BMJ Open

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Journal:	*BMJ Open*
Manuscript ID	bmjopen-2020-046835
Article Type:	Protocol
Date Submitted by the Author:	13-Nov-2020
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Keywords:	Protocols & guidelines < HEALTH SERVICES ADMINISTRATION & MANAGEMENT, Health & safety < HEALTH SERVICES ADMINISTRATION & MANAGEMENT, Immunology < TROPICAL MEDICINE

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Open-label, Randomized Controlled, Phase 2 Clinical Trial to Evaluate the Immunogenicity and Safety of a Prophylactic Vaccination of Health Care Providers by Administration of a Heterologous Vaccine Regimen Against Ebola in the Democratic Republic of the Congo: the Study protocol

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0032 3 265 9716

Word count excluding title page, abstract, references, figures, tables and acknolewdgements: 3731

ABSTRACT

Introduction: This article describes the protocol of an Ebola vaccine clinical trial which investigates the safety and immunogenicity of a booster vaccination with Ad26.ZEBOV after a prophylactic Ebola vaccine regimen comprised of 2 candidate Ebola vaccines (Ad26.ZEBOV and MVA-BN-Filo). This clinical trial is part of the EBOVAC3 project (an Innovative Medicines Initiative 2 Joint Undertaking), and is the first to evaluate the safety and immunogenicity of two different booster vaccination arms in adults.

Methods and analysis: This study is an open-label, monocentric (in Tshuapa province, DRC), phase 2, randomized controlled trial evaluating the immunogenicity and safety of a booster dose of Ad26.ZEBOV offered at respectively 1 year or 2 years (randomization 1:1) after a heterologous 2-dose vaccine regimen with Ad26.ZEBOV as first dose and MVA-BN-Filo as second dose at a 56-day interval. A total number of 700 HCP and front-liners are planned to be recruited from the Tshuapa province in DRC. The primary and secondary objectives of the study assess the immunogenicity of each vaccine dose through the evaluation of binding antibody responses after vaccination and the safety of the vaccines through the collection of serious adverse events until six months post booster vaccination and solicited and unsolicited adverse events for one week after the booster dose.

Ethics and dissemination: The protocol was approved by the National Ethics Committee of the Ministry of Health of the Democratic Republic of Congo (n°121/CNES/BN/PMMF/2019). The clinical trial was registered on the 4th of December 2019 on ClinicalTrials.gov (NCT04186000). Trial activities are planned to finish in July 2022. All participants are required to provide written informed consent and no study-related procedures will be performed until consent is obtained. The results of the trial will be added on ClinicalTrials.gov, published in peer-reviewed journals and presented at international conferences.

Key words: Clinical Trial Protocol, Ebola Vaccines, Safety, Immunogenicity, Health Care Providers

STRENGTHS AND LIMITATIONS OF THIS STUDY

- With this randomized controlled vaccine trial, being the first to evaluate the safety and immunogenicity in two different booster vaccine arms 1 or 2 years after the prime dose, new contributions will be added to already existing safety and immunogenicity data.
- Vaccination of HCP and front-liners can potentially help protect a community which is at risk for future outbreaks.
- Innovative use of iris scanning biometric material to identify participants enrolled in the trial.
- This study takes place in a resource poor setting, impacting logistical set-up of the trial.
- Long duration of the trial (2.5 years) may lead to considerable loss to follow up.

INTRODUCTION

Ebolaviruses (negative stranded RNA viruses) belong to the *Filoviridae* family and cause Ebola virus disease (EVD), which often leads to severe haemorrhagic fever in humans and nonhuman primates[1]. Contact with infected wild animals (such as fruit bats, gorillas, apes, monkeys, etc.) is often reported as the source of animal-to-human transmission[2-4] and once among humans, these public health pathogens spread via direct (body fluids) or indirect (contaminated surfaces) human-to-human contact[2, 3]. While they do not spread via air or water[3], *Ebolaviruses* bring along a severe public health burden with case fatality rates that can range up to 90%[5]. Since the discovery of the Ebola viruses in 1976[6], more than 20 outbreaks (most are endemic to regions in equatorial and western Africa) have taken place[7]. To date, the Democratic Republic of the Congo (DRC) has been the most affected country and is currently battling its 11th outbreak[8]. However, it is only recently that the search for a safe and effective vaccine against Ebola was accelerated when the epidemic potential of *Ebolaviruses*, and more specifically the species *Zaïre Ebolavirus* (virus name: Ebola virus; abbreviation: EBOV[9]), became clear through the West African Ebola epidemic (2013-2016; 28,616 cases with 11,310 deaths[10]).

One of the initiatives to develop such a vaccine came from an international consortium, funded by the Innovative Medicines Initiative 2 (IMI2) Joint Undertaking, dedicated to evaluate a prophylactic Ebola

vaccine regimen comprised of 2 candidate Ebola vaccines (Ad26.ZEBOV and MVA-BN-Filo) and aiming to bring this prophylactic Ebola vaccine to licensure[11]. Ad26.ZEBOV is a monovalent vaccine developed to provide active EBOV-specific immunity. MVA-BN-Filo, which is administered 56 days after the Ad26.ZEBOV vaccine, is a multivalent vaccine developed to establish active immunity to EBOV, *Sudan Ebolavirus*, *Taï Forest Ebolavirus* and the Marburg virus (also part of the *Filoviridae* family). In July 2020, the 2-dose prophylactic vaccine regimen was granted market authorisation[12].

Several projects (PREVAC, EBOVAC1, EBOVAC2 and EBOVAC3) within the IMI2 Joint Undertaking, of which the first in-human clinical trials started in 2014, were at the basis of this successful authorisation[11]. Within these projects, multiple clinical trials assessed or are assessing the timing, tolerability, safety, and immunogenicity of different Ad26.ZEBOV and MVA-BN-Filo vaccine regimen in healthy adults (≥18 years old) and children/adolescents (1-17 years old) via phase 1, 2, 2B and 3 studies. Initial trials showed that healthy adult participants had higher geometric mean concentrations of binding antibodies for the regimen where Ad26.ZEBOV vaccination was followed by an MVA-BN-Filo vaccination 56 days later. Moreover, 100% of them had detectable Ebola glycoprotein-specific Immunoglobulin G (IgG) antibodies up to at least 240 days after vaccination[13-15]. Even though some local (erythema, swelling and pain at injection site) and systemic (headache, nausea, pyrexia, myalgia and fatigue) solicited adverse events were recorded, the vaccine regimen was generally well tolerated across studies[13-17].

While it is of utmost importance that the 2-dose prophylactic vaccine regimen is safe and leads to an immune response, it is also crucial to find out whether or not this regimen can lead to induced immune memory at the time of imminent risk (i.e. an outbreak) through a booster vaccination. To evaluate this induced immune memory, three previous studies within EBOVAC projects have administered a booster vaccine with Ad26.ZEBOV at either 1 year (NCT02325050; NCT02564523) or 2 years (NCT02509494) post Dose 1. However, it still has to be determined whether the induced immune memory response differs if a booster vaccination is given 1 or 2 years after Dose 1.

Healthcare settings play an important role in the control of EVD and therefore health care providers (HCP) and front-liners, due to occupational exposure, are not only more at risk of disease acquisition but also facilitate the spread of the virus[18-21]. Knowing that outbreaks of EVD often occur in regions where there is already a shortage of HCP and front-liners, this further depletes a weak health care system and the quality of care. Consequently, the Strategic Advisory Group of Experts stated in 2018 that for preventive strategies, vaccination of HCP as part of an emergency preparedness plan has significant potential of reducing the scale and duration of outbreaks[22].

This phase 2 clinical trial compares two booster arms with an Ad26.ZEBOV vaccine administered either 1 or 2 years post first dose of a heterologous vaccine regimen with Ad26.ZEBOV followed by MVA-BN-Filo 56 days later. The trial is conducted in a cohort of HCP and front-liners in DRC, a well-known population at risk from clinical and epidemiological perspective.

METHODS

Study design and setting

This study is an open-label, monocentric, phase 2 randomized controlled trial to evaluate the immunogenicity and safety of Ad26.ZEBOV ($5x10^{10}$ viral particles) as first dose and MVA-BN-Filo ($1x10^{8}$ infectious units) as second dose vaccination at a 56-day interval in HCP and front-liners who may be exposed to Ebola in the event of a future Ebola outbreak in DRC. Additionally, after randomization (1:1) a booster of Ad26.ZEBOV ($5x10^{10}$ viral particles) will be offered at respectively 1 year or 2 years after the first dose (Figure 1). As this study is designed to provide descriptive information regarding immunogenicity and safety, an open-label design was preferred.

The study site is located in Boende, Tshuapa province, DRC (Figure 2), at approximately 750km north-west of Kinshasa. Study participants are enrolled at the General Reference Hospital in Boende.

Objectives

The primary, secondary and exploratory objectives and endpoints of this study are described in Table 1.

Table 1. Objectives and endpoints

Objectives	Endpoints
Primary	
• To assess binding antibody responses post-dose 2 vaccination with MVA-BN-Filo.	• Binding antibody levels against the EBOV GP using FANG ELISA at 21 days post-dose 2 (Day 78) vaccination with MVA-BN-Filo.
Secondary	
• To assess binding antibody responses after booster vaccination with Ad26.ZEBOV given at 1 or 2 years after first dose.	• Binding antibody levels against the EBOV GP using FANG ELISA at 7 days (excluding the day of vaccination) post booster.
• To assess the safety of a heterologous vaccine regimen utilizing Ad26.ZEBOV and MVA-BN-Filo administered at a 56-day interval and a booster vaccine with Ad26.ZEBOV at one or two years post first dose.	• Serious adverse events from first dose vaccination until 6 months post booster. • Solicited and unsolicited local and systemic adverse events until 7 days post booster vaccination (day of vaccination and subsequent 7 days) with Ad26.ZEBOV.
Exploratory	
• To assess binding antibody responses at different time points as indicated in the Study time and events overview (Figure 1).	• Binding antibody levels against the EBOV GP using FANG ELISA at different time points as indicated in the Study time and events overview (Figure 1).
• To assess neutralizing antibody response directed against the Adenovirus vector prior to vaccination.	• Neutralizing antibody levels against Ad26 using Ad26 VNA at the first visit.
• To assess neutralizing antibody response directed against the MVA vector prior to vaccination.	• Neutralizing antibody levels against MVA-BN-Filo using MVA PRNT assay at the first visit.
• To assess seroprevalence of Ebola virus disease prior to vaccination.	• Presence of pre-existing Human anti-EBOV GP IgG and anti-EBOV NP IgG using LUMINEX assay.

ELISA: enzyme-linked immunosorbent assay; EU/mL: ELISA units/mL; FANG: Filovirus Animal Nonclinical Group. VNA: Virus Neutralization Assay; PRNT: Plaque Reduction Neutralization Test

Participant population

A total number of 700 Registered HCP and front-liners in DRC (working in the Boende General Reference Hospital, Health Centres or Health Posts in the Boende health district) are planned to be recruited from the Tshuapa province in DRC. This number is defined upon the feasibility of recruitment of HCP in the region.

Inclusion and exclusion criteria that determine the eligibility of participants are reported in Table 2.

Table 2. Inclusion and exclusion criteria

Inclusion criteria
Each potential participant must satisfy all of the following criteria to be enrolled in the study: 1. The participant must pass the Test of Understanding. *Note: If the participant fails the Test of Understanding on the first attempt, he/she must be retrained on the purpose of the study and must take the test again (2 repeats are allowed). If participants fail on the third attempt, they should not continue with screening or consenting procedures.* 2. Each participant must sign an informed consent form indicating that he or she understands the purpose of, and procedures required for, the study and is willing to participate in the study. In case the participant cannot read or write, the procedures must be explained and informed consent must be witnessed by a trusted literate third party not involved with the conduct of the study. 3. The participant must be a man or women aged 18 years or older. 4. The participant must be a documented HCP in DRC. 5. The participant must be healthy in the investigator's clinical judgement and on the basis of vital signs assessed at day 1 screening.

Note: HIV-positive subjects can be enrolled as long as their general condition is good, i.e. they are on antiretroviral treatment or have no signs or symptoms of immunodepression, diagnosed on the basis of physical examination, medical history, and the investigator's clinical judgment.

6. Before vaccination, a woman must be either:

   - Of childbearing potential and practicing (or intending to practice) a highly effective method of birth control consistent with local regulations and/or local culture regarding the use of birth control methods for participants in clinical studies, beginning at least 28 days prior to vaccination and during the study up to at least 3 months after the first (or only) vaccination (Ad26.ZEBOV) and 1 month after the MVA-BN-Filo vaccination (if applicable); and then starting again 14 days before the booster vaccination until 3 months after the booster vaccination. The sponsor considers the following methods of birth control to be highly effective: established use of oral, injected or implanted hormonal methods of contraception; placement of an intrauterine device or intrauterine system; barrier methods: condom or occlusive cap (diaphragm or cervical/vault caps) with or without spermicidal foam/gel/film/cream/suppository; male partner sterilization (the vasectomized partner should be the sole partner for that participant); true abstinence (when this is in line with the preferred and usual lifestyle of the participant); OR
   - Not of childbearing potential: postmenopausal (amenorrhea for at least 12 months without alternative medical cause); permanently sterilized (e.g. bilateral tubal occlusion [which includes tubal ligation procedures as consistent with local regulations], hysterectomy, bilateral salpingectomy, bilateral oophorectomy); or otherwise be incapable of pregnancy.

Note: If the social situation of a woman of childbearing potential changes (e.g. woman who is not heterosexually active becomes active), she must begin a highly effective method of birth control, as described above.

7. Woman of childbearing potential must have a negative urine β-human chorionic gonadotropin pregnancy test immediately prior to each study vaccine administration.
8. Participant must be available and willing to participate for the duration of the study.
9. Participant must be willing and able to comply with protocol requirements (including certain prohibitions and restrictions such as the use of anticonception and the discouragement of concomitant treatment that may alter the immune response).
10. Participant must be willing to provide verifiable identification.
11. Participant must have a means to be contacted.

Exclusion criteria

Participants will be excluded from study participation in case the following criteria apply:

1. The participant has a known history of Ebola virus disease.
2. The participant has received any experimental candidate Ebola vaccine less than 3 months prior to the first study visit.
3. The participant has received any experimental candidate Ad26-vaccine in the past.

   Note: Receipt of any approved or experimental vaccinia/smallpox vaccine or experimental Ad-vector vaccine other than Ad26 prior to study entry is allowed.
4. The participant has a known allergy or history of anaphylaxis or other serious adverse reactions to vaccines or vaccine products (including any of the constituents of the study vaccines [e.g. polysorbate 80, ethylenediaminetetraacetic acid (EDTA) or L-histidine for Ad26.ZEBOV vaccine; and tris (hydroxymethyl)-amino methane (THAM) for MVA BN-Filo vaccine]), including known allergy to egg, egg products and aminoglycosides.
5. The participant has an acute illness (this does not include minor illnesses such as mild diarrhea or mild upper respiratory tract infection) or temperature ≥38.0°C on Day 1.

Participants with such symptoms will be excluded from enrollment at that time, but may be rescheduled for enrollment at a later date if feasible.

6. The participant is a pregnant or breastfeeding women, or women planning to become pregnant while enrolled in this study until at least 3 months after the Ad26.ZEBOV vaccination or 1 month after MVA-BM-Filo.
7. The participant has significant conditions or clinically significant findings at screening or vital signs for which, in the opinion of the investigator, participation would not be in the best interest of the participant (e.g. compromise the safety or well-being) or that could prevent, limit, or confound the protocol-specified assessments.

   Note: Participants who have recently received treatment for acute, uncomplicated malaria are eligible for participation if at least 3 days have elapsed from the conclusion of a standard, recommended course of therapy for malaria; participants who are acutely ill with malaria at the time of screening should complete therapy and wait an additional 3 days after completion before screening for the study.

   Note: Participants with sickle cell trait can be included.
8. The participant had major surgery (per the investigator's judgment) within the 4 weeks prior to screening, or has planned major surgery during the study (from the start of screening onwards).
9. The participant had a post-organ and/or stem cell transplant whether or not with chronic immunosuppressive therapy.
10. The participant received an investigational drug or investigational vaccines or used an invasive investigational medical device within 3 months prior to screening, or current or planned participation in another clinical study during the study.

    Note: Participation in an observational clinical study is allowed.
11. The participant has a history of chronic urticaria (recurrent hives).

Study procedures (Figure 1)

At Day 1, interested participants are informed about the study and are required to pass a test of understanding before providing written consent. No study activities are performed before the participant has signed the informed consent form. Afterwards, the study medical doctor evaluates his/her general health based on the inclusion criteria, vital signs (blood pressure, pulse/heart rate [both at rest] and body temperature) are collected and a urine pregnancy test for women of childbearing potential is performed. Further during this first visit, a blood sample is taken for baseline testing of binding antibody level (i.e. humoral immune response) against EBOV glycoprotein (GP) using Filovirus Animal Non-Clinical Group (FANG) Ebola virus enzyme-linked immunosorbent assay (ELISA) and the presence of pre-existing Human anti-EBOV GP IgG and anti-EBOV nucleoprotein (NP) IgG using LUMINEX assay. For the first 100 enrolled participants an additional test on the collected serum is performed to measure the neutralizing antibody level against Ad26 and MVA vectors using respectively Ad26 Virus Neutralizing Assay (VNA) and MVA Plaque Reduction Immunogenicity Test (PRNT). Subsequently, a blood sample for baseline safety assessment is collected to test haemoglobin, haematocrit, blood cell count (white and red), platelet count, urea, creatinine and transaminases. Then, participants are vaccinated with the first dose (Ad26.ZEBOV) and they are given instructions to contact the study team for any occurring serious adverse events (SAEs), or in case of pregnancy of a participant during the study. After vaccination, participants remain at the study site for an observation period of 30 minutes to make sure no SAEs occur. SAEs are collected from first dose vaccination until 6 months post booster (PB). Lastly on Day 1, randomization is performed (1:1) using sealed envelopes (developed based on the created study randomization list) to determine the timing of the booster vaccine at either 1 year or 2 years after the first dose. Contact information is verified, an appointment for the second dose on Day 57 is arranged and a participant card is printed. Innovatively, next to a participant card, a biometric identification tool via iris scanning is foreseen to ensure correct identification of the participants during all study related visits.

At Day 57, participants return to the study site for urine pregnancy testing (for women of childbearing potential), vital signs measurement, assessment of safety (SAEs), a blood sample for immunogenicity assessment (the binding antibody levels against EBOV GP using FANG ELISA) and afterwards administration of the second vaccine (MVA-BN-Filo). After an observation period of 30 minutes, participants are reminded to contact the study team for any SAE that occurs, or in case of pregnancy of a participant during the study. Contact information is verified and an appointment for the 21-day post-dose 2 visit (Day 78) is arranged.

At 21 days post-dose 2 (Day 78), all participants return to the study site for a safety assessment (SAEs) and for the collection of a blood sample for immunogenicity assessment. Contact information is re-verified and they are reminded to contact the study team in case of SAE occurrence, or in case of pregnancy of a participant.

To make sure no valuable information is missed, participants are contacted by phone to inquire about any occurrence of pregnancies (female participants) and SAEs at approximately 6 months post-dose 2 vaccination. At 1 year after the first vaccine dose, when all participants return to the site, the clinical trial staff inquires after the occurrence of SAEs and a blood sample is collected for immunogenicity assessment of all participants (where applicable pre-administration of the booster dose).

At 1 year or 2 years post first dose, depending on the study arm, a booster vaccination with Ad26.ZEBOV is given. After vaccination, participants remain at the study site for a 30 minute observation period. Participants are asked to collect solicited and unsolicited adverse events (AEs) in a participant diary starting on the day of the vaccination and continuing for the subsequent 7 days. At Day 8 PB the safety data including solicited and unsolicited AEs is reviewed and a blood sample for immunogenicity assessment is taken to document the immune response. At 6 months PB, all participants are contacted by phone and questioned about any SAEs or pregnancies (female participants) that have occurred since the last vaccination. For all participants at 2 years after first dose, a sample is collected for immunogenicity assessment (where applicable pre-administration of

the booster dose) and a safety assessment (SAEs) is performed for those returning for their booster vaccination.

The total duration of the study is 2 years and 6 months post-first dose. The study is considered completed when the last participant has been contacted for the 6 months PB phone call or has left the study.

Study intervention

According to the predefined schedule (Figure 1), participants receive a 0.5 mL intramuscular injection into the deltoid muscle of the upper arm with Ad26.ZEBOV or MVA-BN-Filo. The injection site should be free from any injury, local skin conditions, or other issues that might interfere with the evaluation of local reactions. Every vaccination is given in the opposite arm of the previous vaccination (unless the opposite arm has a condition that prevents evaluating the arm after injection). No local or topical anaesthetic is used prior to the injection.

The second or booster vaccination is not administered if any of the following events occur at any time after the first dose vaccination:

- A participant experiences anaphylaxis clearly attributable to vaccination with the study vaccine; OR
- A participant experiences generalized urticaria within 72 hours of vaccination considered to be related to study vaccine; OR
- A participant experiences a serious adverse event considered to be related to the study vaccine; OR
- A participant experiences injection site ulceration, abscess or necrosis considered to be related to the study vaccine; OR
- A participant has confirmed EVD; OR

- A female participant of childbearing potential has a positive urine β-human chorionic gonadotropin pregnancy test before vaccination (on Day 57, Year 1 or Year 2 [depending on the randomization group]); OR
- A female participant of childbearing potential has a positive urine β-human chorionic gonadotropin pregnancy test between Dose 2 and the booster dose and is still pregnant or breastfeeding at the time of the booster dose; OR
- A participant takes a concomitant treatment with drugs that may alter the immune response; OR
- The principal investigator believes that for safety reasons it is in the best interest of a participant to discontinue the study intervention.

Participants experiencing any of the events described above are still followed up for safety and immunogenicity according to the protocol. The decision to discontinue the study intervention is at the discretion of the principal investigator (University of Kinshasa) and after consultation with the sponsor (University of Antwerp) for any of the events described above.

Patient and public involvement

Difficulties were expected when setting up a clinical trial in Boende, a remote and resource-limited area of DRC. However, to avoid and anticipate some of these challenges and in order to support vaccination compliance, a collaboration is established between the study team and the Provincial Division of Health. Throughout the trial, workshops are organized for HCP in the health district of Boende to sensitize and inform on EVD and other relevant medical topics. These gatherings do not only facilitate enrollment in the trial but also increase the engagement of participants by enhancing their understanding on the clinical trial and the importance of adherence. During these workshops time is available for questions and discussions. In addition to these gatherings for trial participants, community engagement activities and the training and capacity building of the local clinical trial team

that is executing the trial (under supervision of UNIKIN as Principal Investigator (PI)) are organised for the duration of the trial.

Each participant receives an individual visit schedule upon enrollment in the trial and when participants miss a planned study visit, community health workers of the Ministry of Health trace the individual subject. Consent is asked in the informed consent form for this mode of contact.

Furthermore, prior to the start of the clinical trial, a pilot study was performed whereby potential participants were interviewed on the feasibility and acceptability of the use of a biometric iris scanning tool for participant identification during the trial and the use of telephone messaging with visit reminders for participant adherence.

Data management

All information is collected during study visits on source documents by study staff. These source documents with confidential information are transcribed into the clinical database by site data managers. To make sure that all entered data (collected in DFexplore version 5.2.1) is correct, the principal investigator reviews each source document and confirms its correct transcription in the database. Additionally, the sponsor performs quality checks of the entered data in the database and during monitoring visits source data verification is performed.

Statistical analysis

A differentiation in analysis is made according to: 1) the *Full Analysis Set* (FAS; all participants who received at least one dose, regardless of the occurrence of protocol deviations), 2) *Per Protocol Set for primary vaccination series* (all vaccinated subjects, who received both dose 1 and dose 2 [administered within the protocol-defined visit window] vaccinations, have at least 1 post-vaccination [i.e. after the date of dose 1] evaluable immunogenicity sample, and have no major protocol deviations influencing the immune response) and 3) *Per Protocol Set for the Booster/Dose 3 vaccination* (includes all subjects in the per protocol set for the primary vaccination series who received Dose 3 and have at least 1 post-

dose 3 [i.e. after the date of booster vaccination] evaluable immunogenicity sample, and have no major protocol deviations influencing the immune response).

Subject information (i.e. demographics and baseline characteristics, disposition information, treatment compliance, extent of exposure, protocol deviations and concomitant medications) is planned to be tabulated and summarized with descriptive statistics for all subjects. For continuous data such as age, the mean and standard deviation will be provided if applicable, otherwise geometric mean, related standard deviations or median and interquartile range will be used.

For the immunogenicity analysis, two *Per-Protocol Sets* will be used, i.e., the *Per-Protocol Set for primary vaccination series* and the *Per-Protocol Set for the booster*. If more than 10% of participants from the FAS are excluded from the per protocol immunogenicity set, the immunogenicity analysis will be repeated on the FAS to evaluate the robustness of the analysis results. A subgroup analysis of the immune response at different time points will be performed stratified by age (18-40, 40-60 and >60), gender, prior vaccinia/smallpox vaccination, pre-existing Ebola antibodies (positive or negative for pre-existing Human anti-EBOV GP IgG and anti-EBOV NP IgG, and for both), baseline immunogenicity (positivity versus negativity for antibody levels against EBOV GP using FANG ELISA) and the presence of neutralizing antibody levels against Ad26 and MVA vectors using Ad26 VNA and MVA PRNT assay (only the first 100 enrolled participants). For these planned subgroup analyses, N (%), Geometric Mean Concentrations and 95% confidence intervals will be provided as appropriate.

Safety analyses include SAEs collected during the whole study and solicited and unsolicited AEs for 1 week post booster vaccination. The analysis of SAEs will be performed using the FAS and the solicited and unsolicited AEs will be analysed for the participants who received the booster vaccination. Continuous variables will be summarized using the following statistics: number of observations; arithmetic or geometric mean/median (if applicable) with their related measures of dispersion (95% CI for the mean, standard deviation or inter quartile range (Q1-Q3)). Minimum and maximum frequencies and percentages (one decimal place) will be generated for categorical variables.

The primary interim analysis is planned to be performed when all participants have completed the 21-day post dose 2 visit (Day 78) or discontinued earlier. This analysis includes all available immunogenicity and safety data up to this point (date cut-off). Additional interim analyses may be performed during the study for the purpose of informing future vaccine-related decisions in a timely manner.

The final analysis will be performed when all participants have completed the last study-related phone call (6 months PB) or left the study.

DISCUSSION

The aim of this phase 2 trial is assess the safety and immunogenicity of a booster dose with Ad26.ZEBOV administered either 1 or 2 years post first dose of a prophylactic heterologous Ebola vaccine regimen. By doing so, this study will boost the immunogenicity and safety databases of the Ad26.ZEBOV and MVA-BN-Filo vaccines.

Boende (the capital city of Tshuapa province) was selected as the trial site for several reasons. First, the Tshuapa province recovered from an EBOV outbreak that occurred in the Boende Health District in 2014[18]. Following this outbreak, a study (n=565) conducted in the Tshuapa region in 2015 found that 41.4% of the tested HCP were seroreactive to at least one EBOV protein and 2.8% of the HCP showed a neutralizing capacity while never having developed EVD symptoms[21]. This observation suggests a possible endemic EBOV exposure in the Tshuapa province of DRC. These are interesting observations for future ecologic research as the ecology and reservoir(s) of EBOV and other filoviruses remain largely unknown[23, 24]. Second, in addition to the previous outbreak of EVD, Boende was chosen to perform the current clinical trial as there was expertise available after carrying out a phase 3 monkey pox vaccine trial that took place in 2017[25].

In conclusion, because EVD remains a very deadly disease, effective and safe preventive measures will play a crucial role to protect vulnerable communities. While the prophylactic heterologous 2-dose regimen was recently granted market authorisation by the European Commission, further research

into the safety and immunogenicity of the booster dose with Ad26.ZEBOV is still required. This is the first randomized vaccine trial that looks into the safety and immunogenicity of two different booster arms in a large cohort.

ETHICS AND DISSEMINATION

This protocol was submitted and approved by the National Ethics Committee of the Ministry of Health of the Democratic Republic of Congo (approval number: n°121/CNES/BN/PMMF/2019). Prior to being enrolled in the trial, all participants are required to provide written informed consent by singing the informed consent form after having performed a test of understanding. If the participant is unable to read or write, an impartial witness should be present for the entire informed consent process (which includes reading and explaining all written information) and should personally date and sign the informed consent form after the oral consent of the participant is obtained. No study-related procedures are performed until the participant has signed the informed consent form.

The trial was registered on Clinicaltrial.gov on December 4th, 2019 (NCT04186000) and recruitment started on December 18th, 2019. All participants were recruited by the 8th of February 2020 and the study is planned to finish in July, 2022. Results of the trial will be entered on Clinicaltrial.gov, published in peer-reviewed journals and presented at international conferences.

DECLARATIONS

Author contributions

YL wrote the manuscript. TZ, ES, VM, JM, PM, HMM, JPVG and PVD wrote the initial English protocol on which this manuscript is based. TZ, VM, PM, JM and HMM translated it into French for submission to the National Ethics Committee and the “Direction de la Pharmacie et des Médicaments” of the Ministry of Health of the Democratic Republic of Congo as well as the National Scientific committee against Ebola. All authors reviewed and contributed to the final manuscript.

Funding

The EBOVAC3 project has received funding from the Innovative Medicines Initiative 2 Joint Undertaking under grant agreement No 800176 (IMI-EU). This Joint Undertaking receives support from the European Union's Horizon 2020 research and innovation programme, European Federation of Pharmaceutical Industries and Associations (EFPIA) and the Coalition for Epidemic Preparedness Innovations (CEPI). For this trial, the Contract Research Organisation and part of the FANG ELISA analyses are funded by the Coalition for Epidemic Preparedness Innovations. All other trial activities are funded by the Innovative Medicines Initiative 2 Joint Undertaking grant. All vaccines and Neutralizing antibody level analyses against Ad26 at the first visit are provided by Janssen Vaccines & Prevention B.V..

Acknowledgements

We acknowledge Janssen Vaccines & Prevention B.V. (in collaboration with Bavarian Nordic GmbH), the London School of Hygiene and Tropical Medicine (LSHTM), the Institut National de la Santé et de la Recherche Médicale (INSERM), and the College of Medicine and Allied Health Sciences (COMAHS) for their contribution in the EBOVAC3 project. We are grateful to the Division Provinciale de la Santé and, the political-administrative authorities of the Tshuapa province for a trustful collaboration. We acknowledge the reliability and motivation of the study site team and are grateful.

Competing interests

The authors declare that they have no competing interests.

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FIGURE TITLES AND LEGENDS

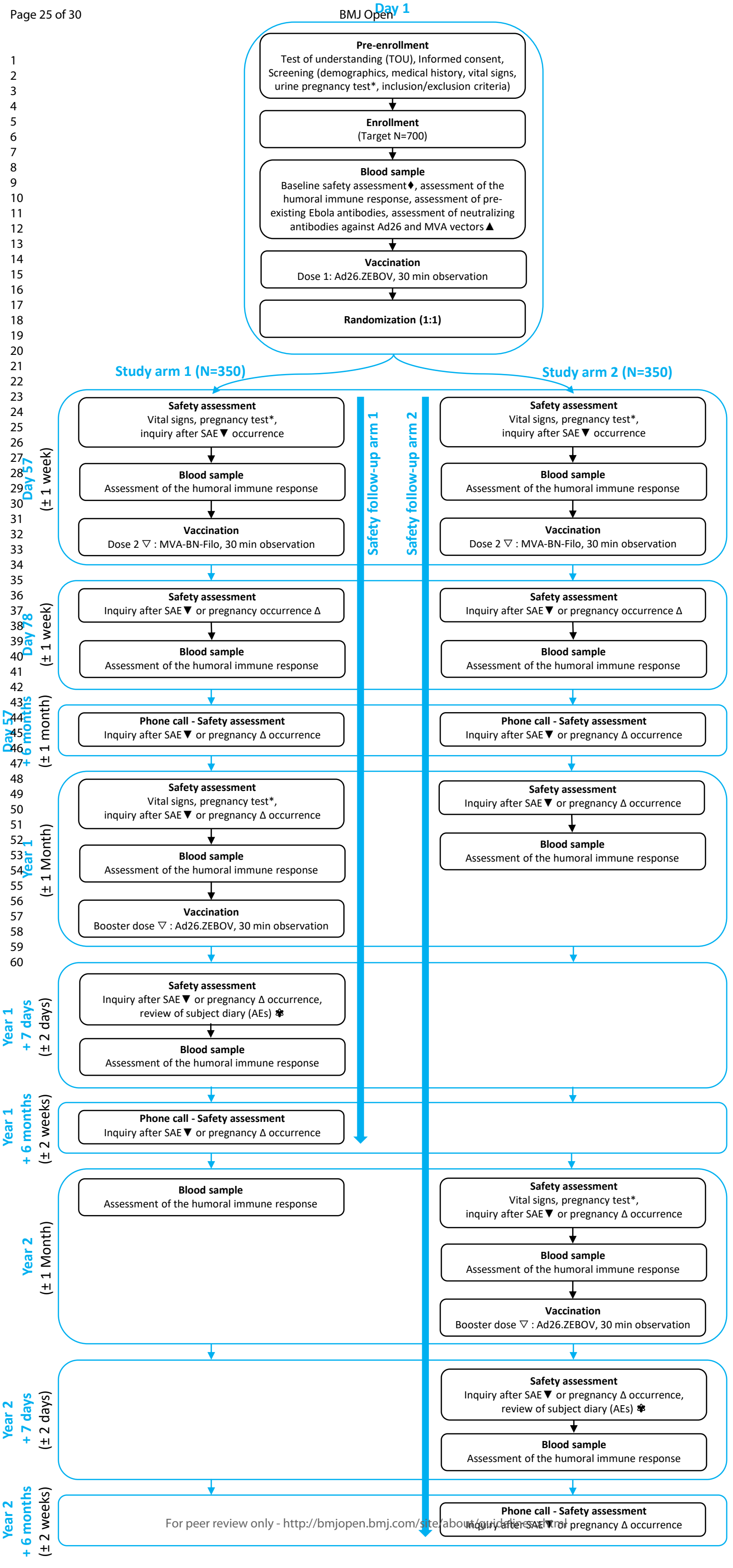
Figure 1. Study time and events overview

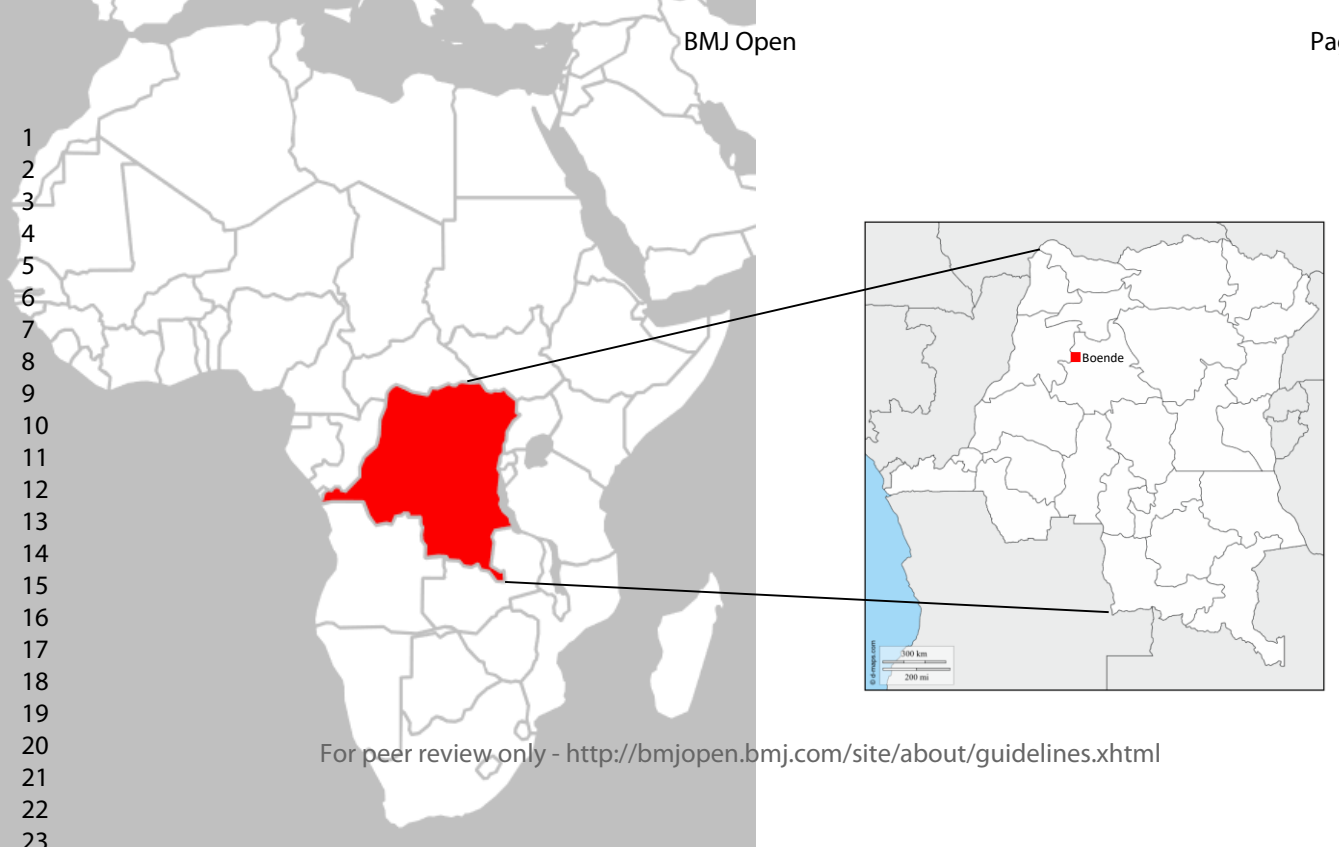
SAE: Serious Adverse Event; * Only for female participants of childbearing potential; ♦ Abnormal results will not exclude a participant, as results will not be reviewed prior to enrollment; ▲ Only the first 100 participants enrolled will be tested for Neutralizing antibody response against ad26 VNA and MVA vectors. Other blood analyses are for all 700 participants; ▼ Concomitant therapies given in conjunction with a serious adverse event (SAE) should be recorded from signing of the ICF onwards until 6 months post booster; ▽ The investigator may withhold the second vaccine or booster dose if a

participant's clinical status changes prior to vaccination. The participant should continue to be followed for safety and immunogenicity according to the protocol; Δ only for female participants; ✽ Solicited and unsolicited Adverse Events (AEs) will be collected in a participant diary during 1 week post booster vaccination.

Figure 2. Study site location

On the left, the Democratic Republic of Congo (DRC) is highlighted on a map of the African continent. On the right, the study site location (Boende, Tshuapa province) is marked on a map of DRC indicating its provinces[26].





SPIRIT
STANDARD PROTOCOL ITEMS: RECOMMENDATIONS FOR INTERVENTIONAL TRIALS

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	ItemNo	Description	Location in manuscript
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	Page 1, line 1-4
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	Page 2, abstract>Ethics and dissemination, line 36-37
	2b	All items from the World Health Organization Trial Registration Data Set	N/A
Protocol version	3	Date and version identifier	N/A
Funding	4	Sources and types of financial, material, and other support	Page 19, declarations>funding, line 321-330
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	Page 1, title page: line 5-11; Page 18, declarations>Author contributions, line 315-320.
	5b	Name and contact information for the trial sponsor	N/A
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	Page 18, Declarations>Authors contribution, line 315-320
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	N/A
Introduction			
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	Page 3-4, Introduction, paragraph 1-6, line 53-104
	6b	Explanation for choice of comparators	Page 4, Introduction, paragraph 4, line 87-93

Section/item	ItemNo	Description	Location in manuscript
Objectives	7	Specific objectives or hypotheses	Page 6, methods>objectives, line 116-119, Table 1
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	Page 5, methods>study design and setting, line 107-113
Methods: Participants, interventions, and outcomes			
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	Page 5, methods>study design and setting, line 114-115; Page 17, Discussion, paragraph 2, line 285-294; Figure titles and legends, Figure 2, line 422-425; see also additional file figure 2
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	Page 6, Methods>Participant population, line 125-126, Table 2
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	Page 11-13, Methods>procedures & study intervention, line 127-188; Page 22, figure titles and legends, Figure 1, line 411-421; see also additional file Figure 1
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	Page 13-14, Methods>Study intervention, paragraph 2, line 189-213
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	Page 14, Methods>Patient and public involvement, paragraph 1, line 216-222
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	Page 14, Methods>Study intervention, paragraph 2, line 206-207; Page 9, Table 2, inclusion criterium 9
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	Page 6, Table 1, endpoints; Page 16, Methods>statistical analysis, paragraph 2-4, line 250-272

Section/item	ItemNo	Description	Location in manuscript
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	Page 11-13, Methods>Study procedures, paragraph 1-6, line 127-181; Page 22, figure titles and legends, Figure 1, line 411-421; see also additional file Figure 1
Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	Page 7, Methods>participant population, line 121-124
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	Page 14, Methods>Patient and public involvement, paragraph 1, line 216-222
Methods: Assignment of interventions (for controlled trials)			
Allocation:			
Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	Page 11, Methods>Study procedures, paragraph 1, line 146-148
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	Page 11, Methods>Study procedures, paragraph 1, line 146-148
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	Page 11, Methods>Study procedures, paragraph 1, line 146-148
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	N/A
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	N/A
Methods: Data collection, management, and analysis			
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with	Page 11-13, Methods>Study procedures, paragraph 1-6, line 127-181; Page 15, methods>data management, line 233-239

Section/item	ItemNo	Description	Location in manuscript
		their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	Page 14, Methods>Patient and public involvement, paragraph 1, line 216-222
Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	Page 15, methods>data management, line 233-239
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	Page 16, Methods>statistical analysis, paragraph 3-4, line 255-272
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	Page 16, Methods>statistical analysis, paragraph 3, line 255-265
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	Page 15-16, Methods>Statistical analysis, paragraph 1, line 241-249 and paragraph 3, line 256-258
Methods: Monitoring			
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	N/A
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	Page 17, Methods>statistical analysis, paragraph 5, line 273-277
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	Page 11-13, Methods>Study procedures, paragraph 1-5, line 128-178
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	Page 15, Methods>Data management, paragraph 1, line 239
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	Page 18, Ethics and dissemination, paragraph 1, line 302-303

Section/item	ItemNo	Description	Location in manuscript
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	N/A
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	Page 18, Ethics and dissemination, paragraph 1, line 303-309
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	Page 14, Methods>Patient and public involvement, paragraph 2, line 226-228
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	Page 15, methods>data management, line 233-239
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	Page 19, Declarations>Competing interests, line 339
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	Page 18, Ethics and dissemination, paragraph 2, line 310-313
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	N/A
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	Page 18, Ethics and dissemination, paragraph 2, line 310-313
	31b	Authorship eligibility guidelines and any intended use of professional writers	Page 18, Declarations>Author contributions, line 315-320
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	N/A
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	N/A
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	N/A

BMJ Open

Open-label, Randomized Controlled, Phase 2 Clinical Trial to Evaluate the Immunogenicity and Safety of a Prophylactic Vaccination of Health Care Providers by Administration of a Heterologous Vaccine Regimen Against Ebola in the Democratic Republic of the Congo: the Study protocol

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2020-046835.R1
Article Type:	Protocol
Date Submitted by the Author:	22-Mar-2021
Complete List of Authors:	Larivière, Ynke; University of Antwerp, Centre for the Evaluation of Vaccination; University of Antwerp, Global Health Institute Zola, Trésor; University of Kinshasa, Tropical Medicine Department Stoppie, Elke; University of Antwerp, Centre for the Evaluation of Vaccination; University of Antwerp, Global Health Institute Maketa, Vivi; University of Kinshasa, Tropical Medicine Department Matangila, Junior ; University of Kinshasa, Tropical Medicine Department Mitashi, Patrick; University of Kinshasa, Tropical Medicine Department De Bie, Jessie; University of Antwerp, Centre for the Evaluation of Vaccination; University of Antwerp, Global Health Institute Muhindo-Mavoko, Hypolite; University of Kinshasa, Tropical Medicine Department Van geertruyden, Jean-Pierre; University of Antwerp, Global Health Institute Vandamme, Pierre; University of Antwerp, Centre for the Evaluation of Vaccination
Primary Subject Heading:	Infectious diseases
Secondary Subject Heading:	Immunology (including allergy), Public health, Global health
Keywords:	Protocols & guidelines < HEALTH SERVICES ADMINISTRATION & MANAGEMENT, Health & safety < HEALTH SERVICES ADMINISTRATION & MANAGEMENT, Immunology < TROPICAL MEDICINE

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Open-label, Randomized Controlled, Phase 2 Clinical Trial to Evaluate the Immunogenicity and Safety of a Prophylactic Vaccination of Health Care Providers by Administration of a Heterologous Vaccine Regimen Against Ebola in the Democratic Republic of the Congo: the Study protocol

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Word count excluding title page, abstract, references, figures, tables and acknolewdgements: 4299

ABSTRACT

Introduction: This article describes the protocol of an Ebola vaccine clinical trial which investigates the safety and immunogenicity of a prophylactic Ebola vaccine regimen comprised of 2 Ebola vaccines (Ad26.ZEBOV and MVA-BN-Filo) administered 56 days apart, followed by a booster vaccination with Ad26.ZEBOV offered at respectively 1 year or 2 years (randomization 1:1) after the first dose. This clinical trial is part of the EBOVAC3 project (an Innovative Medicines Initiative 2 Joint Undertaking), and is the first to evaluate the safety and immunogenicity of two different booster vaccination arms in a large cohort of adults.

Methods and analysis: This study is an open-label, monocentric, phase 2, randomized controlled vaccine trial. A total of 700 HCP and front-liners are planned to be recruited from the Tshuapa province in the Democratic Republic of the Congo (DRC). The primary and secondary objectives of the study assess the immunogenicity of the first (Ad26.ZEBOV), second (MVA-BN-Filo) and booster (Ad26.ZEBOV) dose through the evaluation of binding antibody responses after vaccination and the safety of the vaccines through the collection of serious adverse events from the first dose until six months post booster vaccination and solicited and unsolicited adverse events for one week after the booster dose.

Ethics and dissemination: The protocol was approved by the National Ethics Committee of the Ministry of Health of the DRC (n°121/CNES/BN/PMMF/2019). The clinical trial was registered on the 4th of December 2019 on ClinicalTrials.gov (NCT04186000). Trial activities are planned to finish in July 2022. All participants are required to provide written informed consent and no study-related procedures will be performed until consent is obtained. The results of the trial will be added on ClinicalTrials.gov, published in peer-reviewed journals and presented at international conferences.

Key words: Clinical Trial Protocol, Ebola Vaccines, Safety, Immunogenicity, Health Care Providers

STRENGTHS AND LIMITATIONS OF THIS STUDY

- With this randomized controlled vaccine trial, being the first to evaluate the safety and immunogenicity in two different booster vaccine arms 1 or 2 years after the prime dose, new contributions will be added to already existing safety and immunogenicity data.
- Vaccination of HCP and front-liners can potentially help protect a community which is at risk for future outbreaks.
- Innovative use of iris scanning biometric material to identify participants enrolled in the trial.
- This study takes place in a resource poor setting, impacting logistical set-up of the trial.
- Long duration of the trial (2.5 years) may lead to considerable loss to follow up.

INTRODUCTION

Ebolaviruses (negative stranded RNA viruses) belong to the *Filoviridae* family and cause Ebola virus disease (EVD), which often leads to severe haemorrhagic fever in humans and nonhuman primates[1]. Contact with infected wild animals (such as fruit bats, gorillas, apes, monkeys, etc.) is often reported as the source of animal-to-human transmission[2-4] and once among humans, these public health pathogens spread via direct (body fluids) or indirect (contaminated surfaces) human-to-human contact(2, 3). While they do not spread via air or water[3], *Ebolaviruses* bring along a severe public health burden with case fatality rates that can range up to 90%[5]. Since the discovery of the Ebola viruses in 1976[6], more than 20 outbreaks (most are endemic to regions in equatorial and western Africa) have taken place[7]. To date, the Democratic Republic of the Congo (DRC) has been the most affected country and is currently battling its 12th outbreak[8]. However, it is only recently that the search for a safe and effective vaccine against Ebola was accelerated when the epidemic potential of *Ebolaviruses*, and more specifically the species *Zaïre Ebolavirus* (virus name: Ebola virus; abbreviation: EBOV[9]), became clear through the West African Ebola epidemic (2013-2016; 28,616 cases with 11,310 deaths[10]).

One of the initiatives to develop such a vaccine came from an international consortium, funded by the Innovative Medicines Initiative 2 (IMI2) Joint Undertaking, dedicated to evaluate a prophylactic Ebola

vaccine regimen comprised of 2 candidate Ebola vaccines (Ad26.ZEBOV and MVA-BN-Filo) and aiming to bring this prophylactic Ebola vaccine to licensure[11]. Ad26.ZEBOV is a monovalent vaccine developed to provide active EBOV-specific immunity. MVA-BN-Filo, which is administered 56 days after the Ad26.ZEBOV vaccine, is a multivalent vaccine developed to establish active immunity to EBOV, *Sudan Ebolavirus*, *Taï Forest Ebolavirus* and the Marburg virus (also part of the *Filoviridae* family). In July 2020, the 2-dose prophylactic vaccine regimen was granted market authorisation by the European Commission[12].

Several projects (PREVAC, EBOVAC1, EBOVAC2 and EBOVAC3) within the IMI2 Joint Undertaking, of which the first in-human clinical trials started in 2014, were at the basis of this successful authorisation[11]. Within these projects, multiple clinical trials assessed or are assessing the timing, tolerability, safety, and immunogenicity of different Ad26.ZEBOV and MVA-BN-Filo vaccine regimen in healthy adults (≥18 years old) and children/adolescents (1-17 years old) via phase 1, 2, 2B and 3 studies. Initial trials showed that healthy adult participants had higher geometric mean concentrations of binding antibodies for the regimen where Ad26.ZEBOV vaccination was followed by an MVA-BN-Filo vaccination 56 days later. Moreover, 100% of them had detectable Ebola glycoprotein-specific Immunoglobulin G (IgG) antibodies up to at least 240 days after vaccination[13-15]. Even though some local (erythema, swelling and pain at injection site) and systemic (headache, nausea, pyrexia, myalgia and fatigue) solicited adverse events were recorded, the vaccine regimen was generally well tolerated across studies[13-17].

While it is of utmost importance that the 2-dose prophylactic vaccine regimen is safe and leads to an immune response, it is also crucial to find out whether or not this regimen can lead to induced immune memory at the time of imminent risk (i.e. an outbreak) through a booster vaccination. To evaluate this induced immune memory, three previous studies within EBOVAC projects have administered a booster vaccine with Ad26.ZEBOV at either 1 year (NCT02325050; NCT02564523) or 2 years (NCT02509494) post Dose 1. Results from the NCT02325050 trial have already shown that an immunological memory

was rapidly induced via booster vaccination with Ad26.ZEBOV, indicating that booster vaccination can be considered for at risk individuals (e.g. when an outbreak occurs) that were previously vaccinated with the 2-dose heterologous prophylactic regimen[18]. However, these trials only evaluated booster vaccination in a small amount of participants and it still has to be explored whether the induced immune memory response differs depending on the timing of the booster dose (i.e. 1 or 2 years after Dose 1).

Healthcare settings play an important role in the control of EVD and therefore health care providers (HCP) and front-liners, due to occupational exposure, are not only more at risk of disease acquisition but also facilitate the spread of the virus[19-22]. Knowing that outbreaks of EVD often occur in regions where there is already a shortage of HCP and front-liners, this further depletes a weak health care system and the quality of care. Consequently, the Strategic Advisory Group of Experts stated in 2018 that for preventive strategies, vaccination of HCP as part of an emergency preparedness plan has significant potential of reducing the scale and duration of outbreaks[23].

This phase 2 randomized controlled trial aims to determine the safety and immunogenicity of the 2-dose heterologous vaccine regimen with Ad26.ZEBOV followed by MVA-BN-Filo 56 days later. Additionally, this trial aims to assess the safety and immunogenicity of a booster Ad26.ZEBOV vaccine administered either 1 or 2 years post first dose. The trial is conducted in a cohort of HCP and front-liners from the Boende health district in DRC, a well-known population at risk from clinical and epidemiological perspective.

METHODS

Study design and setting

This study is an open-label, monocentric, phase 2 randomized controlled trial to evaluate the immunogenicity and safety of Ad26.ZEBOV ($5x10^{10}$ viral particles) as first dose and MVA-BN-Filo ($1x10^{8}$ infectious units) as second dose vaccination at a 56-day interval in HCP and front-liners who may be exposed to Ebola in the event of a future Ebola outbreak in DRC. Additionally, after randomization (1:1)

a booster of Ad26.ZEBOV ($5x10^{10}$ viral particles) will be offered at respectively 1 year or 2 years after the first dose (Figure 1). As this study is designed to provide descriptive information regarding immunogenicity and safety, an open-label design was preferred.

The study site is located in Boende, Tshuapa province, DRC (Figure 2), at approximately 750km north-west of Kinshasa. Study participants will be enrolled at the General Reference Hospital in Boende.

Objectives

The primary, secondary and exploratory objectives and endpoints of this study are described in Table 1.

Table 1. Objectives and endpoints

Objectives	Endpoints
Primary	
• To assess binding antibody responses post-dose 2 vaccination with MVA-BN-Filo.	• Binding antibody levels against the EBOV GP using FANG ELISA at 21 days post-dose 2 (Day 78) vaccination with MVA-BN-Filo.
Secondary	
• To assess binding antibody responses after booster vaccination with Ad26.ZEBOV given at 1 or 2 years after first dose.	• Binding antibody levels against the EBOV GP using FANG ELISA at 7 days (excluding the day of vaccination) post booster.
• To assess the safety of a heterologous vaccine regimen utilizing Ad26.ZEBOV and MVA-BN-Filo administered at a 56-day interval and a booster vaccine with Ad26.ZEBOV at one or two years post first dose.	• Serious adverse events from first dose vaccination until 6 months post booster. • Solicited and unsolicited local and systemic adverse events until 7 days post booster vaccination (day of vaccination and subsequent 7 days) with Ad26.ZEBOV.
Exploratory	
• To assess binding antibody responses at different time points as indicated in the Study time and events overview (Figure 1).	• Binding antibody levels against the EBOV GP using FANG ELISA at different time points as indicated in the Study time and events overview (Figure 1).

• To assess neutralizing antibody response directed against the Adenovirus vector prior to vaccination.	• Neutralizing antibody levels against Ad26 using Ad26 VNA at the first visit.
• To assess neutralizing antibody response directed against the MVA vector prior to vaccination.	• Neutralizing antibody levels against MVA-BN-Filo using MVA PRNT assay at the first visit.
• To assess seroprevalence of Ebola virus disease prior to vaccination.	• Presence of pre-existing Human anti-EBOV GP IgG and anti-EBOV NP IgG using LUMINEX assay.
ELISA: enzyme-linked immunosorbent assay; EU/mL: ELISA units/mL; FANG: Filovirus Animal Nonclinical Group. VNA: Virus Neutralization Assay; PRNT: Plaque Reduction Neutralization Test	

Participant population

A total of 700 Registered HCP and front-liners in DRC (working in the Boende General Reference Hospital, Health Centres or Health Posts in the Boende health district) are planned to be recruited from the Tshuapa province in DRC. This assessment was based on information obtained from an ongoing (monkeypox) vaccine trial in the same area at the time the protocol was being written[24]. From discussions with the monkeypox research group, it became clear that a high enrolment rate and retention rate (>90% after two years) could be expected among HCP and front-liners in the Boende health district. Based on this ongoing monkeypox trial, it was estimated that enrolling approximately 50% of the HCP and front-liners working in the Boende health district would be feasible. This sample size was thus defined upon the feasibility of recruitment of HCP and front-liners in the region.

Inclusion and exclusion criteria that determine the eligibility of participants are reported in Table 2.

Table 2. Inclusion and exclusion criteria

Inclusion criteria
Each potential participant must satisfy all of the following criteria to be enrolled in the study: 1. The participant must pass the Test of Understanding. *Note: If the participant fails the Test of Understanding on the first attempt, he/she must be retrained on the purpose of the study and must take the test again (2 repeats are allowed).*

If participants fail on the third attempt, they should not continue with screening or consenting procedures.

2. Each participant must sign an informed consent form indicating that he or she understands the purpose of, and procedures required for, the study and is willing to participate in the study. In case the participant cannot read or write, the procedures must be explained and informed consent must be witnessed by a trusted literate third party not involved with the conduct of the study.
3. The participant must be a man or women aged 18 years or older.
4. The participant must be a documented HCP in DRC.
5. The participant must be healthy in the investigator's clinical judgement and on the basis of vital signs assessed at day 1 screening.

   Note: HIV-positive subjects can be enrolled as long as their general condition is good, i.e. they are on antiretroviral treatment or have no signs or symptoms of immunodepression, diagnosed on the basis of physical examination, medical history, and the investigator's clinical judgment.
6. Before vaccination, a woman must be either:
   - Of childbearing potential and practicing (or intending to practice) a highly effective method of birth control consistent with local regulations and/or local culture regarding the use of birth control methods for participants in clinical studies, beginning at least 28 days prior to vaccination and during the study up to at least 3 months after the first (or only) vaccination (Ad26.ZEBOV) and 1 month after the MVA-BN-Filo vaccination (if applicable); and then starting again 14 days before the booster vaccination until 3 months after the booster vaccination. The sponsor considers the following methods of birth control to be highly effective: established use of oral, injected or implanted hormonal methods of contraception; placement of an intrauterine device or intrauterine system; barrier methods: condom or

occlusive cap (diaphragm or cervical/vault caps) with or without spermicidal foam/gel/film/cream/suppository; male partner sterilization (the vasectomized partner should be the sole partner for that participant); true abstinence (when this is in line with the preferred and usual lifestyle of the participant); OR

- Not of childbearing potential: postmenopausal (amenorrhea for at least 12 months without alternative medical cause); permanently sterilized (e.g. bilateral tubal occlusion [which includes tubal ligation procedures as consistent with local regulations], hysterectomy, bilateral salpingectomy, bilateral oophorectomy); or otherwise be incapable of pregnancy.

Note: If the social situation of a woman of childbearing potential changes (e.g. woman who is not heterosexually active becomes active), she must begin a highly effective method of birth control, as described above.

7. Woman of childbearing potential must have a negative urine β-human chorionic gonadotropin pregnancy test immediately prior to each study vaccine administration.
8. Participant must be available and willing to participate for the duration of the study.
9. Participant must be willing and able to comply with protocol requirements (including certain prohibitions and restrictions such as the use of anticonception and the discouragement of concomitant treatment that may alter the immune response).
10. Participant must be willing to provide verifiable identification.
11. Participant must have a means to be contacted.

Exclusion criteria

Participants will be excluded from study participation in case the following criteria apply:

1. The participant has a known history of Ebola virus disease.
2. The participant has received any experimental candidate Ebola vaccine less than 3 months prior to the first study visit.
3. The participant has received any experimental candidate Ad26-vaccine in the past.

Note: Receipt of any approved or experimental vaccinia/smallpox vaccine or experimental Ad-vector vaccine other than Ad26 prior to study entry is allowed.

4. The participant has a known allergy or history of anaphylaxis or other serious adverse reactions to vaccines or vaccine products (including any of the constituents of the study vaccines [e.g. polysorbate 80, ethylenediaminetetraacetic acid (EDTA) or L-histidine for Ad26.ZEBOV vaccine; and tris (hydroxymethyl)-amino methane (THAM) for MVA BN-Filo vaccine]), including known allergy to egg, egg products and aminoglycosides.
5. The participant has an acute illness (this does not include minor illnesses such as mild diarrhea or mild upper respiratory tract infection) or temperature ≥38.0°C on Day 1. Participants with such symptoms will be excluded from enrollment at that time, but may be rescheduled for enrollment at a later date if feasible.
6. The participant is a pregnant or breastfeeding women, or women planning to become pregnant while enrolled in this study until at least 3 months after the Ad26.ZEBOV vaccination or 1 month after MVA-BM-Filo.
7. The participant has significant conditions or clinically significant findings at screening or vital signs for which, in the opinion of the investigator, participation would not be in the best interest of the participant (e.g. compromise the safety or well-being) or that could prevent, limit, or confound the protocol-specified assessments.

   Note: Participants who have recently received treatment for acute, uncomplicated malaria are eligible for participation if at least 3 days have elapsed from the conclusion of a standard, recommended course of therapy for malaria; participants who are acutely ill with malaria at the time of screening should complete therapy and wait an additional 3 days after completion before screening for the study.

   Note: Participants with sickle cell trait can be included.

8. The participant had major surgery (per the investigator's judgment) within the 4 weeks prior to screening, or has planned major surgery during the study (from the start of screening onwards).
9. The participant had a post-organ and/or stem cell transplant whether or not with chronic immunosuppressive therapy.
10. The participant received an investigational drug or investigational vaccines or used an invasive investigational medical device within 3 months prior to screening, or current or planned participation in another clinical study during the study.

    Note: Participation in an observational clinical study is allowed.
11. The participant has a history of chronic urticaria (recurrent hives).

Randomization procedure

The study randomization list will be developed using an algorithm in the Statistical Analysis System software. This algorithm will randomly assign a treatment group (1:1) to a sequential randomization number. Once established, the list will be shared with the principal investigator (University of Kinshasa), who is in charge of creating sealed envelopes under sponsor (University of Antwerp) supervision. A total of at least 700 randomization envelopes will be created. Thirty envelopes will be grouped into one larger envelope, referred to as a "booklet". The booklets and envelopes will be numbered sequentially by a unique sequence of numbers. The booklets will be labelled in a sequential order (i.e. 01-24) and the envelopes will be labelled with the study number "VAC52150-EBL-2007" and a sequential randomization number (i.e. 001-700) to which a treatment group is linked via the algorithm. The staff delegated to make the envelopes will use the *Envelope Assembly Record Worksheet*, on which the randomization number, initials of the assembler, date on which the assembly took place, and the initials of the staff member(s) that performed the quality control are collected. The randomization booklets with envelopes will be stored and used in the study clinic.

Delegated site staff will assign and open booklets and envelopes in sequential order during study visits. Each envelope will contain two stickers. The first will contain space for writing the subject ID and subject's initials, the second will contain the randomization number and treatment description (pre-printed based on the study randomization list). Upon opening the sealed envelope, the subject ID and initials must be written in the space provided on the first sticker and the subject ID sticker must be placed on the outside of the envelope. To ensure proper source documentation, the sticker with the treatment information must be placed on the corresponding *Randomization worksheet*. Thereafter, the empty envelope, with the subject ID sticker on the outside, must be placed back in the booklet. These booklets are to be stored by the principal investigator.

Study procedures (Figure 1)

At Day 1, interested participants are informed about the study and are required to pass a test of understanding before providing written consent. No study activities are performed before the participant has signed the informed consent form. Afterwards, the study medical doctor evaluates his/her general health based on the inclusion criteria, vital signs (blood pressure, pulse/heart rate [both at rest] and body temperature) are collected and a urine pregnancy test for women of childbearing potential is performed. Further during this first visit, a blood sample is taken for baseline testing of binding antibody level (i.e. humoral immune response) against EBOV glycoprotein (GP) using Filovirus Animal Non-Clinical Group (FANG) Ebola virus enzyme-linked immunosorbent assay (ELISA) and the presence of pre-existing Human anti-EBOV GP IgG and anti-EBOV nucleoprotein (NP) IgG using LUMINEX assay. For Day 1 samples, both FANG ELISA and LUMINEX assay will be carried out. FANG ELISA is performed for all EBOVAC trials in the same laboratory (for consistency and comparability) and LUMINEX assay will provide a more detailed array of IgG (and IgM) antibodies that are not obtained via FANG ELISA. For the first 100 enrolled participants an additional test on the collected serum is performed to measure the neutralizing antibody level against Ad26 and MVA vectors using respectively Ad26 Virus Neutralizing Assay (VNA) and MVA Plaque Reduction Immunogenicity Test (PRNT).

Subsequently, a blood sample for baseline safety assessment is collected to test haemoglobin, haematocrit, blood cell count (white and red), platelet count, urea, creatinine and transaminases. Then, participants are vaccinated with the first dose (Ad26.ZEBOV) and they are given instructions to contact the study team for any occurring serious adverse events (SAEs), or in case of pregnancy of a participant during the study. After vaccination, participants remain at the study site for an observation period of 30 minutes to make sure no SAEs occur. SAEs are collected from first dose vaccination until 6 months post booster (PB). Lastly on Day 1, randomization is performed to determine the timing of the booster vaccine at either 1 year or 2 years after the first dose. Contact information is verified, an appointment for the second dose on Day 57 is arranged and a participant card is printed. Innovatively, next to a participant card, a biometric identification tool via iris scanning is foreseen to ensure correct identification of the participants during all study related visits.

At Day 57, participants return to the study site for urine pregnancy testing (for women of childbearing potential), vital signs measurement, assessment of safety (SAEs), a blood sample for immunogenicity assessment (the binding antibody levels against EBOV GP using FANG ELISA) and afterwards administration of the second vaccine (MVA-BN-Filo). After an observation period of 30 minutes, participants are reminded to contact the study team for any SAEs that occurs, or in case of pregnancy of a participant during the study. Contact information is verified and an appointment for the 21-day post-dose 2 visit (Day 78) is arranged.

At 21 days post-dose 2 (Day 78), all participants return to the study site for a safety assessment (SAEs) and for the collection of a blood sample for immunogenicity assessment. Contact information is re-verified and they are reminded to contact the study team in case of SAE occurrence, or in case of pregnancy of a participant.

To make sure no valuable information is missed, participants are contacted by phone to inquire about any occurrence of pregnancies (female participants) and SAEs at approximately 6 months post-dose 2 vaccination. At 1 year after the first vaccine dose, when all participants return to the site, the clinical

trial staff inquires after the occurrence of SAEs and a blood sample is collected for immunogenicity assessment of all participants (where applicable before administration of the booster dose).

At 1 year or 2 years post first dose, depending on the study arm, a booster vaccination with Ad26.ZEBOV is given. Prior to vaccination, the general well-being of the participant will be evaluated and urine pregnancy testing (for women of childbearing potential), as well as a vital signs measurement will be performed. After vaccination, participants remain at the study site for a 30 minute observation period. Participants are asked to collect solicited and unsolicited adverse events (AEs) in a participant diary starting on the day of the vaccination and continuing for the subsequent 7 days. At Day 8 PB the safety data including solicited and unsolicited AEs is reviewed and a blood sample for immunogenicity assessment is taken to document the immune response.

At 6 months PB, all participants are contacted by phone and questioned about any SAEs or pregnancies (female participants) that have occurred since the last vaccination. For all participants at 2 years after first dose, a sample is collected for immunogenicity assessment (where applicable before administration of the booster dose) and a safety assessment (SAEs) is performed for those returning for their booster vaccination.

The total duration of the study is 2 years and 6 months post-first dose. The study is considered completed when the last participant has been contacted for the 6 months PB phone call or has left the study.

Study intervention

According to the predefined schedule (Figure 1), participants receive a 0.5 mL intramuscular injection into the deltoid muscle of the upper arm with Ad26.ZEBOV or MVA-BN-Filo. The injection site should be free from any injury, local skin conditions, or other issues that might interfere with the evaluation of local reactions. Every vaccination is given in the opposite arm of the previous vaccination (unless the opposite arm has a condition that prevents evaluating the arm after injection). No local or topical anaesthetic is used prior to the injection.

The second or booster vaccination is not administered if any of the following events occur at any time after the first dose vaccination:

- A participant experiences anaphylaxis clearly attributable to vaccination with the study vaccine; OR
- A participant experiences generalized urticaria within 72 hours of vaccination considered to be related to study vaccine; OR
- A participant experiences a serious adverse event considered to be related to the study vaccine; OR
- A participant experiences injection site ulceration, abscess or necrosis considered to be related to the study vaccine; OR
- A participant has confirmed EVD; OR
- A female participant of childbearing potential has a positive urine β-human chorionic gonadotropin pregnancy test before vaccination (on Day 57, Year 1 or Year 2 [depending on the randomization group]); OR
- A female participant of childbearing potential has a positive urine β-human chorionic gonadotropin pregnancy test between Dose 2 and the booster dose and is still pregnant or breastfeeding at the time of the booster dose; OR
- A participant takes a concomitant treatment with drugs that may alter the immune response; OR
- The principal investigator believes that for safety reasons it is in the best interest of a participant to discontinue the study intervention.

Participants experiencing any of the events described above are still followed up for safety and immunogenicity according to the protocol. The decision to discontinue the study intervention is at the discretion of the principal investigator (University of Kinshasa) and after consultation with the sponsor (University of Antwerp) for any of the events described above.

Patient and public involvement

Difficulties were expected when setting up a clinical trial in Boende, a remote and resource-limited area of DRC. However, to avoid and anticipate some of these challenges and in order to support vaccination compliance, a collaboration is established between the study team and the Provincial Division of Health. Throughout the trial, workshops are organized for HCP in the health district of Boende to sensitize and inform on EVD and other relevant medical topics. These gatherings should not only facilitate enrollment in the trial but also increase the engagement of participants by enhancing their understanding on the clinical trial and the importance of adherence. During these workshops time is available for questions and discussions. In addition to these gatherings for trial participants, community engagement activities and the training and capacity building of the local clinical trial team that is executing the trial (under supervision of UNIKIN as Principal Investigator) are organised for the duration of the trial.

Each participant receives an individual visit schedule upon enrollment in the trial and when participants miss a planned study visit, community health workers of the Ministry of Health trace the individual subject. Consent is asked in the informed consent form for this mode of contact.

Furthermore, prior to the start of the clinical trial, a pilot study was performed whereby potential participants were interviewed on the feasibility and acceptability of the use of a biometric iris scanning tool for participant identification during the trial and the use of telephone messaging with visit reminders for participant adherence.

Data management

All information is collected during study visits on source documents by study staff. These source documents with confidential information are transcribed into the clinical database by site data managers. To make sure that all entered data (collected in DFexplore version 5.2.1) is correct, the principal investigator reviews each source document and confirms its correct transcription in the

database. Additionally, the sponsor performs quality checks of the entered data in the database and during monitoring visits source data verification is performed.

Statistical analysis

A differentiation in analysis will be made according to: 1) the *Full Analysis Set* (FAS; all participants who received at least one dose, regardless of the occurrence of protocol deviations), 2) *Per Protocol Set for primary vaccination series* (all vaccinated subjects, who received both dose 1 and dose 2 [administered within the protocol-defined visit window] vaccinations, have at least 1 post-vaccination [i.e. after the date of dose 1] evaluable immunogenicity sample, and have no major protocol deviations influencing the immune response) and 3) *Per Protocol Set for the Booster/Dose 3 vaccination* (includes all subjects in the per protocol set for the primary vaccination series who received Dose 3 and have at least 1 post-dose 3 [i.e. after the date of booster vaccination] evaluable immunogenicity sample, and have no major protocol deviations influencing the immune response).

Subject information (i.e. demographics and baseline characteristics, disposition information, treatment compliance, extent of exposure, protocol deviations and concomitant medications) is planned to be tabulated and summarized with descriptive statistics for all subjects. For continuous data such as age, the mean and standard deviation will be provided if applicable, otherwise the geometric means, related standard deviations or median and interquartile ranges will be used.

For the immunogenicity analysis, two *Per-Protocol Sets* will be used, i.e., the *Per-Protocol Set for primary vaccination series* and the *Per-Protocol Set for the booster*. If more than 10% of participants from the FAS are excluded from the per protocol immunogenicity set, the immunogenicity analysis will be repeated on the FAS to evaluate the robustness of the analysis results. A subgroup analysis of the immune response at different time points will be performed stratified by age (18-40, 40-60 and >60), gender, prior vaccinia/smallpox vaccination, pre-existing Ebola antibodies (positive or negative for pre-existing Human anti-EBOV GP IgG and anti-EBOV NP IgG, and for both), baseline immunogenicity (positivity versus negativity for antibody levels against EBOV GP using FANG ELISA) and the presence

of neutralizing antibody levels against Ad26 and MVA vectors using Ad26 VNA and MVA PRNT assay (only the first 100 enrolled participants). For these planned subgroup analyses, N (%), Geometric Mean Concentrations and 95% confidence intervals (CI) will be provided as appropriate.

Safety analyses include SAEs collected during the whole study and solicited and unsolicited AEs for 1 week post booster vaccination. The analysis of SAEs will be performed using the FAS and the solicited and unsolicited AEs will be analysed for the participants who received the booster vaccination. Continuous variables will be summarized using the following statistics: number of observations; arithmetic or geometric mean/median (if applicable) with their related measures of dispersion (95% CI for the mean, standard deviation or inter quartile range (Q1-Q3)). Minimum and maximum frequencies and percentages (one decimal place) will be generated for categorical variables.

The primary endpoint analysis is planned to be performed when all participants have completed the 21-day post dose 2 visit (Day 78) or discontinued earlier. This analysis includes all available immunogenicity and safety data up to this point (date cut-off). Additional interim analyses may be performed during the study for the purpose of informing future vaccine-related decisions in a timely manner.

The final analysis will be performed when all participants have completed the last study-related phone call (6 months PB) or left the study.

DISCUSSION

The aim of this phase 2 trial is to obtain further safety and immunogenicity data on the 2-dose prophylactic heterologous Ebola vaccine regimen and to assess the safety and immunogenicity of a booster dose with Ad26.ZEBOV administered either 1 or 2 years post first dose in a larger group. By doing so, this study will feed the immunogenicity and safety databases of the Ad26.ZEBOV and MVA-BN-Filo vaccines.

Boende (the capital city of Tshuapa province) was selected as the trial site for several reasons. First, the Tshuapa province recovered from an EBOV outbreak that occurred in the Boende Health District

in 2014[21]. Following this outbreak, a study (n=565) conducted in the Tshuapa region in 2015 found that 41.4% of the tested HCP were seroreactive to at least one EBOV protein and 2.8% of the HCP showed a neutralizing capacity while never having developed EVD symptoms[20]. This observation suggests a possible endemic EBOV exposure in the Tshuapa province of DRC. These are interesting observations for future ecologic research as the ecology and reservoir(s) of EBOV and other filoviruses remain largely unknown[25, 26]. Second, in addition to the previous outbreak of EVD, Boende was chosen to perform the current clinical trial as there was expertise available after carrying out a phase 3 monkey pox vaccine trial that took place in 2017[24].

The initial protocol aimed to provide descriptive information on immunogenicity and safety of the heterologous 2-dose vaccine regimen contributing to licensure of the 2 dose heterologous vaccine regimen. The sample size was determined based on the feasibility assessment obtained from the ongoing monkeypox vaccine trial[24]. Approximately 50% of the total HCP and front-liners in the Boende health district would be included in the Ebola vaccine trial. After finalizing the protocol, a secondary objective comparing two booster arms was added. For this objective a power a power of 0.99 was calculated based on the following parameters: two-sided t-test equal samples of 350 participants, significance level of 0.05, an effect size of 0.35 in antibody response and a retention rate of 90%. The effect size was based on former documented trial data. It is important to note that a varying antibody response after booster vaccination is not directly correlated with protective vaccine efficacy.

In conclusion, because EVD remains a very deadly disease, effective and safe preventive measures will play a crucial role to protect vulnerable communities. While the prophylactic heterologous 2-dose regimen was recently granted market authorisation by the European Commission, further research into the safety and immunogenicity of the 2-dose regimen is still required to obtain worldwide licensure of the regimen. Furthermore, limited data has previously been collected on the safety and

immunogenicity of a booster dose with Ad26.ZEBOV. This is the first randomized vaccine trial that looks into the safety and immunogenicity of two different booster arms in a large cohort.

ETHICS AND DISSEMINATION

This protocol was submitted and approved by the National Ethics Committee of the Ministry of Health of the Democratic Republic of Congo (approval number: n°121/CNES/BN/PMMF/2019). Prior to being enrolled in the trial, all participants are required to provide written informed consent by singing the informed consent form after having performed a test of understanding. If the participant is unable to read or write, an impartial witness should be present for the entire informed consent process (which includes reading and explaining all written information) and should personally date and sign the informed consent form after the oral consent of the participant is obtained. No study-related procedures are performed until the participant has signed the informed consent form.

The trial was registered on Clinicaltrial.gov on December 4th, 2019 (NCT04186000) and recruitment started on December 18th, 2019. All participants were recruited by the 8th of February 2020 and the study is planned to finish in July, 2022. Results of the trial will be entered on Clinicaltrial.gov, published in peer-reviewed journals and presented at international conferences.

DECLARATIONS

Author contributions

YL wrote the manuscript. TZ, ES, YL, VM, JM, PM, HMM, JPVG and PVD wrote the initial English protocol on which this manuscript is based. TZ, VM, PM, JM and HMM translated it into French for submission to the National Ethics Committee and the “Direction de la Pharmacie et des Médicaments” of the Ministry of Health of the Democratic Republic of Congo as well as the National Scientific committee against Ebola. JDB, TZ, ES, VM, JM, PM, HMM, JPVG and PVD reviewed and contributed to the final manuscript.

Funding

The EBOVAC3 project has received funding from the IMI2 Joint Undertaking under grant agreement No 800176 (IMI-EU). This Joint Undertaking receives support from the European Union's Horizon 2020 research and innovation programme, European Federation of Pharmaceutical Industries and Associations (EFPIA) and the Coalition for Epidemic Preparedness Innovations (CEPI). For this trial, the Contract Research Organisation and part of the FANG ELISA analyses are funded by the CEPI. All other trial activities are funded by the IMI2 Joint Undertaking grant. All vaccines and Neutralizing antibody level analyses against Ad26 at the first visit are provided by Janssen Vaccines & Prevention B.V..

Acknowledgements

We acknowledge Janssen Vaccines & Prevention B.V. (in collaboration with Bavarian Nordic GmbH), the London School of Hygiene and Tropical Medicine (LSHTM), the Institut National de la Santé et de la Recherche Médicale (INSERM), and the College of Medicine and Allied Health Sciences (COMAHS) for their contribution in the EBOVAC3 project. We are grateful to the Division Provinciale de la Santé and, the political-administrative authorities of the Tshuapa province for a trustful collaboration. We acknowledge the reliability and motivation of the study site team and are grateful.

Competing interests

The authors declare that they have no competing interests.

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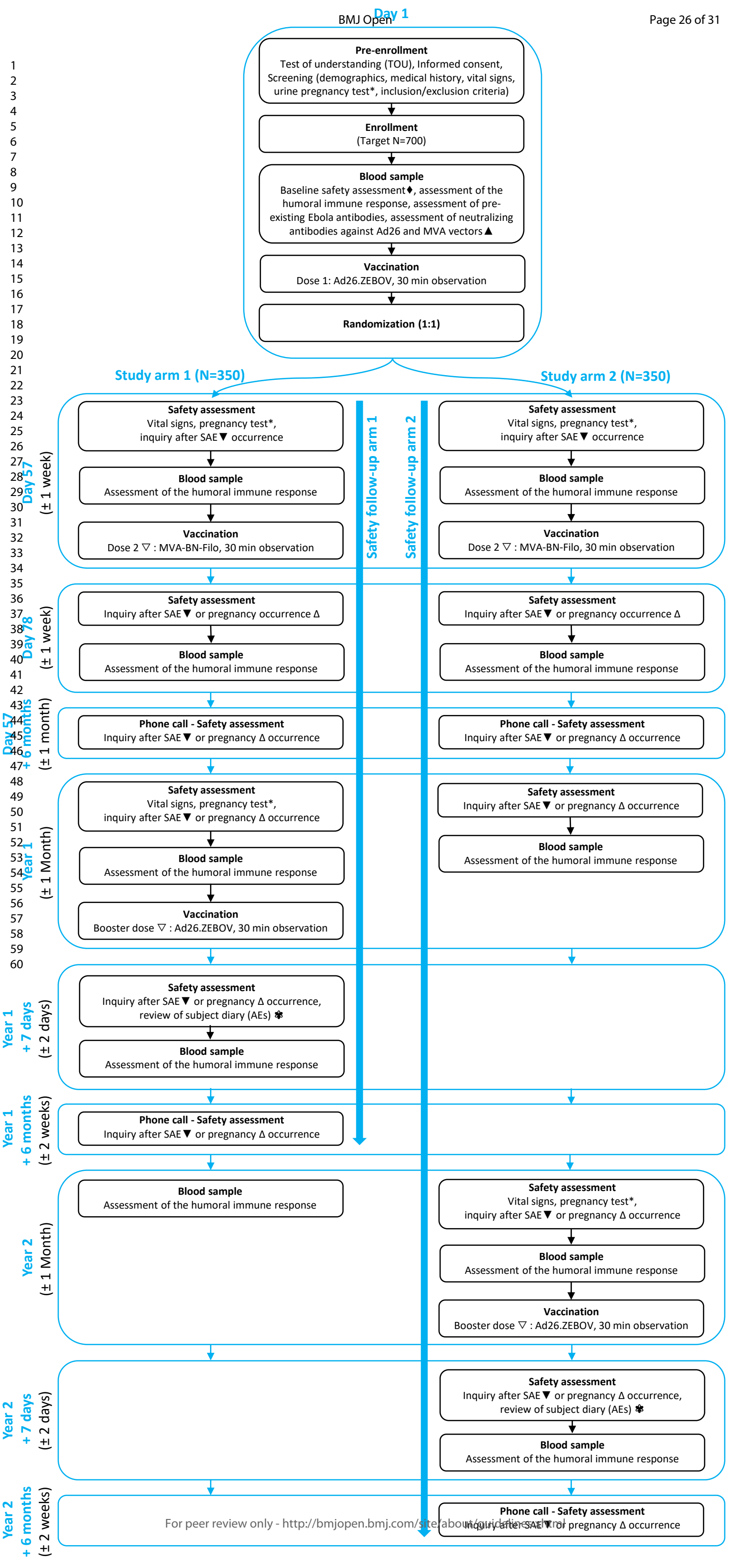
FIGURE TITLES AND LEGENDS

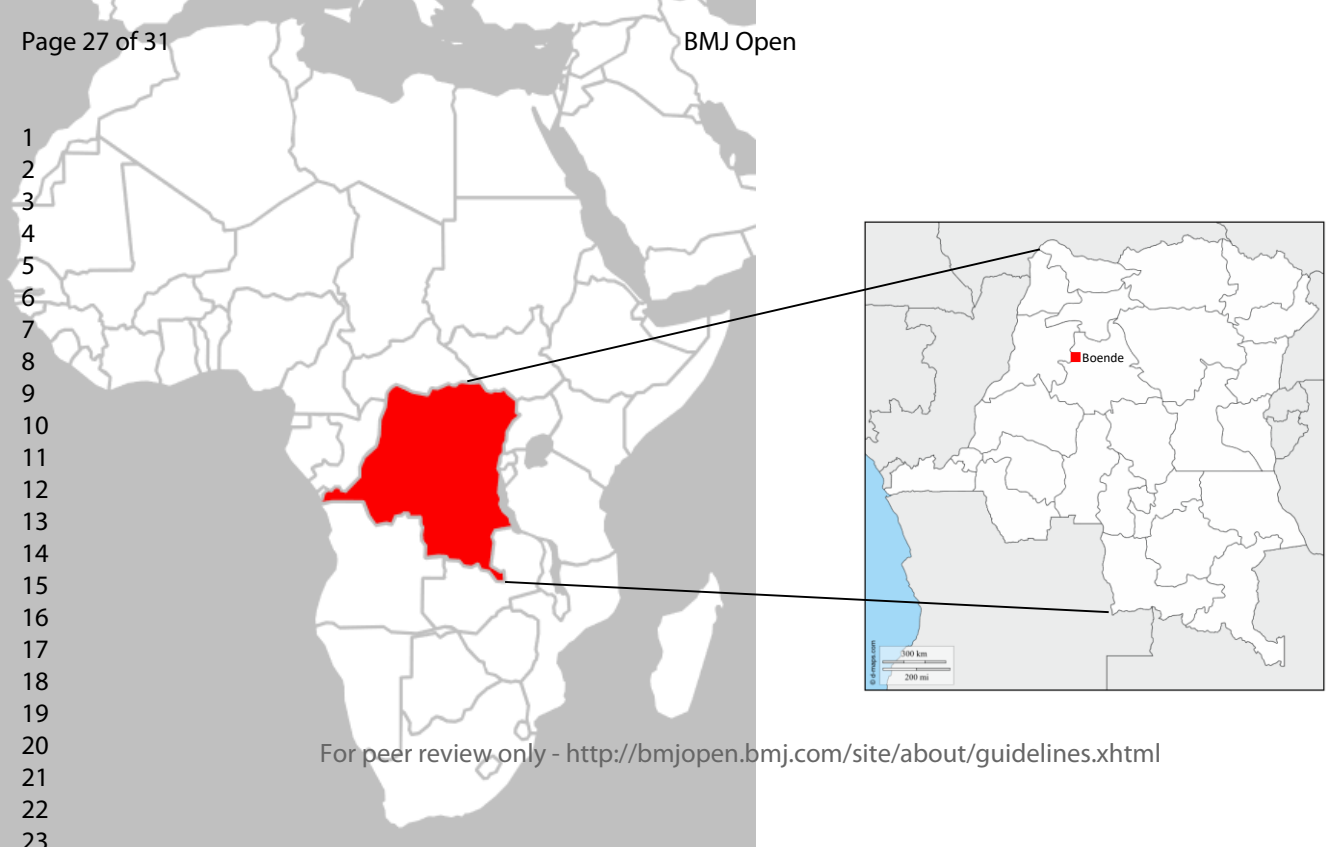
Figure 1. Study time and events overview

SAE: Serious Adverse Event; * Only for female participants of childbearing potential; ♦ Abnormal results will not exclude a participant, as results will not be reviewed prior to enrollment; ▲ Only the first 100 participants enrolled will be tested for Neutralizing antibody response against ad26 VNA and MVA vectors. Other blood analyses are for all 700 participants; ▼ Concomitant therapies given in conjunction with a serious adverse event (SAE) should be recorded from signing of the ICF onwards until 6 months post booster; ∇ The investigator may withhold the second vaccine or booster dose if a participant's clinical status changes prior to vaccination. The participant should continue to be followed for safety and immunogenicity according to the protocol; Δ only for female participants; ✽ Solicited and unsolicited Adverse Events (AEs) will be collected in a participant diary during 1 week post booster vaccination.

Figure 2. Study site location

On the left, the Democratic Republic of Congo (DRC) is highlighted on a map of the African continent. On the right, the study site location (Boende, Tshuapa province) is marked on a map of DRC indicating its provinces[27].





SPIRIT

STANDARD PROTOCOL ITEMS: RECOMMENDATIONS FOR INTERVENTIONAL TRIALS

SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	ItemNo	Description	Location in manuscript
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	Page 1, line 1-4
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	Page 2, abstract>Ethics and dissemination, line 40-41
	2b	All items from the World Health Organization Trial Registration Data Set	N/A
Protocol version	3	Date and version identifier	N/A
Funding	4	Sources and types of financial, material, and other support	Page 20, declarations>funding, line 377-384
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	Page 1, title page: line 10-15; Page 20, declarations>Author contributions, line 371-376
	5b	Name and contact information for the trial sponsor	Name: Page 11, line 147 Contact information: Corresponding author, line 16-21
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	Page 20, declarations>Author contributions, line 371-376
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	N/A
Introduction			
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	Page 3-5, Introduction, paragraph 1-6, line 55-115
	6b	Explanation for choice of comparators	Page 4, Introduction, paragraph 4, line 91-102

Section/item	ItemNo	Description	Location in manuscript
Objectives	7	Specific objectives or hypotheses	Page 6, methods>objectives, line 127-130, Table 1
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	Page 5, methods>study design and setting, line 117-126
Methods: Participants, interventions, and outcomes			
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	Page 5-6, methods>study design and setting, line 117-126; Page 18-19, Discussion, paragraph 2, line 329-338; Figure titles and legends, Figure 2, line 469-472; see also additional file figure 2
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	Page 7, Methods>Participant population, line 141-142, Table 2
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	Page 12-14, Methods>procedures & study intervention, line 166-224; Page 24, figure titles and legends, Figure 1, line 4578-468; see also additional file Figure 1
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	Page 14-15, Methods>Study intervention, paragraph 2, line 232-256
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	Page 16, Methods>Patient and public involvement, paragraph 1, line 258-268
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	Page 14-15, Methods>Study intervention, paragraph 2, line 232-256; Page 9, Table 2, inclusion criterium 9
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	Page 6, Table 1, endpoints; Page 17, Methods>statistical analysis, paragraph 2-4, line 293-320

Section/item	ItemNo	Description	Location in manuscript
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	Page 12-14, Methods>Study procedures, paragraph 1-7, line 166-224; Page 24, figure titles and legends, Figure 1, line 458-468; see also additional file Figure 1
Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	Page 7, Methods>participant population, line 131-140; Page 19, Discussion, paragraph 3, line 339-349
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	Page 16, Methods>Patient and public involvement, paragraph 1, line 258-268
Methods: Assignment of interventions (for controlled trials)			
Allocation:			
Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	Page 11-12, Methods>Randomization procedure, line 143-165
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	Page 11-12, Methods>Randomization procedure, line 143-165
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	Page 11-12, Methods>Randomization procedure, line 143-165
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	N/A
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	N/A
Methods: Data collection, management, and analysis			
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with	Page 12-14, Methods>Study procedures, paragraph 1-7, line 166-224; Page 16-17, methods>data management, line 276-282

Section/item	ItemNo	Description	Location in manuscript
		their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	Page 16, Methods>Patient and public involvement, paragraph 1, line 258-268
Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	Page 16-17, methods>data management, line 276-282
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	Page 17-18, Methods>statistical analysis, paragraph 3-4, line 298-315
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	Page 17, Methods>statistical analysis, paragraph 3, line 298-308
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	Page 17, Methods>Statistical analysis, paragraph 1, line 284-292 and paragraph 3, line 298-308
Methods: Monitoring			
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	N/A
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	Page 18, Methods>statistical analysis, paragraph 5, line 316-320
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	Page 12-13, Methods>Study procedures, paragraph 1-6, line 166-221
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	Page 16, Methods>Data management, line 276-282
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	Page 20, Ethics and dissemination, paragraph 1, line 358-365

Section/item	ItemNo	Description	Location in manuscript
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	N/A
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	Page 20, Ethics and dissemination, paragraph 1, line 358-365
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	Page 16, Methods>Patient and public involvement, paragraph 2, line 269-271
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	Page 16, Methods>Data management, line 276-282
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	Page 21, Declarations>Competing interests, line 392-393
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	Page 20, Ethics and dissemination, paragraph 2, line 366-369
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	N/A
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	Page 20, Ethics and dissemination, paragraph 2, line 366-369
	31b	Authorship eligibility guidelines and any intended use of professional writers	Page 20, Declarations>Author contributions, line 371-376
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	N/A
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	N/A
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	N/A

BMJ Open

Open-label, Randomized, Clinical Trial to Evaluate the Immunogenicity and Safety of a Prophylactic Vaccination of Health Care Providers by Administration of a Heterologous Vaccine Regimen Against Ebola in the Democratic Republic of the Congo: the Study protocol

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2020-046835.R2
Article Type:	Protocol
Date Submitted by the Author:	20-Aug-2021
Complete List of Authors:	Larivière, Ynke; University of Antwerp, Centre for the Evaluation of Vaccination; University of Antwerp, Global Health Institute Zola, Trésor; University of Kinshasa, Tropical Medicine Department Stoppie, Elke; University of Antwerp, Centre for the Evaluation of Vaccination; University of Antwerp, Global Health Institute Maketa, Vivi; University of Kinshasa, Tropical Medicine Department Matangila, Junior ; University of Kinshasa, Tropical Medicine Department Mitashi, Patrick; University of Kinshasa, Tropical Medicine Department De Bie, Jessie; University of Antwerp, Centre for the Evaluation of Vaccination; University of Antwerp, Global Health Institute Muhindo-Mavoko, Hypolite; University of Kinshasa, Tropical Medicine Department Van geertruyden, Jean-Pierre; University of Antwerp, Global Health Institute Vandamme, Pierre; University of Antwerp, Centre for the Evaluation of Vaccination
Primary Subject Heading:	Infectious diseases
Secondary Subject Heading:	Immunology (including allergy), Public health, Global health
Keywords:	Protocols & guidelines < HEALTH SERVICES ADMINISTRATION & MANAGEMENT, Health & safety < HEALTH SERVICES ADMINISTRATION & MANAGEMENT, Immunology < TROPICAL MEDICINE

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Open-label, Randomized, Clinical Trial to Evaluate the Immunogenicity and Safety of a Prophylactic Vaccination of Health Care Providers by Administration of a Heterologous Vaccine Regimen Against Ebola in the Democratic Republic of the Congo: the Study protocol

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0032 3 265 9716

Word count excluding title page, abstract, references, figures, tables and declarations: 4960

ABSTRACT

Introduction: This article describes the protocol of an Ebola vaccine clinical trial which investigates the safety and immunogenicity of a 2-dose prophylactic Ebola vaccine regimen comprised of two Ebola vaccines (Ad26.ZEBOV and MVA-BN-Filo) administered 56 days apart, followed by a booster vaccination with Ad26.ZEBOV offered at either 1 year or 2 years (randomization 1:1) after the first dose. This clinical trial is part of the EBOVAC3 project (an Innovative Medicines Initiative 2 Joint Undertaking), and is the first to evaluate the safety and immunogenicity of two different booster vaccination arms in a large cohort of adults.

Methods and analysis: This study is an open-label, monocentric, phase 2, randomized vaccine trial. A total of 700 health care providers and front-liners are planned to be recruited from the Tshuapa province in the Democratic Republic of the Congo (DRC). The primary and secondary objectives of the study assess the immunogenicity of the first (Ad26.ZEBOV), second (MVA-BN-Filo) and booster (Ad26.ZEBOV) dose. Immunogenicity is assessed through the evaluation of EBOV GP binding antibody responses after vaccination. Safety is assessed through the collection of serious adverse events from the first dose until 6 months post booster vaccination and the collection of solicited and unsolicited adverse events for one week after the booster dose.

Ethics and dissemination: The protocol was approved by the National Ethics Committee of the Ministry of Health of the DRC (n°121/CNES/BN/PMMF/2019). The clinical trial was registered on the 4th of December 2019 on ClinicalTrials.gov (NCT04186000). Trial activities are planned to finish in October 2022. All participants are required to provide written informed consent and no study-related procedures will be performed until consent is obtained. The results of the trial will be added on ClinicalTrials.gov, published in peer-reviewed journals and presented at international conferences.

Key words: Clinical Trial Protocol, Ebola Vaccines, Safety, Immunogenicity, Health Care Providers

STRENGTHS AND LIMITATIONS OF THIS STUDY

- With this randomized vaccine trial, being the first to evaluate the safety and immunogenicity in two different booster vaccine arms 1 or 2 years after the prime dose, new contributions will be added to already existing safety and immunogenicity data. Additionally, it is the first trial to assess the antibody response and (serious) adverse event occurrence of two different booster arms in a large adult cohort.
- Vaccination of health care providers and front-liners can potentially help protect a community which is at risk for future outbreaks.
- Innovative use of iris scanning biometric material to identify participants enrolled in the trial.
- This study takes place in a resource poor setting, impacting logistical set-up of the trial.
- Long duration of the trial (2.5 years) may lead to considerable loss to follow up.

INTRODUCTION

Ebolaviruses (negative stranded RNA viruses) belong to the *Filoviridae* family and cause Ebola virus disease (EVD), which often leads to severe haemorrhagic fever in humans and nonhuman primates[1]. Contact with infected wild animals (such as fruit bats, gorillas, apes, monkeys, etc.) is often reported as the source of animal-to-human transmission[2-4] and once among humans, these public health pathogens spread via direct (body fluids) or indirect (contaminated surfaces) human-to-human contact[2, 3). While they do not spread via air or water[3], *Ebolaviruses* bring along a severe public health burden with case fatality rates that can range up to 90%[5]. Since the discovery of the Ebola viruses in 1976[6], more than 20 outbreaks (most are endemic to regions in equatorial and western Africa) have taken place[7]. To date, the Democratic Republic of the Congo (DRC) has been the most affected country with its 12th outbreak taking place between February and May 2021[8]. However, it is only recently that the search for a safe and effective vaccine against Ebola was accelerated when the epidemic potential of *Ebolaviruses*, and more specifically the species *Zaïre Ebolavirus* (virus name: Ebola virus; abbreviation: EBOV[9]), became clear through the West African Ebola epidemic (2013-2016; 28,616 cases with 11,310 deaths[10]).

One of the initiatives to develop such a vaccine came from an international consortium, funded by the Innovative Medicines Initiative 2 (IMI2) Joint Undertaking, dedicated to evaluate a prophylactic Ebola vaccine regimen comprised of 2 candidate Ebola vaccines (Ad26.ZEBOV and MVA-BN-Filo) and aiming to bring this prophylactic Ebola vaccine to licensure[11]. Ad26.ZEBOV is a monovalent vaccine developed to provide active EBOV-specific immunity. MVA-BN-Filo, which is administered 56 days after the Ad26.ZEBOV vaccine, is a multivalent vaccine developed to establish active immunity to EBOV, *Sudan Ebolavirus*, *Taï Forest Ebolavirus* and the Marburg virus (also part of the *Filoviridae* family). In July 2020, the 2-dose prophylactic vaccine regimen was granted market authorisation by the European Commission[12].

Several projects (PREVAC, EBOVAC1, EBOVAC2 and EBOVAC3) within the IMI2 Joint Undertaking, of which the first in-human clinical trials started in 2014, were at the basis of this successful authorisation[11]. Within these projects, multiple clinical trials assessed or are assessing the timing, tolerability, safety and immunogenicity of different Ad26.ZEBOV and MVA-BN-Filo vaccine regimen in healthy adults (≥18 years old) and children/adolescents (1-17 years old) via phase 1, 2, 2B and 3 studies. Initial trials showed that healthy adult participants had higher geometric mean concentrations of binding antibodies for the regimen where Ad26.ZEBOV vaccination was followed by an MVA-BN-Filo vaccination 56 days later. Moreover, 100% of participants had detectable Ebola glycoprotein-specific Immunoglobulin G (IgG) antibodies up to at least 240 days after vaccination[13-15]. Even though some local (erythema, swelling and pain at injection site) and systemic (headache, nausea, pyrexia, myalgia and fatigue) solicited adverse events were recorded, the vaccine regimen was generally well tolerated across studies[13-17].

While it is of utmost importance that the 2-dose prophylactic vaccine regimen is safe and leads to an immune response, it is also crucial to find out whether or not this regimen can lead to induced immune memory at the time of imminent risk (i.e. an outbreak) through a booster vaccination. To evaluate this induced immune memory response, three previous studies within EBOVAC projects have administered

a booster vaccine with Ad26.ZEBOV at either 1 year (NCT02325050; NCT02564523) or 2 years (NCT02509494) post-dose 1. Results from the NCT02325050 trial have already shown that an immunological memory was rapidly induced via booster vaccination with Ad26.ZEBOV, indicating that booster vaccination can be considered for at risk individuals (e.g. when an outbreak occurs) that were previously vaccinated with the 2-dose heterologous prophylactic regimen[18]. However, these trials only evaluated booster vaccination in a small amount of participants (n ≤ 39) and it still has to be explored whether the induced immune memory response differs depending on the timing of the booster dose (i.e. 1 or 2 years after dose 1).

Healthcare settings play an important role in the control of EVD and therefore health care providers (HCP) and front-liners, due to occupational exposure, are not only more at risk of disease acquisition but also facilitate the spread of the virus[19-22]. Knowing that outbreaks of EVD often occur in regions where there is already a shortage of HCP and front-liners, this further depletes a weak health care system and the quality of care. Consequently, the Strategic Advisory Group of Experts stated in 2018 that for preventive strategies, vaccination of HCP as part of an emergency preparedness plan has significant potential of reducing the scale and duration of outbreaks[23].

This phase 2 randomized clinical trial aims to determine the safety and immunogenicity of the 2-dose heterologous vaccine regimen with Ad26.ZEBOV followed by MVA-BN-Filo 56 days later. Additionally, this trial aims to assess the safety and immunogenicity of a booster Ad26.ZEBOV vaccine administered either 1 or 2 years post first dose and to compare the induced immune memory response between both booster arms. The trial is conducted in a cohort of HCP and front-liners from the Boende health district in DRC, a well-known population at risk from clinical and epidemiological perspective.

METHODS

Study design and setting

This study is an open-label, monocentric, phase 2, randomized trial to evaluate the immunogenicity and safety of Ad26.ZEBOV ($5x10^{10}$ viral particles) as first dose and MVA-BN-Filo ($1x10^{8}$ infectious units)

as second dose vaccination at a 56-day interval in HCP and front-liners who may be exposed to Ebola in the event of a future Ebola outbreak in DRC. Additionally, after randomization (1:1) a booster of Ad26.ZEBOV ($5x10^{10}$ viral particles) will be offered at either 1 year or 2 years after the first dose (Figure 1).

The study site is located in Boende, Tshuapa province, DRC (Figure 2), at approximately 750km north-west of Kinshasa. Study participants will be enrolled at the General Reference Hospital in Boende.

Objectives

The primary, secondary and exploratory objectives and endpoints of this study are described in Table 1.

Table 1. Objectives and endpoints

Objectives	Endpoints
Primary	
• To assess binding antibody responses post-dose 2 vaccination with MVA-BN-Filo.	• Binding antibody levels against the EBOV GP using FANG ELISA at 21 days post-dose 2 (Day 78) vaccination with MVA-BN-Filo.
Secondary	
• To assess binding antibody responses after booster vaccination with Ad26.ZEBOV given at 1 or 2 years after first dose.	• Binding antibody levels against the EBOV GP using FANG ELISA at 7 days (excluding the day of vaccination) post booster.
• To assess the safety of a heterologous vaccine regimen utilizing Ad26.ZEBOV and MVA-BN-Filo administered at a 56-day interval and a booster vaccine with Ad26.ZEBOV at one or two years post first dose.	• Serious adverse events from first dose vaccination until 6 months post booster. • Solicited and unsolicited local and systemic adverse events until 7 days post booster vaccination (day of vaccination and subsequent 7 days) with Ad26.ZEBOV.
Exploratory	
• To assess binding antibody responses at different time points as indicated in the Study time and events overview (Figure 1).	• Binding antibody levels against the EBOV GP using FANG ELISA at different time points as indicated in the Study time and events overview (Figure 1).

• To assess neutralizing antibody response directed against the Adenovirus vector prior to vaccination.	• Neutralizing antibody levels against Ad26 using Ad26 VNA at the first visit.
• To assess neutralizing antibody response directed against the MVA vector prior to vaccination.	• Neutralizing antibody levels against MVA-BN-Filo using MVA PRNT assay at the first visit.
• To assess seroprevalence of Ebola virus disease prior to vaccination.	• Presence of pre-existing Human anti-EBOV GP IgG and anti-EBOV NP IgG using LUMINEX assay.
ELISA: enzyme-linked immunosorbent assay; EU/mL: ELISA units/mL; FANG: Filovirus Animal Nonclinical Group. VNA: Virus Neutralization Assay; PRNT: Plaque Reduction Neutralization Test	

Participant population and sample size

A total of 700 Registered HCP and front-liners in DRC (working in the Boende General Reference Hospital, Health Centres or Health Posts in the Boende health district) are planned to be recruited from the Tshuapa province. This assessment was based on information obtained from an ongoing (monkeypox) vaccine trial in the same area at the time the protocol was being written[24]. From discussions with the monkeypox research group, it became clear that a high enrollment rate and retention rate (>90% after two years) could be expected among HCP and front-liners in the Boende health district. Based on this ongoing monkeypox trial, it was estimated that enrolling approximately 50% of the HCP and front-liners working in the Boende health district would be feasible. The participant population is thus a convenience sample and the sample size is defined upon the feasibility of recruitment of HCP and front-liners in the region.

However, to determine whether it would be possible to compare the induced immune responses of the two booster arms, a power analysis was performed. A power of 0.99 was calculated based on the following parameters: two-sided t-test, equal samples of 350 participants, significance level of 0.05, an effect size of 0.49 in antibody response. The effect size was calculated based on trial data (NCT02564523 and NCT02509494) available in the first edition of the combined Investigator's Brochure of the vaccines with samples from 64 participants vaccinated either 1 year or 2 years after

the first dose[25]. To obtain the effect size, the difference in geometric mean concentrations (log scale) of the EBOV GP-specific antibody responses between the two groups was divided by the pooled standard deviations[26]. With a power of 0.99 it will thus be possible to perform a formal comparative analysis of the induced immune memory response of the two booster arms.

Unfortunately no power analysis could be performed to determine whether the sample size is sufficiently large to perform a formal statistical comparison of safety response (AEs and SAEs) from both arms. In the current combined Investigator's Brochure of the vaccines[25], safety information is pooled for all booster doses independent of the timing of its administration (1 year or 2 years post-dose 1) and thus no effect size can be calculated until the unpooled data from the different trials is obtained.

Inclusion and exclusion criteria that determine the eligibility of participants are reported in Table 2.

Table 2. Inclusion and exclusion criteria

Inclusion criteria
Each potential participant must satisfy all of the following criteria to be enrolled in the study: 1. The participant must pass the Test of Understanding. *Note: If the participant fails the Test of Understanding on the first attempt, he/she must be retrained on the purpose of the study and must take the test again (2 repeats are allowed). If participants fail on the third attempt, they should not continue with screening or consenting procedures.* 2. Each participant must sign an informed consent form indicating that he or she understands the purpose of, and procedures required for, the study and is willing to participate in the study. In case the participant cannot read or write, the procedures must be explained and informed consent must be witnessed by a trusted literate third party not involved with the conduct of the study. 3. The participant must be a man or women aged 18 years or older. 4. The participant must be a documented HCP in DRC.

5. The participant must be healthy in the investigator's clinical judgement and on the basis of vital signs assessed at day 1 screening.

   Note: HIV-positive subjects can be enrolled as long as their general condition is good, i.e. they are on antiretroviral treatment or have no signs or symptoms of immunodepression, diagnosed on the basis of physical examination, medical history, and the investigator's clinical judgment.

6. Before vaccination, a woman must be either:

   - Of childbearing potential and practicing (or intending to practice) a highly effective method of birth control consistent with local regulations and/or local culture regarding the use of birth control methods for participants in clinical studies, beginning at least 28 days prior to vaccination and during the study up to at least 3 months after the first (or only) vaccination (Ad26.ZEBOV) and 1 month after the MVA-BN-Filo vaccination (if applicable); and then starting again 14 days before the booster vaccination until 3 months after the booster vaccination. The sponsor considers the following methods of birth control to be highly effective: established use of oral, injected or implanted hormonal methods of contraception; placement of an intrauterine device or intrauterine system; barrier methods: condom or occlusive cap (diaphragm or cervical/vault caps) with or without spermicidal foam/gel/film/cream/suppository; male partner sterilization (the vasectomized partner should be the sole partner for that participant); true abstinence (when this is in line with the preferred and usual lifestyle of the participant); OR
   - Not of childbearing potential: postmenopausal (amenorrhea for at least 12 months without alternative medical cause); permanently sterilized (e.g. bilateral tubal occlusion [which includes tubal ligation procedures as consistent with local

regulations], hysterectomy, bilateral salpingectomy, bilateral oophorectomy); or otherwise be incapable of pregnancy.

Note: If the social situation of a woman of childbearing potential changes (e.g. woman who is not heterosexually active becomes active), she must begin a highly effective method of birth control, as described above.

7. Woman of childbearing potential must have a negative urine β-human chorionic gonadotropin pregnancy test immediately prior to each study vaccine administration.
8. Participant must be available and willing to participate for the duration of the study.
9. Participant must be willing and able to comply with protocol requirements (including certain prohibitions and restrictions such as the use of anticonception and the discouragement of concomitant treatment that may alter the immune response).
10. Participant must be willing to provide verifiable identification.
11. Participant must have a means to be contacted.

Exclusion criteria

Participants will be excluded from study participation in case the following criteria apply:

1. The participant has a known history of Ebola virus disease.
2. The participant has received any experimental candidate Ebola vaccine less than 3 months prior to the first study visit.
3. The participant has received any experimental candidate Ad26-vaccine in the past.

   Note: Receipt of any approved or experimental vaccinia/smallpox vaccine or experimental Ad-vector vaccine other than Ad26 prior to study entry is allowed.
4. The participant has a known allergy or history of anaphylaxis or other serious adverse reactions to vaccines or vaccine products (including any of the constituents of the study vaccines [e.g. polysorbate 80, ethylenediaminetetraacetic acid (EDTA) or L-histidine for Ad26.ZEBOV vaccine; and tris (hydroxymethyl)-amino methane (THAM) for MVA BN-Filo vaccine]), including known allergy to egg, egg products and aminoglycosides.

5. The participant has an acute illness (this does not include minor illnesses such as mild diarrhea or mild upper respiratory tract infection) or temperature ≥38.0°C on Day 1. Participants with such symptoms will be excluded from enrollment at that time, but may be rescheduled for enrollment at a later date if feasible.
6. The participant is a pregnant or breastfeeding women, or women planning to become pregnant while enrolled in this study until at least 3 months after the Ad26.ZEBOV vaccination or 1 month after MVA-BM-Filo.
7. The participant has significant conditions or clinically significant findings at screening or vital signs for which, in the opinion of the investigator, participation would not be in the best interest of the participant (e.g. compromise the safety or well-being) or that could prevent, limit, or confound the protocol-specified assessments.

   Note: Participants who have recently received treatment for acute, uncomplicated malaria are eligible for participation if at least 3 days have elapsed from the conclusion of a standard, recommended course of therapy for malaria; participants who are acutely ill with malaria at the time of screening should complete therapy and wait an additional 3 days after completion before screening for the study.

   Note: Participants with sickle cell trait can be included.
8. The participant had major surgery (per the investigator's judgment) within the 4 weeks prior to screening, or has planned major surgery during the study (from the start of screening onwards).
9. The participant had a post-organ and/or stem cell transplant whether or not with chronic immunosuppressive therapy.
10. The participant received an investigational drug or investigational vaccines or used an invasive investigational medical device within 3 months prior to screening, or current or planned participation in another clinical study during the study.

    Note: Participation in an observational clinical study is allowed.

11. The participant has a history of chronic urticaria (recurrent hives).

Randomization procedure

The study randomization list will be developed using an algorithm in the Statistical Analysis System software. This algorithm will randomly assign a treatment group (1:1) to a sequential randomization number. Once established, the list will be shared with the principal investigator (University of Kinshasa), who is in charge of creating sealed envelopes under sponsor (University of Antwerp) supervision. A total of at least 700 randomization envelopes will be created. Thirty envelopes will be grouped into one larger envelope, referred to as a "booklet". The booklets and envelopes will be numbered sequentially by a unique sequence of numbers. The booklets will be labelled in a sequential order (i.e. 01-24) and the envelopes will be labelled with the study number "VAC52150-EBL-2007" and a sequential randomization number (i.e. 001-700) to which a treatment group is linked via the algorithm. The staff delegated to make the envelopes will use the *Envelope Assembly Record Worksheet*, on which the randomization number, initials of the assembler, date on which the assembly took place, and the initials of the staff member(s) that performed the quality control are collected. The randomization booklets with envelopes will be stored and used in the study clinic.

Delegated site staff will assign and open booklets and envelopes in sequential order during study visits. Each envelope will contain two stickers. The first will contain space for writing the subject ID and participant's initials, the second will contain the randomization number and treatment description (pre-printed based on the study randomization list). Upon opening the sealed envelope, the subject ID and initials must be written in the space provided on the first sticker and the subject ID sticker must be placed on the outside of the envelope. To ensure proper source documentation, the sticker with the treatment information must be placed on the corresponding *Randomization worksheet*. Thereafter, the empty envelope, with the subject ID sticker on the outside, must be placed back in the booklet. These booklets are to be stored by the principal investigator.

Study procedures (Figure 1)

At Day 1, interested participants are informed about the study and are required to pass a test of understanding before providing written consent. No study activities are performed before the participant has signed the informed consent form. Afterwards, the study medical doctor evaluates his/her general health based on the inclusion criteria, vital signs (blood pressure, pulse/heart rate [both at rest] and body temperature) are collected and a urine pregnancy test for women of childbearing potential is performed. Further during this first visit, a blood sample is taken for baseline testing of binding antibody level (i.e. humoral immune response) against EBOV glycoprotein (GP) using Filovirus Animal Non-Clinical Group (FANG) Ebola virus enzyme-linked immunosorbent assay (ELISA) and the presence of pre-existing Human anti-EBOV GP IgG and anti-EBOV nucleoprotein (NP) IgG using LUMINEX assay. For Day 1 samples, both FANG ELISA and LUMINEX assay will be carried out. FANG ELISA is performed for all EBOVAC trials in the same laboratory (for consistency and comparability) and LUMINEX assay will provide a more detailed array of IgG antibodies that are not obtained via FANG ELISA. For the first 100 enrolled participants an additional test on the collected serum is performed to measure the neutralizing antibody level against Ad26 and MVA vectors using the Ad26 Virus Neutralizing Assay (VNA) and MVA Plaque Reduction Immunogenicity Test (PRNT), respectively. Subsequently, a blood sample for baseline safety assessment is collected to test haemoglobin, haematocrit, blood cell count (white and red), platelet count, urea, creatinine and transaminases. Then, participants are vaccinated with the first dose (Ad26.ZEBOV) and they are given instructions to contact the study team for any occurring serious adverse events (SAEs), or in case of pregnancy of a participant during the study. After vaccination, participants remain at the study site for an observation period of 30 minutes to make sure no SAEs occur. SAEs are collected from first dose vaccination until 6 months post booster. Lastly on Day 1, randomization is performed to determine the timing of the booster vaccine at either 1 year or 2 years after the first dose. Contact information is verified, an appointment for the second dose on Day 57 is arranged and a participant card is printed. Innovatively,

next to a participant card, a biometric identification tool via iris scanning is foreseen to ensure correct identification of the participants during all study related visits.

At Day 57, participants return to the study site for urine pregnancy testing (for women of childbearing potential), vital signs measurement, assessment of safety (SAEs), a blood sample for immunogenicity assessment (the binding antibody levels against EBOV GP using FANG ELISA) and afterwards administration of the second vaccine (MVA-BN-Filo). After an observation period of 30 minutes, participants are reminded to contact the study team for any SAEs that occurs, or in case of pregnancy of a participant during the study. Contact information is verified and an appointment for the 21-day post-dose 2 visit (Day 78) is arranged.

At 21 days post-dose 2 (Day 78), all participants return to the study site for a safety assessment (SAEs) and for the collection of a blood sample for immunogenicity assessment. Contact information is re-verified and they are reminded to contact the study team in case of SAE occurrence, or in case of pregnancy of a participant.

To make sure no valuable information is missed, participants are contacted by phone to inquire about any occurrence of pregnancies (female participants) and SAEs at approximately 6 months post-dose 2 vaccination.

At 1 year and 2 years after the first vaccine, when all participants return to the site, the clinical trial staff inquires after the occurrence of SAEs and a blood sample is collected for immunogenicity assessment of all participants (where applicable before administration of the booster dose). Depending on the study arm, a booster vaccination with Ad26.ZEBOV is given either 1 or 2 years after the first dose. Prior to vaccination, the general well-being of the participant is evaluated and urine pregnancy testing (for women of childbearing potential), as well as a vital signs measurement are performed. After vaccination, participants remain at the study site for a 30 minute observation period. Participants are asked to collect solicited and unsolicited adverse events (AEs) in a participant diary starting on the day of the vaccination and continuing for the subsequent 7 days.

At Day 8 post booster the safety data including solicited and unsolicited AEs is reviewed and a blood sample for immunogenicity assessment is taken to document the immune response. Should any solicited AEs persist at Day 8 post booster, participants are asked to continue monitoring these in their participant diary. Once the solicited AEs have resolved, they are asked to make an unscheduled visit at the site so this information can be reported.

At 6 months post booster, all participants are contacted by phone and questioned about any SAEs or pregnancies (female participants) that have occurred since the last vaccination.

The total duration of the study is 2 years and 6 months post-first dose. The study is considered completed when the last participant has been contacted for the 6 months post booster phone call or has left the study.

Study intervention

According to the predefined schedule (Figure 1), participants receive a 0.5 mL intramuscular injection into the deltoid muscle of the upper arm with Ad26.ZEBOV or MVA-BN-Filo. The injection site should be free from any injury, local skin conditions, or other issues that might interfere with the evaluation of local reactions. Every vaccination is given in the opposite arm of the previous vaccination (unless the opposite arm has a condition that prevents evaluating the arm after injection). No local or topical anaesthetic is used prior to the injection.

The second or booster vaccination is not administered if any of the following events occur at any time after the first dose vaccination:

- A participant experiences anaphylaxis clearly attributable to vaccination with the study vaccine; OR
- A participant experiences generalized urticaria within 72 hours of vaccination considered to be related to study vaccine; OR

- A participant experiences a serious adverse event considered to be related to the study vaccine; OR
- A participant experiences injection site ulceration, abscess or necrosis considered to be related to the study vaccine; OR
- A participant has confirmed EVD; OR
- A female participant of childbearing potential has a positive urine β-human chorionic gonadotropin (β-HCG) pregnancy test before vaccination (on Day 57, Year 1 or Year 2 [depending on the randomization group]); OR
- A female participant of childbearing potential has a positive urine β-HCG pregnancy test between dose 2 and the booster dose and is still pregnant or breastfeeding at the time of the booster dose; OR
- A participant takes a concomitant treatment with drugs that may alter the immune response; OR
- The principal investigator believes that for safety reasons it is in the best interest of a participant to discontinue the study intervention.

Participants experiencing any of the events described above are still followed up for safety and immunogenicity according to the protocol. The decision to discontinue the study intervention is at the discretion of the principal investigator (University of Kinshasa) and after consultation with the sponsor (University of Antwerp) for any of the events described above.

Patient and public involvement

Difficulties were expected when setting up a clinical trial in Boende, a remote and resource-limited area of DRC. However, to avoid and anticipate some of these challenges and in order to support vaccination compliance, a collaboration is established between the study team and the Provincial Division of Health. Throughout the trial, workshops are organized for HCP in the health district of Boende to sensitize and inform about EVD and other relevant medical topics. These gatherings should

not only facilitate enrollment in the trial but also increase the engagement of participants by enhancing their understanding of the clinical trial and the importance of adherence. During these workshops time is available for questions and discussions. In addition to these gatherings for trial participants, community engagement activities and the training and capacity building of the local clinical trial team that is executing the trial (under supervision of the University of Kinshasa as principal investigator) are organised for the duration of the trial.

Each participant receives an individual visit schedule upon enrollment in the trial and when participants miss a planned study visit, community health workers of the Ministry of Health trace the individual participant. Consent is asked in the informed consent form for this mode of contact.

Furthermore, prior to the start of the clinical trial, a pilot study was performed whereby potential participants were interviewed on the feasibility and acceptability of the use of a biometric iris scanning tool for participant identification during the trial and the use of telephone messaging with visit reminders for participant adherence[27].

Data management

All information is collected during study visits on source documents by study staff. These source documents with confidential information are transcribed into the electronic clinical database by site data managers. To make sure that all entered data (collected in DFexplore version 5.2.1) is correct, the principal investigator reviews each source document and confirms its correct transcription in the database. Additionally, the sponsor performs quality checks of the entered data in the database and, during monitoring visits, source data verification is performed.

Statistical analysis

A differentiation in analysis will be made according to: 1) the *Full Analysis Set* (FAS; all participants who received at least one dose, regardless of the occurrence of protocol deviations), 2) *Per Protocol Set for primary vaccination series* (all vaccinated participants, who received both dose 1 and dose 2

[administered within the protocol-defined visit window] vaccinations, have at least 1 post-vaccination [i.e. after the date of dose 1] evaluable immunogenicity sample, and have no major protocol deviations influencing the immune response) and 3) *Per Protocol Set for the Booster vaccination* (includes all participants in the per protocol set for the primary vaccination series who received a booster dose and have at least 1 post booster vaccination evaluable immunogenicity sample, and have no major protocol deviations influencing the immune response).

Participant information (i.e. demographics and baseline characteristics, disposition information, treatment compliance, extent of exposure, protocol deviations and concomitant medications) is planned to be tabulated and summarized with descriptive statistics for all participants. For continuous data, such as age, the mean and standard deviation will be provided if applicable, otherwise the geometric means, related standard deviations or median and interquartile ranges will be used.

For the immunogenicity analysis, two *Per-Protocol Sets* will be used, i.e., the *Per-Protocol Set for primary vaccination series* and the *Per-Protocol Set for the booster*. If more than 10% of participants from the FAS are excluded from the per protocol immunogenicity set, the immunogenicity analysis will be repeated on the FAS to evaluate the robustness of the analysis results. A subgroup analysis of the immune response at different time points will be performed stratified by age (18-40, 40-60 and >60), gender, prior vaccinia/smallpox vaccination, pre-existing Ebola antibodies (positive or negative for pre-existing Human anti-EBOV GP IgG and anti-EBOV NP IgG, and for both), baseline immunogenicity (positivity versus negativity for antibody levels against EBOV GP using FANG ELISA) and the presence of neutralizing antibody levels against Ad26 and MVA vectors using Ad26 VNA and MVA PRNT assays (only the first 100 enrolled participants), respectively. For these planned subgroup analyses, N (%), Geometric Mean Concentrations and 95% confidence intervals (CI) will be provided as appropriate. Finally, a formal comparative analysis of the induced immune memory response between the two booster arms will be performed.

Safety analyses include SAEs collected during the whole study and solicited and unsolicited AEs for 1 week post booster vaccination. The analysis of SAEs will be performed using the FAS and the solicited and unsolicited AEs will be analysed for the participants who received the booster vaccination. Continuous variables will be summarized using the following statistics: number of observations; arithmetic or geometric mean/median (if applicable) with their related measures of dispersion (95% CI for the mean, standard deviation or inter quartile range (Q1-Q3)). Minimum and maximum frequencies and percentages (one decimal place) will be generated for categorical variables. If the unpooled safety data from the NCT02564523 and NCT02509494 studies can be obtained, a power analysis will be performed to assess whether the safety data of the two booster arms can be compared through formal statistical analysis.

The primary endpoint analysis is planned to be performed when all participants have completed the 21-day post-dose 2 visit (Day 78) or discontinued earlier. This analysis includes all available immunogenicity and safety data up to this point (date cut-off). Additional interim analyses may be performed during the study for the purpose of informing future vaccine-related decisions in a timely manner.

The final analysis will be performed when all participants have completed the last study-related phone call (6 months post booster) or left the study.

DISCUSSION

The aim of this phase 2 trial is to obtain further safety and immunogenicity data on the 2-dose prophylactic heterologous Ebola vaccine regimen and to assess the safety and immunogenicity of a booster dose with Ad26.ZEBOV administered either 1 or 2 years post first dose in a large cohort of HCP and front-liners. By doing so, this study will feed the immunogenicity and safety databases of the Ad26.ZEBOV and MVA-BN-Filo vaccines. It will also be the first study to compare the induced immune memory response between two different booster arms in a large cohort of adults.

Boende (the capital city of Tshuapa province) was selected as the trial site for several reasons. First, the Tshuapa province recovered from an EBOV outbreak that occurred in the Boende Health District in 2014[21]. Following this outbreak, a study (n=565) conducted in the Tshuapa region in 2015 found that 41.4% of the tested HCP were seroreactive to at least one EBOV protein and 2.8% of the HCP showed a neutralizing capacity while never having developed EVD symptoms[20]. This observation suggests a possible endemic EBOV exposure in the Tshuapa province of DRC. These are interesting observations for future ecologic research as the ecology and reservoir(s) of EBOV and other filoviruses remain largely unknown[28, 29]. Second, in addition to the previous outbreak of EVD, Boende was chosen to perform the current clinical trial as there was expertise available after carrying out a phase 3 monkey pox vaccine trial that took place in 2017[24].

Some limitations are present in the current set-up of the trial. First, by focussing on occupation (registered HCP and front-liners) rather than age and gender, in the inclusion and exclusion criteria, the aim is to easily reach the target of 700 participants. However, a recent review by Flanagan et al. (2017) has shown that immune responses to vaccination can differ based on gender and age[30]. To take this limitation into account, stratification for age and gender has been foreseen during statistical analysis. Second, while HIV-positive participants can participate in this trial if their general condition is good, it is not possible to be certain of the HIV-status of all participants as no routine checks prior to enrollment or during the course of the trial are foreseen. It is possible that some participants either are unwilling to share their HIV-positive status as a consequence of the stigma that is often linked to it[31] or are simply unaware of their positive status (e.g. during an asymptomatic phase of the disease[32]). However, due to the low prevalence (0.6%) of HIV-positive people in the province of the trial[31], it was chosen not to perform routine checks and to trust the willingness of a participant to share his/her status as it is not considered an exclusion criterium for the trial. Finally, at the start of the project the protocol initially only included a vaccination strategy with the 2-dose heterologous vaccine regimen (Ad26.ZEBOV followed by MVA-BN-Filo 56 days later) and was later adapted to include a booster vaccination at the request of the vaccine producer. The purpose of the initial observational

trial was, next to obtaining additional immunogenicity data, a way to see if performing a vaccination trial in a remote area of DRC was feasible and accepted by the population. While writing the protocol however, administering a booster dose in this large cohort was added as a novel aspect and thus this was entered as a secondary objective/endpoint. Currently it is unknown whether this booster dose will be required or not at the moment of an outbreak and what its protective effect would be. However, to explore its safety and immunogenicity, this study protocol was transformed and became a randomized clinical trial. Unfortunately, as the comparison of the two booster arm induced immune responses is not required for approval of the licensure of the 2-dose heterologous vaccine regimen and the booster dose was added as a second stage to the study design, no sample size calculations were initially performed for this trial and sample size was selected based on available information from a previous monkeypox vaccine trial in the same area. While this trial thus mainly has a descriptive set-up, scientifically it is interesting to learn if there is a significant difference in the induced immune memory response of the two booster arms. For this reason, a power analysis was retrospectively performed to determine whether it would be possible to compare the induced immune memory response of the two arms. Fortunately this will be possible as a power of 0.99 was calculated and a formal statistical comparison induced immune memory response of the two booster arms has now been foreseen in the Statistical Analysis Plan. It is however important to take into account that a varying antibody response after booster vaccination is not necessarily directly correlated with protective vaccine efficacy[33] and that a high power (99% for this study) can lead to significant differences, even if the difference between both groups is small. Prudent and careful interpretation of the results will thus be crucial[34].

In conclusion, because EVD remains a very deadly disease, effective and safe preventive measures will play a crucial role to protect vulnerable communities. While the prophylactic heterologous 2-dose regimen was recently granted market authorisation by the European Commission, further research into the safety and immunogenicity of the 2-dose regimen is still required to obtain worldwide licensure of the regimen. Furthermore, limited data has previously been collected on the safety and

immunogenicity of a booster dose with Ad26.ZEBOV. This is the first large, randomized vaccine trial that assesses and compares the safety and immunogenicity of two different booster arms in a large cohort.

ETHICS AND DISSEMINATION

This protocol was submitted and approved by the National Ethics Committee of the Ministry of Health of the Democratic Republic of Congo (approval number: n°121/CNES/BN/PMMF/2019). Prior to being enrolled in the trial, all participants are required to provide written informed consent by singing the informed consent form after having performed a test of understanding. If the participant is unable to read or write, an impartial witness should be present for the entire informed consent process (which includes reading and explaining all written information) and should personally date and sign the informed consent form after the oral consent of the participant is obtained. No study-related procedures are performed until the participant has signed the informed consent form.

The trial was registered on Clinicaltrial.gov on December 4th, 2019 (NCT04186000) and recruitment started on December 18th, 2019. All participants were recruited by the 8th of February 2020 and the study is planned to finish in October, 2022. Results of the trial will be entered on Clinicaltrial.gov, published in peer-reviewed journals and presented at international conferences.

DECLARATIONS

Author contributions

YL wrote the manuscript. TZ, ES, YL, VM, JM, PM, HMM, JPVG and PVD wrote the initial English protocol on which this manuscript is based. TZ, VM, PM, JM and HMM translated the English protocol into French for submission to the National Ethics Committee and the “Direction de la Pharmacie et des Médicaments” of the Ministry of Health of the Democratic Republic of Congo as well as the National Scientific committee against Ebola. All authors (YL, TZ, ES, JDB, VM, JM, PM, HMM, JPVG and PVD) reviewed and contributed to the final manuscript.

Funding

The EBOVAC3 project has received funding from the IMI2 Joint Undertaking under grant agreement No 800176 (IMI-EU). This Joint Undertaking receives support from the European Union's Horizon 2020 research and innovation programme, European Federation of Pharmaceutical Industries and Associations (EFPIA) and the Coalition for Epidemic Preparedness Innovations (CEPI). For this trial, the Contract Research Organisation and part of the FANG ELISA analyses are funded by the CEPI. All other trial activities are funded by the IMI2 Joint Undertaking grant. All vaccines and neutralizing antibody level analyses against Ad26 at the first visit are provided by Janssen Vaccines & Prevention B.V..

Acknowledgements

We acknowledge Janssen Vaccines & Prevention B.V. (in collaboration with Bavarian Nordic GmbH), the London School of Hygiene and Tropical Medicine (LSHTM), the Institut National de la Santé et de la Recherche Médicale (INSERM), and the College of Medicine and Allied Health Sciences (COMAHS) for their contribution in the EBOVAC3 project. We are grateful to the Division Provinciale de la Santé and, the political-administrative authorities of the Tshuapa province for a trustful collaboration. We acknowledge the reliability and motivation of the study site team and are grateful.

Competing interests

The authors declare that they have no competing interests.

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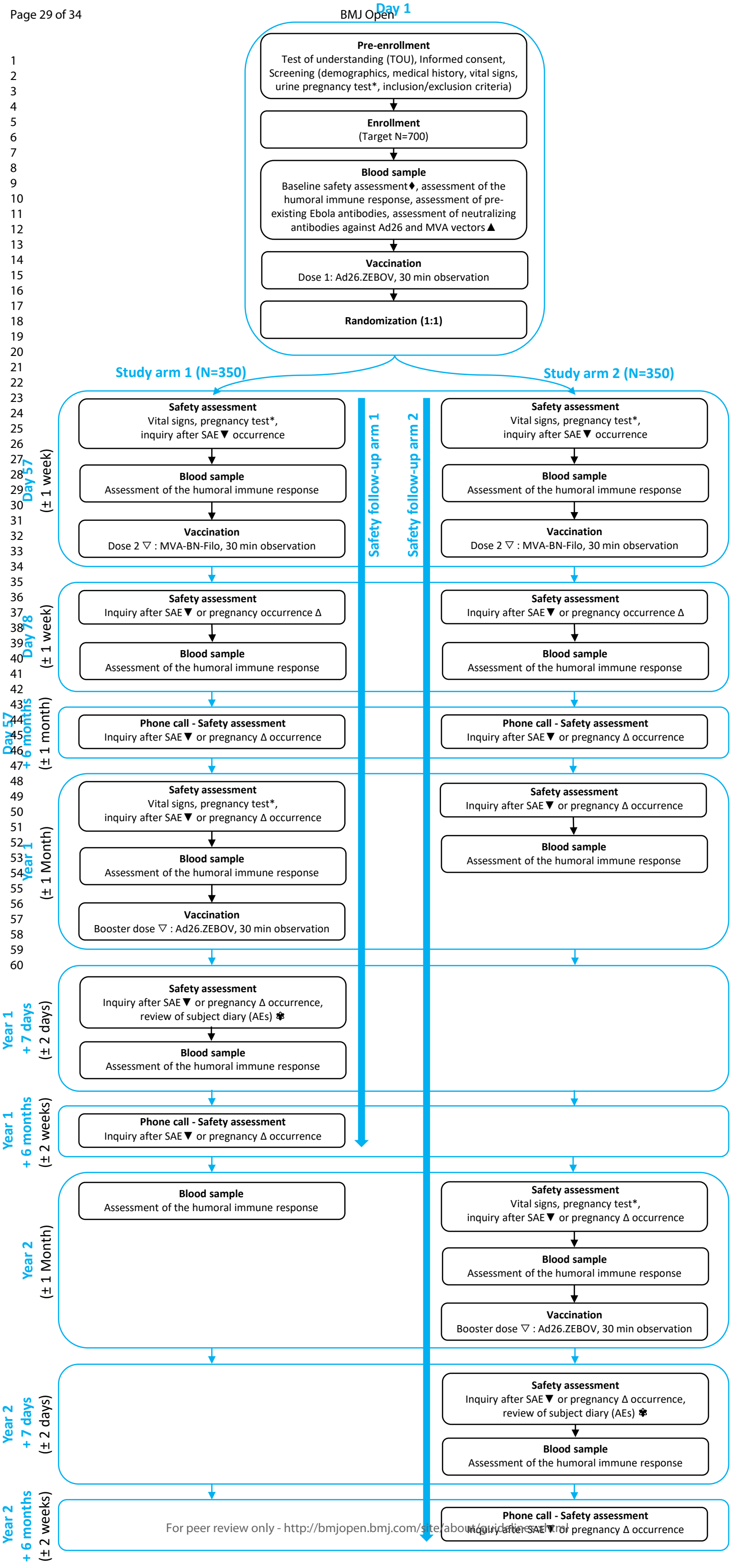
FIGURE TITLES AND LEGENDS

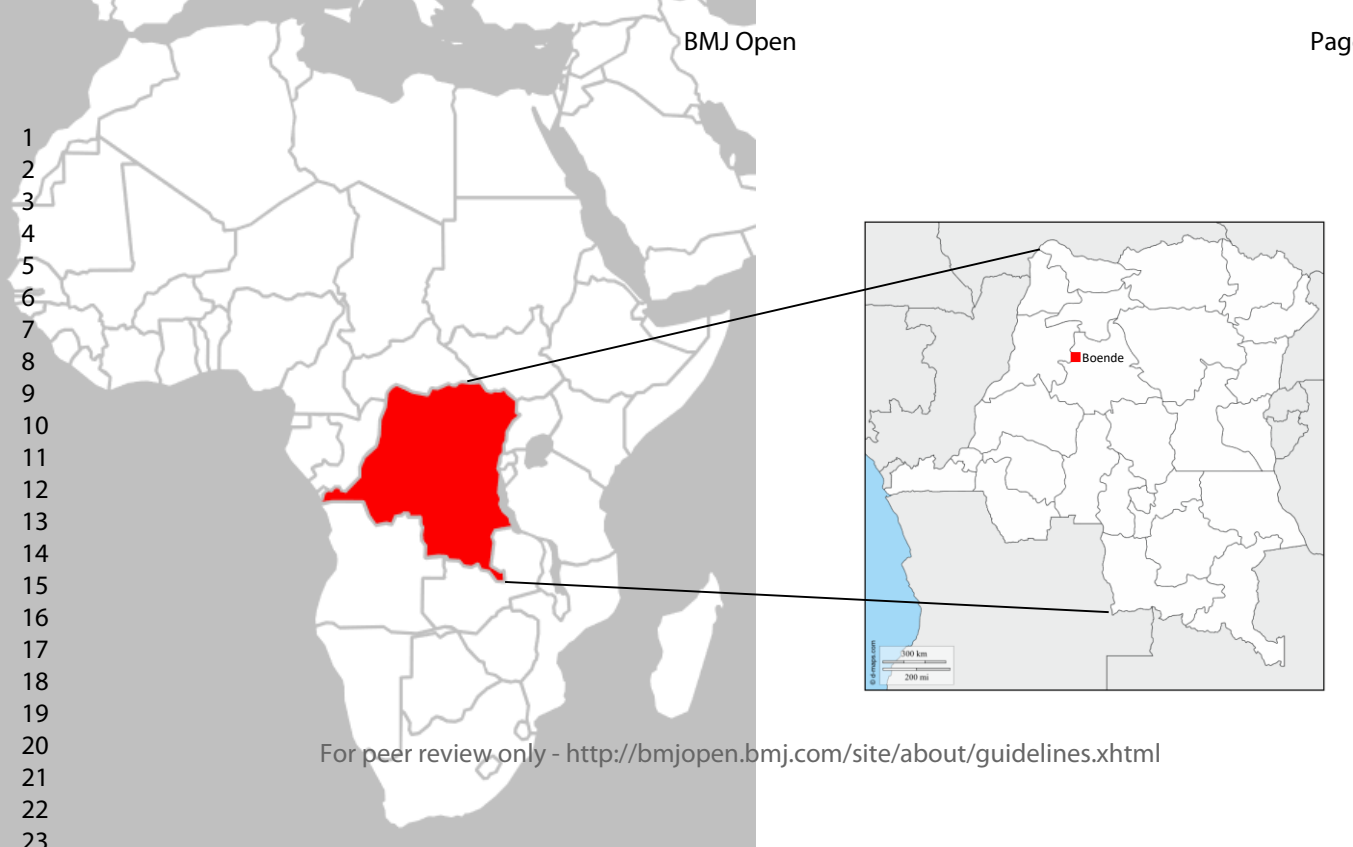
Figure 1. Study time and events overview

SAE: Serious Adverse Event; * Only for female participants of childbearing potential; ♦ Abnormal results will not exclude a participant, as results will not be reviewed prior to enrollment; ▲ Only the first 100 participants enrolled will be tested for neutralizing antibody response against ad26 VNA and MVA vectors. Other blood analyses are for all 700 participants; ▼ Concomitant therapies given in conjunction with a serious adverse event (SAE) should be recorded from signing of the ICF onwards until 6 months post booster; ∇ The Investigator may withhold the second vaccine or booster dose if a participant's clinical status changes prior to vaccination. The participant should continue to be followed for safety and immunogenicity according to the protocol; Δ Only for female participants; ✾ Solicited and unsolicited Adverse Events (AEs) will be collected in a participant diary during 1 week post booster vaccination.

Figure 2. Study site location

On the left, the Democratic Republic of Congo (DRC) is highlighted on a map of the African continent. On the right, the study site location (Boende, Tshuapa province) is marked on a map of DRC indicating its provinces[35].





SPIRIT 2013 Checklist: Recommended items to address in a clinical trial protocol and related documents*

Section/item	ItemNo	Description	Location in manuscript
Administrative information			
Title	1	Descriptive title identifying the study design, population, interventions, and, if applicable, trial acronym	Page 1, line 1-3
Trial registration	2a	Trial identifier and registry name. If not yet registered, name of intended registry	Page 2, abstract>Ethics and dissemination, line 40-41
	2b	All items from the World Health Organization Trial Registration Data Set	N/A
Protocol version	3	Date and version identifier	N/A
Funding	4	Sources and types of financial, material, and other support	Page 23, declarations>funding, line 430-437
Roles and responsibilities	5a	Names, affiliations, and roles of protocol contributors	Page 1, title page: line 4-20; Page 22, declarations>Author contributions, line 423-429
	5b	Name and contact information for the trial sponsor	Name: Page 12, line 165 Contact information: Corresponding author, line 15-20
	5c	Role of study sponsor and funders, if any, in study design; collection, management, analysis, and interpretation of data; writing of the report; and the decision to submit the report for publication, including whether they will have ultimate authority over any of these activities	Page 22, declarations>Author contributions, line 423-429
	5d	Composition, roles, and responsibilities of the coordinating centre, steering committee, endpoint adjudication committee, data management team, and other individuals or groups overseeing the trial, if applicable (see Item 21a for data monitoring committee)	N/A
Introduction			
Background and rationale	6a	Description of research question and justification for undertaking the trial, including summary of relevant studies (published and unpublished) examining benefits and harms for each intervention	Page 3-5, Introduction, paragraph 1-6, line 57-117
	6b	Explanation for choice of comparators	Page 4, Introduction, paragraph 4-5, line 93-104

Section/item	ItemNo	Description	Location in manuscript
Objectives	7	Specific objectives or hypotheses	Page 6, methods>objectives, line 128-131, Table 1
Trial design	8	Description of trial design including type of trial (eg, parallel group, crossover, factorial, single group), allocation ratio, and framework (eg, superiority, equivalence, noninferiority, exploratory)	Page 5-6, methods>study design and setting, line 119-127
Methods: Participants, interventions, and outcomes			
Study setting	9	Description of study settings (eg, community clinic, academic hospital) and list of countries where data will be collected. Reference to where list of study sites can be obtained	Page 5-6, methods>study design and setting, line 119-127; Page 20, Discussion, paragraph 2, line 354-363; Figure titles and legends, Figure 2, line 542-545; see also additional file figure 2
Eligibility criteria	10	Inclusion and exclusion criteria for participants. If applicable, eligibility criteria for study centres and individuals who will perform the interventions (eg, surgeons, psychotherapists)	Page 7, Methods>Participant population, line 159-160, Table 2
Interventions	11a	Interventions for each group with sufficient detail to allow replication, including how and when they will be administered	Page 12-14, Methods>procedures & study intervention, line 166-224; Page 24, figure titles and legends, Figure 1, line 4578-468; see also additional file Figure 1
	11b	Criteria for discontinuing or modifying allocated interventions for a given trial participant (eg, drug dose change in response to harms, participant request, or improving/worsening disease)	Page 15-16, Methods>Study intervention, paragraph 2, line 251-275
	11c	Strategies to improve adherence to intervention protocols, and any procedures for monitoring adherence (eg, drug tablet return, laboratory tests)	Page 16-17, Methods>Patient and public involvement, paragraph 1, line 276-287
	11d	Relevant concomitant care and interventions that are permitted or prohibited during the trial	Page 15-16, Methods>Study intervention, paragraph 2, line 251-275; Page 10, Table 2, inclusion criterium 9
Outcomes	12	Primary, secondary, and other outcomes, including the specific measurement variable (eg, systolic blood pressure), analysis metric (eg, change from baseline, final value, time to event), method of aggregation (eg, median, proportion), and time point for each outcome. Explanation of the clinical relevance of chosen efficacy and harm outcomes is strongly recommended	Page 6, Table 1, line 131, endpoints; Page 18-19, Methods>statistical analysis, paragraph 2-4, line 312-339

Section/item	ItemNo	Description	Location in manuscript
Participant timeline	13	Time schedule of enrolment, interventions (including any run-ins and washouts), assessments, and visits for participants. A schematic diagram is highly recommended (see Figure)	Page 13-15, Methods>Study procedures, paragraph 1-7, line 184-243; Page 27, figure titles and legends, Figure 1, line 530-541; see also additional file Figure 1
Sample size	14	Estimated number of participants needed to achieve study objectives and how it was determined, including clinical and statistical assumptions supporting any sample size calculations	Page 7-8, Methods>participant population, line 132-158; Page 20-21, Discussion, paragraph 3, line 376-400
Recruitment	15	Strategies for achieving adequate participant enrolment to reach target sample size	Page 16-17, Methods>Patient and public involvement, paragraph 1, line 276-287
Methods: Assignment of interventions (for controlled trials)			
Allocation:			
Sequence generation	16a	Method of generating the allocation sequence (eg, computer-generated random numbers), and list of any factors for stratification. To reduce predictability of a random sequence, details of any planned restriction (eg, blocking) should be provided in a separate document that is unavailable to those who enrol participants or assign interventions	Page 12, Methods>Randomization procedure, line 161-183
Allocation concealment mechanism	16b	Mechanism of implementing the allocation sequence (eg, central telephone; sequentially numbered, opaque, sealed envelopes), describing any steps to conceal the sequence until interventions are assigned	Page 12, Methods>Randomization procedure, line 161-183
Implementation	16c	Who will generate the allocation sequence, who will enrol participants, and who will assign participants to interventions	Page 12, Methods>Randomization procedure, line 161-183
Blinding (masking)	17a	Who will be blinded after assignment to interventions (eg, trial participants, care providers, outcome assessors, data analysts), and how	N/A
	17b	If blinded, circumstances under which unblinding is permissible, and procedure for revealing a participant's allocated intervention during the trial	N/A
Methods: Data collection, management, and analysis			
Data collection methods	18a	Plans for assessment and collection of outcome, baseline, and other trial data, including any related processes to promote data quality (eg, duplicate measurements, training of assessors) and a description of study instruments (eg, questionnaires, laboratory tests) along with	Page 13-15, Methods>Study procedures, paragraph 1-7, line 184-243; Page 17, methods>data management, line 295-301

Section/item	ItemNo	Description	Location in manuscript
		their reliability and validity, if known. Reference to where data collection forms can be found, if not in the protocol	
	18b	Plans to promote participant retention and complete follow-up, including list of any outcome data to be collected for participants who discontinue or deviate from intervention protocols	Page 16-17, Methods>Patient and public involvement, paragraph 1, line 276-287
Data management	19	Plans for data entry, coding, security, and storage, including any related processes to promote data quality (eg, double data entry; range checks for data values). Reference to where details of data management procedures can be found, if not in the protocol	Page 17, methods>data management, line 295-301
Statistical methods	20a	Statistical methods for analysing primary and secondary outcomes. Reference to where other details of the statistical analysis plan can be found, if not in the protocol	Page 18-19, Methods>statistical analysis, paragraph 3-4, line 317-339
	20b	Methods for any additional analyses (eg, subgroup and adjusted analyses)	Page 18, Methods>statistical analysis, paragraph 3, line 317-329
	20c	Definition of analysis population relating to protocol non-adherence (eg, as randomised analysis), and any statistical methods to handle missing data (eg, multiple imputation)	Page 17-18, Methods>Statistical analysis, paragraph 1, line 303-311 and paragraph 3, line 317-329
Methods: Monitoring			
Data monitoring	21a	Composition of data monitoring committee (DMC); summary of its role and reporting structure; statement of whether it is independent from the sponsor and competing interests; and reference to where further details about its charter can be found, if not in the protocol. Alternatively, an explanation of why a DMC is not needed	N/A
	21b	Description of any interim analyses and stopping guidelines, including who will have access to these interim results and make the final decision to terminate the trial	Page 19, Methods>statistical analysis, paragraph 5, line 340-344
Harms	22	Plans for collecting, assessing, reporting, and managing solicited and spontaneously reported adverse events and other unintended effects of trial interventions or trial conduct	Page 13-15, Methods>Study procedures, paragraph 1-6, line 184-240;
Auditing	23	Frequency and procedures for auditing trial conduct, if any, and whether the process will be independent from investigators and the sponsor	Page 17, Methods>Data management, line 295-301
Ethics and dissemination			
Research ethics approval	24	Plans for seeking research ethics committee/institutional review board (REC/IRB) approval	Page 22, Ethics and dissemination, paragraph 1, line 409-417

Section/item	ItemNo	Description	Location in manuscript
Protocol amendments	25	Plans for communicating important protocol modifications (eg, changes to eligibility criteria, outcomes, analyses) to relevant parties (eg, investigators, REC/IRBs, trial participants, trial registries, journals, regulators)	N/A
Consent or assent	26a	Who will obtain informed consent or assent from potential trial participants or authorised surrogates, and how (see Item 32)	Page 22, Ethics and dissemination, paragraph 1, line 409-417
	26b	Additional consent provisions for collection and use of participant data and biological specimens in ancillary studies, if applicable	Page 17, Methods>Patient and public involvement, paragraph 2, line 288-290
Confidentiality	27	How personal information about potential and enrolled participants will be collected, shared, and maintained in order to protect confidentiality before, during, and after the trial	Page 17, Methods>Data management, line 295-301
Declaration of interests	28	Financial and other competing interests for principal investigators for the overall trial and each study site	Page 23, Declarations>Competing interests, line 445-446
Access to data	29	Statement of who will have access to the final trial dataset, and disclosure of contractual agreements that limit such access for investigators	Page 22, Ethics and dissemination, paragraph 2, line 418-421
Ancillary and post-trial care	30	Provisions, if any, for ancillary and post-trial care, and for compensation to those who suffer harm from trial participation	N/A
Dissemination policy	31a	Plans for investigators and sponsor to communicate trial results to participants, healthcare professionals, the public, and other relevant groups (eg, via publication, reporting in results databases, or other data sharing arrangements), including any publication restrictions	Page 22, Ethics and dissemination, paragraph 2, line 418-421
	31b	Authorship eligibility guidelines and any intended use of professional writers	Page 22, Declarations>Author contributions, line 423-429
	31c	Plans, if any, for granting public access to the full protocol, participant-level dataset, and statistical code	N/A
Appendices			
Informed consent materials	32	Model consent form and other related documentation given to participants and authorised surrogates	N/A
Biological specimens	33	Plans for collection, laboratory evaluation, and storage of biological specimens for genetic or molecular analysis in the current trial and for future use in ancillary studies, if applicable	N/A