Evaluating the Long- Term Immunogenicity of AD / MVA Ebola Virus Vaccines following late boosting with AD26.ZEBOV vaccine administered after heterologous prime/boost schedules of adenoviral and MVA vectored Ebola vaccines in healthy Senegalese adult volunteers aged 18-50 years: an open-label clinical trial

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Confidentiality Statement

This document contains confidential information that must not be disclosed to anyone other than the Sponsor, the Investigator Team, and members of the Independent Ethics Committee. This information cannot be used for any purpose other than the evaluation or conduct of the clinical investigation without the prior written consent of Assoc. Prof Matthew Snape.

Investigator Agreement

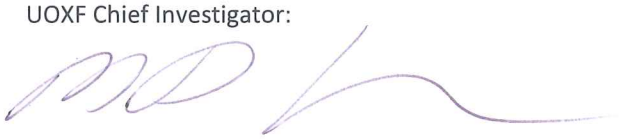
"I have read this protocol and agree to abide by all provisions set forth therein.

I agree to comply with the principles of the International Conference on Harmonisation Tripartite Guideline on Good Clinical Practice."

SIGNATURES

Principal Investigator: Date:

UOXF Chief Investigator: Date:



25TH JUNE 2019.

Conflict of Interest

"According to the Declaration of Helsinki, 2008, I have read this protocol, and declare no/the following (delete as appropriate) conflict of interest"

Principal Investigator: Date:

UOXF Chief Investigator: Date:

25TH JUNE 2019

Details of conflict of interest

Assoc. Prof. Matthew Snape acts, on behalf of the University of Oxford, as Investigator for vaccine studies funded and sponsored by vaccine manufacturers including Janssen, Glaxosmithkline, Novavax, MCM, Pfizer and Medimmune. He receives no personal financial benefit from this work.

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KEY ROLES AND GENERAL INFORMATION

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1. SYNOPSIS

Title	Evaluating the Long- Term Immunogenicity of AD / MVA Ebola Virus Vaccines following late boosting with AD26.ZEBOV vaccine administered after heterologous prime/boost schedules of adenoviral and MVA vectored Ebola vaccines in healthy Senegalese adult volunteers aged 18-50 years: an open-label clinical trial
Short Title	Re-evaluating in Senegal Optimal Vaccine Schedules against Ebola (**RESOLVE**)
Trial Centre(s)	Institut de Recherche en Santé, de Surveillance Epidemiologique et de Formation (**IRESSEF),** Dakar, Senegal
Funders	Innovate UK
Sponsor	University of Oxford
Trial Identifier	RESOLVE
Clinical Phase	II
Active Ingredients of Vaccines	**AD26.ZEBOV** – This is a viral vectored vaccine using an adenovirus as a vector expressing the Mayinga variant glycoprotein of the Ebola virus.
Design	Open- label clinical trial design
Population	Healthy adult volunteers aged 18 – 50 years from previous EBL 06 clinical trial led by IRESSEF and University of Oxford (UOXF) Investigators
Planned Trial Period	Study visits: April 2019 to December 2020
Sample Size	Up to 40 (50% recruitment anticipated, approximately 20)
Finished Products	AD26.ZEBOV at a dose of 5×10^{10} vp
Form	Liquid
Route of Administration	Intramuscular needle injection into the deltoid region of the non-dominant arm
Follow-up Duration	1 year from the date of the booster dose (52 weeks)
Primary Objective	To assess humoral and cellular immunity against Ebola virus glycoprotein at 1 year following a late booster dose of AD26.ZEBOV administered 3 to 4 years after receiving heterologous prime/boost of ChAd3- EBO Z /MVA –EBO Z administered at a 7 day interval
Secondary Objectives	**Safety** Safety and reactogenicity of late booster dose of AD26.ZEBOV administered 3 to 4 years after heterologous prime/boost schedules of adenoviral and MVA vectored Ebola vaccines **Immunogenicity** Humoral and cellular immunity against Ebola virus glycoprotein at 1 month following a late

	booster dose of AD26.ZEBOV administered 3 to 4 years after heterologous prime/boost schedules of adenoviral and MVA vectored Ebola vaccines
Safety Evaluation	Local and systemic solicited and unsolicited adverse events will be assessed. Safety blood tests will be obtained on Days 0, 7 and 28. Solicited adverse events will be recorded daily for 7 days post-vaccination Unsolicited AEs of all severities will be recorded from receipt of vaccination through 28 days post-vaccination. After study Day 28, only SAEs or new chronic medical conditions that require ongoing medical management will be recorded through to the last study visit.
Immunogenicity Evaluation	Immunogenicity will be assessed by a variety of immunological assays and will include: • Humoral- Ebola GP specific IgG as measured by ELISA • Cellular- Ebola GP specific T cell cytokine response measured using ex vivo interferon-γ enzyme-linked immunosorbent spot (ELISPOT) Exploratory outcome measures will include Ebola GP specific T cell cytokine response measured using intracellular staining technique, multicolour flow cytometry and neutralisation assays for antibodies Bleeding time points for immunological assays will be: Day 0, 7, 28 and 365
Statistical Analysis	Descriptive

2. ABBREVIATIONS

AE	Adverse event
aPTT	Activated Partial Thromboplastin Time
ChAd3 EBO Z	Chimapnzee Adenovirus 3 encoding the Ebola Zaire glycoprotein
CBF	Clinical Bio-manufacturing Facility
CCVTM	Centre for Clinical Vaccinology and Tropical Medicine
CI	Confidence Interval
CRF	Case Report Form
DSMB	Data Safety Monitoring Board
DSUR	Development Safety Update Report
ELISA	Enzyme-linked immunosorbant assay
ELISPOT	Enzyme-Linked Immunospot
GCP	Good Clinical Practice
GMO	Genetically Modified Organisms
GP	Glycoprotein
GSK	GlaxoSmithKline
HBsAg	Hepatitis B surface Antigen
HBV	Hepatitis B virus
HCG	Human Chorionic Gonadotrophin
HCV	Hepatitis C virus
HIV	Human Immunodeficiency Virus
HLA	Human Leucocyte Antigen
IB	Investigator Brochure
ICH	International Conference on Harmonisation
ICS	Intracellular Cytokine Staining
IFNγ	Interferon gamma
IM	Intramuscular
IMP	Investigational Medicinal Product
IMPD	Investigational Medicinal Product Dossier
ISR	Internal Safety Review
IUD	Interuterine Device
IUS	Interuterine System
LSM	Local Safety Monitor
mcg	Microgram
MHRA	Medicines and Healthcare Regulatory Authority
MVA	Modified vaccinia virus Ankara
NIH	National Institute of Health
OVG	Oxford Vaccine Group
PBMC	Peripheral Blood Mononuclear Cell
PCR	Polymerase Chain Reaction
PI	Principal Investigator
pfu	Plaque forming unit
PIS	Partcipant Information Sheet
PT	Prothrombin Time
QA	Quality Assurance
QP	Qualified Person
SAE	Serious Adverse Event
SmPc	Summary of Product characteristics
SOP	Standard Operating Procedure
SUSAR	Suspected Unexpected Serious Adverse Reaction
vp	Viral particle
VRC	Vaccine Reasearch Centre, NIH
WHO	World Health Organisation

3. BACKGROUND AND RATIONALE

3.1 Background

Ebolavirus is one of three genera in the family Filoviridae, which along with Marburgvirus and Cuevavirus, are known to induce viral haemorrhagic fever. There are five distinct species in the genus Ebolavirus including Bundibugyo (BDBV), Reston (RESTV), Sudan (SUDV), Taï Forest (TAFV), and Zaire (EBOV).(1) ZEBOV and SUDV are the most pathogenic and have been associated with the major EVD outbreaks.(2) The Ebola virus is a large, negative-strand RNA virus composed of 7 genes encoding viral proteins, including a single glycoprotein (GP). (3-5)

Ebola virus causes outbreaks through person-to-person transmission. Infection results from direct contact with blood, secretions, organs or other bodily fluids of infected people, and indirect contact with environments contaminated by such fluids (2). People remain infectious with EVD as long as their blood and secretions contain the virus; the virus was isolated from semen 61 days after onset of illness in a man who was infected in a laboratory.(2) The high infectivity of blood and secretions puts healthcare workers at particularly high risk during outbreaks, and direct contact with the bodies of deceased victims also has a role in the transmission of the virus.

The incubation period of EVD is 2 to 21 days (7 days on average, depending on the strain) followed by a severe acute viral illness often characterised by the rapid onset of non-specific symptoms such as fever, extreme fatigue, pharyngitis, gastrointestinal complaints, abdominal pain, anorexia, headache, myalgia and/or arthralgia. These initial symptoms last for about 2 to 7 days after which more severe symptoms related to haemorrhagic fever occur, including haemorrhagic rash, epistaxis, mucosal bleeding, hematuria, hemoptysis, hematemesis, melena, conjunctival haemorrhage, tachypnoea, confusion, somnolence, and hearing loss. Laboratory findings include leucopenia, thrombocytopenia and elevated liver enzymes. (2) As the early symptoms are non-specific, diagnosis is difficult and it may take up to three days after symptoms start for the virus to reach detectable levels. Laboratory diagnostic tests such as PCR, ELISA testing and virus isolation can be carried out. In general, the symptoms last for about 7 to 14 days after which recovery may occur. Death can occur 6 to 16 days after the onset of symptoms, and mortality rate has been reported in some outbreaks to be as high as 90% (2, 6, 7).

A primary antibody response (IgM) can be detected in the blood of infected persons 2 to 9 days after infection whereas IgG antibodies appear approximately 17 to 25 days after infection. This IgG response coincides with the recovery phase. In survivors of EVD, both humoral and cellular immunity are detected, however, their relative contribution to protection is unknown (8).

3.1.1 The need for an Ebola vaccine

EVD has occurred in numerous sporadic outbreaks since it was discovered in 1976. The recent EVD outbreak in West Africa in 2014, was the largest in history and recorded a total of 28,616 confirmed, probable, and suspected EVD cases, including 11,310 deaths in Guinea, Liberia, and Sierra Leone, between January 2014 and January 2016.(9) This outbreak was caused by the ZEBOV species of the virus. The recent Ebola virus outbreaks have been characterised by a high mortality rate ranging from 50% to 90%. Treatment options are limited and no specific antiviral drugs or preventative vaccines are currently licensed for use.

Almost all human cases of EVD are due to emergence or re-emergence of the Zaire and Sudan subtype of the virus. The persistence of the virus, evident from various serological surveys, makes this a serious global public health concern in an interconnected word.. Emergence of the Reston subtype in animals poses a potential health risk in parts of Asia such as Philippines.

Future Ebola vaccines will be required to fulfil different needs (10). A vaccine to protect the frontline staff involved in providing care in future outbreaks will need to deliver highly effective and lasting protection (11). Countries with risk of future outbreaks can maintain a pool of vaccinated staff with sustained immunity which can be checked periodically. Such vaccines should prevent infection and virus shedding thereby interrupting onward transmission of the virus during an outbreak.

Vaccines for use during an outbreak in an EVD endemic zone will need to provide rapid protection from a single dose preferably, but would not need to provide long-lasting protection. The main purpose of such a vaccine would be to

reduce disease severity and death, as well as prevention of virus shedding to stop onward transmission. An assessment of sustained immunogenicity of a potential Ebola vaccine will, therefore, be necessary to determine its clinical utility.

Effective control of future Ebola virus outbreaks will require immediate availability of a sufficient cohort of healthcare workers and other frontline staff who are immunized to the infection and will not be a source of viral transmission. The duration of a persistent immunological response from a vaccine will determine whether additional booster dosage will be required to maintain a protective level of immunity in such a cohort. The sustainability of an immune response will also indicate how many such boosters will be required and at what intervals. All such information will be crucial in determining the Ebola vaccination policy by WHO and countries in the Ebola endemic zone.

3.2 Development of Ebola Vaccines

Ebola virus is a filovirus wih a 19-kb, non-segmented, negative-strand RNA genome that encodes 7 viral proteins.(12) The surface glycoprotein mediates viral entry into host cells.(8) This has been the primary antigenic target for vaccine development.(13) 3 early generation Ebola vaccine candidates were evaluated in clinical trials between 2003 and 2009.(14-16) There were no safety concerns but immunogenicity and preclinical efficacy data was deemed inadequate. (16)

The Vaccine Research Center (VRC), National Institute of Allergy and Infectious Diseases (NIAID), National Institutes of Health (NIH) developed a recombinant chimpanzee adenovirus Type 3-vectored Ebolavirus vaccine (cAd3-EBO), VRC-EBOADC069-00-VP. The vaccine encodes wild type (WT) GP from Zaire [GP (Z)] and Sudan [GP (S)] species of Ebola virus. The rationale for the development of this vaccine was based on pre-clinical studies (see Investigator Brochure), previous human experience with other investigational Filovirus vaccines and previous human experience with adenovirus vaccines and the cAd3 vaccine vector.(17) This single vaccination was shown to be safe and immunogenic. (18)

A vaccination strategy to achieve protective immunity in most recipients with a single vaccination would be desirable in an outbreak setting. Vaccination strategies that achieve durable protective immunity would be desirable for populations in areas of the world where outbreaks occur sporadically. Optimally, one approach would serve both needs, but a different approach may be needed for rapid immunity than is needed for durable immunity. The Phase 2 studies and efficacy trials are in progress.

In 2017, the results from a Phase 3 efficacy trial in Guinea testing the rVSV-vectored vaccine for preventing Ebola were published. This was a viral-vectored candidate expressing a surface glycoprotein of the Zaira strain. It was an open-label, cluster-randomised ring vaccination trial. The results suggested substantial protection against the Ebola virus, with no cases among vaccinated individuals in both randomised and non-randomised clusters from day 10 after vaccination. The results gathered from this trial suggest that such a vaccination may be effective in contributing to controlling an Ebola virus disease outbreak and that ring vaccination may also be highly effective.(19)

This trial differs from the previous EBL06 study in that the adenovirus we will be using is AD26. This is because ChAd3 is no longer manufactured by GSK. A previous Phase I study conducted in Oxford showed that AD26 ZEBOV is safe and well tolerated, with a similar reactogenicity profile to ChAd3 EBO Z, and induced comparable immune responses in schedules using an MVA vectored booster dose of vaccine.(20, 21)

3.2.1 AD26.ZEBOV

Pre-Clinical studies

A study to evaluate immunogenicity and protective efficacy of heterologous prime-boost regimens involving AD26, AD35 and MVA-BN-Filo vectors expressing different Ebola and Marburg proteins study in NHPs (Cynomolgus macaques) was performed in a small number of animals (2 per vaccine regimen). Full protection from Ebola virus disease after wild-type Ebola Kikwit 1995 challenge was observed in all recipients of heterologous regimens, with survivors showing only minor to no symptoms. All heterologous prime-boost regimens induced comparable immune responses against the EBOV Mayinga GP. Independently of the vaccine regimen, a strong boost effect was observed after heterologous prime-boost immunisation. As part of this study, safety assessments (non-GLP) were performed. Preliminary findings indicate that the NHPs appear to tolerate the vaccines, without signs of adverse effects. Two additional studies were performed involving more animals, to strengthen the robustness of the nonclinical efficacy data, and also to assess shorter schedules to be evaluated in the clinical program. Overall, the best protection against lethal outcome and Ebola virus disease was obtained with an 8 week prime/boost interval and AD26 as prime immunisation.

Toxicity testing in rabbits addressed general toxicity and reproductive toxicity of different heterologous prime-boost regimens using AD26.ZEBOV and MVA-BN-Filo, and a homologous prime-boost regimen using AD26.ZEBOV.

In a GLP-compliant, repeated dose toxicity study in rabbits, the different dose regimens were well tolerated when administered by IM injection with a 14-day interval period. All vaccine regimens elicited detectable EBOV GP-specific antibody titres. The immune response was associated with local test-article-related inflammatory changes at the injection sites, transient disturbances in blood and serum inflammatory parameters and microscopic findings of increased lymphoid cellularity in the draining iliac lymph nodes and spleen. The findings were noted to be recovering over a 2-week treatment-free period and were considered to reflect a non-adverse physiological response associated with vaccination. There were no effects noted that were considered to be adverse.

In a GLP-compliant embryo-foetal and pre- and postnatal development study in female rabbits, there was no maternal or developmental toxicity following maternal exposure to the different vaccine regimens during the premating and gestation period. All vaccine regimens elicited detectable EBOV GP-specific maternal antibody titres that were transferred to the foetuses. The non clinical data support suitability of AD26.ZEBOV and MVA-BN-Filo as prime-boost regimens.

Clinical studies

The first-in-human phase 1 clinical trial with various prime/boost regimes of the AD26.ZEBOV vaccine was undertaken at the Oxford Vaccine Group. Of the 87 participants recruited, 75 participants were randomised to receive this vaccine in various prime boost regimes with MVA-Bn-Filo, and 12 participants received placebo (saline injection).

The Phase 1 study revealed that more than 90% of healthy adults generated Ebola GP specific IgG 4 weeks after a priming dose with AD26.ZEBOV, increasing to 100% following a booster dose with the MVA-BN-Filo vaccine. 55% developed Ebola specific T cells which were sustained at 8 months following vaccination.(20) Sustenance of the humoral immune responses was evident from detectable GP specific antibodies in all recipients at 1 year following prime immunisation. (21)

There is limited clinical experience with AD26.ZEBOV and there are no known specific precautions or warnings.

To date, no significant safety issues have been identified.

One participant in the study mentioned above (VAC52150EBL2001) (in which participants received a dose of AD26.ZEBOV followed by a boost vaccination with MVA-BN-Filo) developed “Miller Fisher syndrome” presenting approximately one week after suffering from an upper respiratory tract infection. The event happened about a month after boost vaccination with MVA-BN-Filo. The participant was treated in hospital and recovered. After an extensive investigation, the event has been considered to be doubtfully related to vaccine and most likely related to the recent respiratory infection.

Another participant in the same study experienced paraesthesia of the palms and soles approximately two weeks after vaccination with AD26.ZEBOV. After 6 weeks post-vaccination, the case was upgraded to serious, and considered to be a SUSAR again since the participant was experiencing some impact on his daily activities. Although this AE is possibly related to study vaccine, an expert neurology panel consulted by the sponsor concluded that the evolution of this event is atypical for a possible vaccine-related neuropathy. The symptoms improved and the participant was able to return to work.

Study recruitment was paused following each of these adverse events, however was resumed in the UK after approval from the MHRA.

Hypokalemia, anaemia and neutropenia were noted following administration of AD26.ZEBOV but these were not associated with any complaints or symptoms.

4. OBJECTIVES AND ENDPOINTS

4.1 Primary Objective

To assess humoral and cellular immunity against Ebola virus glycoprotein at 1 year following a late booster dose of AD26.ZEBOV administered 3 to 4 years after receiving heterologous prime/boost of ChAd3- EBO Z /MVA –EBO Z administered at a 7 day interval

4.2 Primary Outcome Measures

- Ebola GP specific IgG as measured by ELISA
- Ebola GP specific T cell cytokine response measured using ex vivo interferon-γ enzyme-linked immunosorbent spot (ELISPOT)

4.3 Secondary Objectives

Safety
Safety and reactogenicity of late booster dose of AD26.ZEBOV administered 3 to 4 years after heterologous prime/boost schedules of adenoviral and MVA vectored Ebola vaccines

Immunogenecity

Humoral and cellular immunity against Ebola virus glycoprotein at 1 month following a late booster dose of AD26.ZEBOV administered 3 to 4 years after heterologous prime/boost schedules of adenoviral and MVA vectored Ebola vaccines

4.4 Secondary Outcome Measures

Safety
The specific endpoints for safety and reactogenicity will be actively and passively collected data on adverse events.
The following parameters will be assessed for both groups:

- Occurrence of solicited local reactogenicity signs and symptoms for 7 days following the vaccination
- Occurrence of solicited systemic reactogenicity signs and symptoms for 7 days following the vaccination
- Occurrence of unsolicited adverse events for 28 days following the vaccination
- Change from baseline for safety laboratory measures
- Occurrence of serious adverse events during the whole study duration

Immunogenicity

- Humoral- Ebola GP specific IgG as measured by ELISA
- Cellular- Ebola GP specific T cell cytokine response measured using ex vivo interferon-γ enzyme-linked immunosorbent spot (ELISPOT)

Ebolavirus specific immunogenicity will be assessed by a variety of immunological assays. The primary immunogenicity outcome measures are ELISA IFN-gamma ELISPOT for T cell responses.

Exploratory outcome measures will include Ebola GP specific T cell cytokine response measured using intracellular staining technique (ICS) and multicolour flow cytometry performed with research samples collected at different study timepoints as well as other immunogenicity assays throughout the study. Antibody function will also be assessed using assays to measure neutralisation ability of antibodies to the Ebola glycoprotein. An evaluation of genetic factors associated with immune responses may also be undertaken. These may include:

- Assays to assess immunological aging, dysregulation and senescence, such as telomere length or expression of relevant markers and transcription factors.
- Assays to assess presence or absence of other factors affecting vaccine immunogenicity, such as antibodies against viral pathogens including cytomegalovirus.
- Determination of HLA-type

Both primary and exploratory immunology may involve collaboration with other specialist laboratories, including laboratories outside of Senegal (e.g. Europe and USA). This would involve transfer of serum/plasma and/or peripheral blood mononuclear cells (PBMC), but samples would be anonymised. Volunteers will be consented for this during the process of obtaining informed consent for the study.

5. INVESTIGATIONAL PRODUCTS

5.1 AD26.ZEBOV

This is a monovalent, recombinant, adenovirus vectored vaccine expressing the Mayinga variant GP of the Ebola virus.

5.2 Storage of vaccines

All vaccines will be stored at or below -60°C and not lower than -85°C. All movements of the study vaccines will be documented. Vaccine accountability, storage, shipment and handling will be in accordance with local SOPs.
If deviations in storage temperature occur from the normal allowance for the pharmacy freezer, the storage temperature deviation will be reported promptly to the Sponsor. The deviation must be evaluated and investigated and action must be taken to restore and maintain the desired temperature limits. Pending the outcome of the investigation, the Sponsor will notify the PI if continued clinical use of the product is acceptable.

5.3 Administration of vaccines

Preparation should be done in a temperature controlled preparation unit on a clean table with limited access using aseptic technique.

All vaccines will be administered intramuscularly according to IRESSEF CT site standard SOP. For all groups, the vaccine will be administered intramuscularly into the deltoid muscle of either arm. Participants who consent to receiving a vaccine will receive a 0.5 ml dose of AD26.ZEBOV, containing 5 x 10^{10} vp. The vaccinating Investigator will wear gloves and eye protection. During administration of the vaccines, Advanced Life Support drugs, resuscitation equipment and trained personnel will be immediately available for the management of anaphylaxis. On vaccination day, vaccines will be allowed to thaw to room temperature and administered within 4 hours from the start of thawing.

On the vaccination day, before the injection, study participants will undergo clinical evaluation and samples will be collected for laboratory tests as per schedule of evaluations (Table 3). Pregnancy test results for women of reproductive age must be obtained prior to the study injection on Day 0. A volunteer who arrives at the clinic with fever (axillary temperature ≥37.5°C) or evidence of an acute illness, which precludes administration of the vaccine, may be rescheduled to enroll on a different date. This is outlined in further detail in Section 6.4.4.

Injections will be administered IM by needle and syringe. When choosing an arm for the injection, the investigator should consider whether there is an arm injury or a local skin problem that precludes administering the injection or will interfere with evaluating the arm after injection. The vaccine will be administered by a senior member of the clinical trial team who is experienced in administering similar vaccines. There will also be another study personnel who will confirm that the right doses of the vaccine are given to the volunteers in each group. The volume, date and time of administration will also be documented in a vaccine dispensing log that will be regularly monitored by the PI and an External Monitor.

In keeping with good medical practice, acute medical care will be provided to volunteers for any immediate allergic reactions or other injury resulting from participation in this research study. Procedures for follow-up in the clinic after vaccination are described in detail in section 7 and Table 3.

5.4 Minimising Environmental Contamination with Genetically Modified Organisms (GMO)

The IMP will be handled according to the relevant IRESSEF CT site SOPs. In order to minimise dissemination of the recombinant vectored vaccine viruses into the environment, the inoculation site will be covered with a dressing after vaccination. This should absorb any virus that may leak out through the needle track, and will be removed from the injection site after 30 minutes. Vaccine administrators and destructors will follow precautions for the safe handling of GMOs (including the use of eye protection and gloves).

5.5 Vaccine Supply

The AD26.ZEBOV drug substance and drug product are produced by or under the responsibility of Janssen Vaccines & Prevention B.V., Leiden, the Netherlands. The drug substance is produced in the human cell line PER.C6®. All raw materials used for the production of the AD26.ZEBOV vaccine are chemically defined and of non-animal origin. The manufacturing JNJ-61210474 (AD26.ZEBOV) process is based on applicable guidelines, current Good Manufacturing Practices (cGMP) and previous experience with AD26 vaccine manufacturing for clinical studies.

6. RECRUITMENT AND WITHDRAWAL OF TRIAL VOLUNTEERS

6.1 Volunteer recruitment

The IRESSEF study team will approach approximately 40 participants from a previous Ebola Vaccine study (EBL 06) led at the CHUD. We anticipate that approximately 50% of participants will be subsequently recruited into this study. These volunteers have previously received ChAd3-EBO Z (2.5-3.7 x 10^{10} vp) with a boost of MVA-EBO Z (1.0 x 10^{8} pfu) in 2015.

Although HIV prevalence in Senegal is low, HIV testing will be conducted for potential volunteers. A potential ethical danger of excluding volunteers who are HIV positive is the risk of stigmatisation. Only the investigators will be able to link HIV test results to individuals.

Those who are interested in taking part will be invited to the IRESSEF CT site for further discussion. The study will be further explained to eligible participants on an individual basis. During these discussions, the CT team will continue to stress that this is part of a long process of vaccine development, which will be accelerated by the conduct of such small trials in Africa. Potential volunteers will be informed that they are free to withdraw from the CT at any time without giving any reason. Individuals who feel that the trial is appropriate for them will be invited to attend an appointment where informed consent will be taken, followed by the formal screening visit. Screening will only take place if they sign the Informed Consent Form.

6.2 Informed consent

Detailed pertinent information about the study will be provided in a comprehensible language in a Participant Information Sheet (PIS) that will be given to the participant at least 24 hours prior to the consent being undertaken. The informed consent process will start absolutely before any screening visit evaluation. 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. The investigators will ensure that the volunteers are briefed on all the contents of the PIS in the language they understand. The investigators will also ensure that all volunteers fully understand the risks. Any volunteer who appears to have less than complete understanding after the full process of consent will not be enrolled. As with any experimental vaccine, the volunteers must understand that the vaccines have not yet shown efficacy to prevent infection and this will be stressed during the recruitment stage.

They must also understand there is a very small chance of anaphylactic reactions and that other adverse reactions to the study vaccine may occur, thereby the importance of complying with the observation period after vaccination will be emphasised. The PIS covers these points in detail, and each volunteer will have the contents of the sheet explained in individual meetings.

All volunteers will sign and date the informed consent form before any study specific procedures (including screening visit) are performed. If the volunteer is illiterate, s/he will sign the informed consent form; in the latter case a literate, adult impartial witness will be present throughout the whole consenting process. They will write the participant's name and date of signature and will sign and date the consent form. Volunteers will sign and date two copies of the consent form, one for them to take away and keep, and one to be stored in the participant's medical records.

To ensure that the autonomy of study participants in the trial is not undermined, we will ensure that the informed consent is provided to all participants in a comprehensible manner. We will also disclose to the participants in clear language the risks that are likely to affect their decision to participate or not to participate in the trial. We will emphasise that they have the right to choose not to participate in the trial and the right to withdraw from the study at any time without giving a reason. We will encourage participants to ask questions and discuss areas of concern with the investigators and/or people they trust and ensure that all questions are satisfactorily answered/concerns addressed before the decision to participate in the trial is made.

6.3 Volunteer screening

At the screening visit, the volunteer will again be fully informed of all aspects of the trial, the potential risks and their obligations. In summary, 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 of an investigational vaccine

- There may be no direct benefit from participating in the study
- The volunteer's blood samples taken as part of the study will be stored indefinitely and samples may be sent outside of Senegal and to laboratories collaborating with the University of Oxford. These samples will be anonymised.

The investigators will ensure that the volunteer has signed the consent form and continues to consent for enrolment in the trial before any study specific procedures commence.

6.4 Inclusion and Exclusion criteria

6.4.1 Inclusion criteria

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

- Participants must have completed one of the Ebola vaccine immunisation schedules outlined above (see section 6.1) as part of the EBL 06 study
- Able and willing (in the Investigator's opinion) to comply with all study requirements
- For females only, willingness to practice continuous effective contraception (see section 6.4.3) during the study and a negative pregnancy test on the day(s) of screening and vaccination
- Agreement to refrain from blood donation during the course of the study
- Provide written informed consent

6.4.2 Exclusion criteria

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

- Participation in another research study involving receipt of an investigational product in the 30 days preceding enrolment, or planned participation during the study period
- Receipt of any live, attenuated vaccine within 28 days prior to enrolment
- Receipt of any subunit or killed vaccine within 14 days prior to enrolment
- Administration of immunoglobulins and/or any blood products within the three months preceding the planned administration of the vaccine candidate
- Any confirmed or suspected immunosuppressive or immunodeficient state, including HIV infection; asplenia; recurrent, severe infections and chronic (more than 14 days) immunosuppressant medication within the past 6 months (inhaled and topical steroids are allowed)
- History of allergic disease or reactions likely to be exacerbated by any component of the vaccine, (e.g. egg products) including urticaria, respiratory difficulty or abdominal pain
- Any history of hereditary angioedema, acquired angioedema, or idiopathic angioedema.
- Any history of anaphylaxis in reaction to vaccination
- Pregnancy, lactation or willingness/intention to become pregnant during the study
- History of cancer (except basal cell carcinoma of the skin and cervical carcinoma in situ)
- History of current or previous psychiatric illness.
- Poorly controlled asthma or thyroid disease
- Seizure in the past 3 years or treatment for seizure disorder in the past 3 years
- Bleeding disorder (eg. Factor deficiency, coagulopathy or platelet disorder), or prior history of significant bleeding or bruising following IM injections or venepuncture
- Any other serious chronic illness
- Current anti-tuberculosis prophylaxis or therapy
- Suspected or known current alcohol abuse as defined by an alcohol intake of greater than 42 units every week
- Suspected or known injecting drug abuse in the 5 years preceding enrolment
- Seropositive for hepatitis B surface antigen (HBsAg)
- History of contact with suspected, probable or confirmed cases of Ebola in the previous 21 days
- Any clinically significant abnormal finding on screening biochemistry or haematology blood tests or urinalysis (see Appendix A & B)
- 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

6.4.3 Effective contraception for female volunteers

Female volunteers are required to use an effective form of contraception from the time of consent until 3 months after they have had the vaccine. Previous investigations looking at excretion of similar adenoviral viral vectors after

vaccination in urine for males demonstrated no detectable virus, and therefore males are not required to use barrier contraception whilst taking part in this study as the risk of excretion of the virus is very low.

Acceptable forms of contraception for female volunteers include:

- Injectable or implanted hormonal methods of contraception.
- Placement of an intrauterine device (IUD) or intrauterine system (IUS).

6.4.4 Exclusion criteria at the time of vaccination

The following conditions constitute contraindications to administration of vaccine at the time of vaccination; if any one of these occurs at the time scheduled for vaccination, the participant may be vaccinated at a later date, or withdrawn at the discretion of the Investigator:

- Acute disease at the time of vaccination defined as the presence of a moderate or severe illness with or without fever.
- Temperature of ≥37.5°C at the time of vaccination.
- Positive pregnancy test on the day of vaccination.

6.5 Withdrawal of volunteers

In accordance with the principles of the current revision of the Declaration of Helsinki and any other applicable regulations, a volunteer has the right to withdraw from the study at any time and for any reason, and is not obliged to give his or her reasons for doing so. The 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. Any volunteer who is withdrawn from the study may be replaced on a case by case basis, if that is possible within the specified time frame. The Local Safety Monitor (LSM)/DSMB may recommend withdrawal of volunteers.

If a volunteer withdraws from the study, blood samples collected before their withdrawal from the trial will be used/ stored unless the volunteer specifically requests otherwise. In all cases of volunteer withdrawal every effort will be made by the investigators to continue to collect protocol defined safety assessments, including the safety bloods, unless the participant declines such safety assessment. The study team will ensure the participant understands the purpose and importance of the safety follow up under these circumstances. Safety assessment will continue in this way until the expected end of the study for that participant. In the same way, all adverse events will continue to be collected.

6.6 Compliance with dosing regime

All doses in this vaccine study will be administered by the Investigator and/or designated person and recorded in the CRF.

6.7 Pregnancy

Should a volunteer become pregnant during the trial, she will be followed up as other volunteers and in addition will be followed until pregnancy outcome. No further immunogenicity assessments will be undertaken under these circumstances but safety bloods and other safety assessments will continue as planned unless this is felt to compromise the participants health (or the viability of the pregnancy). Such assessments will be made on a case by case basis and discussion with the DSMB will be considered under these circumstances.

7. TREATMENT OF TRIAL VOLUNTEERS

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

7.1 Study Procedures

Procedures will be performed on the visit time points indicated in the schedules of attendance (Table 1). Additional procedures or laboratory tests may be performed, at the discretion of the Investigators, e.g. urine microscopy in the event of positive urinalysis.

7.2 Observations

Vital signs: pulse, respiratory rate, blood pressure and temperature will be measured at the time-points indicated in the schedule of procedures and may also be measured as part of a physical examination if indicated at other time-points.

7.3 Blood tests and Urinalysis

Blood will be drawn for the following laboratory tests and processed at the IRESSEF, Dakar, Senegal or a designated laboratory using standard procedures.

- **Haematology;** Full Blood Count
- **Biochemistry;** Creatinine and ALT
- **Diagnostic serology;** HBsAg antibodies, HIV antibodies (specific consent will be gained prior to testing blood for these blood-borne viruses)
- **Immunology assays**; Ebola GP specific IgG will be assessed by a variety of immunological assays. These may include *ex vivo* ELISpot assays for interferon gamma as well as antibody ELISAs, functional antibody assays and B cell analyses. Other exploratory immunological assays including cytokine analysis and multicolour flow cytometry
- **Urinalysis;** Urine will be tested for protein, blood and glucose at screening. For female volunteers only, urine will be tested for beta-human chorionic gonadotrophin (β-HCG) immediately prior to vaccination.

Ebola GP specific T cell cytokine response measured using ex vivo interferon-γ enzyme-linked immunosorbent spot (ELISPOT) will be carried out in Senegal and Ebola GP specific IgG as measured by ELISA will be carried out in Oxford.

Collaboration with other specialist laboratories outside of Senegal for further exploratory immunological tests may occur. This would involve the transfer of serum or plasma and/or PBMC and/or RNA to these laboratories, but these would remain anonymised. Informed consent for this will be obtained from volunteers.

Immunological assays will be conducted according to the procedures established in the test laboratories. With the volunteers' informed consent, any leftover cells and serum/plasma will be frozen indefinitely in the OVG Biobank for future immunological analysis of Ebola-specific or vaccine-related responses. This may include human DNA and RNA analysis to search for correlates of vaccine immunogenicity and efficacy.

7.4 Vaccinations

Vaccinations will be administered to eligible volunteers as described in section 6. This is an open-label study.

7.5 Study Visits

The study visits and procedures will be undertaken by a member of IRESSEF CT team. The procedures to be included in each visit are documented in the schedule of attendances. Each visit is assigned a time-point and a window period, within which the visit will be conducted. Deviations from the visit windows in completing study visits are discouraged, but are permitted at the discretion of the investigators in the interest of completing the study schedule and obtaining volunteer safety and immunogenicity evaluations.

7.5.1 Screening Visit

Screening visits for potential volunteers may take place up to 14 days prior to vaccination. Informed consent will be taken before screening, as described in section 6.2. If consent is obtained, the screening procedures indicated in the schedule of attendances will be undertaken.

Abnormal clinical findings from the medical history, physical examination, urinalysis or blood tests at screening will be assessed as detailed in Appendix A and B. Abnormal blood tests following enrolment will be assessed according to site-specific laboratory adverse event grading tables which are filed in the trial master file (TMF). If a test is deemed clinically significant it may be repeated to ensure it is not the result of a laboratory/sampling error. 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, otherwise the individual will be referred to government health facilities. 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. As above, all per-protocol safety assessments will continue to be undertaken under these circumstances unless the participant withdraws consent for this.

7.5.2 Vaccinations

Before the vaccination, the on-going eligibility of the volunteer will be reviewed. The vaccine will be administered as described above in sections 5. The injection site will be covered with a sterile dressing and the volunteer will stay in the IRESSEF Site 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. The sterile dressing will be discarded according to local SOPs at IRESSEF.

Diary cards will collect information on the timing and severity of the following solicited AEs:

Local solicited AEs	Systemic solicited AEs
Pain	Fever
Redness	Feverishness/Chills
Warmth	Joint pains
Swelling	Muscle pains
Itch	Fatigue
	Headache
	Nausea/vomiting
	Malaise

7.5.3 Study Visits

Volunteers will be visited daily at home by a study field worker or nurse, or visit the IRESSEF clinic, to record adverse events (solicited and unsolicited for six consecutive days after vaccination). Additional scheduled visits at the IRESSEFclinic will be at day 7, 28 and 365 post-vaccination during which interim history wil be collected, physical examination and blood tests performed at the time-points indicated in the schedule of attendances (Table 1). Blood will also be taken for exploratory immunology analysis.

7.6 Trial Site

Screening, vaccinations and follow-up visits will take place at IRESSEF, Dakar, Senegal.

Table 1: Schedule of attendances for all participants.

Attendance study clinic	1*	2	-	3	4	5
Timeline (days)	-14	0	1-6	7	28	365
Window period (days)				-1/+3	-1/+3	±14
Informed Consent	X					
Inclusion/Exclusion criteria	X					
Physical exam^	X	X		X		
Medical history	X	X		X		
Urinalysis	X					
BHCG – Urine test[%]		X				
Review contraindications	X	X				
Study Vaccination		X				

AEs reviewed		X~	X	X	X	
SAEs reviewed		X~	X	X	X	X
Daily home visits			X			
HIV, HBV	2					
Haematology	2			2		
Biochemistry*	4			4		
Exploratory Immunology[$]		30		30	30	30
Daily Volume (mL)	**8**	**30**		**36**	**30**	**30**
Max. Cumulative Volume (mL)	**8**	**38**		**74**	**104**	**134**

* = Screening visit; ^ = Physical observations include pulse, blood pressure, temperature; [%] = females only; * = Biochemistry will include Creatinine and ALT; ~ = 30 mins after vaccination. In the event of any abnormality with these or haematology tests, repeat tests will be performed. [$] = Exploratory Immunology will include ELISA, IFN-γ T cell ELISPOT and ICS. These will be outlined in detail in the separate immunomonitoring plan.

8. ASSESSMENT OF SAFETY

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

8.1 Adverse Events

An AE is any untoward medical occurrence in a volunteer, which may occur during or after administration of an Investigational Medicinal Product (IMP) and does not necessarily have a causal relationship with the intervention. An AE can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding), symptom or disease temporally associated with the study intervention, whether or not considered related to the study intervention.

Each adverse event will be graded according to the table for grading severity of adverse events (see **Section 8.9**). The following guidelines will be used to determine whether or not an adverse event is recorded in the study database. Solicited adverse event (*i.e*, reactogenicity parameters) between day 2 and day 6 after vaccination will be recorded by a study field worker or nurse during daily home visits. Volunteers will be encouraged to attend the study clinic in case of any illness. After Study Day 28, only SAEs, as defined in section 8.4 or new chronic medical conditions that require ongoing medical management will be recorded through the last study visit.

8.2 Adverse Reaction

An AR is any untoward or unintended response to an IMP. This means that a causal relationship between the IMP and an AE is at least a reasonable possibility, i.e., the relationship cannot be ruled out. All cases judged by the reporting medical Investigator as having a reasonable suspected causal relationship to an IMP (i.e. possibly, probably or definitely related to an IMP) will qualify as adverse reactions.

8.3 Serious Adverse Events

An SAE is an AE that results in any of the following outcomes, whether or not considered related to the study intervention.

- Death
- Life-threatening event (i.e., the volunteer was, in the view of the Investigator, at immediate risk of death from the event that occurred). This does not include an AE that, if it occurred in a more severe form, might have caused death.
- Persistent or significant disability or incapacity (i.e., substantial disruption of one's ability to carry out normal life functions).
- Hospitalisation, regardless of length of stay, even if it is a precautionary measure for continued observation. Hospitalisation (including inpatient or outpatient hospitalisation for an elective procedure) for a pre-existing condition that has not worsened unexpectedly does not constitute a serious AE.
- An important medical event (that may not cause death, be life threatening, or require hospitalisation) that may, based upon appropriate medical judgment, jeopardise the volunteer and/or require medical or surgical intervention to prevent one of the outcomes listed above. Examples of such medical events include allergic reaction requiring intensive treatment in an emergency room or clinic, blood dyscrasias, or convulsions that do not result in inpatient hospitalisation.

- Congenital anomaly or birth defect.

8.4 Serious Adverse Reaction (SAR)
An adverse event (expected or unexpected) that is both serious and, in the opinion of the reporting investigator or Sponsors, believed to be possibly, probably or definitely due to an IMP or any other study treatments, based on the information provided.

8.5 Suspected Unexpected Serious Adverse Reactions (SUSARs)
A SUSAR is a SAE that is unexpected and thought to be possibly, probably or definitely related to an IMP.

8.6 Causality Assessment
For every unsolicited AE, an assessment of the relationship of the event to the administration of the vaccine will be undertaken. An intervention-related AE refers to an AE for which there is a 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 2).

Table 2: Guidelines for assessing the relationship of vaccine administration to an AE.

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
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
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
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

8.7 Reporting Procedures for All Adverse Events
All AEs occurring within the 28 days following vaccination observed by the investigators or reported by the volunteer, whether or not attributed to study medication, will be reported in the CRF. All AEs that result in a volunteer's withdrawal from the study will be followed up until a satisfactory resolution occurs, or until a non-study related causality is assigned (if the volunteer consents to this). Serious adverse events (SAEs) will be collected throughout the entire trial period.

8.7.1 Reporting procedures for Serious Adverse Events
Every SAE occurring throughout the trial must be reported by telephone, email or fax to the Sponsor, local Ethics Committee, LSM and DSMB by the investigators within 24 hours of (s)he being aware of the occurrence, even if the investigator considers that the adverse event is not related to vaccination. The SAE will also be reported to Janssen Vaccines & Prevention B.V. at the same time.

Any relevant information concerning the adverse event that becomes available after the SAE report form has been sent (outcome, precise description of medical history, results of the investigation, copy of hospitalisation report, etc.) will be forwarded to the Sponsor in a timely manner. The anonymity of the participant shall be respected when forwarding this information.

A Trial specific SAE reporting form will be used which will include specific reporting contacts and time lines (for both SAEs and SUSARs), and further instruction for the management of the SAE documentation.

8.7.2 Reporting procedures for SUSARS

Suspected unexpected serious adverse reactions (SUSARs) will be reported by the Sponsor according to national regulatory guidelines and to the vaccine manufacturer, Janssen Vaccines & Prevention B.V. The Sponsor pledges to inform the Authorities of any trial discontinuation and specify the reason for discontinuation.

8.7.3 Development Update Safety Report

A Development Safety Update Report (DSUR) for each vaccine will be submitted by the Sponsor to the UK competent authority, Ethics Committee and Janssen Vaccines & Prevention B.V. on the anniversary of the first approval date from the regulatory authority.

8.7.4 Pregnancy Safety Reporting

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

8.8 Assessment of severity

The severity of clinical and laboratory adverse events will be assessed according to the scales in Tables 5, 6 and 7 below.

GRADING THE SEVERITY OF ADVERSE EVENTS

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

Adverse event	Grade	Definition (in degrees Celsius)
Temperature	0	< 37.6
	1	37.6 – 38.0
	2	38.1 – 39.0
	3 (severe)	> 39.0

Table 3: Grading of fever AE

Adverse event	Grade	Definition
Any symptom	0	Absence or resolution of symptom
	1	Mild; Awareness of symptom but tolerated; transient or mild discomfort; little or no medical intervention required
	2	Moderate: Discomfort enough to cause limitation of usual activity (some assistance may be needed); some medical intervention or therapy required
	3	Severe: Significant interference with daily activity, some assistance usually required; medical intervention/therapy required, hospitalisation possible
Injection Site	0	No reaction

reaction (erythema, induration and swelling)	1	>3 to ≤50mm
	2	>50 to ≤100mm
	3	>100mm

Table 4: Grading of solicited symptom AE's

Observation	Grade 1	Grade 2	Grade 3 (severe)
Oral temperature (°C)	37.6 – 38.0	38.1 – 39.0	39.1 or greater
Tachycardia (beats/min)	101-115	116-130	>130
Bradycardia (beats/min)	50-54	40-49	<40
Systolic hyper-tension (mmHg)	141-159	160-179	≥180
Diastolic hyper-tension (mmHg)	91-99	100-109	>110
Systolic hypo-tension (mmHg)	85-89	80-84	<80

Table 5. Grading of visit observations AE's

The following ranges are considered normal physiological ranges and are recorded as Grade 0:

Oral temperature between 35.5 and 37.5°Celsius

Resting heart rate between 55 and 100 beats/minute

Systolic blood pressure between 90 and 140 mmHg

8.9 Procedures to be followed in the event of abnormal findings

Eligibility for enrolment in the trial in terms of laboratory findings will be assessed as detailed in Appendix A and B. Abnormal clinical findings from medical history, examination or blood tests will be assessed as to their clinical significance throughout the trial. Laboratory adverse events will be assessed using the site-specific tables in the TMF. 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.

8.9.1 Specific Haematologic criteria for further evaluation

If, following vaccination, there is a 3 femtoliter (fL) or greater change in the mean corpuscular volume (MCV); or a change in severity by one or more grades from baseline for platelet count, or two or more grades from baseline for hemoglobin; or a clinical condition suggesting a coagulopathy, then a thorough hematologic and coagulation evaluation will occur to include at least the following:

- targeted physical exam
- fibrinogen
- blood smear reviewed by a hematologist for evidence of microangiopathic hemolysis.

8.10 Data Safety Monitoring Board

A Data Safety Monitoring Board (DSMB) will be appointed by the Sponsor to provide real-time safety oversight. The DSMB will review all adverse event data generated from the clinical trials in Oxford and Senegal. In addition, all SAEs deemed possibly, probably or definitely related to study interventions will be reviewed by the DSMB. The DSMB will be notified within 24 hours of the Investigators' being aware of the occurrence of any SAE in both clinical trials. The DSMB will determine whether to stop the study for safety concerns on the basis of thorough reviews of interim safety data.

The DSMB will be comprised of a minimum of three (3) members. There will be a minimum of two other independent, appropriately qualified committee members and a statistician, appointed on the basis of their qualifications and experience to serve on a DSMB. Details of the DSMB for this trial can be found in Appendix C.

The DSMB will be complemented by an independent Local Safety Monitor (LSM) at IRESSEF, Dakar, Senegal clinical trial site. All correspondence between the PI and the DSMB will be conveyed by the PI to the trial Sponsor.

The chair of the DSMB may be contacted for advice and independent review by the PI 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 PI or trial Sponsor feels independent advice or review is important.

8.10.1 Safety Report Review

The safety profile will be assessed on an on-going basis by the Investigators. The DSMB will perform independent external safety reviews at specified time periods during the CT. The UOXF Chief investigator, Principal Investigator, and relevant Investigators (as per the trial delegation log) will also review safety issues and SAEs as they arise.

9. STATISTICS

9.1 Description of statistical methods

Statistics will be reported in the form of percentages, frequencies, geometric mean titres and geometric mean fold rises with 95% confidence intervals, per group and per visit. For geometric mean titres and fold rises, data will be log transformed (base 10), and confidence intervals will be calculated on this log scale, prior to transforming back into the original scale for reporting and interpretation. Confidence intervals for proportions will be calculated using the binomial exact method.

9.2 Safety analysis

Safety analysis will be carried out for all vaccinated participants, regardless of whether or not they complete the study.

- Occurrence of each solicited adverse event within 7-day follow-up period (day of vaccination and 6 subsequent days) after vaccination
- Occurrence of unsolicited adverse events within 28 days (day of vaccination and 27 subsequent days) after vaccination
- Occurrence of serious adverse events within 28 days (day of vaccination and 27 subsequent days) after vaccination and over the whole study duration
- Occurrence of a serious adverse event from vaccination to the end of the study

Solicited and unsolicited AE data will be collected at each clinic visit. It will be collected by home visits, clinical review, clinical examination (including observations) and laboratory results. (See Table 3-5).

Hematological (hemoglobin, WBC and platelets) and biochemical (creatinine and ALT) laboratory values will be presented according to toxicity grading scales and tabulated by group.
SAEs, AEs of special interest and withdrawal due to AE(s)/SAE(s) will be described in detail.

9.3 Immunogenicity Analysis

The following statistical parameters and their 95% confidence intervals will be determined and appropriate statistical tests will be carried out to compare between booster groups:

- Percentage of participants with an IgG titre ≥ 166 Elisa units at Baseline (Visit 1), 1 month and 12 months post immunisation
- Geometric mean of pre-boost IgG titres
- Geometric mean of post- boost IgG titres
- Geometric mean of fold rise in IgG titres from pre-boost to 1 and 12 months post-boost

Exploratory outcomes:

- Ebola GP specific T cell cytokine response will be presented as means and confidence intervals, or medians and interquartile ranges if non-normally distributed. Comparisons between vaccine groups will be carried out using the appropriate parametric or non-parametric methods based on the distribution of the data.

The same will be done for IgG (parametric) and T cell (non-parametric) responses for:

- The baseline measures, between each of the three 'feeder' studies
- As sub groups for each of the three studies, comparing
    - The cell frequency/IgG titres in those boosted with AD26.ZEBOV at 1 month post boost
    - The fold rise in these measures, and proportion with three fold increase from baseline to 1 month
    - The cell frequency/IgG titres in those boosted with AD26.ZEBOV at 1 year

10. QUALITY CONTROL AND QUALITY ASSURANCE PROCEDURES

10.1 Direct Access to Source Data/Documents

The PI will provide direct access to the source documents to the EC, to the regulatory agency for inspection, and to authorised representatives nominated by the Sponsor, permitting trial-related monitoring and audits.

10.2 Quality Assurance

10.2.1 Modifications to the Clinical Trial Protocol

Any amendments to the trial that appear necessary during the course of the trial must be discussed by the investigator and Sponsor concurrently unless to eliminate an immediate hazard(s) to study participants. If agreement is reached concerning the need for an amendment, it will be produced in writing by the Sponsor and/or the investigator and will be made a formal part of the protocol. An amendment requires Ethics Committee approval. All amendments must also be transmitted to Regulatory Authorities, if applicable.

An administrative change to the protocol is one that modifies administrative and logistical aspects of a protocol but does not affect the participant's safety, the objectives of the trial and its progress. An administrative change does not require Ethics Committee approval. However, the Ethics committee must be notified whenever an administrative change is made. The PI is responsible for ensuring that changes to an approved CTP, during the period for which Ethics Committee approval has already been given, are not initiated without Ethics Committee review and approval except to eliminate apparent immediate hazards to the participant.

10.2.2 Monitoring

The Sponsor will appoint an appropriately qualified Clinical Monitor (CM) for this CT. Operations of the CM will be outlined in a Monitoring Plan agreed with the PI.

10.2.3 Initiation visit

An initiation visit will be performed before the inclusion of the first volunteer in the study. The Clinical Monitor will verify and document that the vaccines to be used for the trial has been received and that the investigational team has been properly informed about the CT and the applicable regulatory requirements.

10.2.4 Interim Visits

The CM will carry out regular interim visits. The PI or delegate commits to being available for these visits and to allow the monitoring staff direct access to participant's source documents, where existing, laboratories and CRFs. The CM is committed to professional secrecy.

During the visits, the CM may:

- Carry out evaluation of trial progress: in respect of protocol and SOP's, operating guidelines, data collection/source data verification, signature/thumb printing of consent forms, completion of documents, SAE, sample and product management, cold chain monitoring
- Inspect the CRFs, TMF and corresponding correction sheets

The CM will discuss any problem with the investigator and define with him the actions to be taken.

10.2.5 Close-out visit

A close-out visit will be performed at the end of the trial. Its goals are to ensure that:

- The centre has all the documents necessary for archiving
- All unused material has been recovered
- All vaccines have been accounted for

11. ETHICS

11.1 Declaration of Helsinki

The Investigators will ensure that this study is conducted according to the principles of the current revision of the Declaration of Helsinki

11.2 ICH GCP guidelines for Good Clinical Practice (GCP)

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

11.3 Ethical Review

Before the inclusion of the first participant in the CT, the protocol must be approved by the Senegalese Ethics and Oxford Tropical Research Ethics Committee (OxTREC).

11.4 Informed Consent

Although consent from an individual volunteer is sufficient, participants will be encouraged to discuss the study with their husbands/wives (if married) and to have his/her agreement before consent is obtained.

The written Participant Information Sheet (PIS) is provided in French only. When the participant is iliterate, the investigator will interpret the written information in a language the volunteers understand in the presence of a indepdendant witness. When the participant is literate, he/she will read the Participant Information Sheet (PIS). The investigator involved in the informed consent discussion are trained on the study, the information sheet and consent form, and are trained to discuss the trial in the local language the volunteers understand (Wolof). The language of the consent process is documented on the consent form. If the voluteer is not able to read and write in French, an adult witness chosen by him/her, who is not part of the trial, will be present through the whole consent process and sign and date the consent form. The witness will also add the name of the volunteer and his/her date of signature.

The volunteer must give signed informed consent before being included in the trial, after having been informed of the nature of the trial, the potential risks and their obligations. There will be 2 signed informed consent forms provided (one kept by the PI or delegate and the other will be given to the volunteer).

11.5 Confidentiality

All blood tests will be identified by a unique code number only. The code key will be kept by the PI or designee who will be responsible for holding these files securely. All blood results and adverse event data will be encoded in an electronic database and stored securely by the PI or designee.

11.6 Maintaining confidentiality

Only the PI or a designated person will be able to link HIV test results. All information collected as part of the trial will be stored such that confidentiality of study participants is maintained and will not be made available to individuals outside the trial except for monitoring, auditing or required regulatory inspections. The possibility that such information could be reviewed by individuals external to the trial under these circumstances will be specified as part of the informed consent procedure.

11.7 Inducement

There may be a perception amongst volunteers of benefit from physical examination, laboratory screening and HIV testing in the current study, in addition to free health care provided during the study period for non-vaccine related medical problems. The provision of such care is necessary to ensure the required safety data is reliably collected throughout the trial. We will reimburse for transport expenses for all study participants according to standard IRESSEF CT site guidelines (Cf 11.9)

11.8 Indemnity

Compensation for any injury caused by taking part in this study will be in accordance with the guidelines of the Association of the British Pharmaceutical Industry (ABPI). Broadly speaking the ABPI guidelines recommend that 'the Sponsor', without legal commitment, should compensate participants without them having to prove that the Sponsor is at fault. This applies in cases where it is likely that such injury results from giving any new drug or any other procedure carried out in accordance with the CTP for the study. 'The Sponsor' will not compensate participants where such injury

results from any procedure carried out which is not in accordance with the CTP for the study. Where negligence can be proven, participants are still able to seek compensation. In this instance the University of Oxford is the Research Sponsor Institution.

11.9 Compensation

Volunteers will be reimbursed 10,000 F CFA for transport expenses, food and for their time lost at any scheduled study visit.

12. DATA HANDLING AND RECORD KEEPING

12.1 Data Management

The PI will be responsible for receiving, entering, cleaning, querying and storing all data that accrues from the study. The PI may delegate this responsibility to relevant staff at IRESSEF, Dakar, Senegal. The data will be entered into the participants CRFs. Data will be subsequently transferred to an electronic database for analysis.

If any changes to the Clinical Trial Protocol (CTP) are necessary during the course of the CT, a formal amendment will be presented to the Sponsor prior to submission to the relevant EC for approval unless to eliminate an immediate hazard(s) to study participant without prior ethics approval.

12.1.1 Data Capture Methods

Data capture will be on paper CRFs. The CRFs will be considered source documents. Adverse events will be tabulated in an electronic database (OpenClinica®) for descriptive analysis. Immunological data will be transferred to an electronic database for analysis without any volunteer identifier apart from the unique volunteer number.

12.1.2 Types of Data

Data collected will include solicited and non-solicited adverse event data, concomitant medications, clinical laboratory and exploratory immunology data. Source documents will include laboratory results and the case record file containing the case report forms for each volunteer.

12.2 Timing/Reports

Annual Safety Report: Due on anniversary of Regulatory Approval – sent to the Oxford Ethics Committee and the Senegalese Ethics Committee (CNERS).

Annual Progress Report: Due on anniversary of Ethical Approval – sent to Oxford Ethics Committee and the Senegalese Ethics Committee CNERS).

12.3 Archiving

The PI must ensure that arrangements are in place for the clinical trial site keep all clinical trial documents for at least 5 years after the completion or discontinuation of the trial, but this will be extended to meet regulatory requirements if the trial data is included in a Marketing Authorisation (MA) application.

12.4 Protocol deviations

Any unforeseen and unavoidable deviations from the protocol will be documented and filed in the study file with explanation.

12.5 Data ownership, storage, processing, sharing and transference:

The sample data will be jointly owned by all partners in the international consortium involved in the conduct of this vaccine trial. The data will be processed by a dedicated Data Manager using OpenClinica software. The data transfer will be done according to IRESSEF policy and SOP's. The sample data will be stored at Bio-bank Unit of IRESSEF and only authorised personnel including investigators, Sponsor representatives, local ethics committee members, monitors, auditors and inspectors will have access to them.

To better understand the complex antigenicity of Ebola virus and how this affects the efficacy of the vaccine, anonymised blood samples collected from the study participants may be stored for a longer period of time in the OVC Biobank ('Oxford Vaccine Centre Biobank' Southampton & South West Hampshire LREC (B) 10/H0504/25). These samples will only be used for future research purposes e.g genetic studies such as human DNA and RNA analysis to search for correlates of vaccine immunogenicity and efficacy. The samples will not be used for any commercial purpose. This information has been included in the informed consent document and the participants will be provided the option to agree or not to agree to indefinite storage of their samples. Any samples which do not have consent will be destroyed.

13. FINANCING AND INSURANCE

13.1 Financing

The study is funded by an INNOVATE UK grant.

13.2 Insurance

Oxford University Investigators participating in this trial will receive insurance coverage from the University clinical trials insurance policy. The University has a specialist insurance policy in place: - Newline Underwriting Management Ltd, at Lloyd's of London – which would operate in the event of any Participant suffering harm as a result of their involvement in the research.

14. REFERENCES

1. Kuhn JH, Bao Y, Bavari S, Becker S, Bradfute S, Brister JR, et al. Virus nomenclature below the species level: a standardized nomenclature for natural variants of viruses assigned to the family Filoviridae. Archives of virology. 2013;158(1):301-11.
2. WHO. Ebola Virus Disease 2014 [updated April 2014; cited 2014 June 13]. Fact sheet N°103]. Available from: http://www.who.int/mediacentre/factsheets/fs103/en/.
3. Hart MK. Vaccine research efforts for filoviruses. Int J Parasitol. 2003;33(5-6):583-95.
4. Sanchez A, Kiley MP, Holloway BP, Auperin DD. Sequence analysis of the Ebola virus genome: organization, genetic elements, and comparison with the genome of Marburg virus. Virus Res. 1993;29(3):215-40.
5. Sanchez A, Kiley MP, Klenk HD, Feldmann H. Sequence analysis of the Marburg virus nucleoprotein gene: comparison to Ebola virus and other non-segmented negative-strand RNA viruses. J Gen Virol. 1992;73 (Pt 2):347-57.
6. Geisbert TW, Jahrling PB. Exotic emerging viral diseases: progress and challenges. Nat Med. 2004;10(12 Suppl):S110-21.
7. Hensley LE, Jones SM, Feldmann H, Jahrling PB, Geisbert TW. Ebola and Marburg viruses: pathogenesis and development of countermeasures. Curr Mol Med. 2005;5(8):761-72.
8. Sullivan N, Yang ZY, Nabel GJ. Ebola virus pathogenesis: implications for vaccines and therapies. J Virol. 2003;77(18):9733-7.
9. Prevention CfDCa. 2014 Ebola Outbreak in West Africa - Case Counts 2016 [updated 14/04/2016. Available from: https://www.cdc.gov/vhf/ebola/outbreaks/2014-west-africa/case-counts.html.
10. Gilbert SC. Adenovirus-vectored Ebola vaccines. Expert review of vaccines. 2015;14(10):1347-57.
11. Coltart CE, Johnson AM, Whitty CJ. Role of healthcare workers in early epidemic spread of Ebola: policy implications of prophylactic compared to reactive vaccination policy in outbreak prevention and control. BMC Med. 2015;13:271.
12. Zampieri CA, Sullivan NJ, Nabel GJ. Immunopathology of highly virulent pathogens: insights from Ebola virus. Nature immunology. 2007;8(11):1159-64.
13. Marzi A, Feldmann H. Ebola virus vaccines: an overview of current approaches. Expert review of vaccines. 2014;13(4):521-31.
14. Ledgerwood JE, Costner P, Desai N, Holman L, Enama ME, Yamshchikov G, et al. A replication defective recombinant Ad5 vaccine expressing Ebola virus GP is safe and immunogenic in healthy adults. Vaccine. 2010;29(2):304-13.
15. Martin JE, Sullivan NJ, Enama ME, Gordon IJ, Roederer M, Koup RA, et al. A DNA vaccine for Ebola virus is safe and immunogenic in a phase I clinical trial. Clin Vaccine Immunol. 2006;13(11):1267-77.
16. Sarwar UN, Costner P, Enama ME, Berkowitz N, Hu Z, Hendel CS, et al. Safety and immunogenicity of DNA vaccines encoding Ebolavirus and Marburgvirus wild-type glycoproteins in a phase I clinical trial. J Infect Dis. 2015;211(4):549-57.
17. Stanley DA, Honko AN, Asiedu C, Trefry JC, Lau-Kilby AW, Johnson JC, et al. Chimpanzee adenovirus vaccine generates acute and durable protective immunity against ebolavirus challenge. Nat Med. 2014;20(10):1126-9.
18. Ledgerwood JE, DeZure AD, Stanley DA, Coates EE, Novik L, Enama ME, et al. Chimpanzee Adenovirus Vector Ebola Vaccine. The New England journal of medicine. 2017;376(10):928-38.
19. Henao-Restrepo AM, Longini IM, Egger M, Dean NE, Edmunds WJ, Camacho A, et al. Efficacy and effectiveness of an rVSV-vectored vaccine expressing Ebola surface glycoprotein: interim results from the Guinea ring vaccination cluster-randomised trial. Lancet (London, England). 2015;386(9996):857-66.
20. Milligan ID, Gibani MM, Sewell R, Clutterbuck EA, Campbell D, Plested E, et al. Safety and Immunogenicity of Novel Adenovirus Type 26- and Modified Vaccinia Ankara-Vectored Ebola Vaccines: A Randomized Clinical Trial. Jama. 2016;315(15):1610-23.
21. Winslow RL, Milligan ID, Voysey M, Luhn K, Shukarev G, Douoguih M, et al. Immune Responses to Novel Adenovirus Type 26 and Modified Vaccinia Virus Ankara-Vectored Ebola Vaccines at 1 Year. Jama. 2017;317(10):1075-7.
22. FDA. Toxicity Grading Scale for Healthy Adult & Adolescent Volunteers Enrolled in Preventative Vaccine Clinical Trials.

APPENDIX A: Grading of Laboratory Adverse Events

Severity grading criteria for clinically significant laboratory abnormalities; adapted from FDA guidelines (22) using Oxford University Hospitals NHS Trust laboratory reference ranges.

Laboratory Test	Grade 1	Grade 2	Grade 3
Hgb (female) – gm/dL Ref range 12.0 – 15.0	10.5 – 11.5	9.0 – 10.4	<9.0
Hgb (male) – gm/dL Ref range 13.0 – 17.0	11.5 – 12.5	10.0 – 11.4	<10.0
WBC- elevated (x10*9/L) Ref range 4.0-11.0	11.50 – 15.00	15.01 – 20.00	>20.0
WBC- low (x10*9/L) Ref range 4.0-11.0	2.50 – 3.50	1.50 – 2.49	<1.50
Neutrophils decrease (x10*9/L) Ref range 2.0-7.0	1.00 – 1.49	0.50 – 0.99	<0.50
Lymphocytes decrease (x10*9/L) Ref range 1.0-4.0	0.75 – 1.00	0.50 – 0.74	<0.50
Eosinophils (x10*9/L) Ref range 0.0-0.5	0.65 – 1.50	1.51 – 5.00	>5.00
Platelets (x10*9/L) Ref range 150-400	125 – 135	100 – 124	<100
Bilirubin – when accompanied by any increase in Liver Function Test increase by factor Ref range 3-17 (umol/L)	1.1 – 1.25 x ULN	>1.25 – 1.5 x ULN	>1.5 – 1.75 x ULN
Bilirubin- when LFTs normal; increase by factor Ref range 3-17 (umol/L)	1.3 – 1.5 x ULN	1.6 – 2.0 x ULN	>2.0 x ULN
ALT, AST; increase by factor Ref range 10-45 (IU/L)	1.25 – 2.5 x ULN	>2.5 – 5.0 x ULN	>5.0 x ULN
Alkaline phosphate- increase by factor Ref range 95-280 (IU/L)	1.1 – 2.0 x ULN	2.1 – 3.0 x ULN	>3.0 x ULN
Albumin- low (g/L) Ref range 35-50	28 – 31	25 – 27	<25
Creatinine Ref range 54-145 (umol/L)	1.1–1.5 x ULN	>1.6–3.0 x ULN	>3.0 x ULN
Urea (mmol/L) Ref range 2.5-6.7	8.2 – 8.9	9.0 – 11.0	>11.0
Sodium- elevated (mmol/L) Ref range 135-145	146 – 147	148 – 149	≥150
Sodium- low (mmol/L) Ref range 135-145	132 – 134	130 – 131	≤129
Potassium- elevated (mmol/L) Ref range 3.5-5.0	5.1 – 5.2	5.3 – 5.4	≥5.5
Potassium- low (mmol/L) Ref range 3.5-5.0	3.2 –3.3	3.0 – 3.1	≤2.9

APPENDIX B: Laboratory Values for exclusion

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. In general, volunteers will be excluded if a result at screening constitutes what would qualify as a grade 1 (or higher) laboratory adverse event, according to the site-specific laboratory adverse event tables (0). Urinalysis at screening will be assessed as per the table below:

URINE ANALYSIS (using MULTISTIX)	
Protein*	2+ or Protein creatinine ratio of ≥50mg/mmol
Blood[£]	2+ on two dipstick tests
Glucose	1+

**In the event of the dipstick testing positive for protein with ≥1+ protein urine should be sent for a protein creatinine ratio.*

[£] In the event of urine dipstick testing positive for ≥1+ blood with, or without, protein in volunteers a repeat dipstick test will be carried out to confirm haematuria. In female volunteers, a menstrual history will be taken to elicit whether the participant is currently menstruating and if they are, urine dipstick will be repeated after 1 - 2 weeks. If blood and/or proteinuria persist in any volunteer, they will be excluded from the trial, and the appropriate follow-up arranged.

APPENDIX C: DSMB

The members of the DSMB for this clinical trial are as follows:

Chair: Professor Blaise Genton (Professor of Tropical Medicine, Lausanne)

Members: Professor Roger Tine (Professor of parasitology and epidemiology, UCAD, Dakar)
Professor Deborah Watson-Jones (Professor of Clinical Epidemiology and International Health, LSHTM)
Dr Alfred Tiono (Clinical Trials physician, CNRFP, Ouagadougou)
Dr Paul Milligan (Reader in Epidemiology and Medical Statistics, LSHTM)