/- 4	+/- 14				+/-14	0
Informed consent	x																
Biobank consent		x															
Confirmation of consent		x	x	x	X	x	x				x	x				x	x
Obtain 24 hr contact details		x															
Medical history (including demographics and medication)	x																
Interim medical history (including concurrent medication, AEs)		x	x	x	X	x	x				x	x				x	x
Physical examination	x																
Vital signs	x	x	x	x	X	x	x				x	x				x	x
ECG	X																
Urine pregnancy test	x	x															
Urine sample	x																
Blood sample	x	x		x	X	x	x				x	x				x	x
Mucosal sample		x		x	X	x	x				x	x				x	x
Vaccination		x															
eDiary entries		x	x	x													
Intervention arm allocation		x															

Table 4. Scheduled visits per participant and procedures performed at each visit (Group 2)

Visit Name	Screening (V0)	V1	V2	V3	V4	V5	V6	V7	V8	V9	V10	V11	V12	V13	V14	V15	V16
Indicative Study Day		**1**	**2**	**7**	**14**	**28**	**56**	**57**	**63**	**70**	**84**	**182**	**183**	**189**	**196**	**210**	**250 - 365**
Day post last vaccine		0	1	7	14	28	56	1	7	14	28	126	127	133	140	154	194 - 309
Visit Window (days)		N/A	0	+/- 1	+ 7	+/- 4	+/- 4	0	+/- 1	+7	+/- 4	+/- 14				+/-14	0
Informed consent	x																
Biobank consent		x															
Confirmation of consent		x	x	x	X	x	x	X	x	x	x	x				x	x
Obtain 24 hr contact details		x															
Medical history (including demographics and medication)	x																
Interim medical history (including concurrent medication, AEs)		x	x	x	X	x	x	X	x	x	x	x				x	x
Physical examination	x																
Vital signs	x	x	x	x	X	x	x	X	x	x	x	x				x	x
ECG	x																
Urine pregnancy test	x	x					x										
Urine sample	x																
Blood sample	x	x	x	x	X	x	x	X	x	x	x	x				x	x
Mucosal sample		x	x	x	X	x	x	X	x	x	x	x				x	x
Vaccination		x					x										
eDiary entries		x	x	X			x	X	x								
Intervention arm allocation		x															

Table 5. Scheduled visits per participant and procedures performed at each visit (Group 3)

Visit Name	Screening (V0)	V1	V2	V3	V4	V5	V6	V7	V8	V9	V10	V11	V12	V13	V14	V15	V16
Indicative Study Day		**1**	**2**	**7**	**14**	**28**	**56**	**57**	**63**	**70**	**84**	**182**	**183**	**189**	**196**	**210**	**250 - 365**
Day post last vaccine		0	1	7	14	28	56	57	63	70	84	0	1	7	14	28	194 - 309
Visit Window (days)		N/A	0	+/- 1	+ 7	+/- 4	+/- 4				+/- 4	+/- 14	0	+/-1	+7	+/-4	0
Informed consent	x																
Biobank consent		x															
Confirmation of consent		x	x	x	X	x	x				x	x	x	x	x	x	x
Obtain 24 hr contact details		x															
Medical history (including demographics and medication)	x																
Interim medical history (including concurrent medication, AEs)		x	x	x	X	x	x				x	x	x	x	x	x	x
Physical examination	x																
Vital signs	x	x	x	x	X	x	x				x	x	x	x	x	x	x
ECG	x																
Urine pregnancy test	x	x										x					
Urine sample	x																
Blood sample	x	x	x	x	X	x	x				x	x	x	x	x	x	x
Mucosal sample		x	x	x	X	x	x				x	x	x	x	x	x	x
Vaccination		x										x					
eDiary entries		x	x	X								x	X	x			
Intervention arm allocation		x															

7.2. Dosing

We will use the target dose of 5 x 10^{10} VP IM of the ChAdOx1 Plague vaccine for all 3 Groups.

1. Single dose 5 x 10^{10} VP IM ('target dose' – group 1)
2. Two doses 5 x 10^{10} VP IM - second dose at D56 ('target dose with two month boost' – group 2)
3. Two doses of 5 x 10^{10} VP IM – second dose at D182 ('target dose with six month boost' – group 3)

The rationale for this dose is based on experience from previous ChAdOx1 phase 1 trials (see section 5.8 for more details: This dose has been used and was well tolerated for several ChAdOx1-based vaccines containing different antigens). A de-escalation process, as detailed below, will be implemented if recommended by DSMC after the triggering of a holding rule as per Section 11.10 .A reduced dose of 2.5 x 10^{10} VP will be used instead, and all subsequent dosing plans reviewed.

CBF have produced a Quality Control (QC) data report using the Replication Competent Adenovirus (RCA) assay for ChAdOx1 Plague, as part of a safety assessment. This is a cell-based assay in which an amount of product, equivalent to at least the top dose, is added to detector cells. The results from the testing of ChAdOx1 Plague at the 5 x 10^{10} VP dose are available and have been approved by the Qualified Person (QP).

7.2.1 Dosing process

Participants will be enrolled and vaccinated as follows:

Group 1

For safety reasons, the first volunteer to receive 5 x 10^{10} VP of ChAdOx1 Plague will be vaccinated ahead of any other participants and the profile of adverse events will be reviewed after 48 hours post vaccination. Provided there are no safety concerns, as assessed by a medically qualified investigator and/or chair of DSMC, another 2 volunteers will be vaccinated with the IMP after at least 48 hours (+24h) has elapsed following first vaccination and at least 1 hour apart from each other (bringing the total number of participants receiving that dose to 3). The profile of AEs will be assessed by a medically qualified investigator in real time and after 72 hours of the first 3 participants receiving the IMP, further vaccinations will proceed provided there are no safety concerns. Relevant investigators and chair of DSMC will be asked to provide a decision on whether further vaccinations can go ahead after the first 3 participants received the IMP. A full DSMC may also be consulted should safety concerns arise at this point. If no concerns are raised, then the rest of the group will be enrolled up to 15 participants

A DSMC review will be triggered once at least 7 days of data are available for all enrolled Group 1 participants. This review will include an assessment of the profile of adverse events from D0 – D6 and the results of the safety blood tests from D0 (V1), and D7(V3).

Group 2

Recruitment to Group 2 will commence following a successful safety report from the enrolled participants of Group 1. The Group 2 participants will be vaccinated with ChAdOx1 Plague at the target dose (5 x 10^{10} VP) unless the dose de-escalation process was implemented, in which case they will receive the low-dose of ChAdOx1 Plague (2.5 x 10^{10} VP). We have successfully administered 2 doses of ChAdOx1 vaccine in a previous trial (ChAdOx1 nCoV-19 (14)) with no safety concerns; this is the justification for not doing staggered recruitment of the first 3 volunteers in Group 2. Group 2 participants will be boosted with the same dose of ChAdOx1 Plague at D56 as received at D0.

Group 3

Recruitment to Group 3 will commence alongside Group 2. If there have been no significant safety concerns from the target dose of ChAdOx1 Plague, then Group 3 participants be vaccinated with ChAdOx1 Plague at the target dose (5 x 10^{10} VP) at D0. Group 3 participants will then be boosted at D182.

If dose de-escalation from 5 x 10^{10} VP to 2.5 x 10^{10} VP was required in Group 1, then Groups 2 and 3 will receive the low-dose of ChAdOx1 Plague (2.5 x 10^{10} VP). Group 3 participants will be boosted with the same dose of ChAdOx1 Plague at D56 that they received at D0.

7.2.2 DSMC reviews

After all enrolled participants in Group 1 have accumulated at least 7 days of data, the DSMC will review the safety data. If a favourable opinion is obtained following review, then vaccination of Group 2 and Group 3 participants can commence.

For further details of what these safety rules entail and the stopping rules therein, please see section 11.9.

7.3. Group Allocation

This is an open-label, non-randomized trial. Allocation to each arm will be decided in order of recruitment. The numbers allocated to each group will be as outlined in section 7.1.

7.4. Safety Monitoring

Throughout the study, the safety outcomes of the participants will be monitored.

Solicited AR's (see section 11.811.3) occurring within the first 7 days after vaccination, will be recorded in a diary (either electronic or paper) or during visits in the CRF.

Unsolicited AE's are adverse events other than the foreseeable AR's and will be recorded in a diary or during visits in the CRF, from visit 1 to visit 16 in Groups 1, 2 and 3.

Safety blood tests are performed at specific visits (see

Table 3, Table 4 and Table 5) and will be monitored by the clinical team. Laboratory AE's (see APPENDIX A: GRADING THE SEVERITY OF ADVERSE EVENTS) that are assessed as clinically significant will be recorded in the CRF.

The clinical team may call or email the participants as part of the follow up or if any concerns arise. If there is clinical information to be shared with the participant or the GP through email, then these will be sent through the OVG NHSmail account, which creates encrypted emails.

Full details about the reporting of any adverse events or serious adverse events can be found in section 11, and the role of the DSMC beyond the dose de-escalation reviews is discussed in section 11.9.

7.5. Potential risks to the volunteers

The main potential risks are those associated with phlebotomy and vaccination

Venepuncture

Localised bruising and discomfort can occur at the site of venepuncture. Infrequently fainting may occur.

The total volume of blood drawn over a twelve-month period will be 505-645ml. This should not compromise these otherwise healthy volunteers, however there is a low risk of mild anaemia. In the UK, volunteers are permitted to donate 470mL during a single blood donation for the National Blood

transfusion Service over a 3-4 month period. Volunteers will be asked to refrain from blood donation for the duration of their involvement in the trial.

Allergic reactions

Allergic reactions from mild to severe may occur in response to any constituent of a medicinal product's preparation. Anaphylaxis is extremely rare (about 1 in 1,000,000 vaccine doses) but can occur in response to any vaccine or medication.

Vaccination

Local reactions from IM vaccination

The typical local reaction as a result of IM injection is temporary pain, tenderness, redness, and swelling at the site of the injection.

Systemic reactions

Constitutional influenza-like symptoms such as feverishness can occur with any vaccination and last for 2-3 days. Pre-syncopal and syncopal episodes may occur at the time of vaccination which rapidly resolve. As with any other vaccine, temporary ascending paralysis (Guillain-Barré syndrome, GBS) or immune mediated reactions that can lead to organ damage may occur, but this should be extremely rare (1 in 100,000-1,000,000 vaccine doses). Neurological symptoms such as limb weakness and changed sensations have also been reported in relation to vaccination.

Mucosal sampling

Validated synthetic absorptive matrix (SAM) strips are used to sample the mucosal lining fluid from the anterior nasal cavity. Due to the sensitivity of the nasal cavity, insertion of the SAM strip may cause involuntary head movement e.g sneezing. If there are abnormalities in the nasal passage, such as polyps or epistaxis, then insertion of the strips may cause damage and minor bleeding.

7.6. Potential benefits to the volunteers

Recipients of ChAdOx1 Plague do not have any guaranteed benefit. However, it is hoped that the information gained from this study will contribute to the development of a safe and effective vaccine against Plague disease. The only benefits for participants would be information about their general health status.

7.7. Risk/benefit assessment in context of ongoing SARS-CoV-2 pandemic

7.7.1 Risks to participants in relation to COVID-19

To reduce the risk of participants acquiring SARS-CoV-2 in the process of attending study visits, strict infection control precautions will be followed. This will include (as per Section 9.8):

- Strict infection control procedures to limit the risk of staff acquiring and transmitting infection in the workplace (detailed in Section 9.8)
- Participants will be reminded, via text and email, not to come to the centre if they are experiencing any symptoms of COVID-19, have tested positive for SARS-CoV-2, or are self-isolating/in quarantine.

- Participants will be asked to come at staggered times to avoid unnecessary mixing
- Social distancing is enforced in waiting areas
- Participants are required to wear face-coverings on entering the building
- Hand hygiene is encouraged through the placement of alcohol gel stations in key contact areas
- All clinic visits will be conducted by staff in appropriate PPE, as per national guidance (e.g. mask, gloves, disposable gown), who have been trained in specific infection control measures.
- The clinic room surfaces will be disinfected with anti-viral products after every participant.

There is no known or proposed biological mechanism by which receipt of the ChadOx1 Plague vaccine would increase the risk of a participant acquiring a SARS-CoV-2 infection; or developing more severe COVID-19 in the case of subsequent infection, as this is a replication-deficient vaccine platform with a known safety profile from previous trials including Phase III, against an unrelated pathogen.

For participants who are yet to receive an approved or licensed COVID-19 vaccine but become eligible for this as per UK policy, we would discuss this with the participant. Participants would not be impeded in taking up an offer of COVID-19 deployment vaccine if offered through the national rollout. COVID-19 vaccination would not be offered through the trial. If a rollout offer coincided with a planned trial vaccine, the trial vaccine would be rescheduled to 3 weeks before or after the COVID-19 vaccine (whichever was closest to trial schedules). This is in line with the UK Green Book COVID-19 vaccination recommendations for other vaccines which recommends “at least 7 days” interval. It would also minimise the risk of any cross-attribution of reactogenicity, and minimise impact on immunology investigations.

7.7.2 Risk/benefit of conducting trial in the UK during pandemic

There is a strong international effort to provide an incentive for companies to take on vaccines against outbreak pathogens on the WHO list, with funding from governments and several organizations such as the Welcome Trust and the Bill and Melinda Gates Foundation. There is a clear benefit in stockpiling a safe and efficient plague vaccine and phase I trials are essential to progress this.

- The risk to participants from the ChadOX1 Plague vaccine is not increased by being given during the pandemic (as above)
- The conduct of the trial will not impact on routine or pandemic related NHS activity and will not divert resources away from the NHS
- The risk of epidemic plague disease remains a real one, and is likely increased by the weakening of public health and healthcare infrastructure in the wake of the economic instability caused by the COVID pandemic

The benefit of conducting this Phase I trial in the UK (as opposed to in a country where the target population reside) on the basis that:

- A first-in-man clinical trial is a pre-requisite prior to performing Phase 1b trial in the affected populations in developing countries (e.g. Madagascar, Democratic Republic of Congo)
- Novel vaccines are developed and produced in developed countries, and low- and middle-income countries (LMIC) typically follow industrialised nations in the introduction of new vaccines. LMIC often rely on product registration and regulatory reviews in developed countries to progress clinical trials.
- Western sponsors consider the conduct of FIH trials in LMICs to be unacceptable because of the concern that they may be perceived to be exploiting the population in these countries for their own product development.
- There is a risk of having difficulty in discriminating adverse events caused by the investigational product from the generally much higher frequency of all types of undiagnosed symptoms and untreated morbidities in developing countries.

The sponsor will ensure protocol compliance as detailed in Section 14.

7.7.3 Thrombosis with thrombocytopaenia associated with ChAdOx1 nCoV-19

In April 2021, MHRA and JCVI conducted a review of extremely rare reports of cerebral venous sinus thrombosis (and thrombosis of other major veins) with concurrent thrombocytopenia occurring after vaccination with ChAdOx1 nCoV-19 in the national COVID-19 vaccination programme. The potential mechanism of these events, and whether they are related to the ChAdOx1 viral vector or to the SARS-CoV-2 spike protein, is yet to be determined. All volunteers will be provided with this information via the study information booklet and will be kept updated should further information become available. They will also be specifically counselled on side-effects that could be warning signs of serious clotting events, as per public health advice being given to people receiving ChAdOx1 nCoV-19.

8. PARTICIPANT IDENTIFICATION

8.1. Trial Participants

Male or female participants aged 18-55 years inclusive who are in good health. Volunteers will be considered enrolled immediately following administration of first vaccination.

8.2. Inclusion criteria

Participants must satisfy all of the following criteria to be considered eligible for the study:

- Willing and able to give written informed consent for participation in the study
- Aged between 18 and 55 years inclusive at the time of first visit
- In good health as determined by
    - Medical history (as determined by verbal medical history)
    - Physical examination
    - Clinical judgment of the investigators
- Female participants (of childbearing potential) who are willing to ensure that they or their partner use effective contraception during the vaccination period and for the months after vaccination and have a negative pregnancy test on the day(s) of screening and vaccination
- Able to attend the scheduled visits and to comply with all study procedures, including internet access for the recording of diary cards
- Willing to allow his or her General Practitioner (GP) to be notified of participation
- Willing to allow the investigators to discuss the volunteer's medical history with their General Practitioner and access all medical records, including electronic patient records, when relevant to study procedures
- Agrees to refrain from donating blood for the duration of the trial
- Agrees to be registered on the Trial Over-Volunteering Prevention Service (TOPS) and agree to provide their National Insurance number or passport number (if not a British citizen) for the purposes of registration
- Agrees to provide National Insurance number and Bank details for reimbursement purposes
- Normal baseline/screening laboratory (blood/urine) results

8.3. Exclusion Criteria

The participant may not be enrolled in the study if any of the following apply:

- History of significant organ/system disease that could interfere with trial conduct or completion. This includes any history of significant disease in the following:

  - Cardiovascular disease including congenital heart disease, previous myocardial infarction, valvular heart disease (or history of rheumatic fever), previous bacterial endocarditis, history of cardiac surgery (including pacemaker insertion), personal or family history of cardiomyopathy or sudden adult death
  - Respiratory disease such as uncontrolled asthma and chronic obstructive pulmonary disease
  - Endocrine disorders such as diabetes mellitus and Addison's disease
  - Significant renal or bladder disease
  - Biliary tract disease
  - Gastro-intestinal disease such as inflammatory bowel disease, abdominal surgery within the last two years, coeliac disease and liver disease (including hepatitis B or C infection)
  - Neurological disease such as seizures and myasthenia gravis
  - Haematological problems such as coagulation problems or anaemia (haemoglobin < 125g/L and < 135 g/L for females and males, respectively)
  - Metabolic disease such as glucose-6-phosphate dehydrogenase deficiency
  - Psychiatric illness requiring hospitalisation or depression if severity is deemed clinically significant by the study Investigators
  - Known or suspected drug and/or alcohol misuse (defined as an intake exceeding 42 units per week)
  - Non-benign cancer, except squamous cell or basal cell carcinoma of the skin and cervical carcinoma in situ
- History of allergy or anaphylaxis to a vaccine or any component within the vaccines used in this study
- Have any known or suspected impairment or alteration of immune function, resulting from, for example:
  - Congenital or acquired immunodeficiency
  - Human Immunodeficiency Virus infection or symptoms/signs suggestive of an HIV-associated condition
  - Autoimmune disease
  - Receipt of immunosuppressive therapy such as anti-cancer chemotherapy or radiation therapy within the preceding 12 months or long-term systemic corticosteroid therapy (including for more than 7 days consecutively within the previous 3 months).
- Any significant abnormalities on screening investigations that are either unlikely to resolve or do not resolve on repeat testing (at the discretion of an Investigator) within the recruitment timeline of the study
- Weight <50 kg[1]
- Donation of blood within the last 3 (male) or 4 (female) months or plans on giving blood within the next year
- Receipt of a live vaccine within 4 weeks prior to vaccination
- Plan to receive any vaccine other than the study vaccine within 3 weeks following vaccination
- Scheduled procedures requiring general anaesthesia during the study
- Receipt of immunoglobulin or any blood product transfusion within 3 months of study start

[1] Or a Body Mass Index (BMI) that, in the opinion of the study doctors, may adversely impair the interpretation of the study results or affect the safe performance of any study procedures.

- Current active participation in another research study involving an investigational product or where involvement in this study could impact the results
- Previous occurrence of disease caused by *Y. pestis* or vaccine against plague
- Inability, in the opinion of the Investigator, to comply with all study requirements
- Female participants who are pregnant, lactating or who are unwilling to ensure that they or their partner use effective contraception throughout the trial period[2]
- Participant unwilling to allow contact with their GP or is not registered with a GP
- Any other significant disease or disorder which, in the opinion of the Investigator, may
    - Put the participants at risk because of participation in the study;
    - Influence the result of the study; and/or
    - Impair the participant's ability to participate in the study
- Tattoo at injection site that would interfere with assessment of injection site

8.4. Temporary exclusion criteria for vaccination

The following applies to both initial enrolment (point at which prime dose is given) and subsequent vaccination visit (boost visit). If the temporary exclusion resolves within the time constraints of the trial, they can be enrolled and/or progression in the trial can continue.

- Receipt of any systemic corticosteroid (or equivalent) treatment within 14 days prior to vaccination, or for more than 7 days consecutively within the previous 3 months.
- Febrile illness (oral temperature ≥37.5°C) or systemically unwell on the day of vaccination
- If a participant is taking systemic antibiotics, then the vaccination is postponed until 7 days after the last dose. This does not apply to topical antibiotic preparations
- Use of antipyretics in the 4 hours prior to vaccination
- A laboratory AE considered, in the opinion of the Investigator, requiring of further time and/or investigation to resolve or stabilise prior to another dose of vaccine being administered
- Symptoms of COVID-19, without confirmation of infection (as per current government guidelines) 14 days prior to vaccination visit; as per guidance in Section 9.11
- Validated positive SARS-CoV-2 test (NAAT or antigen) within 4 weeks prior to vaccination visit

8.5. Pregnancy and contraception

The possible adverse effects of ChAdOx1 Plague on the outcome of pregnancy are unknown; therefore, pregnant women will be excluded from the study. Women of childbearing potential will be required to use an effective contraceptive measure. Contraception should be maintained during the vaccination period and for the duration of the study. Should a volunteer become pregnant during the trial, she will be followed up for clinical safety assessment with her ongoing consent and in addition will be followed until pregnancy outcome is determined. We would not routinely perform venepuncture in a pregnant volunteer unless there is clinical need. Male participants with female partners are not required to use barrier methods for the purposes of contraception, as the risks of vaccine excretion are negligible.

Female volunteers of childbearing potential are required to use an effective form of contraception until their last follow-up visit. Acceptable forms of contraception for female volunteers include:

- Established use of oral, injected or implanted hormonal methods of contraception.

[2] As defined by CTFG Recommendations related to contraception and pregnancy testing in clinical trials, current document: https://www.hma.eu/fileadmin/dateien/Human_Medicines/01-About_HMA/Working_Groups/CTFG/2014_09_HMA_CTFG_Contraception.pdf [accessed 23rd July 2019]

- Placement of an intrauterine device (IUD) or intrauterine system (IUS).
- Total abdominal hysterectomy.
- Bilateral tubal occlusion.
- Barrier methods of contraception (condom or occlusive cap with spermicide).
- Male sterilisation, if the vasectomised partner is the sole partner for the subject.
- True abstinence, when this is in line with the preferred and usual lifestyle of the subject. Periodic abstinence (e.g. calendar, ovulation, symptothermal, post-ovulation methods), declaration of abstinence for the duration of exposure to IMP, and withdrawal are not acceptable methods of contraception.

9. TRIAL PROCEDURES

9.1. Recruitment

All potential participants may be contacted by methods including but not limited to email, telephone, posters, leaflets, websites, advertisements in university and hospital staff communication circulars, advertisements in newspaper, radio and on social media and/or mail using a REC approved invitation letter or other advertising material using wording from REC approved study documents in the first instance to invite them to participate in the study. Where mail-outs are used, participants may be identified via the electoral open register, or through National Health Service databases. These include the National Health Applications and Infrastructure Services (NHAIS) via a NHAIS data extract or equivalent. For the NHS databases initial contact to potential participants will not be made by the study team. Instead study invitation material will be sent out on our behalf by an external company, CFH Docmail Ltd, in order to preserve the confidentiality of potential participants. CFH Docmail Ltd is accredited as having exceeded standards under the NHS Digital Data Security and Protection Toolkit (ODS ID – 8HN70). For mail-outs via the electoral register, we will have access to the names and addresses of individuals who are on the open electoral register (only contains the names of registered voters who have not opted out). In this instance, the study team will upload the mailing list to the CFH Docmail system, and the study invitation pack will be sent out by CFH Docmail.

The study may be advertised on the electronic newsletter sent out to those potential participants signed up to the Oxford Vaccine Centre's Healthy Volunteers Database. Members of the public who have registered on this secure database have given their consent to be contacted when studies open for recruitment and understand that this is not a commitment to participate. Potential participants who are interested in study participation will be able to contact the Oxford Vaccine Group by telephone, email or online. Once an expression of interest has been received by Oxford Vaccine Group staff, participants will be directed to the appropriate website, where the information booklet will be available. Participants will be directed to complete the online screening process. This is to ensure that participants do not meet any major exclusions to being in the study. This will also record consent to collect their personal information, in order to facilitate further contact from the trial team. There will also be a consent to collect medical information on the website form and at telephone pre-screening.

9.2. Screening and Eligibility Assessment

Telephone Screening

Individuals whose responses have been received from the website will be contacted by telephone, as part of the pre-screening and asked to answer a series of questions to assess their eligibility based on the inclusion and exclusion criteria outlined on the SIB.

The following areas will be assessed during the telephone consultation:

- Participant demographics; age, sex and ethnicity
- Medical history
- Vaccination history including of any COVID-19 vaccines received
- Discussion about contraceptive requirements during the study
- Use of concomitant medication (including over the counter medications, vitamins, illicit drug use and herbal supplements)
- History of alcohol and/or smoking/vaping use
- History of prior vaccination with any *Yersinia pestis* vaccine

Individuals who are eligible and willing to proceed are invited for their first in-person visit (in-person screening). GP records will be requested with consent of the volunteer. This will be done in one of two ways:

1) At the end of the telephone screening the screener will email the volunteer a form to confirm consent for the study team to contact their GP and request a medical and vaccination summary. The volunteer may complete this electronically and return it via email to the study team. Permitted forms of electronic signature are a stylus or finger drawn signature, a typed name, or an inserted image of a wet-ink signature.

OR

2) Should process (1) not be completed, signature of the same consent form in paper format may be sought at the in-person screening visit, and the GP summary requested after that visit.

This consent relates only to the requesting of a GP medical and vaccination summary and is not the final study consent. A separate consent is taken for inclusion of the volunteer in the trial.

In-Person Screening Visit

Once a participant has successfully completed Telephone Screening, they will be invited to attend an in-person screening visit.

Once informed written consent is obtained, the following baseline assessments and information is collected by an appropriately trained member of the study team (usually a nurse or doctor) as part of the assessment of inclusion/exclusion criteria:

- Review of inclusion and exclusion criteria assessed at telephone screening
- Confirmation of medical history
- Recording of resting pulse and blood pressure
- 12 lead ECG
- BMI (height and weight)
- Physical examination; cardiovascular, respiratory, abdominal and gross neurological Examination
- Urinalysis
- Urine pregnancy test (females of childbearing potential only)
- Blood samples for: haemoglobin count, white cell indices, platelet count, serum sodium, serum potassium, serum urea, serum creatinine, liver function tests, C-reactive protein, HIV, Hepatitis B and C
- Point-of-care testing: measurement of blood glucose

The medical history, vaccination history and prescribed medication lists are based primarily on participant recall. With prior participant approval, the GP surgery will be asked to return a medical summary containing the participants medical and immunisation history. These will be requested and received via secure NHS.net email accounts. The GP will not be required to give an assessment of a participant's suitability for enrolment, however they may be contacted to clarify a medical record. The GP must be reachable throughout the trial, in case an abnormal finding or adverse reaction occurs that requires

medical follow-up outside of the trial. A medical doctor will review the information provided, with other screening information obtained, to determine eligibility.

Participants will be informed that they would also be eligible for BioBank ('Oxford Vaccine Centre Biobank' South Central Hampshire B REC - 21/SC/0161). BioBank is a separate study and optional to all participants of studies conducted by OVC. Separate consent is sought for this at screening or a later visit.

9.3. Trial Over-volunteering Prevention System (TOPS)

Consent will be taken to register the participant onto The Over-volunteering Prevention System (TOPS) database to guard against the potential for harm that can result from excessive volunteering in clinical trials involving IMPs and blood donations. This will be done using the participants National Insurance number or passport number (if not a British citizen). The participant will be excluded from the trial if found to be on another trial drug or vaccine trial.

The database will be updated with vaccination doses, if the participant receives the IMP, as the study progresses. The system will be updated in the event of the participant being withdrawn or excluded. Alternatively, TOPS will be updated on the participant's last visit.

9.4. Informed Consent

The participant must personally sign and date the latest approved version of the informed consent form before any study specific procedures are performed. Consent will be sought as described in relevant SOPs.

Written and verbal versions of the study information booklet and informed consent form will be presented by means of a voiced-over digital presentation, detailing no less than:

- The exact nature of and the rationale for performing the study
- Implications and constraints of the protocol
- The risks and benefits involved in taking part

The participant will then be able to discuss any queries arising from this with a member of the study team. The SIB will also be available for participant review.

It will be clearly stated that the participant is free to withdraw from the study at any time, for any reason and that they are under no obligation to give the reason for withdrawal. The participant will be allowed at least 24 hours to consider the information from when they receive it, and the opportunity to question the researcher, their GP or other independent parties to decide whether they will participate in the study.

The participant will have the opportunity to discuss the study with a medically qualified investigator. Written informed consent will be obtained by means of a dated signature of the participant and a signature of the appropriately trained and delegated clinician. A copy of the signed informed consent will be given to the participant and the original signed form will be retained at the study site.

9.5. Vaccination Visit

Vaccination visits (V1 and V6) are held at the CCVTM building or in its surrounding mobile units. The visit procedure for the vaccination visits will be as follows:

- Ensure that participant consent remains valid and, ensure they are still happy to continue with the study
- Obtain and document interim medical history since the last visit and check eligibility criteria (specifically temporary exclusion to vaccination)
- Set up the electronic diary access
- Review laboratory AE profile

- Record oral temperature, pulse and blood pressure
- Perform urinary pregnancy test for females of child bearing potential
- Perform blood draw as per Tables 3 and 4
- Mucosal sample collection
- Administer vaccine by IM injection
- Obtain biobank consent
- Observe for immediate adverse events for 60 minutes following ChAdOx1 Plague.
- Provide participant with a Medic Alert Contact Card with 24 hour telephone number to reach a study doctor
- Obtain 24 hour contact details. The study team will obtain the name and number of a close friend, relative or housemate. This person may be contacted if study staff is unable to contact the participant in the case of an emergency.
- Remind participants to contact the study team if they have any concerns regarding their wellbeing, symptoms and/or admission to hospital.
- Participants will be provided with eDiary log-on details and training on how to complete Additional paper backup copies of the eDiary will also be provided.
- Schedule next visit and re-iterate participant requirements such as completion of the electronic diary entries
- Participant GPs will be notified of their participation in the study shortly after enrolment to all groups is complete. This is to ensure medical records are updated.

9.6. Subsequent Visits

Other visits (as detailed in

Table 3,

Table 4, Table 5) will require the following procedures:

- Ensure that participant consent remains valid and ensure they are still happy to continue with the study
- Check latest safety bloods
  Perform blood draw as per Table 6, Table 7, Table 8
- Mucosal sample collection as per
- Table 3,
- Table 4, Table 5
- Pregnancy test according to
- Table 3,
- Table 4 Table 5
- Review electronic diary entries, laboratory tests, any adverse events and use of any concomitant medication since the last visit
- Record oral temperature, pulse and blood pressure
- Schedule next visit and re-iterate participant requirements such as return of the Diary Card entries
- Remind participants to contact the study team if they have any concerns regarding their wellbeing, symptoms and/or admission to hospital.

9.7. Unscheduled Visits

In the event of an unexpected or serious adverse event, a participant may need to have unscheduled clinic visits. These visits would have the same format as “subsequent visits” in section 9.6. Physical examination

may be performed and safety bloods may be sent at these visits, at the discretion of the investigator. These visits will be recorded in the participant CRFs.

9.8. COVID-19 Infection Control Measures at Visits

The CCVTM was one of the sentinel sites used by the University of Oxford to try out its COVID-19 infection control policies and act as a model for securing a workplace. The flow through the building has been altered to create a one-way system and reduce contact between people moving around. There is a policy in place that masks must be worn by every member of staff and every participant that enters the building. Strict social distancing guidance is in place and maintaining a 2m distance between people is a rule that must be followed. The on-going Oxford COVID-19 Vaccine Trials have been conducted at the CCVTM, therefore a lot of experience has been gained regarding the safe handling of clinic visits during this pandemic.

Participants will be reminded, via text and email, not to come to the centre if they are experiencing any symptoms of COVID-19, have tested positive for SARS-COV-2, or are in quarantine or self-isolating. Participants will be asked to come at staggered times to avoid unnecessary mixing and will be required to wear a face covering upon entering the building. Alcohol gel stations have been placed in key contact areas. All clinic visits will be conducted by staff in appropriate PPE, as per national guidance (e.g. mask, gloves, disposable gown), who have been trained in specific infection control measures. This will all be detailed in the Clinical Study Plan and will take into account NHS policies. The clinic room surfaces will be disinfected with anti-viral products after every participant.

9.9. Diary Monitoring

Following vaccination, participants will have access to an eDiary system (internally created and managed by OVG). Participants will be asked to record their temperature, rate a list of solicited symptoms, and record any other unsolicited symptoms for seven days after vaccination. They will also be asked to record any new concurrent medications taken during the trial. Any new medications and/or symptoms beyond the 7-day monitoring period may be reported to clinical team and recorded in the eCRF during the follow-up period. Participants will be able to use the paper diary to record any new medications and any illness or injuries that occur throughout the study period, as a memory aid to take to visits.

Each participant will be given unique log-in details associated with their study number. Training for this will be given at the first visit as described above. A paper copy of the diary will be provided to allow for completion in the event of inability to access the online version for whatever reason. The study team will be following up on compliance.

9.10. Participants under quarantine

Given the evolving epidemiological situation both globally and in the UK, should a participant be under quarantine and unable to attend any of the scheduled visits, a telephone/video consultation may be arranged in order to obtain safety and the visit may be re-scheduled, depending on the timelines.

9.11. Participants with COVID-19 symptoms

Participants who become symptomatic during follow-up will be instructed to call the study team who will then advise on how to proceed with clinical testing for COVID-19, through the community testing programme, if necessary, as per the trial working instructions. Participants will get weekly reminders (email or text messages) to get in touch with the study team if they have symptoms of COVID-19, experience any new event requiring medical attendance, or if they are admitted to hospital for any reason. Participants would be expected to report a transient, flu-like illness within 24hours of vaccination. If this reaction should include a fever, we would expect this to resolve within 48hours. If a fever starts and

resolves within 48hours of vaccination it will be attributed to the vaccine. If a fever persists for more than 48hours, or starts more than 48hours after vaccination, it will be considered unlikely to be related to vaccination and the participant will be advised to proceed with clinical testing for COVID-19, outside of the study.

Participants who develop COVID-19 symptoms and have a positive SARS-CoV-2 test (appropriately validated NAAT or antigen test) after the first vaccination can only receive a booster dose after a minimum 4 weeks interval from their positive test, provided they have had only a mild illness and have fully recovered. Moderate-Severe illness will be defined as 4 or above as per the WHO Clinical Progression Scale (See 22 Appendix B: WHO Clinical Progression Scale for clinical studies of COVID-19)). Those who have had moderate-severe disease will not receive further IMP.

In cases of mild or asymptomatic disease, the decision to proceed with booster vaccinations will be at clinical discretion of the investigators, and each case will be evaluated by a study doctor before proceeding (including physical examination and peripheral oxygen saturation recording [Sp02]). The trial clinician must assess that the participant has fully recovered from their illness. Participants must have no ongoing symptoms that could be attributable to their COVID-19 illness and feel that they have fully recovered and are well.

For participants who are asymptomatic and have a positive SARS-CoV-2 test (and who remain asymptomatic), a minimum of 4 weeks from positivity will be required before boosting. They will also undergo a physical examination and peripheral oxygen saturation recording before proceeding with IMP.

Participants who have had mild or asymptomatic disease and have been deemed to have fully recovered, with normal physical examination and normal peripheral oxygen saturation recordings (Sp02), will be counselled when they are offered a booster that the risks involved in receiving the ChAdOx1 Plague vaccine after a SARS-CoV-2 infection or COVID-19 illness are unknown.

9.12. Sample Handling

See below for schedule of blood sampling, by group. * Sampling will be up to the volumes (505ml for Group 1; 645ml for Group 2 and 3). Additional samples may be required for safety investigations.

Table 6. Blood Sampling Schedule for Groups 1

Group 1									
Visit	**Week of Study visit**	**Day of visit**	Serum (ml)	PBMCs (ml)	Functional genomics (ml)	Full Blood Count (ml)	LFTs, U&Es &CRP (ml)	HIV/Hep B/C (ml)	Visit volume (ml) *
V0	Pre-study	Screening visit				2	3	5	10
V1	Week 0	D0	20	40	2.5	2	3		67.5
V2									
V3	Week 1	D7	10	40	2.5	2	3		57.5
V4	Week 2	D14	10	40					50

V5	Week 4	D28	10	40		2	3		55
V6	Week 8	D56	10	40					50
V7									
V8									
V9									
V10	Week 12	D84	10	40		2	3		55
V11	Week 26	D182	10	40		2	3		55
V12									
V13									
V14									
V15	Week 30	D210	10	40					50
V16	Weeks 36-52	D250 - D365	10	40		2	3		55
Unscheduled	Additional safety visit (if needed)					0-2	0-3		0-5
						Total			505ml (+2-3ml per additional safety blood)

Table 7. Blood Sampling Schedule for Group 2

Group 2									
Visit	**Week of Study visit**	**Day of visit**	Serum (ml)	PBMCs (ml)	Functional genomics (ml)	Full Blood Count (ml)	LFTs, U&Es &CRP (ml)	HIV/Hep B/C (ml)	Visit volume (ml) *
V0	Pre-study	Screening visit				2	3	5	10
V1	Week 0	D0	20	40	2.5	2	3		67.5
V2	D1	D1		10	2.5				12.5
V3	Week 1	D7	10	40	2.5	2	3		57.5
V4	Week 2	D14	10	40					50

V5	Week 4	D28	10	40		2	3		55
V6	Week 8	D56	10	40	2.5	2	3		57.5
V7	Week 8 + D1	D57		10	2.5				12.5
V8	Week 9	D63	10	40	2.5	2	3		57.5
V9	Week 10	D70	10	40					50
V10	Week 12	D84	10	40		2	3		55
V11	Week 26	D182	10	40		2	3		55
V12	Week 26+1	D183							
V13	Week 27	D189							
V14	Week 28	D196							
V15	Week 30	D210	10	40					50
V16	Weeks 36-52	D250 - D365	10	40		2	3		55
Unscheduled	Additional safety visit (if needed)	-				0-2	0-3		0-5
						Total			645 (+2-3ml per additional safety blood)

Table 8. Blood Sampling Schedule for Group 3

Group 3									
Visit	**Week of Study visit**	**Day of visit**	Serum (ml)	PBMCs (ml)	Functional genomics (ml)	Full Blood Count (ml)	LFTs, U&Es &CRP (ml)	HIV/Hep B/C (ml)	Visit volume (ml) *
V0	Pre-study	Screening visit				2	3	5	10
V1	Week 0	D0	20	40	2.5	2	3		67.5
V2	D1	D1		10	2.5				12.5
V3	Week 1	D7	10	40	2.5	2	3		57.5
V4	Week 2	D14	10	40					50

V5	Week 4	D28	10	40		2	3		55
V6	Week 8	D56	10	40					50
V7									
V8									
V9									
V10	Week 12	D84	10	40		2	3		55
V11	Week 26	D182	10	40	2.5	2	3		57.5
V12	Week 26+1	D183		10	2.5				12.5
V13	Week 27	D189	10	40	2.5	2	3		57.5
V14	Week 28	D196	10	40					50
V15	Week 30	D210	10	40		2	3		55
V16	Weeks 36-52	D250 - D365	10	40		2	3		55
Unscheduled	Additional safety visit (if needed)	-				0-2	0-3		0-5
						Total			645 (+2-3ml per additional safety blood)

9.12.1 Blood testing schedule

Blood samples will be taken by an appropriately trained member of the research team (normally a nurse or doctor). Samples will be handled, processed, stored and shipped according to the lab analysis plan.

Table 6, Table 7, Table 8 include details of which blood tests are performed at each visit for groups 1, 2 and 3; the total volume of blood obtained per participant is up to approximately 505ml for group 1 and 645ml for groups 2 and 3; from screening to the 52 weeks completion of all subsequent visits. If additional safety blood visits are required, a volume of up to 5ml could be taken per visit.

9.12.2 Safety bloods and Screening Investigations

The safety and screening blood tests (full blood count, U&Es, LFTs, CRP, HIV, and Hepatitis B/C) will be sent to the Oxford University Hospitals pathology laboratories (haematology, biochemistry or microbiology labs) for processing as per their standard SOPs. These labels will be labelled with the participants study number and initials, but no other identifying details. The urine tests (urine dip and/or pregnancy test) will be performed by a trained member of the research team (usually a nurse or doctor) in real time during the appropriate study visit. Urine will be tested for protein, blood and glucose at screening. For female volunteers only, urine will be tested for beta-human chorionic gonadotrophin (β-HCG) at screening and immediately prior to vaccination.

9.12.3 Phlebotomy

The volume of blood drawn over the study period should not compromise healthy adult participants. No more than 645mls (+potential 2-3ml additional safety samples up to approx. additional 20ml) of blood will be taken over the course of the study (1 year). As a comparison, women are able to donate a maximum of 1410mls of blood per year, and men 1880mls, to the National Transfusion Service. At enrolment, any recent blood donations will be checked to ensure that the total volume of blood taken is safe to take. Participants will be closely monitored both clinically and by laboratory results for haemoglobin during the study. Should anaemia develop during the study, sample volumes will be reduced or minimised possibly to include only essential safety bloods, according to the OVG venepuncture SOP. Risks from venepuncture include mild tenderness, bruising, light-headedness and, rarely, syncope or arterial puncture.

9.12.4 Mucosal samples

Mucosal samples will be collected using synthetic absorptive matrix (SAM) strips, pressed against the anterior nasal mucosal surface. SAM strips are soft, absorptive, and have rapid wicking for sample collection. Details on the use of the SAM strips will be outlined in the clinical study plan. These samples will be processed by the OVG laboratory.

9.12.5 Laboratory immunology

In addition to blood samples needed for the safe conduct of the trial and assessment of the primary endpoint, blood samples from the participants will also be subjected to laboratory analyses in order to assess the objective defined in the secondary endpoint (serum), and potentially for the exploratory objectives (serum, PBMCs, serum for cytokine samples, FG, mucosal samples). These samples will be relabelled to a laboratory number upon processing in the OVG laboratory, which is linked to the original participant number. The plan for analysis is outlined below, and will be further detailed in a specific lab analysis plan:

ELISA

Assays of particular scientific interest may include the quantification of the relative concentration of serum antibodies against vaccine antigens and adenovirus structural proteins by enzyme linked immunosorbent assay (ELISA).

Exploratory Analysis of immune responses elicited by the vaccines

Cellular responses to quantify the B- and T-cell responses specific to the vaccine components will be performed when feasible using peripheral blood mononuclear cells (PBMCs) derived from study participants sampled before, and after vaccination, using memory B cells, plasma cells and cytokine ELISPOT and flow cytometric assay (phenotyping, intracellular cytokine staining).

The ability of serum antibodies to mediate a functional activity effect may be assessed by an assay tailored for use with a plague, attenuated plague or pseudotuberculosis plague target strains as used in the literature.

Innate immune activation and mucosal immune responses may also be assessed.

Analysis of gene expression may be performed using peripheral blood. This analysis may highlight differences in gene expression induced by vaccination and provide insight into the immunobiology of vaccine responses. In addition, DNA samples obtained from peripheral blood will contribute to a Biobank of samples from multiple different Oxford Vaccine Group studies. These DNA samples will be used to

analyse the genetic factors influencing vaccine responses (immunogenicity and reactogenicity). DNA extraction and storage will only occur with the specific consent of participants, and DNA will not be analysed for any other purpose than to assess factors influencing vaccine responses.

Human serum transfer study into mice, followed by challenge, as a surrogate for measuring the efficacy of the antibodies generated by the vaccine

9.12.6 Optional contribution of samples to other research projects

Participants in the PlaVac clinical trial are eligible to contribute samples towards other research projects as outlined below. The Biobank study is optional; is not a requirement to be enrolled into this study, and participants do not receive additional compensation.

BioBank study (REC reference H/0504/25)

Remaining serum, cells and DNA samples obtained from peripheral blood and mucosal samples will contribute to a BioBank of samples from multiple different OVG studies. DNA will not be analysed for any other purpose than to assess factors influencing vaccine responses (immunogenicity and reactogenicity). DNA extraction and storage will only occur with the specific consent of participants, and DNA will not be analysed for any other purpose than to assess factors influencing vaccine responses. Serum and cells remaining after the planned analyses have been performed will, with specific consent of the participants, be transferred to the OVG BioBank for future analysis for sero-epidemiological and other vaccine related purposes.

9.13. Discontinuation/Withdrawal of Participants from Trial

In accordance with the principles of the current revision of the Declaration of Helsinki and any other applicable regulations, a volunteer has the right to withdraw from the study at any time and for any reason and is not obliged to give his or her reasons for doing so. The reason for withdrawal will be recorded in the CRF, if this information is provided by the volunteer. If withdrawal is due to an AE, appropriate follow-up visits or medical care will be arranged, with the agreement of the volunteer, until the AE has resolved, stabilised or a non-trial related causality has been assigned. If an SAE is ongoing after withdrawal and the participant declines a follow up visit, then the investigators will request that the participant provides follow-up information until resolution.

The DSMC or DSMC chair may recommend withdrawal of volunteers. The investigator may discontinue a participant from the study at any time if the investigator considers it necessary for any reason including, though not exclusive to, the following:

- Pregnancy
- Ineligibility (either arising during the study or in the form of new information not declared or detected at screening)
- Significant protocol deviation
- Significant non-compliance with study requirements
- An adverse event which requires discontinuation of the study vaccinations, puts the participants health or wellbeing at undue risk or results in an inability to continue to comply with study procedures
- Consent withdrawn
- Lost to follow up

Withdrawal from the study will not result in exclusion of the data generated by that participant from analysis. All data and participant samples obtained up to the point of withdrawal will be used in the analysis.

Of the first 10 participants recruited to Group 1, those who have been withdrawn from the study, can be replaced if they have not reached the Day 28 time point. We may replace the other participants in the trial if they do not reach the Day 14 time point. However, in both these scenarios, the participant cannot be replaced if the reason for discontinuation/withdrawal is a safety event or toxicity.

9.14. Definition of End of Trial

The end-of-study is defined as the date of the last visit of the last patient undergoing the trial, or the completion of the last laboratory assay on the last participant sample, whichever is later. At this point, all samples will be destroyed, unless transferred to the Biobank with participant consent.

10. INVESTIGATIONAL MEDICINAL PRODUCT (IMP)

The participants in Group 1 will receive a single dose of ChAdOx1 Plague, by intramuscular route. Participants in Group 2 and 3 will receive two doses of ChAdOx1 Plague, by intramuscular route. Group 2 will be boosted at two months and Group 3 will be boosted at six months.

10.1. IMP Description

ChAdOx1 Plague

The vaccine product ChAdOx1 Plague is formulated in 10 mM Histidine, 35 mM NaCl, 1 mM $MgCl_2$, 0.1 mM EDTA, 0.5 % (v/v) ethanol, 7.5 % (w/v) sucrose, 0.1 % (w/v) PS80, in Water for Injection, at pH 6.6, sterile-filtered. The vaccine is stored frozen (-80°C nominal) in Type 1 glass, particle free (as per USP or Ph. Eur. Method), sterile and depyrogenated vials. Each vial contains an extractable volume of 450µl at 1.49x10e11 vp/ml. The product is manufactured, tested and labelled according to current EMEA guidelines in keeping with Good Manufacturing Practice (GMP). See the IB and IMPD for detailed descriptions of the final drug product.

10.2. Storage and administration of IMP

ChAdOx1 Plague storage

ChAdOx1 Plague has been manufactured under Good Manufacturing Practice (GMP) conditions at the Clinical Biomanufacturing Facility (CBF), University of Oxford. At the CBF the vaccine will be certified and labelled for the trial by a QP before transfer to the clinical site.

The vaccine product will be stored according to GMP at -70 °C to -85 °C at the CBF in a locked, alarmed and temperature monitored freezer until authorised for release. Once released, the IMP will be stored at -80°C at the CCVTM.

Transport of the vaccine to the clinical site will be performed by the CBF, or a representative from the Oxford Vaccine Group Team, with all movements' temperature controlled, maintaining the cold chain at all times. All movements of study medication between the CBF and OVG, will be documented in accordance with relevant SOPs.

The study vaccine will be stored at the OVG in temperature monitored freezers and refrigerators with an auditable temperature record in accordance with the manufacturer's instructions and relevant SOPs. Study

fridges and freezers are connected to a monitoring system with 24-hour access to staff that are able to move the product in the event of significant temperature deviation, for example fridge malfunction.

ChAdOx1 Plague administration

On vaccination day, ChAdOx1 Plague will be thawed to room temperature and will be administered in accordance with trial specific instructions. The vaccine will be administered intramuscularly into the deltoid of the non-dominant arm (preferably). All participants will be observed in the unit for 1 hour (±10 minutes) after vaccination.

During administration of the investigational products, Advanced Life Support drugs and resuscitation equipment will be immediately available for the management of anaphylaxis. Vaccination will be performed and the IMPs handled according to the relevant SOPs. As the vial will be defrosted on participant arrival and immediately administered, there will be no need for further storage.

10.3. Accountability of the Trial Vaccine

The ChAdOx1 Plague vaccine has been manufactured, packaged, labelled and supplied by the Clinical BioManufacturing Facility (CBF), University of Oxford. All vaccines are labelled with a label specifying 'For clinical trial use only' and no less than the following:

- The clinical trial identifier (by reference code)
- The content of each vial
- Dose route
- The batch number
- The chief investigator
- Expiry date

The vaccine will be stored at the CBF pending authorised release for use in the clinical trial.

10.4. Concomitant Medication

As set out by the exclusion criteria, volunteers may not enter the study if they:

- Receive a live vaccine within 4 weeks prior to vaccination
- Receive any other vaccine including a COVID-19 vaccination within 14 days of study vaccine
- Receive any investigational product within 30 days prior to enrolment or if receipt is planned during the study period
- Receive immunosuppressant medication in the 12 months prior to enrolment or are planned to receive them at any time during the study period (except topical steroids and short course of low dose steroids < 14 days).

Apart from the above, there is no restriction on the use of concomitant medication. All concomitant medication, prescribed or over-the-counter, will be recorded in the CRF or diary.

The new prescription of a medication to a participant during the course of the study will be evaluated by a study clinician and may result in the withdrawal of the participant at the discretion of the Investigator. The receipt of antibiotics or antipyretics (specifically within 4 hours of vaccination) may result in a temporary exclusion.

10.5. Genetically Modified Organism (GMO) Approval

As the trial IMP, ChAdOx1 Plague involves use of a GMO, approval of IMP use and associated procedures (e.g. disposal) will be sought from the local GMO committee in line with The Genetically Modified Organisms (Contained Use) Regulations 2014.

The study will be performed in accordance with UK Genetically Modified Organisms (Contained Use) Regulations (2014). In order to minimise dissemination of the recombinant vectored vaccine virus into the environment, ChAdOx1 Plague inoculation sites will be covered with a dressing after immunisation. This should absorb any virus that may leak out through the needle track. The dressing will be removed from the injection site after 30 minutes (+15/- 5 minutes) and will be disposed as GMO waste by autoclaving or by waste disposal companies with appropriate licenses.

11. SAFETY REPORTING

11.1. Safety Reporting Definitions

Adverse Event (AE)	Any untoward medical occurrence in a participant to whom a medicinal product has been administered, including occurrences which are not necessarily caused by or related to that product.
Adverse Reaction (AR)	An untoward and unintended response in a participant to an investigational medicinal product which is related to any dose administered to that participant. The phrase "response to an investigational medicinal product" means that a causal relationship between a trial medication and an AE is at least a reasonable possibility, i.e. the relationship cannot be ruled out. All cases judged by either the reporting medically qualified professional or the Sponsor as having a reasonable suspected causal relationship to the trial medication qualify as adverse reactions.
Serious Adverse Event (SAE)	A serious adverse event is any untoward medical occurrence that: • Results in death • Is life-threatening • Requires inpatient hospitalisation or prolongation of existing hospitalisation • Results in persistent or significant disability/incapacity • Consists of a congenital anomaly or birth defect. Other 'important medical events' may also be considered serious if they jeopardise the participant or require an intervention to prevent one of the above consequences. NOTE: The term "life-threatening" in the definition of "serious" refers to an event in which the participant was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it were more severe.
Serious Adverse Reaction (SAR)	An adverse event that is both serious and, in the opinion of the reporting Investigator, believed with reasonable probability to be due to one of the trial treatments, based on the information provided.

Suspected Unexpected Serious Adverse Reaction (SUSAR)	A serious adverse reaction, the nature and severity of which is not consistent with the information about the medicinal product in question set out: • In the case of a product with a marketing authorisation, in the summary of product characteristics (SmPC) for that product • In the case of any other investigational medicinal product, in the investigator's brochure (IB) relating to the trial in question.

NB: to avoid confusion or misunderstanding of the difference between the terms "serious" and "severe", the following note of clarification is provided: "Severe" is often used to describe intensity of a specific event, which may be of relatively minor medical significance. "Seriousness" is the regulatory definition supplied above. See APPENDIX A: GRADING THE SEVERITY OF ADVERSE EVENTS

Any pregnancy occurring during the clinical trial and the outcome of the pregnancy should be recorded and followed up for congenital abnormality or birth defect, at which point it would fall within the definition of "serious".

11.2. Causality

For every AE, an assessment of the relationship of the event to the administration of the vaccine will be undertaken by the CI-delegated clinician. An interpretation of the causal relationship of the intervention to the AE in question will be made, based on the type of event; the relationship of the event to the time of vaccine administration; and the known biology of the vaccine therapy. Alternative causes of the AE, such as the natural history of pre-existing medical conditions, concomitant therapy, other risk factors and the temporal relationship of the event to vaccination will be considered and investigated. Events reported as having a possible, probable or definite relationship will be managed as being related to the IMP. Causality assessment will take place during planned safety reviews, interim analyses (e.g. if a holding or stopping rule is activated) and at the final safety analysis, except for SAEs, which should be assigned by the reporting investigator, immediately.

No relationship:

- No temporal relationship to vaccine administration
- Alternative aetiology (clinical, environmental or other intervention), and
- Does not follow pattern of recognised response to vaccine administration

Possible:

- Reasonable temporal relationship to vaccine administration, or
- Event not readily explained by alternative aetiology (clinical, environmental or other interventions), or
- Similar pattern of response to that seen to vaccine administration.

Probable

- Reasonable temporal relationship to vaccine administration, and
- Event not readily produced by alternative aetiology (clinical, environment, or other interventions), or
- Known pattern of response with vaccine administration.

Definite

- Reasonable temporal relationship to vaccine administration and

- Event not readily produced by alternative aetiology (clinical, environment, or other interventions), and known pattern of response to vaccine administration

11.3. Procedures for Collecting and Recording Adverse Events

All AEs occurring during the study (from first vaccine administration until the last visit) observed by the investigator or reported by the participant, irrespective of their relatedness to the study medication, will be recorded in the AE section of the CRF.

All AEs that result in a volunteer's withdrawal from the study will be followed up until a satisfactory resolution occurs, or until a non-study related causality is assigned (if the volunteer consents to this). SAEs will be collected throughout the entire trial period. Only AEs occurring in the 28 days post-vaccination (from Visit 1 for Group 1; from Visit 1 and Visit 6 for Group 2 and 3), will be used as an outcome measure for the primary objective.

Participants will be asked to record Foreseeable Adverse Reactions (as detailed in section 11.8) and Unforeseeable Adverse Reactions in an eDiary for 7 days after vaccination (see section 9.9). Participants will be provided with a thermometer and tape measure to accurately record temperature and size of swelling, redness and induration during this period. Any ARs present at the end of the 7-day post-vaccination period, will then be recorded by the study team in the AE section of the CRF and followed up, until resolution. Participants will be encouraged to report any unsolicited AEs occurring during the study period and the study team will record these in the CRF, at each study visit or following a call/email notification from the participant. Vital signs will also be taken and recorded at each visit.

AEs will be recorded using the following guidance:

- Pre-existing medical conditions (present before start of the AE collection period) are considered "concurrent medical conditions" and should not be recorded as AEs. However, if the participant experiences a worsening or complication of such a condition, the worsening or complication should be recorded as an AE. Investigators should ensure that the AE term recorded captures the change in the condition (e.g., "worsening of")
- Each AE should be recorded in the CRF to represent a single diagnosis. Accompanying signs or symptoms (including abnormal laboratory values) should not be recorded as additional AEs.
- Changes in laboratory values are graded Mild, Moderate, Severe, Potentially life threatening (APPENDIX A: GRADING THE SEVERITY OF ADVERSE EVENTS). Significance of laboratory AEs will be ascertained by clinical judgement of the investigator, and reported as AEs as appropriate. If abnormal laboratory values are the result of pathology for which there is an overall diagnosis, they should be reported as one AE; the diagnosis.

The following information will be recorded in the CRF: description of the AE, the date of onset and end date, severity, assessment of relatedness to study vaccine(s) (as judged by a medically qualified investigator) and action taken. Follow-up information should be provided as necessary. AEs considered related to the study vaccine(s) will be followed until resolution, the event is considered stable or until a non-study causality is assigned.

During the trial, laboratory results from the enrolled participants will be entered into the main clinical database (RedCAP), using a double data entry process. Designated clinical staff will have access to this database, which will be monitored and maintained by the lead doctor, the lead nurse and the senior research nurse. This database will be maintained according to the relevant OVG Database SOP.

It will be left to the investigator's clinical judgment whether or not an AE is of sufficient severity to require the participant's removal from study. A participant may also voluntarily withdraw from treatment due to what he or she perceives as an intolerable AE. If either of these occurs, the participant must undergo an end of study assessment and be given appropriate care under medical supervision, by referral to their GP, until symptoms cease, or the condition becomes resolved or is stable. If required, the investigator can refer the participant directly to hospital if the AE warrants it.

11.4. Reporting Procedures for Adverse Events

All AE's will be reported to the CI and DSMC chair as outlined in the DSMC charter. All SUSARs will be reported by the CI as outline in section 11.7.

11.5. Reporting Procedures for Serious Adverse Events

All SAEs must be recorded on an SAE form and reported to the CI and DSMC Chair (or their nominated deputy) within 24 hours of awareness of the event (DSMC contact details will be available in the DSMC charter and on the SAE form). Additional information received for a case (follow-up or corrections to the original case) need to be detailed on a new SAE form and faxed or emailed to the CI and DSMC Chair (or their nominated deputy). If the SAE is also a SUSAR, additional procedures for reporting are described below in section 11.7

The chair of the DSMC (or a deputy nominated by the chair) will perform an independent review of SAEs and request any further information required in a manner adherent to the regulatory requirements for the expedited reporting of SUSARs to the MHRA & the REC. Documentation of this review will be kept in the TMF.

The DSMC will provide independent real-time safety assessment throughout the study as described in section 11.9.

11.6. Expectedness

Expectedness of AEs will be determined according to the reference safety information section of the Investigators' Brochure for ChAdOx1 Plague. No IMP related SAEs are expected in this study. All SAEs at least possibly related to ChAdOx1 Plague will be considered unexpected and be reported to the MHRA and REC as SUSARs within the regulatory timelines, as in section 11.7.

11.7. SUSAR Reporting

All SUSARs will be reported by the CI to the relevant Competent Authority and to the REC and other parties as applicable. For fatal and life-threatening SUSARS, this will be done no later than 7 calendar days after the Sponsor or delegate is first aware of the reaction. Any additional relevant information will be reported within 8 calendar days of the initial report. All other SUSARs will be reported within 15 calendar days.

Principal Investigators will be informed of all SUSARs for the relevant IMP for all studies with the same Sponsor, whether or not the event occurred in the current trial.

11.8. Foreseeable Adverse Reactions

The foreseeable ARs following vaccination with ChAdOx1 Plague include, locally; injection site pain/ tenderness, redness, swelling, hardness, itch, warmth; and systemically, headache, malaise, fever, feverishness, nausea, fatigue, vomiting, muscle pain, chills, joint pain and rash.

11.9. Safety Monitoring Committee

The DSMC will be chaired by Professor Graham Cooke.

The DSMC is independent and will review safety data throughout the study according to the DSMC Charter. The specific role of the committee will be as follows:

1. Formal review of the safety profile after 7 days of safety data has been collected from all enrolled participants of Group 1

2. Independent review following any SAE deemed to be possibly, probably, or definitely related to the trial vaccine will be carried out in real-time.
3. Unscheduled reviews on request of the study management committee

From these reviews the DSMC will make recommendations to the study investigators on whether there are any ethical or safety reasons why the trial should not continue. A summary of all AEs and SAEs to date will be provided to the DSMC on request.

The outcome of each DSMC review will be communicated directly to the study investigators and documentation of all reviews will be kept in the TMF.

The Chair of the DSMC will also be contacted for advice where the Chief Investigator feels independent advice or review is required.

11.10. Group holding rules

Group holding rules are as follows:

Solicited/unsolicited/laboratory adverse events

Solicited local adverse events:

- If more than 25% of doses of the vaccine at a given time point (Day 0, Day 56, Day 182) in a study group are followed by the same Grade 3 solicited local adverse event beginning within 2 days after vaccination (day of vaccination and one subsequent day) and persisting at Grade 3 for >72 hrs

Solicited systemic adverse events:

- If more than 25% of doses of the vaccine at a given time point (Day 0, Day 56, Day 182) in a study group are followed by the same Grade 3 solicited systemic adverse event beginning within 2 days after vaccination (day of vaccination and one subsequent day) and persisting at Grade 3 for >72 hrs

Unsolicited adverse events:

- If more than 25% of doses of the vaccine at a given time point (Day 0, Day 56, Day 182) in a study group are followed by the same Grade 3 unsolicited adverse event beginning within 2 days after vaccination (day of vaccination and one subsequent day) and persisting at Grade 3 for >72 hrs

Laboratory adverse event:

- If more than 25% of doses of the vaccine at a given time point (Day 0, Day 56, Day 182) in a study group are followed by the same Grade 3 laboratory adverse event beginning within 3 days after vaccination and persisting at Grade 3 for >72 hrs

SAEs

If an SAE occurs in any one individual, which is possibly, probably or definitely related to vaccination this would trigger a holding rule (at group and individual level).

Safety Review

If any of the group holding rules are activated, then further vaccinations in any of the groups will not occur until a safety review by the DSMC, study sponsor and the chief investigator has been conducted and it is deemed appropriate to restart dosing, or dose de-escalate.

The safety review will consider:

- The relationship of the AE or SAE to the vaccine.
- The relationship of the AE or SAE to the vaccine dose, or other possible causes of the event.
- If appropriate, additional screening or laboratory testing for other participants to identify those who may develop similar symptoms and alterations to the current Study Information Booklet (SIB) are discussed.
- New, relevant safety information from ongoing research programs on the various components of the vaccine.
- The implementation of a dose de-escalation process

Any further dosing in the clinical trial will be halted in case of severe adverse reactions, in particular (which are considered at least, possibly related to IMP administration), in two subjects at the same dose level or cohort, independent of whether these occur within the same system-organ-class. This group holding rule applies to the dose level group in which the events were observed and also to any higher dose level group. The Regulatory Authority will be informed and a request to restart dosing with pertinent data will be submitted as a substantial amendment.

The Sponsor, the Research Ethics Committee, MHRA and CBF as the vaccine manufacturers will be notified if a holding rule is activated or released.

All vaccinated participants will be followed for safety until resolution or stabilization (if determined to be chronic sequelae) of their AEs.

11.11. Individual holding rules

In addition to the above stated group holding rules, stopping rules for individual participants will apply (i.e., indications to withdraw individuals from further blood draws, with the exception of blood draws for safety monitoring)

- **Laboratory AEs:**
  - The participant develops a Grade 3 laboratory adverse event considered possibly, probably or definitely related within 7 days after vaccination, persisting continuously at Grade 3 for > 72hrs.

- **Solicited adverse events:**
  - The participant develops a Grade 3 systemic solicited adverse event considered possibly, probably or definitely related to vaccination within 2 days after vaccination (day of vaccination and one subsequent day), persisting continuously at Grade 3 for > 72hrs.

- **Unsolicited adverse events:**
  - Injection site ulceration, abscess or necrosis
  - The participant has a Grade 3 adverse event, considered possibly, probably or definitely related to vaccination, persisting continuously at Grade 3 for >72hrs,
  - The participant has a serious adverse event considered possibly, probably or definitely related to vaccination, or
  - The participant has an acute allergic reaction or anaphylactic shock following the administration of vaccine investigational product.

If a participant fulfils any of the temporary exclusion criteria related to vaccination (see section 8.4) at the scheduled time of a second administration of investigational product, the participant will not receive the vaccine at that time. The vaccine may be administered to that participant at a later date within the time window specified in the protocol (see Tables 3 and 4) or they may be withdrawn from the study at the discretion of the Investigator.

All vaccinated participants will be followed for safety until the end of their planned participation in the study or until resolution or stabilisation (if determined to be chronic sequelae) of their AEs, providing they consent to this.

11.12. Stopping Rules

The trial will be discontinued in the event of any of the following:

- New scientific information is published to indicate that subjects in the trial are being exposed to undue risks as a result of administration of the IMP, or as a result of the trial procedures or follow-up schedule.
- Serious concerns about the safety of the IMP arise as a result of one or more vaccine related SAE(s) occurring in the subjects enrolled in this or any other on-going trial of vaccines containing the ChAdOx1 vector.
- For any other reason at the discretion of the Chief Investigator or DSMC.

Additionally, the DSMC can temporarily pause the trial if time is required to reach a decision regarding stopping the trial e.g. to determine causality for SAE.

11.13. Development Safety Update Reports

The CI will submit (in addition to the expedited reporting above) DSURs once a year throughout the clinical trial, or on request, to the Competent Authority (MHRA in the UK), Ethics Committee, Host NHS Trust and Sponsor. The trial DSUR will be a standalone document.

11.14. Updating participants and GPs on safety signals during trial

Should any safety signals relating to the IMP, or other ChAdOx1-based vaccines, become apparent during the trial participants and their registered medical practitioners (GPs) will be notified. Any significant clinical updates would also be notified.

12. STATISTICS

12.1. Description of Statistical Methods

The analyses for this study will be descriptive in purpose and will not include any hypothesis testing or presentation of p values for group comparisons or power calculations needed.

12.2. The Number of Participants

Up to 45 participants will be recruited to the study allocated to groups 1, 2 or 3, as described in section 7.1 The first 10 participants from Group 1 will be replaced if they do not reach the Day 28 time point due to

withdrawal from the study. Pragmatically, it was decided that a minimum of 10 healthy volunteers would be adequate to assess the primary objective. The reasons for withdrawal would be taken into consideration and trial termination would be considered if many participants withdrew because of vaccine-related reactions. The other participants in the trial may be replaced if they do not reach Day 14.

There has been no power calculation to determine these numbers as the study is primarily descriptive. The numbers have been therefore chosen on the maximal number able within budgetary and practical constraints.

12.3. The Level of Statistical Significance

There will be no statistical significance testing. All confidence intervals for descriptive analyses will be set at 95%.

12.4. Interim review of immune responses

We are planning to review the immunogenicity data in all groups when it becomes available after the timepoints: D28, D84, D208. Reviewing the immunogenicity data we can ensure the results are genuine and plan for the next stages of development if an early signal on immunogenicity is observed. Since there is no formal statistical testing involved in the interim review, we believe the interim review of the immunogenicity data will not affect the integrity of the trial.

12.5. Criteria for the Termination of the Trial

The Chief Investigator and Data Safety Monitoring Committee will have the right to terminate the study at any time on grounds of participant safety. If the study is prematurely terminated the investigator will promptly inform the participants and will ensure appropriate therapy and follow-up. If the study is halted, the MHRA and relevant Ethics Committee will be notified within 15 days of this occurring.

In the event of the trial being terminated early, follow-up of enrolled participants will still continue as detailed in tables 3 and 4, for safety reasons.

12.6. Procedure for Accounting for Missing, Unused, and Spurious Data.

All available data will be used in the analyses and there will be no imputations for missing data. Participants will be analysed according to the group to which they were assigned.

12.7. Inclusion in Analysis

All participants with any available data will be included in the analyses.

13. DATA MANAGEMENT

13.1. Source Data

Source documents are where data are first recorded, and from which participants' CRF data are obtained. These include, but are not limited to, hospital records (from which medical history and previous and concurrent medication may be summarised into the CRF), clinical and office charts, laboratory and pharmacy records, diaries, microfiches, radiographs, and correspondence. In this study CRF entries will be considered source data where it is the site of the original recording. All documents will be stored safely

under strict confidentiality and with restricted access. On all study-specific documents, other than the signed consent and the participant contact sheet, the participant will be referred to by the study participant number/code only.

13.2. Access to Data

Direct access will be granted to authorised representatives from the sponsor, host institution and the regulatory authorities to permit trial-related monitoring, audits and inspections.

13.3. Data Recording and Record Keeping

The investigators will populate the content of participants' CRFs and all the study data will be recorded directly into an Electronic Data Capture (EDC) system (e.g. OpenClinica, REDCap, or similar) or onto a paper source document for later entry into EDC if direct entry is not available. Any additional information that needs recording but is not relevant for the CRF (such as signed consent forms etc.) will be recorded on a separate paper source document. All documents will be stored safely and securely in confidential conditions.

The EDC system (CRF data) uses a relational database (MySQL/ PostgreSQL) via a secure web interface with data checks applied during data entry to ensure data quality. The database includes a complete suite of features which are compliant with GCP, EU and UK regulations and Sponsor security policies, including a full audit trail, user-based privileges, and integration with the institutional LDAP server. The MySQL and PostgreSQL database and the webserver will both be housed on secure servers maintained by Oxford Vaccine Group IT personal and local site IT personal. The servers are in a physically secure location in EU and data are backed up on secure servers operated by the University of Oxford IT Services physically located in EU zone. Backups will be stored in accordance with the IT department schedule of daily, weekly, and monthly retained for one month, three months, and six months, respectively. The IT servers provide a stable, secure, well-maintained, and high capacity data storage environment. REDCap and OpenClinica are widely-used, powerful, reliable, well-supported systems. Access to the study's database will be restricted to the members of the study team by username and password.

Participant's personally identifiable information will be stored in a separate password protected Access databased saved on a secure University of Oxford server. Only Oxford staff have access to the Access database and are permitted for data entry.

Each study participant will have a unique participant number which will be allocated at the time a screening visit is booked and all names and/or identifying details are not included in any study data electronic file. After enrolment the participants will be identified by a study specific participants number and/or code. Samples sent to laboratories for processing will be identified by trial number and participant number only.

The study team will use names and contact details to contact participants about the research study, and make sure that relevant information about the study is recorded for their care, in relation to their health during the study and to oversee the quality of the study. At the completion of the study, unless participants consent otherwise (e.g. requesting to be informed of other trials), participant's personal details will not be used to contact them other than exceptional circumstances concerning their safety. If consent is provided by participants to take part in another study carried out by the study site, personal information and medical information including blood test results may be accessed to avoid unnecessary repetition. If participants provide specific consent, we will use personal identifiable data to invite participants for future research.

Bank details will be stored for 7 years in line with University financial policy.

13.3.1 Data integrity

Data collection and storage will be inspected throughout the study by performed by the Oxford Vaccine Group and monitoring will be carried out by the study Sponsor, University of Oxford RGEA.

13.3.2 Data archiving and storage

Study data may be stored electronically on a secure server, and paper notes will be kept in a key-locked filing cabinet at the site. All essential documents will be retained for a minimum of 5 years after the study has finished. Volunteers who complete online screening or telephone screening only (before informed consent) will not have data kept beyond the end of the trial. The need to store study data for longer in relation to licensing of the vaccine will be subject to ongoing review. For effective vaccines that may be licensed, we may store research data securely at the site at least 15 years after the end of the study, subject to adjustments in clinical trials regulations. Participants' bank details will be stored for 7 years in line with the site financial policy. De-identified research data maybe be stored indefinitely.

General archiving procedures will be conducted in compliance to SOP OVC020 Archiving.

14. QUALITY ASSURANCE PROCEDURES

14.1. Investigator procedures

The study will be conducted in accordance with the current approved protocol, GCP, relevant regulations and standard operating procedures (SOPs). Approved and relevant SOPs will be used at all clinical and laboratory sites.

14.2. Risk Assessment

A risk assessment and monitoring plan will be prepared before the study opens and will be reviewed as necessary over the course of the trial to reflect significant changes to the protocol or outcomes of monitoring activities.

14.3. Monitoring

Monitoring will be performed according to Good Clinical Practice (GCP) guidelines by RGEA. Following written SOPs, the monitors will verify that the clinical trial is conducted and data are generated, documented and reported in compliance with the protocol, GCP and the applicable regulatory requirements. The investigator sites will provide direct access to all trial related source data/documents and reports for the purpose of monitoring and auditing by the sponsor and inspection by local and regulatory authorities

14.4. Protocol deviation

A trial related deviation is a departure from the ethically approved trial protocol or other trial document or process (e.g. consent process or IMP administration) or from Good Clinical Practice (GCP) or any applicable regulatory requirements. Any deviations from the protocol will be documented in a protocol deviation form and filed in the trial master file.

14.5. Audit & Inspection

The QA manager conducts systems based internal audits to check that trials are being conducted according to local procedures and in compliance with GCP and applicable regulations.

The Sponsor, trial sites, and ethical committee(s) may carry out audits to ensure compliance with the protocol, GCP and appropriate regulations.

GCP inspections may also be undertaken by the MHRA to ensure compliance with protocol and the Medicines for Human Use (Clinical Trials) Regulations 2004, as amended. The Sponsor will assist in any inspections and will support the response to the MHRA as part of the inspection procedure.

14.6. Trial Progress

The progress of the trial will be overseen by the Chief Investigator.

15. SERIOUS BREACHES

The Medicines for Human Use (Clinical Trials) Regulations contain a requirement for the notification of "serious breaches" to the MHRA within 7 days of the Sponsor becoming aware of the breach.

A serious breach is defined as “A breach of GCP or the trial protocol which is likely to affect to a significant degree –

(a) the safety or physical or mental integrity of the subjects of the trial; or

(b) the scientific value of the trial”.

In the event that a serious breach is suspected the Sponsor must be contacted within 1 working day. In collaboration with the C.I., the serious breach will be reviewed by the Sponsor and, if appropriate, the Sponsor will report it to the REC committee, Regulatory authority and the NHS host organisation within seven calendar days.

16. ETHICAL AND REGULATORY CONSIDERATIONS

16.1. Declaration of Helsinki

The Investigator will ensure that this trial is conducted in accordance with the principles of the Declaration of Helsinki.

16.2. Guidelines for Good Clinical Practice

The Investigator will ensure that this trial is conducted in accordance with relevant regulations and with Good Clinical Practice.

16.3. Approvals

Following sponsor approval, the protocol, informed consent form, participant information sheet and any proposed advertising material will be submitted to an appropriate Research Ethics Committee (REC), Health Research Authority (HRA) (where required), regulatory authorities (MHRA in the UK), and host institution(s) for written approval.

The Investigator will submit and, where necessary, obtain approval from the above parties for all substantial amendments to the original approved documents.

16.4. Reporting

The CI shall submit once a year throughout the clinical trial, or on request, an Annual Progress Report to the REC, HRA (where required), host organisation and Sponsor. In addition, an End of Trial notification and final report will be submitted to the MHRA, the REC, host organisation and Sponsor.

16.5. Participant Confidentiality

The study staff will ensure that participants' anonymity is maintained. Study participants will be identified by initials and a participant ID number on the CRF. Two study identifiers are used to prevent errors in documentation and avoid mislabelling of samples. Any electronic databases and documents with participant identifying details will be stored securely and will only be accessible by study staff and authorised personnel. The study will comply with the UK General Data Protection Regulation (GDPR) and Data Protection Act 2018, which require data to be de-identified as soon as it is practical to do so.

16.6. Participant Reimbursement

Each participant is compensated for their time and for the inconvenience based on the following figures:

- Travel expenses: £15 per visit
- Inconvenience of blood tests: £10 per visit
- Time required for visits: £20 per visit

. Payments will be made in instalments as follows:

- Group 1 - after V0, V5, V10 and V16
- Group 2 – after V0, V5, V8 and V16.
- Group 3 – after V0, V5, V10 and V16

Each participant can therefore receive £495– £630 depending on whether they are in Group 1, 2 or 3. Remuneration is on a *pro rata* basis, should a participant fail to complete all visits and/or study requirements.

Additional reimbursement for unscheduled visits at £45 per visit will be provided up to maximum of £135 (the equivalent of three unscheduled visits). This will not be given unless an unscheduled visit occurs.

16.7. Incidental Findings

It is possible that during the study, we will detect incidental findings at screening or during the trial unrelated to the trial but of potential health concern to the participant (e.g. high blood pressure). The participant will be informed of this and, with their permission, their GP will be informed to provide any necessary follow-up as per local SOPs.

16.8. Transparency in Research

Prior to the recruitment of the first participant, the trial will have been registered on a publicly accessible database. Results will be uploaded to the European Clinical Trial (EudraCT) Database within 12 months of the end of trial declaration by the CI or their delegate. Where the trial has been registered on multiple public platforms, the trial information will be kept up to date during the trial, and the CI or their delegate will upload results to all those public registries within 12 months of the end of the trial declaration.

17. FINANCE AND INSURANCE

17.1. Funding

The clinical trial is funded by Innovate UK.

17.2. Insurance

The University has a specialist insurance policy in place which would operate in the event of any participant suffering harm as a result of their involvement in the research (Newline Underwriting Management Ltd, at Lloyd's of London). NHS indemnity operates in respect of the clinical treatment that is provided.

17.3. Contractual arrangements

Appropriate contractual arrangements will be put in place with all third parties.

18. PUBLICATION POLICY

Authorship of any publications arising from this study should fulfil the following criteria:

- Made a substantial contribution to the conception or design of the work; or the acquisition, analysis or interpretation of data for the work; and
- Drafted or substantively reviewed or revised the publication; and
- Approved the final version of the publication; and
- Agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work could be appropriately investigated and resolved.

The Investigator will co-ordinate dissemination of data from this study. All publications (e.g., manuscripts, abstracts, oral/slide presentations, book chapters) based on this study will be reviewed by each sub-investigator prior to submission.

19. DEVELOPMENT OF A NEW PRODUCT/ PROCESS FOR THE GENERATION OF INTELLECTUAL PROPERTY

Ownership of IP generated by employees of the University vests in the University. The University will ensure appropriate arrangements are in place as regards any new IP arising from the trial.

20. REFERENCES

1. Andrianaivoarimanana V, Piola P, Wagner DM, Rakotomanana F, Maheriniaina V, Andrianalimanana S, et al. Trends of Human Plague, Madagascar, 1998-2016. Emerg Infect Dis. 2019;25(2):220-8.

2. Derbise A, Guillas C, Gerke C, Carniel E, Pizarro-Cerdà J, Demeure CE. Subcutaneous vaccination with a live attenuated Yersinia pseudotuberculosis plague vaccine. Vaccine. 2020;38(8):1888-92.

3. Randremanana R, Andrianaivoarimanana V, Nikolay B, Ramasindrazana B, Paireau J, ten Bosch QA, et al. Epidemiological characteristics of an urban plague epidemic in Madagascar, August–November, 2017: an outbreak report. The Lancet Infectious Diseases. 2019;19(5):537-45.

4. Galimand M, Carniel E, Courvalin P. Resistance of Yersinia pestis to Antimicrobial Agents. Antimicrobial Agents and Chemotherapy. 2006;50(10):3233.

5. Baril L, Vallès X, Stenseth NC, Rajerison M, Ratsitorahina M, Pizarro-Cerdá J, et al. Can we make human plague history? A call to action. BMJ Global Health. 2019;4(6):e001984.

6. Williamson ED, Oyston PC. Protecting against plague: towards a next-generation vaccine. Clin Exp Immunol. 2013;172(1):1-8.

7. Top FH, Jr. Control of adenovirus acute respiratory disease in U.S. Army trainees. Yale J Biol Med. 1975;48(3):185-95.

8. Afolabi MO, Tiono AB, Adetifa UJ, Yaro JB, Drammeh A, Nebie I, et al. Safety and Immunogenicity of ChAd63 and MVA ME-TRAP in West African Children and Infants. Mol Ther. 2016;24(8):1470-7.

9. Morris SJ, Sebastian S, Spencer AJ, Gilbert SC. Simian adenoviruses as vaccine vectors. Future Virol. 2016;11(9):649-59.

10. Xiang Z, Li Y, Cun A, Yang W, Ellenberg S, Switzer WM, et al. Chimpanzee adenovirus antibodies in humans, sub-Saharan Africa. Emerg Infect Dis. 2006;12(10):1596-9.

11. Tatsis N, Blejer A, Lasaro MO, Hensley SE, Cun A, Tesema L, et al. A CD46-binding chimpanzee adenovirus vector as a vaccine carrier. Mol Ther. 2007;15(3):608-17.

12. Dicks MD, Spencer AJ, Edwards NJ, Wadell G, Bojang K, Gilbert SC, et al. A novel chimpanzee adenovirus vector with low human seroprevalence: improved systems for vector derivation and comparative immunogenicity. PLoS One. 2012;7(7):e40385.

13. Milligan ID, Gibani MM, Sewell R, Clutterbuck EA, Campbell D, Plested E, et al. Safety and Immunogenicity of Novel Adenovirus Type 26- and Modified Vaccinia Ankara-Vectored Ebola Vaccines: A Randomized Clinical Trial. Jama. 2016;315(15):1610-23.

14. Folegatti PM, Ewer KJ, Aley PK, Angus B, Becker S, Belij-Rammerstorfer S, et al. Safety and immunogenicity of the ChAdOx1 nCoV-19 vaccine against SARS-CoV-2: a preliminary report of a phase 1/2, single-blind, randomised controlled trial. The Lancet. 2020;396(10249):467-78.

15. Jerald Sadoff, Els De Paepe, Wouter Haazen, Edmund Omoruyi, Arangassery R Bastian, Christy Comeaux, Esther Heijnen, Cynthia Strout, Hanneke Schuitemaker, Benoit Callendret, Safety and Immunogenicity of the Ad26.RSV.preF Investigational Vaccine Coadministered With an Influenza Vaccine in Older Adults, The Journal of Infectious Diseases, jiaa409

16. Zhu FC, Li YH, Guan XH, et al. Safety, tolerability, and immunogenicity of a recombinant adenovirus type-5 vectored COVID-19 vaccine: a dose-escalation, open-label, non-randomised, first-in-human trial. Lancet. 2020;395(10240):1845-1854.

17. Zhu FC, Guan XH, Li YH, Huang JY, Jiang T, Hou LH, Li JX, Yang BF, Wang L, Wang WJ, Wu SP, Wang Z, Wu XH, Xu JJ, Zhang Z, Jia SY, Wang BS, Hu Y, Liu JJ, Zhang J, Qian XA, Li Q, Pan HX, Jiang HD, Deng P, Gou JB, Wang XW, Wang XH, Chen W. Immunogenicity and safety of a recombinant adenovirus type-5-vectored COVID-19 vaccine in healthy adults aged 18 years or older: a randomised, double-blind, placebo-controlled, phase 2 trial. Lancet. 2020 Aug 15;396(10249):479-488. doi: 10.1016/S0140-6736(20)31605-6. Epub 2020 Jul 20.

18. Tapia MD, Sow SO, Mbaye KD, et al. Safety, reactogenicity, and immunogenicity of a chimpanzee adenovirus vectored Ebola vaccine in children in Africa: a randomised, observer-blind, placebo-controlled, phase 2 trial. Lancet Infect Dis. 2020;20(6):719-730.

19. Zoulim F, Fournier C, Habersetzer F, et al. Safety and immunogenicity of the therapeutic vaccine TG1050 in chronic hepatitis B patients: a phase 1b placebo-controlled trial. Hum Vaccin Immunother. 2020;16(2):388-399.

20. Graham, S.P., McLean, R.K., Spencer, A.J. et al. Evaluation of the immunogenicity of prime-boost vaccination with the replication-deficient viral vectored COVID-19 vaccine candidate ChAdOx1 nCoV-19. npj Vaccines 5, 69 (2020).

21. APPENDIX A: GRADING THE SEVERITY OF ADVERSE EVENTS

Labelling of an AE as severe will be defined by the severity threshold highlighted in each table.

Table 9. Grading of Foreseeable and Unforeseeable AEs

Adverse event	Grade	Definition
Foreseeable: Injection site reaction (pain, tenderness, itch, warmth), muscle pain, joint pain, headache, feverishness, fever, chills, malaise, fatigue, nausea, vomiting and rash	0	Absence or resolution of symptom
	1	Awareness of symptom but tolerated; transient or mild discomfort; little or no medical intervention required
	2	Discomfort enough to cause limitation of usual activity; some medical intervention or therapy required
	3 (severe)	Significant interference with daily activity
	4 (severe)	Emergency department visit or hospitalisation
Injection Site reaction (redness, hardness and swelling)	0	No reaction
	1	1 to ≤10mm
	2	11 to ≤25mm
	3 (severe)	26 to ≤50mm
	4 (severe)	51 to ≤ 100mm
	5 (severe)	>100mm
Unforeseeable AEs	0	Absence or resolution of medical issue
	1	Awareness of medical issue but tolerated; transient or mild discomfort; little or no medical intervention required
	2	Discomfort enough to cause limitation of usual activity; some medical intervention or therapy required
	3 (severe)	Significant interference with daily activity

	4 (severe)	Emergency department visit or hospitalisation

Table 10. Grading of observations AEs

Observation	Grade 1	Grade 2	Grade 3 (severe)
Oral temperature (°C)	37.6 – 38.0	38.1 – 39.0	39.1 or greater
Tachycardia (beats/min)	101-115	116-130	>130
Bradycardia (beats/min)	50-54	45-49	<45
Systolic hyper-tension (mmHg)	141-150	151-155	>155
Diastolic hyper-tension (mmHg)	91-95	96-100	>100
Systolic hypo-tension (mmHg)	85-89	80-84	<80

The following ranges are considered normal physiological ranges and are considered grade 0:

Oral temperature between 35.5 and 37.5°C

Resting heart rate between 55 and 100 beats/minute

Systolic blood pressure between 90 and 140 mmHg

Table 11. Grading of laboratory AEs

	Grade 1 (Mild)	Grade 2 (Moderate)	Grade 3 (Severe)	Grade 4 (Potentially life threatening)
Haemoglobin (female): decrease from baseline value (g/l)	10- 15	16-20	21-50	>50
Haemoglobin (male): decrease from baseline value (g/l)	10-15	16-20	21-50	>50
Haemoglobin (female): absolute value (g/l)	110 – 119	95 – 109	80 – 94	< 80
Haemoglobin (male): absolute value (g/l)	125 129	105 – 124	85 – 104	< 85
White Blood Cells elevated ($x10^9$)	11.5 – 15	>15 - 20	>20 - 25	>25
White Blood Cells low ($x10^9$)	2.5 – 3.5	1.5 – 2.49	1 – 1.49	<1
Lymphocytes	0.75 - 0.99	0.5 - 0.74	0.25 – 0.49	<0.25
Eosinophils	0.65 - 1.5	1.51 – 5.00	>5.00	Hypereosinophilia
Neutrophils	1.5 – 1.99	1.0 - 1.49	0.5 – 0.99	<0.50
Platelets	125 - 140	100 - 124	25 - 99	<25
Sodium: hyponatraemia (mmol/L)	132–134	130–131	125–129	<125
Sodium: hypernatraemia (mmol/L)	146	147	148–150	>150
Potassium: hyperkalaemia (mmol/L)	5.1–5.2	5.3–5.4	5.5–5.6	>5.6
Potassium: hypokalaemia (mmol/L)	3.4	3.3	3.1–3.2	<3.1
Urea (mmol/L)	8.2–8.9	9.0–11	>11	RRT
Creatinine (µmol/L)	114-156	157-312	>312	RRT
ALT and/or AST (IU/L)	1.1–2.5 x ULN	>2.5–5.0 x ULN	>5.0-10 x ULN	>10 x ULN
Bilirubin, with increase in LFTs (µmol/L)	1.1–1.25 x ULN	>1.25–1.5 x ULN	>1.5–1.75 x ULN	>1.75 x ULN
Bilirubin, with normal LFTs (µmol/L)	1.1–1.5 x ULN	>1.5–2.0 x ULN	>2.0–3.0 x ULN	>3.0 x ULN
Alkaline phosphatase (IU/L)	1.1–2.0 x ULN	>2.0–3.0 x ULN	>3.0–10 x ULN	>10 x ULN
Albumin: hypoalbuminaemia (g/L)	28–31	25–27	<25	Not applicable
C-reactive protein	>10-30	31-100	101-200	>200

Severity grading criteria for clinically significant laboratory abnormalities (adapted from FDA guidelines using OUH NHS Trust laboratory reference ranges)

22. Appendix B: WHO Clinical Progression Scale for clinical studies of COVID-19

Patient State	Descriptor	Score
Uninfected	Uninfected; no viral RNA detected	0
Ambulatory mild disease	Asymptomatic; viral RNA detected	1
	Symptomatic; independent	2
	Symptomatic; assistance needed	3
Hospitalised: moderate disease	Hospitalised; no oxygen therapy*	4
	Hospitalised; oxygen by mask or nasal prongs	5
Hospitalised: severe diseases	Hospitalised; oxygen by NIV or high flow	6
	Intubation and mechanical ventilation, pO_2/FiO_2 ≥150 or SpO_2/FiO_2 ≥200	7
	Mechanical ventilation pO_2/FIO_2 <150 (SpO_2/FiO_2 <200) or vasopressors	8
	Mechanical ventilation pO_2/FiO_2 <150 and vasopressors, dialysis, or ECMO	9
Dead	Dead	10

23. APPENDIX C: AMENDMENT HISTORY

Amendment No.	Protocol Version No.	Date issued	Author(s) of changes	Details of Changes made
1	V2.0	01-December-2020	Robert Shaw, Arabella Stuart, Christine Rollier	1. Changes as per MHRA comments: Section 5.7.2 amended to provide a rationale for the choice of species used in the toxicology study, as per MHRA comments. Addition of Table 2. Toxicity studies on ChAdOx1-vectored experimental vaccines and clinical studies. 2. Section 5.7.2 and 5.7.8 amended to contain a justification of the safety of the booster dose (MHRA comments)

				3. Section 8.4: Wording changed slightly to clarify that enrolment is when prime is given and 'subsequent vaccination' refers to boosts. Amended wording re "screening abnormalities" to "laboratory AEs" (MHRA comments) 4. Section 9.11: amended to clarify that participants who develop severe COVID-19 will be withdrawn from receiving a boost dose. Each case will be evaluated by a clinician before proceeding to boost (MHRA comments) 5. Section 7.2.1 reworded the dosing schedule section to highlight the role that the senior investigator and DSMC will play in determining whether vaccination should continue, in keeping with the exact approach and protocol used in the most recent FIH with the same vector COV001(MHRA comments) 6. Section 7.2.2 amended in line with the new wording of the dosing schedule section (as per MHRA comments) 7. Section 7.7 to clarify quality assurance procedures can be found detailed in Section 14 (MHRA comments) 8. Section 8.4 and 9.11 amended to replace "positive PCR test" with positive SARS-CoV-2 test 9. Section 8.5 changed "highly effective" to "effective" contraception (as per MHRA comments) 10. Section 9.13 amended to clarify that if the reason for discontinuation/ withdrawal is not safety event or toxicity, of the first 10 participants recruited to Group 1, those who have been withdrawn from the study can be replaced if they have not reached the Day 28 time point. We may replace the other participants in the trial if they do not reach the

				Day 14 time point (MHRA comments) 11. Section 9.14 amended to clarify that the end of the trial will be defined as the date of the last visit of the last patient undergoing the trial, or the completion of the last laboratory assay on the last participant sample, whichever is later 12. Section 10.4 amended to clarify that there is no expected interaction between the currently allowed prescribed concomitant medications (not immunosuppressants) and ChAdOx1 Plague that could justify a restriction on prescribed medication. We would not restrict volunteers from accessing medical care during the study, and would not restrict the use of needed prescribed medication (MHRA comments) 13. Section 11.10: paragraph added to clarify that any further dosing in the clinical trial will be halted in case of severe adverse reactions, as per MHRA comments 14. Section 11.1 amended to clarify that when an individual stopping rule is reached, this will stop further IMP treatment but an adequate safety monitoring will continue as appropriate, as per MHRA comments 15. Section 11.3 amended to clarify that all SAEs will be collected throughout the entire trial period and that also a laboratory abnormality will be considered an AE if considered clinically significant at the investigator judgement. (MHRA comments)
2	V2.1	02-December-2020	Robert Shaw, Arabella Stuart, Christine Rollier	Changes as per REC comments: 1. Change the reference to antibody instead of antigen in the text about immunogenicity as per REC comments in tables 3.Synopsis and 6. Objectives and Outcome measures

3	V2.2	17-December-2020	Robert Shaw, Arabella Stuart	Changes as per MHRA comments: 1. Section 9.11 updated to define moderate-severe illness; to clarify that participants with moderate-severe disease will not receive further IMP; to confirm that a participant is fully recovered the investigator must conduct a complete physical examination and Oxygen Saturation assessment, which must be normal; to clarify that clinical discretion is only relevant in cases of mild or asymptomatic disease; to clarify that participants) will be counselled when they are offered a booster. 2. Section 8.4 amended to align with changes in section 9.11 3. Appendix B. WHO Clinical Progression Scale for clinical studies of COVID-19 added
4	V3.0	22-April-2021	Arabella Stuart, Robert Shaw	1. Change to group 3 with high dose boost removed and dosing interval changed to D182 2. Section 7.1: Figure 7 updated to reflect group changes 3. Section 7.1: Tables 3, 4 and 5 updated to reflect group changes 4. Section 9.12:new tables of blood sampling created (6,7,8) to reflect changes 5. Increase in target sample size for group 3 from 10 to 15 participants. 6. Lengthening of post-asymptomatic SARS-CoV-2 positivity boost delay period to 4 weeks from 2 weeks to align with UK Green Book guidance 7. Correction of various typographical and formatting errors throughout document 8. Addition of vaping history as information to be collected in section 9.2 screening. Updated wording and clarification added to the GP letter request consent process.

				9. Addition of wording re mobile units as possible location for vaccination, Section 9.5 10. Removal of specification that asymptomatic urinalysis findings will be sent for microbiological investigation (not in keeping with national infection guidance). Changes in Section 9.2 and 9.12.2 11. Section 9.12.2 Addition of clarification that urine will be screened for blood protein and glucose at screening. 12. Section 7.5 Removal of requirement to take SAM strip samples before phlebotomy – no rationale. 13. Clarification to Synopsis 3 that duration of follow up per participant is 12 months from prime 14. Removal of exclusion criteria of having had a previous Ad or ChAd vectored vaccine. There is not felt to be any immunological benefit in excluding these participants, and no safety concerns in including them. 15. Removal of "safety bloods" at D1 post vaccination – not felt to be clinically useful timepoint 16. Amendment of temporary exclusion criteria of confirmed SARS-CoV-2 infection from 2 to 4 weeks 17. Section 12.4 – removal of requirement for laboratory staff blinding and relabelling of samples to enable this. This is a difficult procedure that frequently leads to errors and is of no benefit in this un-blinded phase I study. It will not improve the integrity of the results, therefore removed. 18. Section 9.1 – university and hospital circulars added to recruitment strategy. Clarification that volunteers will be directed to submit expressions of interest via a website.

				19. Added specific wording to state that COVID-19 vaccination history will be checked at screening (in addition to all vaccination history) 20. Change in exclusion time period criteria for receipt of another non-study vaccine from 4 to 3 weeks. This is felt to be both a) clinically appropriate in terms of minimising cross reactogenicity and b) immunologically appropriate. This would apply to any COVID-19 vaccinations also. 21. Section 16.6 – reimbursement update 22. Removal of requirement for ECG at screening – not felt to be clinically useful in young healthy adults 23. Tables 3,4,5 visit schedules amended. Revised in light of new optimum scheduling for collection of immunology samples, and rationalisation of some safety blood timepoints. This means there are an increased number of visits for groups 2&3 (14) and a reduced number for group 1 (10 visits) 24. Visit 2 removed for Group 1. Not required for immunology or safety purposes. Participants will be completing ediaries daily which will be checked daily by the clinical team and allow detection of any potential clinical issues. There is no biological rationale for "safety bloods" at this timepoint as vaccine-related changes in blood indices would not be apparent at day 1 post vaccine. 25. Section 13.3.2 – additional statement added clarifying that for volunteers who complete online or telephone screening but progress no further, data will not be kept beyond the end of the trial. 26. Update to the group holding rules in Section 11.10 in order to clarify them and related processes. Removal of statement in relation

				to severe adverse reactions, as contradictory with existing holding rules and lacked clarity. 27. Clarification in section 7.2 in relation to triggers for dose de-escalation. This will now be related to group holding rules. 28. Addition of Section 11.14 – statement to clarify that participants and GPs will be updated should IMP or backbone related safety signals arise during the trial 29. Updates to Table 1 in relation to enrolment numbers for the COV ChAdOx1 nCoV19 trials. New Section 7.7.3 added with information on events of thrombotic thrombocytopaenia in people who have received ChAdOx1 nCoV-19 and relationship to this study.
5	V3.1	24-May-2021	Arabella Stuart, Robert Shaw	Changes as per MHRA GNA for SA1: Re-instated the following sections: 1. Section 7 - Table 3: Visit 2 reinstated (1 day post-dose) in Group 1 to assess as a minimum the participant's health status, AEs and vital signs. All Tables - the ECG listed at screening 2. Section 9.2: Re-instate the ECG assessment at screening in all study groups 3. Section 11.10: a) Reinstate the removed stopping criteria as follows: If an SAE occurs in any one individual, which is possibly, probably or definitely related to vaccination this would trigger a holding rule (at group and individual level). b) Stopping criteria related to severe adverse reactions reinstated as per MHRA request. Non-MHRA response 4. Section 9.12: Amendment of typographical blood volume errors that has led to up to 10ml increase for group 1 and 12ml for groups 2&3 in the total amounts of volume taken

				5. Section 7.1: in table 3, table 4 and table 5, changed 'visit number' to be 'visit name'.
6	V3.2	29-07-2021	Robert Shaw	1. Table 11 – Correction of typographical errors in laboratory AE grading table 2. Section 10.2 – typographical error storage temperature -70 to -85
7	V3.3	27/08/2021	Arabella Stuart	3. Amendment of wording in Section 8.3, change from "enter the study" to "be enrolled" in order to clarify the meaning" and avoid confusion 4. Table 11 Grading of lab AEs – correction of typographical error causing overlap of Grade 3 and Grade 4 classifications
8	V3.4	19/11/2021	Arabella Stuart	1. Section 6, Timepoints: correction of ediary collection days from days 0-6 to days 0-7 in line with current practice across trials. Solicited data for the 8 days has been collected for all participants since the first vaccination of the trial. 2. Removal of D1 in Safety blood tests following each vaccination as per amendment 4. The relevant tables/schedules, and blood sampling volume details in the protocol and SIB were updated with these changes as per Substantial Amendment 1 (12/03/2021) 3. DSMB corrected to DSMC throughout – typographical error.. 4. Section 7.4, correction in wording relating to which visits have safety bloods. Correction of typo from Table 5 to Table 4. 5. Section 7.2.1 – removal of specification of review of "D2" safety blood timepoint. This timepoint had safety bloods removed from it in

				amendment 4, text had not been updated correctly. Visit numbers added to the safety blood test days for clarity. 6. Table 11. Typographical error in Hb decrease from baseline value corrected so that grade 1 has a lower parameter (10).This is to prevent all changes and normal fluctuations from being graded as AEs, and allows only the potentially clinically significant changes to be graded. 7. Section 9.5 updated to include information on GP enrolment notification. 8. Section 16.6. Payment triggers for all groups have been updated, so each group is paid at four timepoints throughout the study. The paragraphs within this section have been rearranged for clarity. 9. Section 7.7.1 – wording re isolation/quarantine clarified to ensure alignment throughout protocol. 10. Section 9.11 – COVID-19 symptom list de-specified, given changes to government testing criteria and potential for future changes. 11. Section 9.8 – wording corrected for clarity in use of terms SARS-CoV-2 and COVID-19, and difference between self-isolation and quarantine. 12. References to CTRG have been replaced with 'RGEA' throughout. 13. Section 9.2 – OVC biobank REC name and number updated due to renewal.