A phase I study to determine the safety and immunogenicity of a bivalent ChAdOx1 vectored vaccine against Zaire and Sudan Ebola virus species in UK healthy adult volunteers

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Full Study Title	A phase I study to determine the safety and immunogenicity of a bivalent ChAdOx1 vectored vaccine against Zaire and Sudan Ebola virus species in UK healthy adult volunteers
Short Study Title	A study of a new vaccine against two types of Ebola
Study Code	EBL07
IRAS Project ID	301075
ClinicalTrials.gov NCT number	NCT05079750
REC Details	REC reference: 21/FT/0108 Fast Track REC Email: fasttrack.rec@hra.nhs.uk
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1 SYNOPSIS

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<tr><td>Trial Site</td><td>Centre for Clinical Vaccinology & Tropical Medicine, University of Oxford, Churchill Hospital, Old Road, Headington, Oxford, OX3 7LE</td></tr>
<tr><td>Trial code</td><td>EBL07</td></tr>
<tr><td>Study Design</td><td>Open–label, non-randomised, dose escalation, first-in-human, single centre, phase I clinical trial</td></tr>
<tr><td>Population</td><td>Healthy adults aged 18 – 55 years</td></tr>
<tr><td>Planned Sample Size</td><td>26 volunteers
<table><tr><th>Group</th><th>Treatment</th></tr><tr><td>Group 1 (n=6)</td><td>Single IM Dose ChAdOx1 biEBOV 5 × 10^9 vp</td></tr><tr><td>Group 2 (n=6-9*)</td><td>Single IM Dose ChAdOx1 biEBOV 2.5 × 10^{10} vp</td></tr><tr><td>Group 3 (n=11-14*)</td><td>Two IM Doses ChAdOx1 biEBOV 5 × 10^{10} vp, 12 weeks apart</td></tr></table>*Group 2 may be increased to 9 participants following interim safety reviews. In this case group 3 will consist of 11 rather than 14 participants.</td></tr>
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<tr><td>Secondary Objective</td><td>To assess the immunogenicity of ChAdOx1 biEBOV in healthy adult volunteers</td></tr>
<tr><td>Investigational Product</td><td>ChAdOx1 biEBOV</td></tr>
<tr><td>Dose per Administration</td><td>ChAdOx1 biEBOV 5 × 10^9 vp
ChAdOx1 biEBOV 2.5 × 10^{10} vp
ChAdOx1 biEBOV 5 × 10^{10} vp</td></tr>
<tr><td>Form</td><td>Liquid</td></tr>
<tr><td>Route</td><td>Intramuscularly (IM) into the deltoid region of the arm</td></tr>
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2 SCHEDULE OF PROCEDURES TABLE

2.1 Schedule of Procedures Table: Screening Visit

		Protocol Section
Visit Number	S	
Visit Type	Screening	7.5.1
Timeline	0 to 90 days before D0	
Visit Procedures		
Informed Consent	X	6.4
ID Check and Photocopy	X	
Review inclusion and exclusion criteria	X	6.5
Record Demographic Data	X	7.2
Medical History	X	7.3
Vital signs (Heart rate, Temperature, Blood Pressure)	X	7.4
Screening Physical Examination	X	7.5
TOPS Registration *www.tops.org.uk*	X	6.5.4
Urine Samples		
Urinalysis	X	7.6.4
Urinary HCG (WOCBP only)	X	7.6.4
Stool Samples (Optional)		
Sample containers and instructions provided	X	3.7 and 7.6.3
Blood Samples		
HBsAg, HCV Ab, HIV serology (mL)	5	7.6.1
Biochemistry, Haematology (mL)	5	7.6.1
Blood volume per visit (mL)	10	
Cumulative blood volume (mL)	10	

2.2 Schedule of Procedures Table: Vaccination and Follow up Visits (single dose schedule)

	Trial Period								Protocol Section
Visit Number	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8†**	
Visit Type	Vac	f/u	f/u	f/u	f/u	f/u	f/u	f/u	
Timeline	D0	D2	D7	D14	D28	D56	D182	D364	
Time window (days)		±1	±2	±3	±3	±7	±60	±60	
Visit Procedures									
Review contraindications, inclusion and exclusion criteria	X								6.5
Enrolment and Group Allocation	X								6.7
ChAdOx1 biEBOV vaccination	X								7.8
Vital signs (Heart rate, Temperature, Blood Pressure)	X	X	X						7.4
Targeted Medical History, Physical Examination	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	7.5
Adverse Event Collection									
Solicited AE collection	X	X	X						9.2
Unsolicited AE collection	X	X	X	X	X				9.3 and 9.5.2
SAEs & AESI collection	X	X	X	X	X	X	X	X	9.4 and 9.7.1
Review Ongoing AEs		X	X	X	X	X	X	X	
Electronic diary (eDiary)									
Electronic diary started	X								7.9
Electronic diary review		X	X	X	X				7.9
Electronic diary closed					X				7.9
Urine Samples									
Urinary HCG (WOCBP only)	X								7.6.4
Stool Samples (Optional)									
Home collection of stool samples	(X)			(X)			(X)	(X)	3.7 and 7.6.3
Blood Samples*									
HLA typing (mL)	4								7.6.1
Biochemistry, Haematology (mL)	5	5	5		5				7.6.1
Immunology (mL)	50		50	50	50	50	50	50	7.6.2
Blood volume per visit (mL)	59	5	55	50	55	50	50	50	
Cumulative blood volume (mL)	69	74	129	179	234	284	334	384	

() = Optional

*Minor differences in blood volumes may occur depending on the collection tubes and equipment used.

† Group 3 only (visit added in SA03)

2.3 Schedule of Procedures Table: Vaccination and Follow up Visits (two dose schedule)

	Trial Period												Protocol Section
Visit Number	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12†**	
Visit Type	Vac	f/u	f/u	f/u	f/u	f/u	Vac2	f/u	f/u	f/u	f/u	f/u	
Timeline (days)	D0	D2	D7	D14	D28	D56	D84	V2 +7	V2 +14	V2 +28	V2 +182	V2 +280	
Time window (days)		±1	±2	±3	±3	±7	D77-126	±2	±3	±3	±60	±60	
Visit Procedures													
Review contraindications, inclusion and exclusion criteria	X						X						6.5
Enrolment and Group Allocation	X												6.7
ChAdOx1 biEBOV vaccination	X						X						7.8
Vital signs (Heart rate, Temperature, Blood Pressure)	X	X	X				X		X				7.4
Targeted Medical History, Physical Examination	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	(X)	7.5
Adverse Event Collection													
Solicited AE collection	X	X	X				X	X					9.2
Unsolicited AE collection	X	X	X	X	X		X	X	X	X			9.3 and 9.5.2
SAEs & AESI collection	X	X	X	X	X	X	X	X	X	X	X	X	9.4 and 9.7.1
Review Ongoing AEs		X	X	X	X	X	X	X	X	X	X	X	
Electronic diary (eDiary)													
Electronic diary started	X						X						7.9
Electronic diary review**		X	X	X	X		X	X	X	X			7.9
Electronic diary closed					X					X			7.9
Urine Samples													
Urinary HCG (WOCBP only)	X						X						7.6.4
Stool Samples (Optional)													
Home collection of stool samples	(X)			(X)			(X)		(X)		(X)	(X)	3.7 and 7.6.3
Blood Samples*													
HLA typing (mL)	4												7.6.1
Biochemistry, Haematology (mL)	5	5	5		5		5	5	5	5			7.6.1
Immunology (mL)	50		50	50	50	50	50	-	50	70	50	50	7.6.2
Blood volume per visit (mL)	59	5	55	50	55	50	55	5	55	75	50	50	
Cumulative blood volume (mL)	69	74	129	179	234	284	339	344	399	474	524	574	

() = Optional *Minor differences in blood volumes may occur depending on the collection tubes and equipment used. ** eDiaries are remotely monitored in (near) real-time for the occurrence of grade ≥3 Aes. A remote review of all eDiary Aes will occur at 48hrs following V2 for the first 3 group 3 volunteers. † Group 3 only (visit added in SA03)

3 BACKGROUND & RATIONALE

3.1 Ebola Virus Disease (EBOD)

Ebola disease (EBOD) is a fatal viral haemorrhagic fever caused by 4 of the 6 known genus Ebolavirus species: Zaire ebolavirus (EBOV), Sudan ebolavirus (SUDV), Bundibugyo ebolavirus and Taï Forest ebolavirus. Low level endemicity of these viruses occurs in tropical regions of sub-Saharan Africa, where periodic outbreaks occur, likely initiated by zoonotic spill over events from local mammalian animal reservoirs prior to ongoing human-to-human transmission.

The WHO records 41 Ebolavirus outbreaks with the first occurring in 1976[1]. The average case fatality rate of EBOD is 50%, ranging between 25% and 90% for individual outbreaks[1]. The 2013-2016 west African outbreak, the largest to-date, was estimated to cause 28,000 cases with 11,000 deaths. In addition to mortality, survivors can face debilitating long term health sequelae[2]. Negative secondary effects on local healthcare systems can be large and persistent, likely contributing to further indirect morbidity and mortality during and after outbreaks[3]. Healthcare workers face very high risks of infection and death with 6 to 8% of the entire healthcare workforce of Liberia and Sierra Leone killed during the 2013-2016 outbreak[4].

To date outbreaks have been contained within relatively limited geographical areas. However, the possibility of imported cases via returning travellers, especially healthcare workers deployed in crises, can require vigilance and health surveillance in distant countries.

Survivors of EBOD are thought to acquire protective immunity to subsequent infection against the same species of ebolavirus[5]. It remains unknown, however, whether convalescent individuals acquire immunity to heterologous ebolavirus species. Cross-reactive binding antibodies[6,7] and T-cells[7] have been observed in some survivors although it is unclear whether these provide any protection against disease.

3.2 Zaire Ebolavirus (EBOV) and Sudan ebolavirus (SUDV)

Zaire ebolavirus and Sudan ebolavirus species have caused the vast majority of EBOD epidemics, with EBOV causing the greatest number of outbreaks (28 total), infections (33,645 total), and deaths (14,495 total)[1]. Five outbreaks have been attributed to SUDV, with the largest in 2000-2001, leading to 425 infections and 224 deaths[1]. Both viruses are within the Ebolavirus genus, part of the filovirus RNA virus family, which contains other more distantly related viruses that also cause viral haemorrhagic fever in humans, such as Marburg virus.

Human infection with either virus is usually catastrophic, although viral pathogenesis is incompletely understood. A suggested key early step in the infective process is the infection of dendritic cells and macrophages, leading to an initial spread of virus to regional lymphatics and immune dysfunction[8]. A wide range of tissues and cell types can become infected, leading to widespread organ dysfunction in addition to a severe systemic inflammatory reaction, shock and disseminated intravascular coagulation.

3.3 Currently Approved Vaccines

Two ebolavirus vaccine regimens are currently approved for use. rVSV-ZEBOV (Ebola Zaire vaccine live; trade name: Ervebo) and Ad26.ZEBOV/MVA-BN-Filo (trade name: Zabdeno/Mvabea). Both vaccine

regimens are approved specifically for preventative use against Zaire ebolavirus only, rather than other Ebolavirus species.

3.3.1 rVSV-ZEBOV (Ebola Zaire vaccine live; trade name: Ervebo)

rVSV-ZEBOV is a live recombinant vesicular stomatitis virus vectored vaccine expressing the native Zaire ebolavirus glycoprotein that received conditional marketing authorisation by the EMA in November 2019 and WHO pre-qualification shortly afterwards. The approval was granted based on several clinical studies conducted in Africa, Europe and North America including an open-label cluster RCT efficacy trial in Guinea during the 2014-2016 outbreak, which demonstrated high efficacy against EBOV[9].

3.3.2 Ad26.ZEBOV/MVA-BN-Filo (trade name: Zabdeno/Mvabea)

Ad26.ZEBOV is replication deficient adenovirus serotype 26 vector encoding the EBOV glycoprotein (Mayinga variant). MVA-BN-Filo is a multivalent MVA vector expressing four filovirus antigens: Zaire ebolavirus, Sudan ebolavirus, and Marburg virus glycoproteins; and nucleoprotein from the Tai Forest virus. The combination of Ad26.ZEBOV/MVA-BN-Filo were approved by the EMA as an 8-week 2-dose heterologous prime-boost vaccination schedule in May 2020[10]. Although MVA-BN-Filo encodes antigens from multiple filovirus species the authorisation is specifically for protection against EBOV only. Additionally, the authorisation was granted under "exceptional circumstances", without human efficacy data, being supported instead by NHP challenge studies, with human efficacy inferred through immunological bridging studies[11].

3.4 ChAdOx1 biEBOV

3.4.1 ChAdOx1 biEBOV vaccine

ChAdOx1 biEBOV consists of the replication-deficient simian adenovirus vector ChAdOx1 encoding two antigens: EBOV glycoprotein and SUDV glycoprotein under the control of a TetR-repressible CMV promoter. The ChAdOx1 vector is replication-deficient as the E1 gene region, essential for viral replication, has been deleted. This means the virus will not replicate in cells within the human body. The E3 locus is additionally deleted in the ChAdOx1 vector. ChAdOx1 propagates only in cells expressing E1, such as HEK293 cells and their derivatives, e.g. T-REx™-293 (InVitrogen) or, other cell lines expressing E1 gene region.

3.4.2 Preclinical studies

ChAdOx1 biEBOV has been shown to be immunogenic in mice, resulting in antibody and T-cell responses against both SUDV and EBOV glycoproteins. Additionally, vaccination has shown complete protection against disease in a guinea pig challenge study, using a guinea pig adapted EBOV strain.

A murine toxicology study (Covance SR79DS) carried out using the toxicology batch of ChAdOx1 biEBOV showed no evidence of ChAdOx1 biEBOV related toxicity after administration. Treatment with the vaccine was safe, well tolerated and not associated with any adverse effects.

Biodistribution studies have not been conducted with ChAdOx1 biEBOV although these have been conducted with two other ChAdOx1 vectored vaccines. In both studies, high levels of detectable vector were observed at the administration site at early timepoints, decreasing over time. Lower levels could also be detected at distant sites (heart, liver, ovary, testes), also decreasing with time.

Full details of preclinical studies using ChAdOx1 biEBOV are contained within the ChAdOx1 biEBOV Investigator Brochure.

3.4.3 Previous clinical experience

EBL07 is a first-in-human study employing ChAdOx1 biEBOV in healthy volunteers recruited in the UK. However, phase I clinical trials of ChAdOx1 vectored vaccines encoding antigens for Influenza (fusion protein NP+M1), Tuberculosis (85A), Prostate Cancer (5T4), Malaria (LS2), Chikungunya (structural polyprotein), Zika (prM and E), group B Meningococcus (outer membrane protein) and SARS-CoV-2 have previously been undertaken (refer to ChAdOx1 biEBOV Investigator Brochure) and shown the vaccine to be safe and immunogenic.

Additionally, ChAdOx1 nCoV-19 has now been approved widely and rolled out in a number of countries. The vaccine has been shown to be safe in the vast majority of individuals, although a rare but serious side effect of thrombosis with thrombocytopenia has been seen in a small number of individuals (see section 3.8.3).

3.5 Rationale for Vaccination

Multivalent vaccines covering multiple different filovirus species are attractive and the preferred approach of the WHO[12], offering the advantage of protection against a number of targeted species as well as decreasing manufacturing cost and reducing the overall risk compared to developing individual monovalent vaccines. NHP and guinea pig challenge studies have demonstrated monovalent Ebolavirus glycoprotein vaccines offer only partial cross-protection against heterologous ebolavirus species[13,14]. Although a multivalent MVA-vectored vaccine has been developed, this is currently only approved for use with a monovalent adenovirus prime and specifically against Zaire ebolavirus only (section 3.3.2). As such, the need for a multivalent vaccine, approved for use against multiple Ebolavirus species, remains unmet.

Adenovirus vaccines are highly scalable, with a well-developed existing manufacturing capability and supply chains that support a continuous uninterrupted supply of vaccine and the potential for rapid scale up in public health emergencies. Wild type replication competent oral human adenovirus vaccines, to protect against acute respiratory illness caused by adenovirus serotype 4 and 7, have been administered to millions of military personnel over decades in the USA with no safety issues[15].

ChAdOx1 nCoV-19 (AKA ChAdOx1-S) was shown to be safe, immunogenic and efficacious against COVID-19 disease in a number of clinical trials[16-19] and has now been approved in multiple territories as well as receiving WHO pre-qualification. Additionally, it has been administered to millions of individuals, including those with serious medical co-morbidities, across diverse populations. Post-approval studies following the vaccines rollout have demonstrated high levels of vaccine effectiveness against severe COVID-19 following a single dose only[20,21]. Safety surveillance of ChAdOx1 nCoV-19 has demonstrated a very rare association with a syndrome consisting of major thrombosis and thrombocytopenia (covered in section 3.8.3). This safety signal remains under intense investigation by regulators and researchers. These events remain rare and the vaccine continues to be recommended for use as the benefits against COVID-19 outweigh the risks of its use for the majority of populations.

In addition to ChAdOx1 nCoV-19, a further 320 trial volunteers have received other ChAdOx1 vectored vaccines encoding antigens against a range of targets in phase 1 trial settings (section 3.4.3). It has been shown to be highly immunogenic after single dose administration against the large number of inserts used across these trials. Further details of ChAdOx1 studies and associated publications are contained within the ChAdOx1 biEBOV IB.

ChAdOx1 has a proven record as a platform technology for the development of vaccines against emerging pathogens. Experience with ChAdOx1 nCoV-19 has shown the vaccine can be rapidly manufactured at high volume for low cost, with storage conditions amenable to use in the developing world. This study will provide valuable data on safety and immunological aspects of a novel multivalent ChAdOx1 vaccine.

3.6 Rationale for Selected Doses

Doses to be administered in this trial have been selected on the basis of clinical experience with the ChAdOx1 adenovirus vector expressing a different insert, and similar adenovirus vectored vaccines (e.g. ChAdOx1 and ChAd63). ChAdOx1 nCoV-19 has temporary authorisation in the UK under Regulation 174 at a dose of 5×10^{10} viral particles. It is well tolerated and immunogenic at this dose. Although authorised as a 2-dose schedule 4 to 12 weeks apart, the vaccine is immunogenic and efficacious following the first dose[19]. Phase III trials and post-authorisation effectiveness studies have shown a single 5×10^{10} vp dose of vaccine is protective against COVID-19 disease. Over 200 million 5×10^{10} viral particles doses of ChAdOx1 nCoV-19 have been administered worldwide as of June 2021.

For safety reasons, the first dose of ChAdOx1 biEBOV proposed in this study (5×10^{9} vp) is one tenth of the expected tolerated dose (5×10^{10} vp). Doses will be gradually increased aiming to identify an optimal dose of ChAdOx1 biEBOV considering the tolerability, reactogenicity and immunogenicity profiles.

3.6.1 Rationale for ChAdOx1 biEBOV in a homologous 12-week two dose schedule (SA02)

Experience with ChAdOx1 nCoV-19 has shown that a second dose of ChAdOx1 significantly boosts binding and neutralising antibody responses to SARS-CoV-2 and vaccine efficacy. A delayed 12-week schedule has been shown to be more immunogenic than a 4-week schedule. A second dose of ChAdOx1 nCoV-19 is well tolerated and produces lower rates of adverse reactions compared with the initial dose [17,22,23].

The ChAdOx1 biEBOV two-week repeat dose pre-clinical toxicology study (Covance SR79DS – summarised within ChAdOx1 biEBOV Investigator Brochure) supports the clinical evaluation of ChAdOx1 biEBOV in a two dose 12-week schedule in this clinical trial, as advised by WHO guidelines on nonclinical evaluation of vaccines[24].

3.7 The Influence of Gut Microbiota on Vaccine Responses

Humans and microbiota, which consist of bacteria, fungi, viruses and eukaryotic species, have co-evolved over millions of years and their coexistence is beneficial to both parties. The human immune system is constitutively exposed to microbial stimulation and any vaccine design and responsiveness needs to be considered in the context of host-microbiota interactions. Manipulation of the microbiota functions and composition through diet, engraftment and/or any other means may thus become a viable strategy for improving vaccine responsiveness as well as treating malfunctions of the immune system[25]. Reports on the influence of gut microbiota diversity and composition on responses to vaccination have been emerging for some time[25-29].

Analysis of faeces microbiota will allow a comprehensive yet non-invasive characterisation of the gut microbiome. A thorough characterisation of the gut microbiome composition and function will be performed using shotgun sequencing. Extensive metabolomic analyses, showing associations with microbial dysbiosis, may also be performed.

3.8 Potential Risks for Participants

The potential risk to participants is considered as low. The potential risks are those associated with phlebotomy and vaccination. In general, recombinant adenoviral vectors are safe. Similar vaccines encoding different antigens have been given to several thousand volunteers (including children) with a good safety profile. Post-marketing experience with ChAdOx1 nCoV-19 has uncovered a very rare but serious side effect of thrombosis with thrombocytopenia following use of that vaccine.

3.8.1 Phlebotomy

The maximum volume of blood drawn over the study period (Section 2) should not compromise these otherwise healthy volunteers. There may be minor bruising, local tenderness or pre-syncopal symptoms associated with venepuncture, which will not be documented as AEs if they occur.

3.8.2 Common vaccine reactions

Potential foreseeable risks from vaccination include local effects such as pain, redness, warmth, swelling, tenderness or itching. Systemic reactions that could potentially occur following immunisation with a recombinant adenovirus vaccine include a flu-like illness with feverishness, fatigue, malaise, arthralgia, myalgia and headache. Side effect profiles have been consistent across different ChAdOx1 vaccines when used in clinical trials. Rates of solicited adverse reactions that occurred with ChAdOx1 nCoV-19 in large scale trials are shown in Table 1.

Table 1 Profile of Reactions to ChAdOx1 nCoV-19 in clinical trial participants[30]

Adverse Reaction	**Frequency (%)**
Injection site tenderness	64%
Injection site pain	54%
Fatigue	53%
Headache	52%
Malaise	44%
Myalgia	44%
Feverishness	34%
Arthralgia	26%
Nausea	22%
Fever over ≥38°C	8%

3.8.3 Thrombosis with Thrombocytopenia

In April 2021, MHRA and JCVI conducted a review of extremely rare reports of cerebral venous sinus thrombosis (and thrombosis of other major veins) occurring with concurrent thrombocytopenia which had been received after large scale use of the ChAdOx1 nCoV-19 vaccine. The potential mechanism of these events is yet to be determined. It is currently unknown whether they are related to the ChAdOx1 vector or to the SARS-CoV-2 spike protein.

Estimates of incidence of thrombosis with thrombocytopenia following ChAdOx1 nCoV-19 vaccination vary and may change further as more cases are gathered and case definitions are finalised. Current UK estimates are of cases occurring at a rate of around 1 case per 100,000 prime vaccinations.

On the basis of their ongoing scientific review the UK Medicines Healthcare Regulatory Agency have concluded: "that the evidence of a link with COVID-19 Vaccine AstraZeneca is stronger"[31]. The European Medicines Agency concluded that unusual blood clots with low blood platelets should be listed as very rare side effects of this vaccine. Both the MHRA and EMA have confirmed that the evidence to date does not suggest that the COVID-19 Vaccine AstraZeneca causes venous thromboembolism *without* a low platelet count[31,32].

The MHRA data shows a higher reported incidence rate in younger adults. The reported incidence rate is also higher in females although this sex difference is small and not consistent across age groups.

Cases of thrombosis with thrombocytopenia have also been reported after COVID-19 Vaccine Janssen, an adenovirus serotype 26 vectored spike-protein based COVID-19 vaccine. The EMA has also conducted a review of these cases and concluded there is a likely causal relationship with the Janssen vaccine[33].

Thrombosis with thrombocytopenia syndrome has not previously been reported following the use of live oral adenovirus serotype 4 and serotype 7 adenovirus vaccines in the United States, where these vaccines have been approved for vaccination of military personnel[15,34,35]. Over 1.3 million doses have been administered between 2011 to 2018 with no reports of thrombosis following vaccination to the US CDC/FDA Vaccine Adverse Event Reporting System (VAERS) post-marketing passive vaccine safety monitoring database[15].

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 symptoms that could be warning signs of serious clotting events, as per public health advice being given to people receiving ChAdOx1 nCoV-19.

3.8.4 Other Potential Rare Vaccine Reactions

As with any vaccine, Guillain-Barré syndrome or immune-mediated reactions that can lead to organ damage may occur, but this should be extremely rare. Serious allergic reactions including anaphylaxis could also occur and for this reason volunteers will be vaccinated in a clinical area where Advanced Life Support trained physicians, equipment and drugs are immediately available for the management of any serious adverse reactions (SAR).

3.8.5 Potential Interaction with Other Adenoviral Vectored Vaccines:

The ChAdOx1 vector used in the ChAdOx1 biEBOV experimental vaccine is the same as that used in the Oxford AstraZeneca and similar to the one used in the Janssen (Johnson and Johnson) COVID-19 vaccines (Adenovirus 26). There is a theoretical risk that receiving the experimental ChAdOX1 biEBOV vaccine may reduce the benefit of subsequent administrations of certain vaccines such as the Janssen (Johnson & Johnson) or the AstraZeneca COVID-19 vaccines. This may be more likely to happen if the vaccines are given at short intervals. However, the immune response to the AstraZeneca COVID-19 vaccine was not affected in a study of individuals who received a previous ChAdOx1-vectored vaccine for a non-spike transgene at least one year earlier[18,36].

Other studies suggest that an interval of three months between administrations of two adenoviral vectored vaccines reduces the risk of this interference[37-41]. For this reason, we will enrol participants who have been vaccinated with an mRNA Covid vaccine or will have their NHS Covid vaccine offer at least three months after the ChAdOx1 biEBOV vaccine. No such theoretical interference will be expected with mRNA or protein COVID-19 vaccines (such as Moderna, Pfizer, Novavax) and these can be given at least 2 weeks before or after the ChAdOx1 biEBOV.

3.9 Potential Benefits

The recruitment population in this study are unlikely to benefit directly from participation in this study. However, it is hoped that the information gained from this study will contribute to the development of a further safe and effective vaccine to protect at-risk populations from ebolavirus disease. The only benefits for participants would be information about their general health status.

4 OBJECTIVES AND ENDPOINTS

4.1 Objectives, Outcome Measures and Evaluation Timepoints

Outcome	Objective	Outcome measure	Evaluation Timepoints
Primary	To assess the safety and tolerability of ChAdOx1 biEBOV in healthy volunteers.	a) Occurrence of solicited local reactogenicity signs and symptoms	7 days following each vaccination (D0 to D7)
		b) Occurrence of solicited systemic reactogenicity signs and symptoms	7 days following each vaccination (D0 to D7)
		c) Occurrence of unsolicited adverse events (AEs)	28 days following each vaccination (D0 to D28)
		d) Change from baseline for safety laboratory measures	**1 dose:** D0, D2, D7, D28 **2 dose:** D0, D2, D7, D28, V2, V2+7 V2+14, V2+28
		e) SAEs and AESIs	Whole duration of the study **1 dose:** D0 to D182 (or D364†) **2 dose:** D0 to V2+182, V2+280
Secondary	To assess the immunogenicity of ChAdOx1 biEBOV in healthy adult volunteers.	a) Filovirus GP specific serological response as measured by ELISA	**1 dose:** D0, D28, D56, D182, D364† **2 dose:** D0, D28, D56, V2, V2+14, V2+28, V2+182, V2+280
		b) Filovirus GP specific T cell response measured by IFN-γ ELISPOT	**1 dose:** D0, D7, D14, D28, D56, D182, D364† **2 dose:** D0, D7, D14, D28, D56, V2, V2+14, V2+28, V2+182, V2+280
Exploratory*	Immunological Profiling	a) Filovirus neutralising antibodies (using either live or pseudoneutralisation assays) b) Cellular immune response to filovirus GP measured by ICS, proliferation and/or whole blood assays	Timepoints will be detailed in the lab analysis plan
	To assess anti-vector immunity following vaccination	a) Binding and/or neutralising antibody responses against ChAdOx1	Timepoints will be detailed in the lab analysis plan
	To investigate a potential relationship between the composition of the gut microbiota and vaccination outcomes	a) Characterisation of the gut microbiome composition and function using shotgun sequencing. b) Metabolomic analyses may also be performed.	**1 dose:** D0, D14, D182, D364† **2 dose:** D0, D14, V2+14, V2+182, V2+280†

*Sample analysis for the completion of exploratory endpoints may be performed under the ethically approved OVC Biobank protocol (REC 16/SC/014). Further exploratory assays/analyses may be carried out (see section 7.6.2).

†Group 3 only

5 STUDY DESIGN

This is a first-in-human, open-label, dose escalation, phase I clinical trial to assess the safety and immunogenicity of the candidate ChAdOx1 biEBOV vaccine in healthy UK volunteers aged 18-55. The vaccine will be administered intramuscularly (IM).

Volunteers will be recruited and vaccinated at the Centre for Clinical Vaccinology and Tropical Medicine (CCVTM), Oxford. There will be 3 study groups and it is anticipated that a total of 26 volunteers will be enrolled. Dose escalation and sentinel participant procedures are detailed in section 5.4. Volunteers will be first recruited into Group 1 and subsequently into Groups 2 and 3 following interim clinical safety reviews (section 5.4). Volunteers will be sequentially allocated to a study group by selecting eligible volunteers for enrolment following screening. Sequential allocation will occur based on the order in which volunteers are enrolled.

5.1 Study Groups

Group	Treatment	Follow up Period
Group 1 (n=6)	Single IM Dose ChAdOx1 biEBOV 5×10^9 vp	6 months (section 2.2)
Group 2 (n=6-9*)	Single IM Dose ChAdOx1 biEBOV 2.5×10^{10} vp	6 months (section 2.2)
Group 3 (n=11-14*)	Two IM Doses ChAdOx1 biEBOV 5×10^{10} vp, 12-weeks apart (second dose optional)	12 months** (section 2.3)

*Group 2 may be increased to 9 participants following interim safety reviews. In this case group 3 will consist of 11 rather than 14 participants.

**Group 3 participants that decline a second dose of ChAdOx1 biEBOV will continue to follow the single dose follow up schedule (section 2.2). The follow up period for group 3 participants was extended to 12 months as part of SA03.

5.2 Trial Duration

The total duration of the study will be 6-12 months from the day of enrolment for each volunteer.

5.3 Definition of Start and End of Trial

The start of the trial is defined as the date of the first vaccination of the first volunteer. The end of the trial is the date of the last visit of the last volunteer (LVLV).

5.4 Sequence of Enrolment

The enrolment sequence is summarised below (Table 2).

5.4.1 Sentinel Participants

The first participant in each group (i.e. at each dose level) will be vaccinated alone, ahead of any other participants in that group. Their profile of adverse events will be reviewed at least 48 hours post-vaccination. Provided there are no safety concerns at that point, as assessed by the CI and local safety monitor, a further two volunteers will be vaccinated using the same dose at least 1 hour apart from each other, bringing the total number of volunteers receiving that dose to 3. The profile of adverse events for the second and third participants will then be reviewed by the Local Safety Monitor (LSM) and CI after a further 48 hours and if this safety profile is deemed acceptable, the remaining (non-sentinel) participants in the group may then be vaccinated.

5.4.2 Dose Escalation

An interim safety review with at least 7 days of safety data from all participants of the preceding dose group, including the results of day 7 clinical laboratory blood tests, will be performed. The decision to proceed to the first participant of the next dose level will be taken on the basis of this review, by the LSM and CI.

Table 2 Sequence of enrolment *The size of group 2 may optionally be extended from n=6 to n=9. If the group size is increased then a further safety review ("Step 8b") will be undertaken.

Sequence	Volunteer groups	Minimum Interval before next step	Local Safety Monitor Interim Safety Review prior to progression
Step 1	Group 1 volunteer 1	48 hours	Y
Step 2	Group 1 volunteer 2	1 hour	N
Step 3	Group 1 volunteer 3	48 hours	Y
Step 4	Group 1 remaining volunteers	7 days	Y
Step 5	Group 2 volunteer 1	48 hours	Y
Step 6	Group 2 volunteer 2	1 hour	N
Step 7	Group 2 volunteer 3	48 hours	Y
Step 8a	Group 2 volunteers 4 to 6	7 days	Y
(Step 8b)*	(Group 2 volunteer 7 to 9)*	(7 days)*	(Y)*
Step 9	Group 3 volunteer 1	48 hours	Y
Step 10	Group 3 volunteer 2	1 hour	N
Step 11	Group 3 volunteer 3	7 days	Y
Step 12	Group 3 remaining volunteers	n/a	n/a

5.4.3 Second Dose (SA02) Administration Sequence

Group 3 second doses will initially be staggered using the following sequence (Table 3 Sequence of second dose administration) and an interim safety reviews as detailed in section 9.11.2.

Table 3 Sequence of second dose administration

Sequence	Volunteer groups	Minimum Interval before next step	Local Safety Monitor Interim Safety Review prior to progression
Step 1	Second-dose administered to volunteer 1	48 hours	Y
Step 2	Second dose administered to volunteer 2	1 hour	N
Step 3	Second dose administered to volunteer 3	48 hours	Y
Step 4	Second doses administered to remaining group 3 volunteers	n/a	n/a

Safety holding rules will continue to apply following the second dose and will include real-time assessment of ePRO eDiary adverse event data.

5.5 Masking / Blinding

This is an open-label trial. Investigators and participants will remain unblinded to study vaccinations including dose/group and whether participants are sentinel or non-sentinel participants.

6 RECRUITMENT AND WITHDRAWAL OF TRIAL VOLUNTEERS

6.1 Participant Flow

1	Recruitment through Advertising and other activities
2	Completion of Online pre-screening eligibility questionnaire
3	Informed Consent (at screening visit, consent form signed)
4	Screening Procedures (at screening visit, after informed consent obtained)
5	Eligibility Assessment by Investigator (following completion of screening investigations)
6	Enrolment & Vaccination of eligible participants
7	Completion of trial follow up visits

6.2 Recruitment

Volunteers may be recruited by use of an advertisement and/or registration form formally approved by the ethics committee(s) and distributed or posted in the following places:

- In public places, including buses and trains, with the agreement of the owner/proprietor.
- In newspapers or other literature for circulation.
- On radio via announcements.
- On a website or social media site operated by our group or with the agreement of the owner or operator (including on-line recruitment through our web-site).
- By e-mail distribution to a group or list only with the express agreement of the network administrator or with equivalent authorisation.
- By email distribution to individuals who have already expressed an interest in taking part in any clinical trial at the Oxford Vaccine Centre.
- On stalls or stands at exhibitions or fairs.
- Via presentations (e.g. presentations at lectures or invited seminars).
- Direct mail-out: This will involve obtaining names and addresses of adults via the most recent Electoral Roll. The contact details of individuals who have indicated that they do not wish to receive postal mail-shots would be removed prior to the investigators being given this information. The company providing this service is registered under the Data Protection Act 2018. Investigators would not be given dates of birth or ages of individuals, but the list supplied would only contain names of those aged between 18-55 years (as per the inclusion criteria).
- Oxford Vaccine Centre databases: We may contact individuals from databases of groups within the CCVTM (including the Oxford Vaccine Centre database) of previous trial participants who have expressed an interest in receiving information about all future studies for which they may be eligible.

SA02 Note: Recruitment of all volunteers into the trial has now been completed.

6.3 Online Pre-screening Eligibility Questionnaire

Prior to invitation for a screening visit, individuals will be directed to an online pre-screening questionnaire covering key eligibility criteria such as age. Respondents that are not ineligible at this stage will be invited to the trial site for the screening visit.

6.4 Informed consent

All volunteers will sign and date the informed consent form before any study specific procedures are performed. The information sheet will be made available to the volunteer at least 24 hours prior to the screening visit. At the screening visit, the volunteer will be fully informed of all aspects of the trial, the potential risks and their obligations. The following general principles will be emphasised:

- Participation in the study is entirely voluntary.
- Refusal to participate involves no penalty or loss of medical benefits.
- The volunteer may withdraw from the study at any time.
- The volunteer is free to ask questions at any time to allow him or her to understand the purpose of the study and the procedures involved.
- The study involves research into an investigational vaccine.
- There is no direct benefit to individuals from participating.
- The volunteer's General Practitioner (GP) will be informed of their participation in the study.
- Confirmation of their medical history will be required e.g. through a medical history summary from their GP practice or equivalent.
- The volunteer's blood may be sent outside of the UK and Europe to laboratories in collaboration with the University of Oxford. These will be de-identified.
- That long term storage of samples after the trial is over is optional and will be covered under the Oxford Vaccine Centre Biobank Study protocol which will be consented to separately.

The aims of the study and all tests to be carried out will be explained. The volunteer will be given the opportunity to ask about details of the trial and will then have time to consider whether or not to participate. If they decide to participate they will sign and date the consent form once informed consent has been obtained. These forms will also be signed and dated by the Investigator. Participants will be provided with a copy of the signed consent form.

6.5 Inclusion and exclusion criteria

This study will be conducted in healthy adults, who meet the following inclusion and exclusion criteria:

6.5.1 Inclusion criteria

The volunteer must satisfy all the following criteria to be eligible for the study:

1. Healthy adults aged 18 to 55 years.
2. Able and willing (in the Investigator's opinion) to comply with all study requirements.
3. Willing to allow confirmation of their past medical history either through: provision of a GP medical record summary, allowing investigators to obtain a copy of their medical history from their GP practice or by providing an alternative acceptable means of confirming their past medical history.
4. Agreement to refrain from blood donation during the course of the study.
5. Provide written informed consent.
6. *For women of childbearing potential only:* Willingness to practice continuous effective contraception for the duration of the trial.
7. *For women of childbearing potential only:* A negative pregnancy test within the screening window and on the day of vaccinations.

6.5.2 Exclusion criteria

The volunteer may not enter the study if any of the following apply:

1. Participation in another research study involving receipt of an investigational product in the 30 days preceding enrolment or during the trial follow up period.
2. Receipt of a recombinant simian adenoviral vaccine prior to enrolment.
3. Planned receipt of another adenoviral vectored vaccine (e.g. Oxford/AstraZeneca or Janssen COVID-19 vaccines) within 90 days after vaccination with the ChAdOx1 biEBOV.
4. Planned or actual receipt of any vaccines administered within 30 days (before or after) enrolment and/or planned receipt of a vaccine ≤30 days after enrolment EXCEPT for protein, RNA (or other non-adenovirus based) COVID-19 vaccinations which may be given within 14 days of the trial vaccine.
5. Previous receipt of an Ebola virus vaccine.
6. Administration of immunoglobulins and/or any blood products within the three months preceding the planned administration(s) of the vaccine candidate.
7. Any confirmed or suspected immunosuppressive or immunodeficient state, including HIV infection; asplenia; recurrent, severe infections and chronic (more than 14 days) systemically active immunosuppressant medication within the past 6 months.
8. History of allergic disease or reactions likely to be exacerbated by any component of the vaccine. Including hypersensitivity to the active substance or to any of the excipients of the IMP or Vaxzevria (i.e. the Oxford/AstraZeneca COVID-19 vaccine).
9. History of hereditary angioedema, acquired angioedema, or idiopathic angioedema.
10. History of anaphylaxis in relation to vaccination.
11. History of cancer (except basal cell carcinoma of the skin and cervical carcinoma in situ).
12. History of serious psychiatric condition likely to affect participation in the study.
13. Ongoing or planned pregnancy or breastfeeding during the trial follow up period.
14. Bleeding disorder (eg. Factor deficiency, coagulopathy or platelet disorder), or prior history of significant bleeding or bruising following IM injections or venepuncture.

15. History of confirmed major thrombotic event (including cerebral venous sinus thrombosis, deep vein thrombosis, pulmonary embolism), history of antiphospholipid syndrome, or history of heparin induced thrombocytopenia.
16. Individuals who have experienced thrombosis with thrombocytopenia syndrome (TTS) following vaccination with Vaxzevria (i.e. the Oxford/AstraZeneca COVID-19 vaccine).
17. Individuals who have previously experienced episodes of capillary leak syndrome.
18. Any other serious chronic illness requiring hospital specialist supervision.
19. Suspected or known current alcohol abuse as defined by an alcohol intake of greater than 42 units every week.
20. Suspected or known injecting drug abuse in the 5 years preceding enrolment.
21. Detectable circulating hepatitis B surface antigen (HBsAg).
22. Seropositive for hepatitis C virus (antibodies to HCV).
23. Any clinically significant abnormal finding on screening biochemistry or haematology blood tests or urinalysis.
24. Any other significant disease, disorder or finding which may significantly increase the risk to the volunteer because of participation in the study, affect the ability of the volunteer to participate in the study or impair interpretation of the study data.

6.5.3 Effective Contraception for Women of Childbearing Potential (WOCBP)

6.5.3.1 Definition of Women of Childbearing Potential (WOCBP)

For the purpose of this trial, a woman is considered of childbearing potential (WOCBP) unless they meet either of the following criteria:

- Surgical sterilisation including:
    - Hysterectomy
    - Bilateral salpingectomy
    - Bilateral oophorectomy.
- Post-menopausal:
    - For women aged ≥ 45 years, post-menopausal is defined as having a history of ≥12 months amenorrhea, without using hormonal contraception or other alternative cause.

6.5.3.2 Acceptable Forms of Contraception for WOCBP:

Female participants of childbearing potential are required to use an effective form of contraception from one month before prime until three months after administration of the IMP.

Acceptable forms of contraception for volunteers of childbearing potential include:

- Established use of oral, injected or implanted hormonal methods of contraception.
- Placement of an intrauterine device (IUD) or intrauterine system (IUS).
- 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 (defined as refraining from heterosexual intercourse) when this is in line with the preferred and usual lifestyle of the subject. Periodic abstinence and withdrawal are *not* acceptable methods of contraception.

6.5.4 Prevention of Over-Volunteering

Volunteers will be excluded from the study if they are concurrently enrolled in another CTIMP trial. In order to check this, volunteers will be asked to provide their National Insurance or Passport number (if they are not entitled to a NI number) and will be registered on a national database of participants in clinical trials (*www.tops.org.uk*).

6.6 Screen Failures

Participants who have signed the informed consent form but are not subsequently enrolled in the trial will be regarded as screen failures. For each of these participants, a minimal set of screen failure data will be recorded including demographic details and the reason for screening failure. These details will be reported as required by Consolidated Standards of Reporting Trials (CONSORT) publishing standards.

6.7 Enrolment and Group Allocation

Participants will be considered enrolled at the point of receiving their vaccination at the D0 visit. Allocation of participants to groups / sentinels will be performed by the investigator and will follow the sequence in section 5.4.

6.8 Withdrawal of volunteers

In accordance with the principles of the Declaration of Helsinki (2008) 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 Investigator may withdraw the volunteer at any time in the interests of the volunteer's health and well-being. In addition, the volunteer may withdraw/be withdrawn for any of the following reasons:

- Administrative decision by the Investigator.
- Ineligibility (either arising during the study or retrospectively, having been overlooked at screening).
- Significant protocol deviation.
- Volunteer non-compliance with study requirements.
- An AE, which requires discontinuation of the study involvement or results in inability to continue to comply with study procedures.

The reason for withdrawal will be recorded in the CRF. 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. The local safety monitor may recommend withdrawal of volunteers on safety grounds.

Any participant who repeatedly fails to attend for follow-up visits during the study may be considered for withdrawal from the study, following an assessment by the trial team and principal investigator. Participants should not be withdrawn as lost to follow-up until all efforts contact them have been exhausted.

If a volunteer withdraws from the study, blood and stool samples collected before their withdrawal from the trial will be used/ stored unless the volunteer specifically requests otherwise.

In all cases of participant withdrawal, excepting those of complete consent withdrawal, long-term safety data collection, including some procedures such as safety bloods, will continue as appropriate if participants have received at least one dose of the trial IMP.

6.9 Compliance with dosing regime

All doses in this vaccine study will be administered by appropriately trained and delegated clinical trial staff and administration details will be recorded in an appropriate CRF. The study medication will not be in the possession of the volunteer at any time and compliance will therefore not be an issue within this trial.

6.10 Pregnancy

Should a volunteer become pregnant during the trial, she will be followed up for safety outcomes. Additionally, she will be followed until pregnancy outcome. We will not routinely perform venepuncture in a pregnant volunteer.

7 CLINICAL TRIAL PROCEDURES

This section describes the procedures for evaluating study participants and follow-up after administration of study vaccine.

7.1 Study procedures

All participants will receive the ChAdOx1 biEBOV vaccine and subsequently attend the predefined follow up visit schedule as indicated in the schedule of procedures table (Section 2). Study procedures will depend on the specific visit and include: vaccination with the study IMP, collection and review of AEs via symptom e-Diaries, collection of SAE and AESI data, blood sampling for clinical safety monitoring and immunogenicity analysis, vital signs and pregnancy testing for WOCBP.

In certain circumstances, additional visits or procedures may be performed at the discretion of investigators. These may include further medical history and targeted physical examinations, urinalysis, confirmatory urine testing in the event of positive urinalysis and additional blood tests if clinically relevant.

7.2 Demographic Data Collection

Demographic data will be collected at the screening visit. This will include:

- Age
- Biological sex
- Ethnicity

7.3 Medical History

7.3.1 Screening Medical History

A past medical history will be recorded at the screening visit which should cover: all significant previous and ongoing medical conditions experienced by the participant and concomitant medications.

7.3.2 Review of Medical Records/ Confirmation of Past Medical History

Investigators will confirm the past medical history of participants through a review of their medical records or by another means. This will enable eligibility criteria to be confirmed and ensure accurate baseline information is recorded. Acceptable methods of confirming past medical histories include:

- Review of a GP medical summary
- Review of electronic GP records if available
- Investigator discussion with a participant's GP
- Any other method of confirming a participant past medical history that is deemed to be acceptable by the investigator.

7.3.3 Targeted Medical History

Participants reporting AEs may also be reviewed and targeted histories may take place throughout the trial as though appropriate by investigators.

7.4 Physical Observations / Vital Signs

The following vital signs will be measured in clinic at the time-points indicated in the schedule of procedures table (Section 2):

- Pulse
- Blood Pressure
- Temperature (Oral)

Additionally, physical observations may also be measured as part of a physical examination if indicated at other timepoints during the study. Pulse oximetry will not be routinely measured unless clinically indicated.

In addition to in-clinic vital signs, participants will self-assess oral temperature daily for 7 days following IMP vaccination as part of solicited AE data collection.

7.5 Physical Examinations

A physical examination will be performed at the screening visit. Targeted physical examinations may also be carried out were indicated at follow up visits. Any abnormal examination findings at screening or during will be recorded in the appropriate CRF.

7.5.1 Screening Physical Examination

A screening physical examination will generally include assessment of:

- Height & weight
- General appearance
- Skin
- Lymph node examination
- Abdominal examination
- Cardiovascular examination
- Respiratory examination

7.5.2 Targeted Physical Examination

A targeted physical examination may include areas suggested by the medical history, clinical signs, and symptoms.

7.6 Participant Samples

7.6.1 Clinical Laboratory Blood Samples

Blood will be drawn for the following laboratory tests. These will be carried out at Oxford University Hospitals NHS Foundation Trust using NHS standard procedures:

- **Haematology:**
    - Full Blood Count (Including: Haemoglobin, platelet count, total white cell count, neutrophil count, lymphocyte count, eosinophil count)
- **Biochemistry:**
    - Urea and Electrolytes (Including: Sodium, Potassium, Urea and Creatinine)
    - Liver Function Tests (Including: ALT, ALP, Bilirubin, Albumin)
- **Diagnostic serology (Screening Only):**
    - Screening tests for Hep B, Hep C and HIV infection (Including: HBsAg, HCV antibodies, HIV antibodies)

- **Immunology:**
    - Human Leukocyte Antigen (HLA) typing

Additional safety blood tests may be performed if clinically relevant at the discretion of the medically qualified investigators. These generally include, but are not limited to AST, GGT and a coagulation screen.

7.6.2 Immunology Samples

7.6.2.1 University of Oxford research laboratories:

Immunogenicity will be assessed by a variety of immunological assays. This may include ex vivo ELISpot assays for interferon gamma and flow cytometry assays, functional antibody assays and B cell analyses. Other exploratory immunological assays including cytokine analysis and other antibody assays, production of monoclonal antibodies, DNA analysis of genetic polymorphisms potentially relevant to vaccine immunogenicity and gene expression studies amongst others may be performed.

7.6.2.2 Other research laboratories:

Collaboration with other specialist laboratories in the UK, Europe and outside of Europe for further exploratory immunological tests may occur. This would involve the transfer of serum, plasma and PBMC to these laboratories, but these would remain de-identified. Informed consent for this will be gained from volunteers. Immunological assays will be conducted according to local SOPs.

7.6.3 Stool Sample Collection

Stool samples will optionally be collected for the study of gut microbiota. Participants will collect faecal samples at home, following an established SOP for stool collection and using specific containers provided by the trial team. Instructions will be provided, and participants will return samples either to the clinic or via post.

In participants that opt in, samples of faeces will be collected at the following timepoints:

- (Single dose recipients) Before vaccination, at D14 (thought to be the peak of the T-cell response), and at the participant's final visit.
- (Two dose recipients) Before the first vaccination, at D14, before the second vaccination, 14 days after the second vaccination (V2+14) and at the participant's final.

Stool samples will be taken prior to the above visits, within 24 hours.

7.6.4 Urine Samples

Urine will be collected at specified timepoints for the following tests:

- **Urinalysis;** Urine will be tested for protein, blood and glucose at screening (via bedside urinalysis strips)
- **Pregnancy Testing;** For female volunteers of childbearing potential only, urine will be tested for human chorionic gonadotrophin (HCG) at screening and immediately prior to each vaccination. β-HCG blood sampling may also alternatively be acceptably used to confirm a female participant is not pregnant.

7.6.5 Retention of Samples

Participants will be informed that they may opt-in to the Oxford Vaccine Centre Biobank study (REC 16/SC/014) to allow long-term storage of biological samples collected under the EBL07 protocol for use in possible future research. The OVC Biobank study is covered by a separate study protocol and

consent process. Participants will be informed that declining to take part in the OVC Biobank study will not affect their participation in the EBL07 study. If a participant elects to decline to take part in the OVC Biobank, all of their leftover samples will be discarded after the required period of storage to meet Good Clinical Practice (GCP) and regulatory requirements.

7.7 Study visits

The study visits and procedures will be undertaken by one of the clinical trials team. The procedures to be included in each visit are documented in the schedule of attendances tables (Section 2). Each visit is assigned a time-point and a window period, within which the visit will be conducted.

7.7.1 Screening visit

All potential volunteers will have a screening visit, which may take place up to 90 days prior to vaccination. Informed consent will be taken before screening, as described in section 6.4. If consent is obtained, the screening procedures indicated in section 2.1 will be undertaken. To avoid unnecessary additional sampling, if the appropriate results for screening are available for the same volunteer from a screening visit for another Jenner Institute Clinical Trials group vaccine study, these results may be used for assessing eligibility (provided the results date is within the 3 months preceding enrolment in EBL07).

The participant's general practitioner will be contacted with the written permission of the participant after satisfactory screening as notification that the participant has volunteered for the study. During the screening the volunteers will be asked to provide their National Insurance or passport number so that this can be entered on to a national database which helps prevent volunteers from participating in more than one clinical trial simultaneously or over-volunteering for clinical trials (www.tops.org.uk).

Abnormal clinical findings from the urinalysis or blood tests at screening will be assessed by the lead clinician according to the relevant SOP. Abnormal blood tests following screening will be assessed according to laboratory adverse event grading tables. Any abnormal test result deemed clinically significant may be repeated to ensure it is not a single occurrence. If an abnormal finding is deemed to be clinically significant, the volunteer will be informed and appropriate medical care arranged with the permission of the volunteer.

The eligibility of the volunteer will be reviewed at the end of the screening visit and again when all results from the screening visit have been considered. Decisions to exclude the volunteer from enrolling in the trial or to withdraw a volunteer from the trial will be at the discretion of the Investigator. If eligible, a day 0 visit will be scheduled for the volunteer to receive the vaccine.

7.7.2 ChAdOx1 biEBOV vaccination visit(s)

Before vaccination, the eligibility of the volunteer will be reviewed. Pulse, blood pressure and temperature will be observed and if necessary, a further medical history and physical examination may be undertaken to determine need to postpone vaccination. Vaccinations will be administered as described below in section 7.8 and in accordance with relevant SOPs.

7.7.3 Follow up Visits

Follow-up visits will take place as per the schedule of procedures table (Section 2). Volunteers will be assessed for local and systemic adverse events, interim history, physical examination and a review of electronic diaries at the indicated time points. Blood will also be taken for both safety assessments (biochemistry and haematology tests listed in section 7.6.1) and immunology purposes at the majority of follow up visits.

If volunteers experience adverse events (laboratory or clinical), which the investigator (physician), CI and/or LSM determine necessary for further close observation, the volunteer may be admitted to an NHS hospital for observation and further medical management under the care of the Consultant on call.

7.7.4 Missed Visits

In exceptional circumstances, where follow up visits would otherwise be missed entirely, visits may alternatively be conducted remotely via phone or video calling. This will allow a minimum set of safety and adverse event data to be collected.

7.8 ChAdOx1 biEBOV vaccinations

Before each vaccination, the on-going eligibility of the volunteer will be reviewed. ChAdOx1 biEBOV will be administered intramuscularly according to vaccine administration SOPs as described in section 8.2. The injection site will be covered with a sterile dressing and the volunteer will stay in the CCVTM for observation, in case of immediate adverse events. Observations will be taken 30 minutes after vaccination (±5 minutes) and the sterile dressing removed and injection site inspected. Observations will also be taken at 60 minutes (±10 minutes), before the volunteer leaves.

7.9 Electronic Participant Symptom Diary

An oral thermometer, tape measure and electronic diary access will be given to each volunteer, with instructions on use, along with a contact card including the emergency 24 hour telephone number to contact the on-call study physician if needed. In the event of technical problems, paper symptom diaries may alternatively be issued.

The eDiary ePRO system collects information on the timing and severity of the solicited AEs listed in section 9.2. Volunteers will be instructed on how to self-assess the severity of these AEs. There will also be space on the eDiary to self-document unsolicited AEs, and whether medication was taken to relieve the symptoms.

The eDiary database automatically monitors for grade ≥3 severity adverse events in (near) real-time, which are then flagged up to key study site personnel. This allows (near) real-time assessment of potential solicited/unsolicited adverse events for holding rules and to ensure individual participant safety. Additional scheduled eDiary automatic data checks are carried as detailed in the EBL07 data management plan.

Backup paper versions of the eDiary can be made available in exceptional circumstances e.g. in case of technical failures.

7.10 COVID-19 Study Considerations

The study will be carried out in line with the latest Public Health England (PHE) advice. Local SOPs for the use of appropriate personal protective equipment (PPE) and social distancing will be followed by clinical staff.

Participants must consent to inform the trial team of COVID-19 infection during the study as part of the informed consent process, in order to be enrolled in the study. Participants will be reminded to follow the up-to-date local public health guidance regarding COVID-19 during the trial. In keeping with PHE guidance on vaccine associated fever during the pandemic[42], participants experiencing fever within the first 48 hours of vaccination, with no other primary symptom of covid-19 (cough, anosmia, ageusia) will not be advised to self-isolate unless there is otherwise clinical suspicion of COVID-19.

Participants will be able to discuss symptoms with a medically qualified on call study clinician via mobile phone at all times during the study. Participants describing potential COVID-19 symptoms may be recommend to seek follow up and testing for COVID-19 through healthcare pathways available to them.

8 INVESTIGATIONAL PRODUCTS

All participants will receive at least one vaccination with ChAdOx1 biEBOV, with a dose according to their allocated group, as outlined in section 5.1.

8.1 Supply (ChAdOx1 biEBOV)

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

The ChAdOx1 biEBOV drug substance is a slightly opaque frozen viral solution, essentially free from visible particulates. The vaccine is supplied as a liquid in glass vials for intramuscular administration and will be stored at nominal -80°C in a secure freezer at the clinical site.

8.2 Administration of Investigational Medicinal Products

On vaccination day, ChAdOx1 biEBOV will be allowed to thaw to room temperature and will be administered within 1 hour of removal from the freezer. The vaccine will be administered intramuscularly into the deltoid of the non-dominant arm (preferably). All volunteers will be observed in the unit for 1 hour (±15 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.

8.3 Minimising environmental contamination with genetically modified organisms (GMO)

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 biEBOV 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.

9 ASSESSMENT OF SAFETY

Safety will be assessed by the frequency, incidence and nature of adverse events and serious adverse events arising during the study.

9.1 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 a serious adverse event when, based upon appropriate medical judgement, the event may jeopardise the participant and may require medical or surgical intervention to prevent one of the outcomes listed above. 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. All serious adverse events deemed possible, probably or definitely related will be considered SARs in this study.
Suspected Unexpected Serious Adverse Reaction (SUSAR)	A serious adverse reaction, the nature and severity of which is not consistent with the Reference Safety Information for the medicinal product in question set out: • in the case of a product with a marketing authorisation, in the approved summary of product characteristics (SmPC) for that product • in the case of any other investigational medicinal product, in the approved investigator's brochure (IB) relating to the trial in question.

9.2 Solicited Adverse Events Assessment

Predefined local and systemic solicited AEs for reactogenicity assessment, as listed below (Table 4 Solicited Adverse Events), will be collected in an electronic diary (details in section 7.9) for 7 days following administration of the vaccine. Participants will measure and record their temperature and the diameter of any injection site redness with the provided thermometer and tape measure and AE severity gradings will be calculated based on these measurements. For all other solicited AEs, solicited AE severity will be self-assessed by participants according to severity grading scales provided to them as defined in section 9.8.

Table 4 Solicited Adverse Events

Local solicited AEs	Systemic solicited AEs
Redness at the injection site (measured)	Fever (measured)
Warmth at the injection site	Chills
Itch at the injection site	Feverishness
Pain at the injection site	Joint pains
	Muscle pains
	Fatigue
	Headache
	Nausea
	Malaise

9.3 Unsolicited Adverse Events Assessment

Unsolicited adverse events i.e. those collected through open questioning e.g. “did you experience any new illnesses?” that do not constitute SAEs or AESIs will be collected for 28 days following administration of the IMP. These will be recorded in the participant eDiary and relevant eCRF.

9.4 Adverse Events of Special Interest (AESI)

AEs of special interest are based on Brighton Collaboration case definitions, clinical experience, and scientific interest. AESIs will be monitored and recorded throughout the study period. These will include the list below (Table 5 EBL07 AESIs). Additionally, other adverse events (i.e. not listed below) may also be categorised by investigators as AESIs if scientifically warranted.

Table 5 EBL07 AESIs

Neurological	Transverse Myelitis
	Generalised convulsion
	Guillain-Barre Syndrome (GBS)
	Acute Disseminated Encephalomyelitis (ADEM)
	Other New Neurological Diagnoses
Haematological / Vascular	Thrombocytopenia
	Thrombosis with Thrombocytopenia Syndrome
	Major thrombosis (without thrombocytopenia)
	Heparin-Induced Thrombocytopenia
Immunological	Anaphylaxis
	Vasculitides
	Other Immune-Mediated Conditions

9.5 Causality assessment

9.5.1 Solicited AE Causality Assessment

Solicited AEs are expected to occur after vaccination and will therefore not be assessed for relationship to study intervention.

9.5.2 Unsolicited AE Causality Assessment

For unsolicited AEs, an assessment of the relationship of the event to the administration of the vaccine will be undertaken by the CI or delegated clinician. An intervention-related AE refers to an AE for which there is a possible, probable or definite relationship to administration of a vaccine. 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 (Table 6). 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. Causality assessment will take place during planned safety reviews, interim analyses (e.g. if a holding rule is activated) and at the final safety analysis, except for SAEs, which should be assigned by the reporting investigator.

Table 6. Guidelines for assessing the relationship of vaccine administration to an AE.

0	No Relationship	No temporal relationship to study product; ***and*** Alternate aetiology (clinical state, environmental or other interventions); ***and*** Does not follow known pattern of response to study product.
1	Unlikely	Unlikely temporal relationship to study product; ***and*** Alternate aetiology likely (clinical state, environmental or other interventions); ***and*** Does not follow known typical or plausible pattern of response to study product.
2	Possible	Reasonable temporal relationship to study product; ***or*** Event not readily produced by clinical state, environmental or other interventions; ***or*** Similar pattern of response to that seen with other vaccines.
3	Probable	Reasonable temporal relationship to study product; ***and*** Event not readily produced by clinical state, environment, or other interventions; ***or*** Known pattern of response seen with other vaccines.
4	Definite	Reasonable temporal relationship to study product; ***and*** Event not readily produced by clinical state, environment, or other interventions; ***and*** Known pattern of response seen with other vaccines.

9.6 Expectedness assessment

All serious adverse reactions related to ChAdOx1 biEBOV administration will be assessed for expectedness by the investigator. Expectedness will be determined according to the information set out in the reference safety section of the ChAdOx1 biEBOV IB.

No SARs are currently expected for ChAdOx1 biEBOV. Therefore, any SARs associated with ChAdOx1 biEBOV will be reported as SUSARs.

9.7 Reporting procedures for all Adverse Events

All local and systemic AEs occurring in the 28 days following each IMP vaccination observed by the Investigator or reported by the volunteer, whether or not attributed to study medication, will be recorded (excluding those expected consequences from venepuncture, described in section 3.8). Recording and reporting of all AEs will take place as detailed in relevant trial SOPs. 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). Serious adverse events (SAEs) will be collected throughout the entire trial period.

9.7.1 Reporting procedures for SAEs

In order to comply with current regulations on serious adverse event reporting to regulatory authorities, the event will be documented accurately and notification deadlines respected. SAEs will be reported on the SAE forms to members of the study team immediately once an Investigator becomes aware of their occurrence, as described in SOP OVC005. Copies of all reports will be forwarded for review to the CI (as the Sponsor's representative) within 24 hours of the Investigator being aware of the suspected SAE. The LSM will be notified of SAEs that are deemed possibly, probably or definitely related to study interventions within 24 hours of the Investigator being aware of their occurrence. SAEs will not normally be reported immediately to the ethics committee(s) unless there is a clinically important increase in occurrence rate, an unexpected outcome, or a new event that is likely to affect safety of trial volunteers, at the discretion of the CI and/or LSM. In addition to the expedited reporting above, the Investigator shall include all SAEs in the annual Development Safety Update Report (DSUR).

9.7.2 Reporting Procedures for SUSARS

The CI will report all SUSARs to the Medicines and Healthcare products Regulatory Authority (MHRA) and ethical committee(s) within required timelines (15 calendar days for all SUSARs from the date of awareness, unless life threatening in which case 7 days, with a final report within a further 8 days (total 15)). The CI will also inform all Investigators concerned of relevant information about SUSARs that could adversely affect the safety of participants. All SUSARs and deaths occurring during the study will be reported to the Sponsor. For all deaths, available autopsy reports and relevant medical reports will be made available for reporting to the relevant authorities.

9.7.3 Development Safety Update Report

The CI will submit (in addition to the expedited reporting above) Development Safety Update Reports (DSURs) once a year throughout the clinical trial, or on request, to the Competent Authority (MHRA in the UK), Ethics Committee, HRA (where required), Host NHS Trust and Sponsor.

9.8 Assessment of severity

The severity of clinical and laboratory adverse events will be assessed according to scales based on FDA toxicity grading scales for healthy and adolescent volunteers enrolled in preventive vaccine clinical trials, listed in

Appendix C: Severity Grading Criteria and study specific SOPs.

9.9 Procedures to be followed in the event of abnormal findings

Laboratory parameters for inclusion/exclusion in the trial will be considered on an individual basis, with investigator discretion for interpretation of results and the need for repeated tests. Abnormal clinical findings from medical history, examination or blood tests will be assessed as to their clinical significance throughout the trial. If a test is deemed clinically significant, it may be repeated, to ensure it is not a single occurrence. If a test remains clinically significant, the volunteer will be informed and appropriate medical care arranged as appropriate and with the permission of the volunteer. Decisions to exclude the volunteer from enrolling in the trial or to withdraw a volunteer from the trial will be at the discretion of the Investigator.

9.10 Local Safety Monitor

An independent Local Safety monitor (LSM) will be appointed to provide real-time safety oversight. The LSM will review SAEs deemed possibly, probably or definitely related to study interventions. The LSM will be notified within 24 hours of the investigators' being aware of their occurrence. The LSM has the power to terminate the study if deemed necessary following a study intervention-related SAE. At the time of writing, the LSM will be Prof Brian Angus, a Clinical Tutor in Medicine, Honorary Consultant Physician and Director, Centre for Tropical Medicine at the University of Oxford. All correspondence between investigator and LSM will be conveyed by the investigator to the trial Sponsor.

The LSM may be contacted for advice and independent review by the investigator or trial Sponsor in the following situations:

- Following any SAE deemed to be possibly, probably, or definitely related to a study intervention.
- Any other situation where the Investigator or trial sponsor feels independent advice or review is important.

9.11 Interim safety reviews

The safety profile of the IMP will be assessed on an on-going basis by the Investigators with communication to the LSM as necessary. The CI and relevant Investigators (as per the trial delegation log) will also review safety issues and SAEs as they arise.

9.11.1 First dose vaccination

The following interim safety reviews will take place. (Also refer to section 5.4 - Sequence of Enrolment):

- 48 hours following the vaccination of the first volunteer in each group to decide on whether to proceed with vaccination of the next 2 volunteers in the group.
- Following vaccination of the first 3 volunteers in each group to decide whether to proceed with vaccinations of the remaining participants in the group.
- Following vaccination of all participants within a group. These reviews will include the results of clinical safety blood tests at the D7 visit and will be required to decide on dose escalation.

Interim safety reports will be produced for interim safety reviews and will contain the relevant adverse event / safety data.

In the event of reactogenicity judged to be significant at the lower doses, 3 more participants may be enrolled into the 2.5 x10^{10} vp dose group (group 2). If utilised, this will enable more reactogenicity data to be collected prior to the 5 x10^{10} vp dose escalation decision.

9.11.2 Second dose vaccination

Reduced reactogenicity has previously been observed following second doses of other ChAdOx1 vaccines (section 3.6.1). However, second dose vaccinations will initially be staggered. Electronic patient-reported outcome (ePRO) eDiary adverse event data will be reviewed remotely after (a minimum of) 48 hours following administration of second doses for the first three initial second dose participants. This data will be presented to the LSM as the following interim safety reports:

- 48 hours following the first second-dose vaccination to decide on whether to proceed with vaccination of the next 2 volunteers in the group.
- Following vaccination of the first 3 volunteers in each group to decide whether to proceed with vaccinations of the remaining participants in the group.

Vaccination of the remaining group 3 volunteers with the second dose may only proceed after this safety review (see section 5.4.3).

9.12 Safety Holding Rules

Safety holding rules are in place as this is a first-in-human dose escalation study. It is expected that a small proportion of participants may report transient grade 3 symptoms within 48 hours of vaccination, at the 5 x 10^{10} vp dose. This is based on experience with ChAdOx1 nCoV-19 (which is now an approved vaccine) and other ChAdOx1 vectored vaccines used at the 5 x 10^{10} vp dose. The following safety holding rules below are designed to reflect this. Safety holding rules also apply following the second vaccination (SA02).

9.12.1 Group holding rules

The group holding rules are as follows;

- Solicited local adverse events:

If 2 or more vaccinations in a 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 >48 hrs.

- Solicited systemic adverse events:

If 2 or more vaccinations in a 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 >48 hrs.

- Unsolicited adverse events:

If 2 or more vaccinations in a group are followed by the same Grade 3 unsolicited adverse event (including the same laboratory adverse event) that is considered possibly, probably or definitely related to vaccination and persists at Grade 3 for > 48hrs.

- A serious adverse event considered possibly, probably or definitely related to vaccination occurs
- Death occurs

- A life-threatening reaction occurs

If a holding rule has been met we will inform the regulatory authority; following an internal safety review, if it is deemed appropriate to restart dosing, a request to restart dosing with pertinent data must be submitted to the regulatory authority as a request for a substantial amendment. The internal 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 volunteers to identify those who may develop similar symptoms and alterations to the current Participant Information Sheet (PIS).
- New, relevant safety information from ongoing research programs on the various components of the vaccine.

The sponsor, LSM, and vaccine manufacturer (CBF) will also be notified if a holding rule is activated or released. All vaccinated volunteers 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.

In addition to these pre-defined criteria, the study can be put on hold upon advice of the LSM, CI, Study Sponsor, Regulatory Authority or Ethical Committee(s), for any single event or combination of multiple events which they deem jeopardise the safety of the volunteers or the reliability of the data.

10 DATA MANAGEMENT

10.1 Data Management Plan

A trial specific data management plan will be generated and adopted for this trial. This will describe relevant aspects of data management related to the trial in detail.

10.2 Data Handling

The Chief Investigator will be responsible for all data that accrues from the study. The data will be entered into CRFs in an electronic format (using the study OpenClinica™ database). Electronic data will be stored on secure servers which are outsourced by OpenClinica™. Data will be entered into the OpenClinica Database via a secure web browser. OpenClinica™ meets FDA part 11B standards. This includes safety data, laboratory data (both clinical and immunological) and outcome data.

Adverse event data will also be entered onto electronic or paper diaries by the volunteer

10.3 Record Keeping

The Investigators will maintain appropriate medical and research records for this trial, in compliance with GCP and regulatory and institutional requirements for the protection of confidentiality of volunteers. The Chief Investigator, co-Investigators and clinical research nurses will have access to records. The Investigators will permit authorised representatives of the Sponsor(s), as well as ethical and regulatory agencies to examine (and when required by applicable law, to copy) clinical records for the purposes of quality assurance reviews, audits and evaluation of the study safety and progress.

With the volunteers' consent, we will keep their contact details after participation in the study is complete, so we may inform them of opportunities to participate in future vaccine related research. This will be entirely optional and participation in this study will not be affected by their decision to allow or not allow storage of their contact details beyond participation in this trial. Details will be stored electronically on a secure server and only authorised individuals at the CCVTM will have access to it. We will not, under any circumstances, share their contact details with any third party institutions without their permission. Volunteers will be informed that being contacted does not oblige them to agree to take part in future research and they can ask us to have their contact details removed from our database at any time.

10.4 Source Data and Case Report Forms (CRFs)

All protocol-required information will be collected in CRFs designed by the Investigator. All source documents will be filed in individual participant files. Source documents are original documents, data, and records from which the volunteer's CRF data are obtained. For this study, these will include, but are not limited to, volunteer consent form, blood results, GP response letters, laboratory records, diaries, and correspondence. In the majority of cases, CRF entries will be considered source data as the CRF is the site of the original recording (i.e. there is no other written or electronic record of data). In this study this will include, but is not limited to medical history, medication records, vital signs, physical examination records, urine assessments, blood results, adverse event data and details of vaccinations. All source data and volunteer CRFs will be stored securely.

10.5 Data Protection

The study protocol, documentation, data and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorised third party, without prior written approval of the sponsor.

10.6 Data Quality

Data collection tools will undergo appropriate validation to ensure that data is collected accurately and completely. Datasets provided for analysis will be subject to quality control processes to ensure analysed data is a true reflection of the source data.

Trial data will be managed in compliance with local data management SOPs (including the overarching SOP OVC007 Data and Database Management) and the trial specific data management plan.

The trial will comply with the UK General Data Protection Regulation (GDPR) and Data Protection Act 2018, which requires data to be de-identified as soon as it is practical to do so.

10.7 Archiving

Study data may be stored electronically on a secure server, and paper notes will be kept in a key-locked filing cabinet at the CCVTM – Churchill Hospital, University of Oxford. All essential documents will be retained for a minimum of 5 years after the study has finished. 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 University of Oxford for 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 University of Oxford financial policy.

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

11 STATISTICS

11.1 Sample Size Selection

This is a descriptive dose-escalation study and as such no formal power calculations have been performed. The numbers have been therefore chosen on the basis of providing adequate descriptive safety information to permit further clinical evaluation in later phase trials.

11.2 Study Analyses

The analyses for this study will be descriptive and will not include any hypothesis testing or presentation of p-values for group comparisons. All confidence intervals for descriptive analyses will be set at 95%. Variables will be summarised using appropriate descriptive statistics.

11.2.1 Recruitment, Enrolment and Withdrawal Data

The number of individuals screened, screening failures, number enrolled and vaccinated within each group and withdrawals will be summarised descriptively.

11.2.2 Demographic and Baseline Data

Demographic and baseline medical histories will be summarised and presented for each group.

11.2.3 Safety Data

All participants that received a study vaccine will be included in the safety analysis. The self-reported solicited AE data collected in the first 7 days after vaccination, will be summarised by severity for each day of the first 7 days after the administration of vaccines. Unsolicited AEs, SAEs and AESIs will be categorised by MedDRA SOC and PT levels. Abnormal clinical safety laboratory values will be graded based on a pre-specified severity scale. The frequency and percentage of unsolicited adverse events will be summarised for 28 days following the last vaccine. Serious adverse events (SAEs) will be summarised during the entire study and also be described narratively.

11.2.4 Immunogenicity Data

Immunogenicity endpoints will be presented by vaccine dose. Details of any immunological analyses to be performed will be described in an immunology analysis plan.

12 ETHICS AND REGULATORY CONSIDERATIONS

12.1 Declaration of Helsinki

The Investigators will ensure that this study is conducted according to the principles of the Declaration of Helsinki (2008).

12.2 Guidelines for Good Clinical Practice

The Investigators will ensure that this study is conducted in full conformity with the Good Clinical Practice (GCP), the requirements of the Medicines for Human Use (Clinical Trials) Regulations 2004, and local regulatory requirements.

12.3 Approvals

The protocol, informed consent form, participant information sheet and any proposed advertising material will be submitted to an appropriate Research Ethics Committee (REC), 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.

No substantial amendments to this protocol will be made without consultation with, and agreement of, the Sponsor. Any substantial amendments to the trial that appear necessary during the course of the trial must be discussed by the Investigator and Sponsor concurrently. If agreement is reached concerning the need for an amendment, it will be produced in writing by the Chief Investigator and will be made a formal part of the protocol following ethical and regulatory approval.

The Investigator is responsible for ensuring that changes to an approved trial, during the period for which regulatory and ethical committee(s) approval has already been given, are not initiated without regulatory and ethical committee(s)' review and approval except to eliminate apparent immediate hazards to the participant.

12.4 Reporting

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

12.5 Volunteer Confidentiality

Study participants are required to consent for us to contact their GP. We will write to their GP to inform them about your enrolment and study completion status, so they can update your medical records accordingly. The GP practice may also be asked to share information about participant's medical history and give access to any other medical records as required to ensure eligibility criteria are met. We would only notify GP practices of results of any medical tests with participant's permission.

All research data will be de-identified: volunteer data will be identified by a unique study number in the study database. Data will be entered electronically using the study OpenClinica™ database. OpenClinica™ is stored on a secure European server – data will be entered in a web browser on PCs in the CCVTM building and then transferred to the OpenClinica Database by encrypted (Https) transfer.

A separate confidential participant file containing identifiable information and source data will be stored in a secured location in accordance with the Data Protection Act 2018. All documents will be stored securely and only accessible by study staff and authorised personnel. The study staff will safeguard the privacy of participants' personal data. Photographs taken of vaccination sites (if required, with the volunteer's written, informed consent) will not include the volunteer's face and will be identified by the date, trial code and participant's unique identifier. Once developed, photographs will be stored as confidential records, as above. This material may be shown to other professional staff, used for educational purposes, or included in a scientific publication.

We will keep identifiable information about volunteers such as contact details for a minimum of 5 years after the study has finished. The need to store this information 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 for at least 15 years after the end of the study, subject to adjustments in clinical trials regulations. In addition to the de-identified scientific data, we will also store documents containing personal information volunteers provide when registering for the trial (including contact details), medical information and signed consent forms during this archiving period.

The study team will use volunteer name and contact details, to contact them 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 volunteers consent otherwise (e.g. if they request to be informed of other trials), volunteer personal details will not be used to contact them other than in exceptional circumstances concerning their safety. If they consent to take part in another study carried out by the Jenner Institute, personal information and medical information including blood test results may be accessed to avoid unnecessary repetition.

A photocopy of volunteer ID (driving licence, passport or national ID card) will be taken at the screening visit and retained until the end of the study.

Volunteer bank details will be stored for 7 years in line with university financial policy.

13 QUALITY CONTROL AND QUALITY ASSURANCE PROCEDURES

13.1 Investigator procedures

Approved site-specific standard operating procedures (SOPs) will be used at all clinical and laboratory sites.

13.2 Monitoring

Monitoring will be performed according to GCP by Clinical Trials Research Governance (CTRG). 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 site 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.

13.3 Protocol deviation

Any deviations from the protocol will be documented in a protocol deviation form and filed in the trial master file. Each deviation will be assessed as to its impact on volunteer safety and study conduct. Significant deviations will be listed in the end of study report.

13.4 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 FINANCING AND INSURANCE

14.1 Financing

The study is funded by Innovate UK.

14.2 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).

14.3 Participant Financial Compensation

Volunteers will be compensated for their time and for the inconvenience caused by procedures. They will be compensated £25 for attending the screening visit. For all other trial visits (as outlined in section 2.2), compensation will be calculated according to the following:

- Travel expenses:
    - £15 per visit. Where travel expenses are greater than £10 per visit because the volunteer lives outside the city of the trial site, the volunteer will be given further reimbursement to meet the cost of travel necessary for study visits following receipt of valid proof of costs incurred.
- Inconvenience of blood tests:
    - £10 per blood donation
- Time required for visit:
    - £20 per hour

The total amount of compensation for an individual participant will depend on the actual number of visits attended and whether any repeat or additional visits were necessary. If a participant withdraws consent for continued participation in the trial or is withdrawn for any other reason, they will still be compensated for any trial visits they attended.

14.4 Contractual Arrangements

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

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 participants of the trial; or

(b) the scientific value of the trial”.

In the event that a serious breach is suspected the Sponsor will be informed within one working day.

16 PUBLICATION POLICY

The Investigators will be involved in reviewing drafts of the manuscripts, abstracts, press releases and any other publications arising from the study. Data from the study may also be used as part of a thesis for a PhD or MD.

17 DEVELOPMENT OF A NEW PRODUCT/PROCESS OR THE GENERATION OF INTELLECTUAL PROPERTY

Ownership of IP generated by employees of the University vests in the University. The protection and exploitation of any new IP is managed by the University’s technology transfer office, Oxford University Innovations.

18 ABBREVIATIONS

Abbreviations	
AdC	Chimpanzee adenovirus
AdC68	Chimpanzee Adenovirus serotype 68
ADEM	Acute Disseminated Encephalomyelitis
AdHu	Human adenovirus
AdHu5	Human adenovirus serotype 5
AE	Adverse event
AESI	Adverse Events of Special Interest
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
AR	Adverse reaction
AST	Aspartate aminotransferase
BAC	Bacterial artificial chromosome
CBF	Clinical Biomanufacturing Facility
CCVTM	Centre for Clinical Vaccinology and Tropical Medicine
CDC	Centers for Disease Control and Prevention
ChAd63	Chimpanzee Adenovirus serotype 63
ChAdOx1	Chimpanzee Adenovirus Ox1
ChAdOx2	Chimpanzee Adenovirus Ox2
ChAdOx1 biEBOV	Recombinant simian adenovirus ChAdOx1 vector expressing the glycoproteins from Sudan ebolavirus and Zaire ebolavirus
CI	Chief Investigator
CMV	Human cytomegalovirus
CRF	Case Report Form
CTIMP	Clinical Trial of an Investigational Medicinal Product
CTRG	Clinical Trials Research Governance (Now renamed as Research Governance, Ethics and Assurance)
DALYs	Disability-adjusted life years
DNA	Deoxyribonucleic acid
DSUR	Development Safety Update Report
EBV	Epstein Barr virus
EBOD	Ebola Virus Disease
EBOV	Zaire Ebolavirus
ELISA	Enzyme linked immunosorbent assay
ELISpot	Enzyme linked immunospot assay
EMA	European Medicines Agency
ePRO	Electronic patient-reported outcomes
FDA	Food and Drug Administration
Filovirus GP	Filovirus Glycoprotein
GBS	Guillain-Barre Syndrome
GCP	Good Clinical Practice
GGT	Gamma-glutamyl Transferase
GMO	Genetically modified organism
GMP	Good Manufacturing Practice
GP	General Practitioner
HBsAg	Hepatitis B surface antigen
HCG	Human Chorionic Gonadotrophin
HCV	Hepatitis C virus

Abbreviations	
HCV Ab	Hepatitis C virus antibody
HEK	Human embryonic kidney
HIV	Human immunodeficiency virus
HLA	Human leukocyte antigen
HRA	Health Research Authority
IB	Investigators Brochure
ICH	International Conference on Harmonisation
ICS	Intracellular Cytokine Staining
IFN	Interferon
IM	Intramuscular/intramuscularly
IMP	Investigational medicinal product
ISF	Investigator Site File
IU	Infectious units
IUD	Intrauterine device
IUS	Intrauterine system
JCVI	Joint Committee on Vaccination and Immunisation
LSM	Local Safety Monitor
LVLV	Last volunteer last visit
MHRA	Medicines and Healthcare products Regulatory Agency
mRNA	Messenger ribonucleic acid
MVA	Modified Vaccinia Virus Ankara
NCT Number	National Clinical Trial number
NHP	Non-human primate
OVC	Oxford vaccine centre
PBMC	Peripheral blood mononuclear cell
PCR	Polymerase Chain Reaction
PEP	Post-exposure prophylaxis
PHE	Public Health England
PrEP	Pre-exposure prophylaxis
PIS	Participant information sheet
PT	Preferred terms
QP	Qualified Person
RCT	Randomised controlled trial
REC	Research Ethics Committee
RGEA	Research Governance, Ethics and Assurance (Formerly named Clinical Trials and Research Governance)
RNA	Ribonucleic acid
RSV	Respiratory syncytial virus
rVSV-ZEBOV	Recombinant vesicular stomatitis virus-Zaire Ebola Virus
SAE	Serious Adverse Event
SAR	Serious Adverse Reaction
SARS-CoV-2	Severe acute respiratory syndrome coronavirus 2
SFC	Spot forming cells
SmPC	Summary of Product Characteristics
SOC	System Organ Classes
SOP	Standard Operating Procedure
SUDV	Sudan Ebolavirus
SUSAR	Suspected Unexpected Serious Adverse Reaction
TMF	Trial Master File

Abbreviations	
TOPS	The Over-Volunteering Prevention System
VAERS	Vaccine Adverse Event Reporting System
VNA	Virus neutralising assay
vp	Viral particles
WHO	World Health Organisation
WOCBP	Women of Childbearing Potential

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20 Appendix A: Investigator Signature and Declarations

Statement of Compliance

The trial will be conducted in compliance with the protocol, the principles of Good Clinical Practice Guideline, Medicines for Human Use (Clinical Trials) Regulations 2004 (as amended) and all other applicable regulatory requirements.

Chief Investigator Approval, Agreement and Conflict of Interest statement

I have read the trial protocol and agree to conduct the trial in compliance with the protocol, the principles of Good Clinical Practice and all applicable regulatory requirements.

Conflict of interest statement:

I declare that there is no conflict of interest.

Paola Cicconi		02NOV2022
Chief Investigator		
Name	Signature	Date

Principal Investigator Approval, Agreement and Conflict of Interest statement

I have read the trial protocol and agree to conduct the trial in compliance with the protocol, the principles of Good Clinical Practice and all applicable regulatory requirements.

Conflict of interest statement:

I declare that there is no conflict of interest.

Daniel Jenkin		02NOV2022
Principal Investigator		
Name	Signature	Date

21 Appendix B: Document History

Version	Author	Changes
1.0	Daniel Jenkin, Teresa Lambe	Document created
2.0	Daniel Jenkin	SA01: Response to MHRA grounds for non-acceptance: • Added additional exclusion criteria based on the SmPC-like document for Vaxzevria [ChAdOx1 nCoV-19]: (hypersensitivity to the IMP, history of thrombosis with thrombocytopenia following Vaxzervria and history of capillary leak syndrome). • Corrected erroneous reference to urinary beta-HCG and replaced with urinary HCG. • Clarified the blood safety assessments referenced in section 7.7.3 are the haematology and biochemistry tests listed in section 7.6.1.
3.0	Daniel Jenkin	SA02: - Addition of second dose • Group 3 to receive two doses 12-weeks apart • Update to schedule of procedures for the new two dose group to add in the following visits: V2, V2+14, V2+28 and V2+182 • Update to study endpoints timings with new follow up schedule timepoints • Additional stool sampling timepoint following the second vaccine dose(V2+14) • Additional interim safety review after the first 3 individuals have received a second dose of ChAdOx1 biEBOV (using remote eDiary data at 48 hours). • Trial planned (end) date updated to Q4 2022 to account for longer follow up period for group 3. - Update to eDiary section with details on (near) real-time remote monitoring of participant entered AEs - Name change of Sponsor and Monitor (from CTRG to RGEA). The organisation remains the same but has rebranded.

Version	Author	Changes
3.1	Daniel Jenkin	NSA01: Correction of minor blood volume typographical errors in section 2.3: Schedule of Procedures Table: Vaccination and Follow up Visits (two dose schedule) • V2+28 immunology blood volume corrected to 70ml (previously stated as 50ml) and V2+14 immunology blood volume corrected to 50ml (previously stated as 70ml). These were typographical errors in protocol version 3.0. • Errors in cumulative blood volume calculations fixed resulting in a new total of 524ml (previously listed as 519ml in error)

4.0	Daniel Jenkin	SA03:
		• Extension of follow up visits for group 3 to assess longer term durability of vaccine responses. This will include an additional follow up visit with the procedures as listed in section 2. These include
		  o SAE/AESI collection
		  o Immunology blood sampling
		  o Optional stool sampling
		  o An increase to the cumulative blood volume
		• Outcome evaluation timepoints amended to add in the new extended group 3 follow up visit
		  o SAE/AESIs now evaluated to D364/V2+280
		  o Secondary immunology outcomes now evaluated to D364/V2+280
		• Clarification of wording around use of screening results and wording of the timing of the inclusion criteria relating to pregnancy testing to clarify a sample within the screening window is acceptable.

22 Appendix C: Severity Grading Criteria

Table 7. Severity grading criteria for local adverse events *erythema ≤2.5cm is an expected consequence of skin puncture and will therefore not be considered an adverse event

Adverse Event	Grade	Intensity
Pain at injection site	1	Pain that is easily tolerated
	2	Pain that interferes with daily activity
	3	Pain that prevents daily activity
	4	A&E visit or hospitalization
Tenderness	1	Mild discomfort to touch
	2	Discomfort with movement
	3	Significant discomfort at rest
	4	A&E visit or hospitalization
Erythema at injection site*	1	2.5 - 5 cm
	2	5.1 - 10 cm
	3	>10 cm
	4	Necrosis or exfoliative dermatitis
Induration/Swelling at injection site	1	2.5 – 5 cm and does not interfere with activity
	2	5.1 - 10 cm or interferes with activity
	3	>10 cm or prevents daily activity
	4	Necrosis

Table 8. Severity grading criteria for local and systemic AEs. NB: A&E assessment in itself does not constitute a SAE. Refer to 9.1.3 for SAE definition

GRADE 0	None
GRADE 1	Mild: Transient or mild discomfort (< 48 hours); No interference with activity; No medical intervention/therapy required
GRADE 2	Moderate: Mild to moderate limitation in activity – some assistance may be needed; no or minimal medical intervention/therapy required
GRADE 3	Severe: Marked limitation in activity, some assistance usually required; medical intervention/therapy required.
GRADE 4	Potentially Life-threatening: requires assessment in A&E or hospitalisation

Table 9. Severity grading criteria for physical observations (applies to adults only). *Taken after ≥10 minutes at rest **When resting heart rate is between 60 – 100 beats per minute. Use clinical judgement when characterising bradycardia among some healthy subject populations, for example, conditioned athletes. ***Only if symptomatic (e.g. dizzy/ light-headed)

Vital Signs	Grade 1 (mild)	Grade 2 (moderate)	Grade 3 (severe)	Grade 4 Potentially Life threatening
Fever (oral)	38.0°C - 38.4°C	38.5°C – 38.9°C	39.0°C - 40°C	> 40°C
Tachycardia (bpm)*	101 - 115	116 – 130	>130	A&E visit or hospitalisation for arrhythmia
Bradycardia (bpm)**	50 – 54	45 – 49	<45	A&E visit or hospitalisation for arrhythmia
Systolic hypertension (mmHg)	141 - 150	151 – 155	≥155	A&E visit or hospitalisation for malignant hypertension
Diastolic hypertension (mmHg)	91 - 95	96 – 100	>100	A&E visit or hospitalisation for malignant hypertension
Systolic hypotension (mmHg)***	85 - 89	80 – 84	<80	A&E visit or hospitalisation for hypotensive shock
Respiratory Rate (breaths per minute)	17 - 20	21-25	>25	Intubation