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# 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

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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 Muhindo-Mavoko, Hypolite; University of Kinshasa, Tropical Medicine Department De Bie, Jessie; University of Antwerp, Centre for the Evaluation of Vaccination; University of Antwerp, Global Health Institute Van geertruyden, Jean-Pierre; University of Antwerp, Global Health Institute Vandamme, Pierre; University of Antwerp, Centre for the Evaluation of Vaccination
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3 1 **Open-label, Randomized Controlled, Phase 2 Clinical Trial to Evaluate the Immunogenicity**  
4 **and Safety of a Prophylactic Vaccination of Health Care Providers by Administration of a**  
5 **Heterologous Vaccine Regimen Against Ebola in the Democratic Republic of the Congo: the**  
6 **Study protocol**  
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12 5 Ynke Larivière<sup>1,2\*</sup>, Trésor Zola<sup>3</sup>, Elke Stoppie<sup>1,2</sup>, Vivi Maketa<sup>3</sup>, Junior Matangila<sup>3</sup>, Patrick Mitashi<sup>3</sup>, Jessie  
13  
14 6 De Bie<sup>1,2</sup>, Hypolite Muhindo-Mavoko<sup>3</sup>, Jean-Pierre Van Geertruyden<sup>1</sup>, Pierre Van Damme<sup>2</sup>  
15  
16

17 7 *<sup>1</sup> Centre for the Evaluation of Vaccination, Vaccine and Infectious Disease Institute, University of*  
18  
19 8 *Antwerp, Wilrijk, Belgium*  
20  
21

22 9 *<sup>2</sup> Global Health Institute, Department of Family Medicine and Population Health, University of Antwerp,*  
23  
24 10 *Wilrijk, Belgium.*  
25  
26

27 11 *<sup>3</sup> Tropical Medicine Department, University of Kinshasa, Kinshasa, Democratic Republic of the Congo*  
28  
29

30 12 Corresponding author:

31 13 \*Ynke Larivière

32 14 Centre for the Evaluation of Vaccination, Vaccine and Infectious Disease Institute, University  
33  
34 15 of Antwerp, Campus Drie Eiken, Universiteitsplein 1, Building S2, 2610 Wilrijk, Belgium  
35  
36

37 16 [ynke.lariviere@uantwerpen.be](mailto:ynke.lariviere@uantwerpen.be)  
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## 20 **ABSTRACT**

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

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

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

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

## 43 STRENGTHS AND LIMITATIONS OF THIS STUDY

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

## 52 INTRODUCTION

53 Ebolaviruses (negative stranded RNA viruses) belong to the *Filoviridae* family and cause Ebola virus  
54 disease (EVD), which often leads to severe haemorrhagic fever in humans and nonhuman primates[1].  
55 Contact with infected wild animals (such as fruit bats, gorillas, apes, monkeys, etc.) is often reported  
56 as the source of animal-to-human transmission[2-4] and once among humans, these public health  
57 pathogens spread via direct (body fluids) or indirect (contaminated surfaces) human-to-human  
58 contact[2, 3]. While they do not spread via air or water[3], *Ebolaviruses* bring along a severe public  
59 health burden with case fatality rates that can range up to 90%[5]. Since the discovery of the Ebola  
60 viruses in 1976[6], more than 20 outbreaks (most are endemic to regions in equatorial and western  
61 Africa) have taken place[7]. To date, the Democratic Republic of the Congo (DRC) has been the most  
62 affected country and is currently battling its 11th outbreak[8]. However, it is only recently that the  
63 search for a safe and effective vaccine against Ebola was accelerated when the epidemic potential of  
64 *Ebolaviruses*, and more specifically the species *Zaire Ebolavirus* (virus name: Ebola virus; abbreviation:  
65 EBOV[9]), became clear through the West African Ebola epidemic (2013-2016; 28,616 cases with  
66 11,310 deaths[10]).

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

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3 69 vaccine regimen comprised of 2 candidate Ebola vaccines (Ad26.ZEBOV and MVA-BN-Filo) and aiming  
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5 70 to bring this prophylactic Ebola vaccine to licensure[11]. Ad26.ZEBOV is a monovalent vaccine  
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7 71 developed to provide active EBOV-specific immunity. MVA-BN-Filo, which is administered 56 days after  
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10 72 the Ad26.ZEBOV vaccine, is a multivalent vaccine developed to establish active immunity to EBOV,  
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12 73 *Sudan Ebolavirus*, *Tai Forest Ebolavirus* and the Marburg virus (also part of the *Filoviridae* family). In  
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14 74 July 2020, the 2-dose prophylactic vaccine regimen was granted market authorisation[12].

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17 75 Several projects (PREVAC, EBOVAC1, EBOVAC2 and EBOVAC3) within the IMI2 Joint Undertaking, of  
18  
19 76 which the first in-human clinical trials started in 2014, were at the basis of this successful  
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21 77 authorisation[11]. Within these projects, multiple clinical trials assessed or are assessing the timing,  
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23 78 tolerability, safety, and immunogenicity of different Ad26.ZEBOV and MVA-BN-Filo vaccine regimen in  
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25 79 healthy adults ( $\geq 18$  years old) and children/adolescents (1-17 years old) via phase 1, 2, 2B and 3  
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27 80 studies. Initial trials showed that healthy adult participants had higher geometric mean concentrations  
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29 81 of binding antibodies for the regimen where Ad26.ZEBOV vaccination was followed by an MVA-BN-Filo  
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31 82 vaccination 56 days later. Moreover, 100% of them had detectable Ebola glycoprotein-specific  
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33 83 Immunoglobulin G (IgG) antibodies up to at least 240 days after vaccination[13-15]. Even though some  
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35 84 local (erythema, swelling and pain at injection site) and systemic (headache, nausea, pyrexia, myalgia  
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37 85 and fatigue) solicited adverse events were recorded, the vaccine regimen was generally well tolerated  
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39 86 across studies[13-17].

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42 87 While it is of utmost importance that the 2-dose prophylactic vaccine regimen is safe and leads to an  
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44 88 immune response, it is also crucial to find out whether or not this regimen can lead to induced immune  
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46 89 memory at the time of imminent risk (i.e. an outbreak) through a booster vaccination. To evaluate this  
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48 90 induced immune memory, three previous studies within EBOVAC projects have administered a booster  
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50 91 vaccine with Ad26.ZEBOV at either 1 year (NCT02325050; NCT02564523) or 2 years (NCT02509494)  
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52 92 post Dose 1. However, it still has to be determined whether the induced immune memory response  
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54 93 differs if a booster vaccination is given 1 or 2 years after Dose 1.  
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3 94 Healthcare settings play an important role in the control of EVD and therefore health care providers  
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5 95 (HCP) and front-liners, due to occupational exposure, are not only more at risk of disease acquisition  
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7 96 but also facilitate the spread of the virus[18-21]. Knowing that outbreaks of EVD often occur in regions  
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10 97 where there is already a shortage of HCP and front-liners, this further depletes a weak health care  
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12 98 system and the quality of care. Consequently, the Strategic Advisory Group of Experts stated in 2018  
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14 99 that for preventive strategies, vaccination of HCP as part of an emergency preparedness plan has  
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16 100 significant potential of reducing the scale and duration of outbreaks[22].  
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19 101 This phase 2 clinical trial compares two booster arms with an Ad26.ZEBOV vaccine administered either  
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21 102 1 or 2 years post first dose of a heterologous vaccine regimen with Ad26.ZEBOV followed by MVA-BN-  
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23 103 Filo 56 days later. The trial is conducted in a cohort of HCP and front-liners in DRC, a well-known  
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26 104 population at risk from clinical and epidemiological perspective.  
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## 29 105 **METHODS**

### 30 106 **Study design and setting**

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34 107 This study is an open-label, monocentric, phase 2 randomized controlled trial to evaluate the  
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36 108 immunogenicity and safety of Ad26.ZEBOV ( $5 \times 10^{10}$  viral particles) as first dose and MVA-BN-Filo ( $1 \times 10^8$   
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38 109 infectious units) as second dose vaccination at a 56-day interval in HCP and front-liners who may be  
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40 110 exposed to Ebola in the event of a future Ebola outbreak in DRC. Additionally, after randomization (1:1)  
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42 111 a booster of Ad26.ZEBOV ( $5 \times 10^{10}$  viral particles) will be offered at respectively 1 year or 2 years after  
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44 112 the first dose (Figure 1). As this study is designed to provide descriptive information regarding  
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46 113 immunogenicity and safety, an open-label design was preferred.  
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51 114 The study site is located in Boende, Tshuapa province, DRC (Figure 2), at approximately 750km north-  
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53 115 west of Kinshasa. Study participants are enrolled at the General Reference Hospital in Boende.  
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116 **Objectives**

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

119 **Table 1. Objectives and endpoints**

Objectives	Endpoints
<b>Primary</b>	
<ul style="list-style-type: none"> <li>To assess binding antibody responses post-dose 2 vaccination with MVA-BN-Filo.</li> </ul>	<ul style="list-style-type: none"> <li>Binding antibody levels against the EBOV GP using FANG ELISA at 21 days post-dose 2 (Day 78) vaccination with MVA-BN-Filo.</li> </ul>
<b>Secondary</b>	
<ul style="list-style-type: none"> <li>To assess binding antibody responses after booster vaccination with Ad26.ZEBOV given at 1 or 2 years after first dose.</li> </ul>	<ul style="list-style-type: none"> <li>Binding antibody levels against the EBOV GP using FANG ELISA at 7 days (excluding the day of vaccination) post booster.</li> </ul>
<ul style="list-style-type: none"> <li>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.</li> </ul>	<ul style="list-style-type: none"> <li>Serious adverse events from first dose vaccination until 6 months post booster.</li> <li>Solicited and unsolicited local and systemic adverse events until 7 days post booster vaccination (day of vaccination and subsequent 7 days) with Ad26.ZEBOV.</li> </ul>
<b>Exploratory</b>	
<ul style="list-style-type: none"> <li>To assess binding antibody responses at different time points as indicated in the Study time and events overview (Figure 1).</li> </ul>	<ul style="list-style-type: none"> <li>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).</li> </ul>
<ul style="list-style-type: none"> <li>To assess neutralizing antibody response directed against the Adenovirus vector prior to vaccination.</li> </ul>	<ul style="list-style-type: none"> <li>Neutralizing antibody levels against Ad26 using Ad26 VNA at the first visit.</li> </ul>
<ul style="list-style-type: none"> <li>To assess neutralizing antibody response directed against the MVA vector prior to vaccination.</li> </ul>	<ul style="list-style-type: none"> <li>Neutralizing antibody levels against MVA-BN-Filo using MVA PRNT assay at the first visit.</li> </ul>
<ul style="list-style-type: none"> <li>To assess seroprevalence of Ebola virus disease prior to vaccination.</li> </ul>	<ul style="list-style-type: none"> <li>Presence of pre-existing Human anti-EBOV GP IgG and anti-EBOV NP IgG using LUMINEX assay.</li> </ul>

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

120 **Participant population**

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

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

126 **Table 2. Inclusion and exclusion criteria**

<b>Inclusion criteria</b>
Each potential participant must satisfy all of the following criteria to be enrolled in the study:
<ol style="list-style-type: none"> <li>1. The participant must pass the Test of Understanding.  <i>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.</i></li> <li>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.</li> <li>3. The participant must be a man or women aged 18 years or older.</li> <li>4. The participant must be a documented HCP in DRC.</li> <li>5. The participant must be healthy in the investigator's clinical judgement and on the basis of vital signs assessed at day 1 screening.</li> </ol>

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*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  $\beta$ -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  $\geq 38.0^{\circ}\text{C}$  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).

### 127 **Study procedures (Figure 1)**

128 At Day 1, interested participants are informed about the study and are required to pass a test of  
129 understanding before providing written consent. No study activities are performed before the  
130 participant has signed the informed consent form. Afterwards, the study medical doctor evaluates  
131 his/her general health based on the inclusion criteria, vital signs (blood pressure, pulse/heart rate  
132 [both at rest] and body temperature) are collected and a urine pregnancy test for women of  
133 childbearing potential is performed. Further during this first visit, a blood sample is taken for baseline  
134 testing of binding antibody level (i.e. humoral immune response) against EBOV glycoprotein (GP) using  
135 Filovirus Animal Non-Clinical Group (FANG) Ebola virus enzyme-linked immunosorbent assay (ELISA)  
136 and the presence of pre-existing Human anti-EBOV GP IgG and anti-EBOV nucleoprotein (NP) IgG using  
137 LUMINEX assay. For the first 100 enrolled participants an additional test on the collected serum is  
138 performed to measure the neutralizing antibody level against Ad26 and MVA vectors using respectively  
139 Ad26 Virus Neutralizing Assay (VNA) and MVA Plaque Reduction Immunogenicity Test (PRNT).  
140 Subsequently, a blood sample for baseline safety assessment is collected to test haemoglobin,  
141 haematocrit, blood cell count (white and red), platelet count, urea, creatinine and transaminases.  
142 Then, participants are vaccinated with the first dose (Ad26.ZEBOV) and they are given instructions to  
143 contact the study team for any occurring serious adverse events (SAEs), or in case of pregnancy of a  
144 participant during the study. After vaccination, participants remain at the study site for an observation  
145 period of 30 minutes to make sure no SAEs occur. SAEs are collected from first dose vaccination until  
146 6 months post booster (PB). Lastly on Day 1, randomization is performed (1:1) using sealed envelopes  
147 (developed based on the created study randomization list) to determine the timing of the booster  
148 vaccine at either 1 year or 2 years after the first dose. Contact information is verified, an appointment  
149 for the second dose on Day 57 is arranged and a participant card is printed. Innovatively, next to a  
150 participant card, a biometric identification tool via iris scanning is foreseen to ensure correct  
151 identification of the participants during all study related visits.

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3 152 At Day 57, participants return to the study site for urine pregnancy testing (for women of childbearing  
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5 153 potential), vital signs measurement, assessment of safety (SAEs), a blood sample for immunogenicity  
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7 154 assessment (the binding antibody levels against EBOV GP using FANG ELISA) and afterwards  
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10 155 administration of the second vaccine (MVA-BN-Filo). After an observation period of 30 minutes,  
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12 156 participants are reminded to contact the study team for any SAE that occurs, or in case of pregnancy  
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14 157 of a participant during the study. Contact information is verified and an appointment for the 21-day  
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16 158 post-dose 2 visit (Day 78) is arranged.

19 159 At 21 days post-dose 2 (Day 78), all participants return to the study site for a safety assessment (SAEs)  
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21 160 and for the collection of a blood sample for immunogenicity assessment. Contact information is re-  
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23 161 verified and they are reminded to contact the study team in case of SAE occurrence, or in case of  
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25 162 pregnancy of a participant.

29 163 To make sure no valuable information is missed, participants are contacted by phone to inquire about  
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31 164 any occurrence of pregnancies (female participants) and SAEs at approximately 6 months post-dose 2  
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33 165 vaccination. At 1 year after the first vaccine dose, when all participants return to the site, the clinical  
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35 166 trial staff inquires after the occurrence of SAEs and a blood sample is collected for immunogenicity  
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37 167 assessment of all participants (where applicable pre-administration of the booster dose).

41 168 At 1 year or 2 years post first dose, depending on the study arm, a booster vaccination with  
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43 169 Ad26.ZEBOV is given. After vaccination, participants remain at the study site for a 30 minute  
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45 170 observation period. Participants are asked to collect solicited and unsolicited adverse events (AEs) in a  
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47 171 participant diary starting on the day of the vaccination and continuing for the subsequent 7 days. At  
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49 172 Day 8 PB the safety data including solicited and unsolicited AEs is reviewed and a blood sample for  
50  
51 173 immunogenicity assessment is taken to document the immune response. At 6 months PB, all  
52  
53 174 participants are contacted by phone and questioned about any SAEs or pregnancies (female  
54  
55 175 participants) that have occurred since the last vaccination. For all participants at 2 years after first  
56  
57 176 dose, a sample is collected for immunogenicity assessment (where applicable pre-administration of  
58  
59  
60

1  
2  
3 177 the booster dose) and a safety assessment (SAEs) is performed for those returning for their booster  
4  
5 178 vaccination.

6  
7  
8 179 The total duration of the study is 2 years and 6 months post-first dose. The study is considered  
9  
10 180 completed when the last participant has been contacted for the 6 months PB phone call or has left the  
11  
12 181 study.

## 16 182 **Study intervention**

17  
18 183 According to the predefined schedule (Figure 1), participants receive a 0.5 mL intramuscular injection  
19  
20 184 into the deltoid muscle of the upper arm with Ad26.ZEBOV or MVA-BN-Filo. The injection site should  
21  
22 185 be free from any injury, local skin conditions, or other issues that might interfere with the evaluation  
23  
24 186 of local reactions. Every vaccination is given in the opposite arm of the previous vaccination (unless  
25  
26 187 the opposite arm has a condition that prevents evaluating the arm after injection). No local or topical  
27  
28 188 anaesthetic is used prior to the injection.

29  
30  
31  
32  
33 189 The second or booster vaccination is not administered if any of the following events occur at any time  
34  
35 190 after the first dose vaccination:

- 36  
37  
38 191 • A participant experiences anaphylaxis clearly attributable to vaccination with the study  
39  
40 192 vaccine; OR
  - 41  
42 193 • A participant experiences generalized urticaria within 72 hours of vaccination considered  
43  
44 194 to be related to study vaccine; OR
  - 45  
46 195 • A participant experiences a serious adverse event considered to be related to the study  
47  
48 196 vaccine; OR
  - 49  
50 197 • A participant experiences injection site ulceration, abscess or necrosis considered to be  
51  
52 198 related to the study vaccine; OR
  - 53  
54 199 • A participant has confirmed EVD; OR
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- 1  
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3 200 • A female participant of childbearing potential has a positive urine  $\beta$ -human chorionic  
4  
5 201 gonadotropin pregnancy test before vaccination (on Day 57, Year 1 or Year 2 [depending  
6  
7 202 on the randomization group]); OR  
8  
9  
10 203 • A female participant of childbearing potential has a positive urine  $\beta$ -human chorionic  
11  
12 204 gonadotropin pregnancy test between Dose 2 and the booster dose and is still pregnant  
13  
14 205 or breastfeeding at the time of the booster dose; OR  
15  
16 206 • A participant takes a concomitant treatment with drugs that may alter the immune  
17  
18 207 response; OR  
19  
20  
21 208 • The principal investigator believes that for safety reasons it is in the best interest of a  
22  
23 209 participant to discontinue the study intervention.

24  
25  
26 210 Participants experiencing any of the events described above are still followed up for safety and  
27  
28 211 immunogenicity according to the protocol. The decision to discontinue the study intervention is at the  
29  
30 212 discretion of the principal investigator (University of Kinshasa) and after consultation with the sponsor  
31  
32 213 (University of Antwerp) for any of the events described above.

### 33 34 35 36 214 **Patient and public involvement**

37  
38 215 Difficulties were expected when setting up a clinical trial in Boende, a remote and resource-limited  
39  
40 216 area of DRC. However, to avoid and anticipate some of these challenges and in order to support  
41  
42 217 vaccination compliance, a collaboration is established between the study team and the Provincial  
43  
44 218 Division of Health. Throughout the trial, workshops are organized for HCP in the health district of  
45  
46 219 Boende to sensitize and inform on EVD and other relevant medical topics. These gatherings do not only  
47  
48 220 facilitate enrollment in the trial but also increase the engagement of participants by enhancing their  
49  
50 221 understanding on the clinical trial and the importance of adherence. During these workshops time is  
51  
52 222 available for questions and discussions. In addition to these gatherings for trial participants,  
53  
54 223 community engagement activities and the training and capacity building of the local clinical trial team  
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3 224 that is executing the trial (under supervision of UNIKIN as Principal Investigator (PI)) are organised for  
4  
5 225 the duration of the trial.  
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7

8 226 Each participant receives an individual visit schedule upon enrollment in the trial and when  
9  
10 227 participants miss a planned study visit, community health workers of the Ministry of Health trace the  
11  
12 228 individual subject. Consent is asked in the informed consent form for this mode of contact.  
13  
14

15 229 Furthermore, prior to the start of the clinical trial, a pilot study was performed whereby potential  
16  
17 230 participants were interviewed on the feasibility and acceptability of the use of a biometric iris scanning  
18  
19 231 tool for participant identification during the trial and the use of telephone messaging with visit  
20  
21 232 reminders for participant adherence.  
22  
23

### 24 233 **Data management**

25  
26  
27 234 All information is collected during study visits on source documents by study staff. These source  
28  
29 235 documents with confidential information are transcribed into the clinical database by site data  
30  
31 236 managers. To make sure that all entered data (collected in DFExplore version 5.2.1) is correct, the  
32  
33 237 principal investigator reviews each source document and confirms its correct transcription in the  
34  
35 238 database. Additionally, the sponsor performs quality checks of the entered data in the database and  
36  
37 239 during monitoring visits source data verification is performed.  
38  
39  
40

### 41 240 **Statistical analysis**

42  
43  
44 241 A differentiation in analysis is made according to: 1) the *Full Analysis Set* (FAS; all participants who  
45  
46 242 received at least one dose, regardless of the occurrence of protocol deviations), 2) *Per Protocol Set for*  
47  
48 243 *primary vaccination series* (all vaccinated subjects, who received both dose 1 and dose 2 [administered  
49  
50 244 within the protocol-defined visit window] vaccinations, have at least 1 post-vaccination [i.e. after the  
51  
52 245 date of dose 1] evaluable immunogenicity sample, and have no major protocol deviations influencing  
53  
54 246 the immune response) and 3) *Per Protocol Set for the Booster/Dose 3 vaccination* (includes all subjects  
55  
56 247 in the per protocol set for the primary vaccination series who received Dose 3 and have at least 1 post-  
57  
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3 248 dose 3 [i.e. after the date of booster vaccination] evaluable immunogenicity sample, and have no major  
4  
5 249 protocol deviations influencing the immune response).

6  
7  
8 250 Subject information (i.e. demographics and baseline characteristics, disposition information,  
9  
10 251 treatment compliance, extent of exposure, protocol deviations and concomitant medications) is  
11  
12 252 planned to be tabulated and summarized with descriptive statistics for all subjects. For continuous  
13  
14 253 data such as age, the mean and standard deviation will be provided if applicable, otherwise geometric  
15  
16 254 mean, related standard deviations or median and interquartile range will be used.

17  
18  
19  
20 255 For the immunogenicity analysis, two *Per-Protocol Sets* will be used, i.e., the *Per-Protocol Set for*  
21  
22 256 *primary vaccination series* and the *Per-Protocol Set for the booster*. If more than 10% of participants  
23  
24 257 from the FAS are excluded from the per protocol immunogenicity set, the immunogenicity analysis will  
25  
26 258 be repeated on the FAS to evaluate the robustness of the analysis results. A subgroup analysis of the  
27  
28 259 immune response at different time points will be performed stratified by age (18-40, 40-60 and >60),  
29  
30 260 gender, prior vaccinia/smallpox vaccination, pre-existing Ebola antibodies (positive or negative for pre-  
31  
32 261 existing Human anti-EBOV GP IgG and anti-EBOV NP IgG, and for both), baseline immunogenicity  
33  
34 262 (positivity versus negativity for antibody levels against EBOV GP using FANG ELISA) and the presence  
35  
36 263 of neutralizing antibody levels against Ad26 and MVA vectors using Ad26 VNA and MVA PRNT assay  
37  
38 264 (only the first 100 enrolled participants). For these planned subgroup analyses, N (%), Geometric Mean  
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40 265 Concentrations and 95% confidence intervals will be provided as appropriate.

41  
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44  
45 266 Safety analyses include SAEs collected during the whole study and solicited and unsolicited AEs for 1  
46  
47 267 week post booster vaccination. The analysis of SAEs will be performed using the FAS and the solicited  
48  
49 268 and unsolicited AEs will be analysed for the participants who received the booster vaccination.  
50  
51 269 Continuous variables will be summarized using the following statistics: number of observations;  
52  
53 270 arithmetic or geometric mean/median (if applicable) with their related measures of dispersion (95%  
54  
55 271 CI for the mean, standard deviation or inter quartile range (Q1-Q3)). Minimum and maximum  
56  
57 272 frequencies and percentages (one decimal place) will be generated for categorical variables.  
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59  
60

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3 273 The primary interim analysis is planned to be performed when all participants have completed the  
4  
5 274 21-day post dose 2 visit (Day 78) or discontinued earlier. This analysis includes all available  
6  
7 275 immunogenicity and safety data up to this point (date cut-off). Additional interim analyses may be  
8  
9  
10 276 performed during the study for the purpose of informing future vaccine-related decisions in a timely  
11  
12 277 manner.

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14  
15 278 The final analysis will be performed when all participants have completed the last study-related phone  
16  
17 279 call (6 months PB) or left the study.

## 20 280 **DISCUSSION**

21  
22 281 The aim of this phase 2 trial is assess the safety and immunogenicity of a booster dose with  
23  
24 282 Ad26.ZEBOV administered either 1 or 2 years post first dose of a prophylactic heterologous Ebola  
25  
26 283 vaccine regimen. By doing so, this study will boost the immunogenicity and safety databases of the  
27  
28 284 Ad26.ZEBOV and MVA-BN-Filo vaccines.

29  
30  
31  
32 285 Boende (the capital city of Tshuapa province) was selected as the trial site for several reasons. First,  
33  
34 286 the Tshuapa province recovered from an EBOV outbreak that occurred in the Boende Health District  
35  
36 287 in 2014[18]. Following this outbreak, a study (n=565) conducted in the Tshuapa region in 2015 found  
37  
38 288 that 41.4% of the tested HCP were seroreactive to at least one EBOV protein and 2.8% of the HCP  
39  
40 289 showed a neutralizing capacity while never having developed EVD symptoms[21]. This observation  
41  
42 290 suggests a possible endemic EBOV exposure in the Tshuapa province of DRC. These are interesting  
43  
44 291 observations for future ecologic research as the ecology and reservoir(s) of EBOV and other filoviruses  
45  
46 292 remain largely unknown[23, 24]. Second, in addition to the previous outbreak of EVD, Boende was  
47  
48 293 chosen to perform the current clinical trial as there was expertise available after carrying out a phase  
49  
50 294 3 monkey pox vaccine trial that took place in 2017[25].

51  
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54  
55 295 In conclusion, because EVD remains a very deadly disease, effective and safe preventive measures will  
56  
57 296 play a crucial role to protect vulnerable communities. While the prophylactic heterologous 2-dose  
58  
59 297 regimen was recently granted market authorisation by the European Commission, further research  
60

1  
2  
3 298 into the safety and immunogenicity of the booster dose with Ad26.ZEBOV is still required. This is the  
4  
5 299 first randomized vaccine trial that looks into the safety and immunogenicity of two different booster  
6  
7 300 arms in a large cohort.  
8  
9

## 10 301 **ETHICS AND DISSEMINATION**

11  
12  
13 302 This protocol was submitted and approved by the National Ethics Committee of the Ministry of Health  
14  
15 303 of the Democratic Republic of Congo (approval number: n°121/CNES/BN/PMMF/2019). Prior to being  
16  
17 304 enrolled in the trial, all participants are required to provide written informed consent by signing the  
18  
19 305 informed consent form after having performed a test of understanding. If the participant is unable to  
20  
21 306 read or write, an impartial witness should be present for the entire informed consent process (which  
22  
23 307 includes reading and explaining all written information) and should personally date and sign the  
24  
25 308 informed consent form after the oral consent of the participant is obtained. No study-related  
26  
27 309 procedures are performed until the participant has signed the informed consent form.  
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31  
32 310 The trial was registered on Clinicaltrial.gov on December 4<sup>th</sup>, 2019 (NCT04186000) and recruitment  
33  
34 311 started on December 18<sup>th</sup>, 2019. All participants were recruited by the 8<sup>th</sup> of February 2020 and the  
35  
36 312 study is planned to finish in July, 2022. Results of the trial will be entered on Clinicaltrial.gov, published  
37  
38 313 in peer-reviewed journals and presented at international conferences.  
39  
40

## 41 314 **DECLARATIONS**

### 42 315 **Author contributions**

43  
44  
45  
46 316 YL wrote the manuscript. TZ, ES, VM, JM, PM, HMM, JPG and PVD wrote the initial English protocol  
47  
48 317 on which this manuscript is based. TZ, VM, PM, JM and HMM translated it into French for submission  
49  
50 318 to the National Ethics Committee and the “Direction de la Pharmacie et des Médicaments” of the  
51  
52 319 Ministry of Health of the Democratic Republic of Congo as well as the National Scientific committee  
53  
54 320 against Ebola. All authors reviewed and contributed to the final manuscript.  
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330 Prevention B.V..

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336 and, the political-administrative authorities of the Tshuapa province for a trustful collaboration. We  
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### 338 **Competing interests**

339 The authors declare that they have no competing interests.

### 340 **REFERENCES**

- 341 1. Baseler L, Chertow DS, Johnson KM, et al. The Pathogenesis of Ebola Virus Disease. *Annu Rev Pathol*  
342 2017;12:387-418. doi: 10.1146/annurev-pathol-052016-100506 [published Online First:  
343 2016/12/14]

- 1  
2  
3 344 2. Muyembe-Tamfum JJ, Mulangu S, Masumu J, et al. Ebola virus outbreaks in Africa: past and present.  
4  
5 345 *Onderstepoort J Vet Res* 2012;79(2):451. doi: 10.4102/ojvr.v79i2.451 [published Online First:  
6  
7 346 2013/01/19]  
8  
9  
10 347 3. Rewar S, Mirdha D. Transmission of ebola virus disease: an overview. *Ann Glob Health*  
11  
12 348 2014;80(6):444-51. doi: 10.1016/j.aogh.2015.02.005 [published Online First: 2015/05/12]  
13  
14 349 4. Rouquet P, Froment JM, Bermejo M, et al. Wild animal mortality monitoring and human Ebola  
15  
16 350 outbreaks, Gabon and Republic of Congo, 2001-2003. *Emerg Infect Dis* 2005;11(2):283-90. doi:  
17  
18 351 10.3201/eid1102.040533 [published Online First: 2005/03/09]  
19  
20  
21 352 5. World Health Organization. Ebola virus disease. Fact sheet N 103. Updated September 2014.  
22  
23 353 6. Report of an International Commission. Ebola haemorrhagic fever in Zaire, 1976. *Bull World Health*  
24  
25 354 *Organ* 1978;56(2):271-93. [published Online First: 1978/01/01]  
26  
27  
28 355 7. Malvy D, McElroy AK, de Clerck H, et al. Ebola virus disease. *The Lancet* 2019;393(10174):936-48.  
29  
30 356 8. World Health Organization. New Ebola outbreak detected in northwest Democratic Republic of the  
31  
32 357 Congo; WHO surge team supporting the response 2020 [Available from:  
33  
34 358 [https://www.who.int/news-room/detail/01-06-2020-new-ebola-outbreak-detected-in-](https://www.who.int/news-room/detail/01-06-2020-new-ebola-outbreak-detected-in-northwest-democratic-republic-of-the-congo-who-surge-team-supporting-the-response)  
35  
36 359 [northwest-democratic-republic-of-the-congo-who-surge-team-supporting-the-response](https://www.who.int/news-room/detail/01-06-2020-new-ebola-outbreak-detected-in-northwest-democratic-republic-of-the-congo-who-surge-team-supporting-the-response).  
37  
38  
39 360 9. International Committee on Taxonomy. Virus Metadata Repository: version May 1, 2020; MSL35.  
40  
41 361 10. World Health Organization. Situation Report Ebola Virus Disease. [http://apps.who.int/ebola/ebola-](http://apps.who.int/ebola/ebola-situation-reports)  
42  
43 362 [situation-reports](http://apps.who.int/ebola/ebola-situation-reports), 10 June 2016.  
44  
45  
46 363 11. Ebovac. EBOVAC3 2020 [Available from: <https://www.ebovac.org/ebovac-3/>.  
47  
48 364 12. European Commission. Vaccine against Ebola: Commission grants new market authorisation July  
49  
50 365 2020 [Available from: [https://ec.europa.eu/commission/presscorner/detail/en/ip\\_20\\_1248](https://ec.europa.eu/commission/presscorner/detail/en/ip_20_1248).  
51  
52 366 13. Milligan ID, Gibani MM, Sewell R, et al. Safety and Immunogenicity of Novel Adenovirus Type 26-  
53  
54 367 and Modified Vaccinia Ankara-Vectored Ebola Vaccines: A Randomized Clinical Trial. *Jama*  
55  
56 368 2016;315(15):1610-23. doi: 10.1001/jama.2016.4218 [published Online First: 2016/04/20]  
57  
58  
59  
60

- 1  
2  
3 369 14. Mutua G, Anzala O, Luhn K, et al. Safety and Immunogenicity of a 2-Dose Heterologous Vaccine  
4  
5 370 Regimen With Ad26.ZEBOV and MVA-BN-Filo Ebola Vaccines: 12-Month Data From a Phase 1  
6  
7 371 Randomized Clinical Trial in Nairobi, Kenya. *J Infect Dis* 2019;220(1):57-67. doi:  
8  
9 372 10.1093/infdis/jiz071 [published Online First: 2019/02/24]  
10  
11  
12 373 15. Anywaine Z, Whitworth H, Kaleebu P, et al. Safety and Immunogenicity of a 2-Dose Heterologous  
13  
14 374 Vaccination Regimen With Ad26.ZEBOV and MVA-BN-Filo Ebola Vaccines: 12-Month Data  
15  
16 375 From a Phase 1 Randomized Clinical Trial in Uganda and Tanzania. *J Infect Dis* 2019;220(1):46-  
17  
18 376 56. doi: 10.1093/infdis/jiz070 [published Online First: 2019/02/24]  
19  
20  
21 377 16. Safety and immunogenicity of a two-dose heterologous Ad26.ZEBOV and MVA-BN®-Filo Ebola  
22  
23 378 vaccine regimen: a phase 2 randomised clinical study in Europe (EBOVAC2). 29th ECCMID;  
24  
25 379 2019; Amsterdam, Netherlands.  
26  
27  
28 380 17. Shukarev G, Callendret B, Luhn K, et al. A two-dose heterologous prime-boost vaccine regimen  
29  
30 381 eliciting sustained immune responses to Ebola Zaire could support a preventive strategy for  
31  
32 382 future outbreaks. *Hum Vaccin Immunother* 2017;13(2):266-70. doi:  
33  
34 383 10.1080/21645515.2017.1264755 [published Online First: 2016/12/08]  
35  
36  
37 384 18. Maganga GD, Kapetshi J, Berthet N, et al. Ebola virus disease in the Democratic Republic of Congo.  
38  
39 385 *N Engl J Med* 2014;371(22):2083-91. doi: 10.1056/NEJMoa1411099 [published Online First:  
40  
41 386 2014/10/16]  
42  
43  
44 387 19. Nanclares C, Kapetshi J, Lionetto F, et al. Ebola Virus Disease, Democratic Republic of the Congo,  
45  
46 388 2014. *Emerg Infect Dis* 2016;22(9):1579-86. doi: 10.3201/eid2209.160354 [published Online  
47  
48 389 First: 2016/08/18]  
49  
50  
51 390 20. Evans DK, Goldstein M, Popova A. Health-care worker mortality and the legacy of the Ebola  
52  
53 391 epidemic. *Lancet Glob Health* 2015;3(8):e439-e40. doi: 10.1016/s2214-109x(15)00065-0  
54  
55 392 [published Online First: 2015/07/15]  
56  
57  
58  
59  
60



- 1  
2  
3 393 21. Hoff NA, Mukadi P, Doshi RH, et al. Serologic Markers for Ebolavirus Among Healthcare Workers in  
4  
5 394 the Democratic Republic of the Congo. *J Infect Dis* 2019;219(4):517-25. doi:  
6  
7 395 10.1093/infdis/jiy499 [published Online First: 2018/09/22]  
8  
9  
10 396 22. World Health Organization. Meeting of the Strategic Advisory Group of Experts on Immunization,  
11  
12 397 October 2018—Conclusions and recommendations. *Weekly Epidemiological Record*  
13  
14 398 2018;93(49):661-79.  
15  
16 399 23. Gryseels S, Mbala-Kingebeni P, Akonda I, et al. Role of Wildlife in Emergence of Ebola Virus in  
17  
18 400 Kaigbono (Likati), Democratic Republic of the Congo, 2017. *Emerg Infect Dis* 2020;26(9):2205-  
19  
20 401 09. doi: 10.3201/eid2609.191552 [published Online First: 2020/08/21]  
21  
22  
23 402 24. Marí Saéz A, Weiss S, Nowak K, et al. Investigating the zoonotic origin of the West African Ebola  
24  
25 403 epidemic. *EMBO Mol Med* 2015;7(1):17-23. doi: 10.15252/emmm.201404792 [published  
26  
27 404 Online First: 2015/01/01]  
28  
29  
30 405 25. Petersen BW, Kabamba J, McCollum AM, et al. Vaccinating against monkeypox in the Democratic  
31  
32 406 Republic of the Congo. *Antiviral Res* 2019;162:171-77. doi: 10.1016/j.antiviral.2018.11.004  
33  
34 407 [published Online First: 2018/11/18]  
35  
36  
37 408 26. Map DR of the Congo: boundaries, provinces. d-maps.com. [https://d-](https://d-maps.com/carte.php?num_car=4886&lang=en)  
38  
39 409 [maps.com/carte.php?num\\_car=4886&lang=en](https://d-maps.com/carte.php?num_car=4886&lang=en) (accessed 10 Nov2020).  
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## 410 **FIGURE TITLES AND LEGENDS**

### 411 **Figure 1. Study time and events overview**

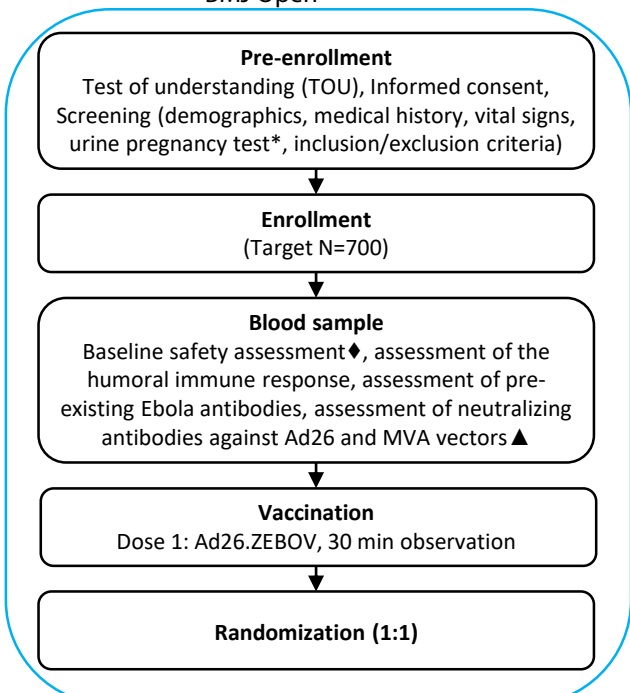
412 SAE: Serious Adverse Event; \* Only for female participants of childbearing potential; ♦ Abnormal  
413 results will not exclude a participant, as results will not be reviewed prior to enrollment; ▲ Only the  
414 first 100 participants enrolled will be tested for Neutralizing antibody response against ad26 VNA and  
415 MVA vectors. Other blood analyses are for all 700 participants; ▼ Concomitant therapies given in  
416 conjunction with a serious adverse event (SAE) should be recorded from signing of the ICF onwards  
417 until 6 months post booster; ▽ The investigator may withhold the second vaccine or booster dose if a  
418

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3 418 participant's clinical status changes prior to vaccination. The participant should continue to be followed  
4  
5 419 for safety and immunogenicity according to the protocol;  $\Delta$  only for female participants; \* Solicited  
6  
7 420 and unsolicited Adverse Events (AEs) will be collected in a participant diary during 1 week post booster  
8  
9 421 vaccination.

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13 422 **Figure 2. Study site location**

14  
15 423 On the left, the Democratic Republic of Congo (DRC) is highlighted on a map of the African continent.  
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17 424 On the right, the study site location (Boende, Tshuapa province) is marked on a map of DRC indicating  
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19 425 its provinces[26].  
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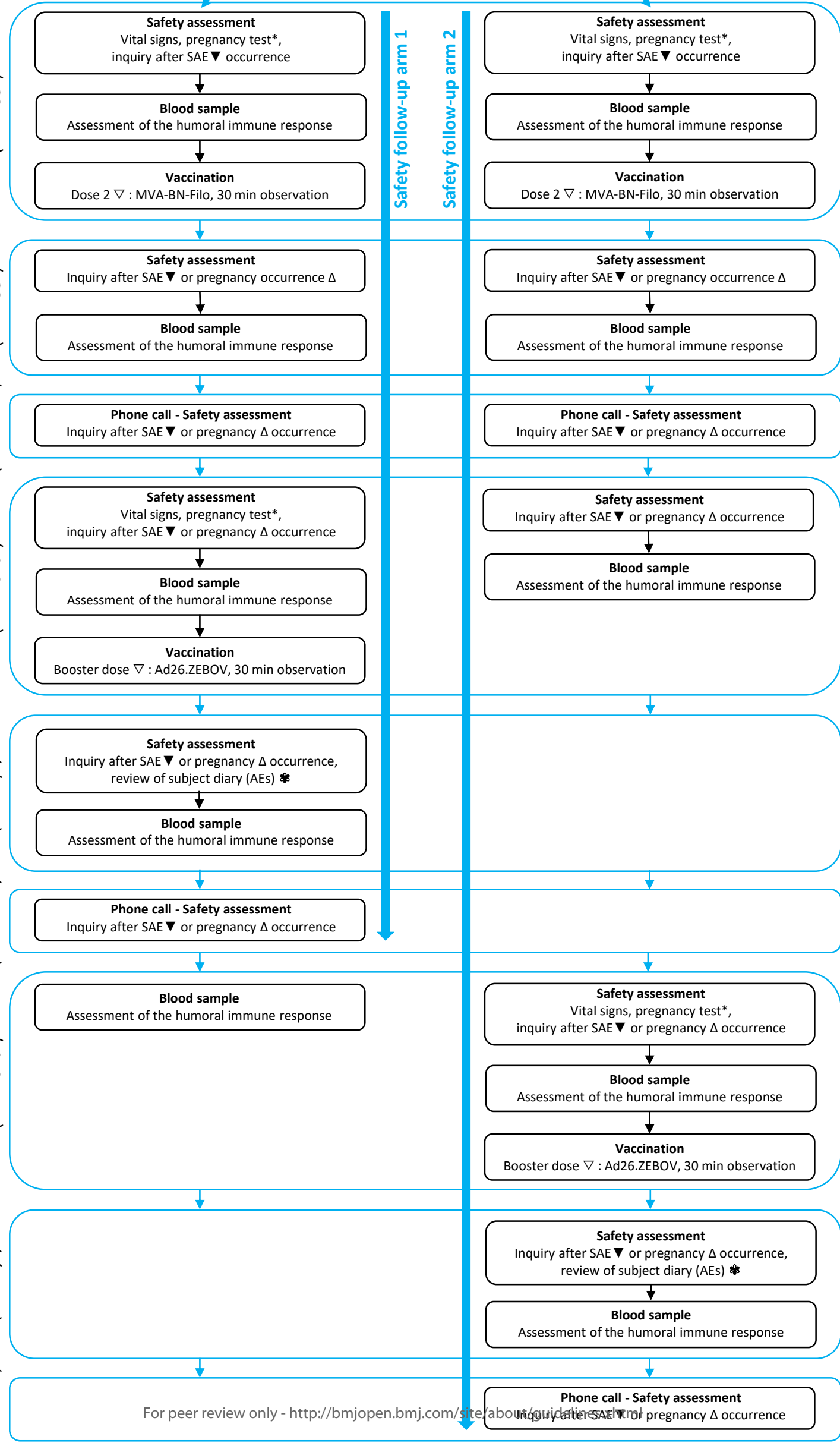


**Study arm 1 (N=350)**

**Study arm 2 (N=350)**

**Safety follow-up arm 1**

**Safety follow-up arm 2**



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

Section/item	ItemNo	Description	Location in manuscript
<b>Administrative information</b>			
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
<b>Introduction</b>			
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
<b>Methods: Participants, interventions, and outcomes</b>			
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

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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
<b>Methods: Assignment of interventions (for controlled trials)</b>			
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
<b>Methods: Data collection, management, and analysis</b>			
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
<b>Methods: Monitoring</b>			
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
<b>Ethics and dissemination</b>			
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
<b>Appendices</b>			
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

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<b>Primary Subject Heading</b>:	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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3 1 **Open-label, Randomized Controlled, Phase 2 Clinical Trial to Evaluate the Immunogenicity**  
4 **and Safety of a Prophylactic Vaccination of Health Care Providers by Administration of a**  
5 **Heterologous Vaccine Regimen Against Ebola in the Democratic Republic of the Congo: the**  
6 **Study protocol**  
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12 5 *Ynke Larivière (0000-0002-5422-0194)<sup>1,2\*</sup>, Trésor Zola (0000-0002-5830-415X)<sup>3</sup>, Elke Stoppie<sup>1,2</sup>, Vivi*  
13  
14 6 *Maketa (0000-0002-9007-1376)<sup>3</sup>, Junior Matangila (0000-0002-9025-3604)<sup>3</sup>, Patrick Mitashi (0000-*  
15  
16 7 *0002-6589-2869)<sup>3</sup>, Jessie De Bie (0000-0001-9035-1549)<sup>1,2</sup>, Hypolite Muhindo-Mavoko (0000-0002-*  
17  
18 8 *3307-3324)<sup>3</sup>, Jean-Pierre Van Geertruyden (0000-0001-5006-6364)<sup>2</sup>, Pierre Van Damme (0000-0002-*  
19  
20 9 *8642-1249)<sup>1</sup>*  
21  
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24 10 <sup>1</sup> *Centre for the Evaluation of Vaccination, Vaccine and Infectious Disease Institute, University of*  
25  
26 11 *Antwerp, Wilrijk, Belgium (Y Larivière MSc, J De Bie PhD, P Van Damme MD PhD)*  
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29 12 <sup>2</sup> *Global Health Institute, Department of Family Medicine and Population Health, University of Antwerp,*  
30  
31 13 *Wilrijk, Belgium (Y Larivière MSc, J De Bie PhD, JP Van geertruyden MD PhD)*  
32

33 14 <sup>3</sup> *Tropical Medicine Department, University of Kinshasa, Kinshasa, Democratic Republic of the Congo (T*  
34  
35 15 *Zola MD, V Maketa MD PhD, J Matangila MD PhD, P Mitashi MD PhD, H Muhindo-Mavoko MD PhD)*  
36  
37

38 16 Corresponding author:

39  
40 17 \*Ynke Larivière

41  
42 18 Centre for the Evaluation of Vaccination, Vaccine and Infectious Disease Institute, University  
43  
44 19 of Antwerp, Campus Drie Eiken, Universiteitsplein 1, Building S2, 2610 Wilrijk, Belgium

45  
46 20 [ynke.lariviere@uantwerpen.be](mailto:ynke.lariviere@uantwerpen.be)

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3 24 **ABSTRACT**  
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5 25 **Introduction:** This article describes the protocol of an Ebola vaccine clinical trial which investigates the  
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7 26 safety and immunogenicity of a prophylactic Ebola vaccine regimen comprised of 2 Ebola vaccines  
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9 27 (Ad26.ZEBOV and MVA-BN-Filo) administered 56 days apart, followed by a booster vaccination with  
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11 28 Ad26.ZEBOV offered at respectively 1 year or 2 years (randomization 1:1) after the first dose. This  
12  
13 29 clinical trial is part of the EBOVAC3 project (an Innovative Medicines Initiative 2 Joint Undertaking),  
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15 30 and is the first to evaluate the safety and immunogenicity of two different booster vaccination arms in  
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17 31 a large cohort of adults.  
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21 32 **Methods and analysis:** This study is an open-label, monocentric, phase 2, randomized controlled  
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23 33 vaccine trial. A total of 700 HCP and front-liners are planned to be recruited from the Tshuapa province  
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25 34 in the Democratic Republic of the Congo (DRC). The primary and secondary objectives of the study  
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27 35 assess the immunogenicity of the first (Ad26.ZEBOV), second (MVA-BN-Filo) and booster (Ad26.ZEBOV)  
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29 36 dose through the evaluation of binding antibody responses after vaccination and the safety of the  
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31 37 vaccines through the collection of serious adverse events from the first dose until six months post  
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33 38 booster vaccination and solicited and unsolicited adverse events for one week after the booster dose.  
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37 39 **Ethics and dissemination:** The protocol was approved by the National Ethics Committee of the Ministry  
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39 40 of Health of the DRC (n°121/CNES/BN/PMMF/2019). The clinical trial was registered on the 4<sup>th</sup> of  
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41 41 December 2019 on ClinicalTrials.gov (NCT04186000). Trial activities are planned to finish in July 2022.  
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43 42 All participants are required to provide written informed consent and no study-related procedures will  
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45 43 be performed until consent is obtained. The results of the trial will be added on ClinicalTrials.gov,  
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47 44 published in peer-reviewed journals and presented at international conferences.  
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51 45 **Key words:** Clinical Trial Protocol, Ebola Vaccines, Safety, Immunogenicity, Health Care Providers  
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## 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 *Zaire 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

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3 72 vaccine regimen comprised of 2 candidate Ebola vaccines (Ad26.ZEBOV and MVA-BN-Filo) and aiming  
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5 73 to bring this prophylactic Ebola vaccine to licensure[11]. Ad26.ZEBOV is a monovalent vaccine  
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7 74 developed to provide active EBOV-specific immunity. MVA-BN-Filo, which is administered 56 days after  
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10 75 the Ad26.ZEBOV vaccine, is a multivalent vaccine developed to establish active immunity to EBOV,  
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12 76 *Sudan Ebolavirus*, *Tai Forest Ebolavirus* and the Marburg virus (also part of the *Filoviridae* family). In  
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14 77 July 2020, the 2-dose prophylactic vaccine regimen was granted market authorisation by the European  
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16 78 Commission[12].

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19 79 Several projects (PREVAC, EBOVAC1, EBOVAC2 and EBOVAC3) within the IMI2 Joint Undertaking, of  
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21 80 which the first in-human clinical trials started in 2014, were at the basis of this successful  
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23 81 authorisation[11]. Within these projects, multiple clinical trials assessed or are assessing the timing,  
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25 82 tolerability, safety, and immunogenicity of different Ad26.ZEBOV and MVA-BN-Filo vaccine regimen in  
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27 83 healthy adults ( $\geq 18$  years old) and children/adolescents (1-17 years old) via phase 1, 2, 2B and 3  
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29 84 studies. Initial trials showed that healthy adult participants had higher geometric mean concentrations  
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31 85 of binding antibodies for the regimen where Ad26.ZEBOV vaccination was followed by an MVA-BN-Filo  
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33 86 vaccination 56 days later. Moreover, 100% of them had detectable Ebola glycoprotein-specific  
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35 87 Immunoglobulin G (IgG) antibodies up to at least 240 days after vaccination[13-15]. Even though some  
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37 88 local (erythema, swelling and pain at injection site) and systemic (headache, nausea, pyrexia, myalgia  
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39 89 and fatigue) solicited adverse events were recorded, the vaccine regimen was generally well tolerated  
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41 90 across studies[13-17].

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47 91 While it is of utmost importance that the 2-dose prophylactic vaccine regimen is safe and leads to an  
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49 92 immune response, it is also crucial to find out whether or not this regimen can lead to induced immune  
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51 93 memory at the time of imminent risk (i.e. an outbreak) through a booster vaccination. To evaluate this  
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53 94 induced immune memory, three previous studies within EBOVAC projects have administered a booster  
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55 95 vaccine with Ad26.ZEBOV at either 1 year (NCT02325050; NCT02564523) or 2 years (NCT02509494)  
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57 96 post Dose 1. Results from the NCT02325050 trial have already shown that an immunological memory  
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3 97 was rapidly induced via booster vaccination with Ad26.ZEBOV, indicating that booster vaccination can  
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5 98 be considered for at risk individuals (e.g. when an outbreak occurs) that were previously vaccinated  
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7 99 with the 2-dose heterologous prophylactic regimen[18]. However, these trials only evaluated booster  
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10 100 vaccination in a small amount of participants and it still has to be explored whether the induced  
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12 101 immune memory response differs depending on the timing of the booster dose (i.e. 1 or 2 years after  
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14 102 Dose 1).

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17 103 Healthcare settings play an important role in the control of EVD and therefore health care providers  
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19 104 (HCP) and front-liners, due to occupational exposure, are not only more at risk of disease acquisition  
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21 105 but also facilitate the spread of the virus[19-22]. Knowing that outbreaks of EVD often occur in regions  
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23 106 where there is already a shortage of HCP and front-liners, this further depletes a weak health care  
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25 107 system and the quality of care. Consequently, the Strategic Advisory Group of Experts stated in 2018  
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27 108 that for preventive strategies, vaccination of HCP as part of an emergency preparedness plan has  
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29 109 significant potential of reducing the scale and duration of outbreaks[23].

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33 110 This phase 2 randomized controlled trial aims to determine the safety and immunogenicity of the 2-  
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35 111 dose heterologous vaccine regimen with Ad26.ZEBOV followed by MVA-BN-Filo 56 days later.  
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37 112 Additionally, this trial aims to assess the safety and immunogenicity of a booster Ad26.ZEBOV vaccine  
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39 113 administered either 1 or 2 years post first dose. The trial is conducted in a cohort of HCP and front-  
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41 114 liners from the Boende health district in DRC, a well-known population at risk from clinical and  
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43 115 epidemiological perspective.

## 44 45 46 47 48 116 **METHODS**

### 49 50 117 **Study design and setting**

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53 118 This study is an open-label, monocentric, phase 2 randomized controlled trial to evaluate the  
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55 119 immunogenicity and safety of Ad26.ZEBOV ( $5 \times 10^{10}$  viral particles) as first dose and MVA-BN-Filo ( $1 \times 10^8$   
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57 120 infectious units) as second dose vaccination at a 56-day interval in HCP and front-liners who may be  
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59 121 exposed to Ebola in the event of a future Ebola outbreak in DRC. Additionally, after randomization (1:1)



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

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

## 127 Objectives

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

130 **Table 1. Objectives and endpoints**

Objectives	Endpoints
<b>Primary</b>	
<ul style="list-style-type: none"> <li>To assess binding antibody responses post-dose 2 vaccination with MVA-BN-Filo.</li> </ul>	<ul style="list-style-type: none"> <li>Binding antibody levels against the EBOV GP using FANG ELISA at 21 days post-dose 2 (Day 78) vaccination with MVA-BN-Filo.</li> </ul>
<b>Secondary</b>	
<ul style="list-style-type: none"> <li>To assess binding antibody responses after booster vaccination with Ad26.ZEBOV given at 1 or 2 years after first dose.</li> </ul>	<ul style="list-style-type: none"> <li>Binding antibody levels against the EBOV GP using FANG ELISA at 7 days (excluding the day of vaccination) post booster.</li> </ul>
<ul style="list-style-type: none"> <li>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.</li> </ul>	<ul style="list-style-type: none"> <li>Serious adverse events from first dose vaccination until 6 months post booster.</li> <li>Solicited and unsolicited local and systemic adverse events until 7 days post booster vaccination (day of vaccination and subsequent 7 days) with Ad26.ZEBOV.</li> </ul>
<b>Exploratory</b>	
<ul style="list-style-type: none"> <li>To assess binding antibody responses at different time points as indicated in the Study time and events overview (Figure 1).</li> </ul>	<ul style="list-style-type: none"> <li>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).</li> </ul>

<ul style="list-style-type: none"> <li>To assess neutralizing antibody response directed against the Adenovirus vector prior to vaccination.</li> </ul>	<ul style="list-style-type: none"> <li>Neutralizing antibody levels against Ad26 using Ad26 VNA at the first visit.</li> </ul>
<ul style="list-style-type: none"> <li>To assess neutralizing antibody response directed against the MVA vector prior to vaccination.</li> </ul>	<ul style="list-style-type: none"> <li>Neutralizing antibody levels against MVA-BN-Filo using MVA PRNT assay at the first visit.</li> </ul>
<ul style="list-style-type: none"> <li>To assess seroprevalence of Ebola virus disease prior to vaccination.</li> </ul>	<ul style="list-style-type: none"> <li>Presence of pre-existing Human anti-EBOV GP IgG and anti-EBOV NP IgG using LUMINEX assay.</li> </ul>
<p><i>ELISA: enzyme-linked immunosorbent assay; EU/mL: ELISA units/mL; FANG: Filovirus Animal Nonclinical Group. VNA: Virus Neutralization Assay; PRNT: Plaque Reduction Neutralization Test</i></p>	

### 131 Participant population

132 A total of 700 Registered HCP and front-liners in DRC (working in the Boende General Reference  
 133 Hospital, Health Centres or Health Posts in the Boende health district) are planned to be recruited from  
 134 the Tshuapa province in DRC. This assessment was based on information obtained from an ongoing  
 135 (monkeypox) vaccine trial in the same area at the time the protocol was being written[24]. From  
 136 discussions with the monkeypox research group, it became clear that a high enrolment rate and  
 137 retention rate (>90% after two years) could be expected among HCP and front-liners in the Boende  
 138 health district. Based on this ongoing monkeypox trial, it was estimated that enrolling approximately  
 139 50% of the HCP and front-liners working in the Boende health district would be feasible. This sample  
 140 size was thus defined upon the feasibility of recruitment of HCP and front-liners in the region.

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

### 142 Table 2. Inclusion and exclusion criteria

<p><b>Inclusion criteria</b></p> <p>Each potential participant must satisfy all of the following criteria to be enrolled in the study:</p> <ol style="list-style-type: none"> <li>The participant must pass the Test of Understanding.</li> </ol> <p><i>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).</i></p>
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2  
3 *If participants fail on the third attempt, they should not continue with screening or*  
4 *consenting procedures.*  
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- 7  
8 2. Each participant must sign an informed consent form indicating that he or she understands  
9 the purpose of, and procedures required for, the study and is willing to participate in the  
10 study. In case the participant cannot read or write, the procedures must be explained and  
11 informed consent must be witnessed by a trusted literate third party not involved with the  
12 conduct of the study.  
13  
14 3. The participant must be a man or women aged 18 years or older.  
15  
16 4. The participant must be a documented HCP in DRC.  
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18 5. The participant must be healthy in the investigator's clinical judgement and on the basis of  
19 vital signs assessed at day 1 screening.  
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27 *Note: HIV-positive subjects can be enrolled as long as their general condition is good, i.e.*  
28 *they are on antiretroviral treatment or have no signs or symptoms of immunodepression,*  
29 *diagnosed on the basis of physical examination, medical history, and the investigator's*  
30 *clinical judgment.*  
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- 36  
37 6. Before vaccination, a woman must be either:  
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39
  - 40 • Of childbearing potential and practicing (or intending to practice) a highly effective  
41 method of birth control consistent with local regulations and/or local culture  
42 regarding the use of birth control methods for participants in clinical studies,  
43 beginning at least 28 days prior to vaccination and during the study up to at least 3  
44 months after the first (or only) vaccination (Ad26.ZEBOV) and 1 month after the  
45 MVA-BN-Filo vaccination (if applicable); and then starting again 14 days before the  
46 booster vaccination until 3 months after the booster vaccination. The sponsor  
47 considers the following methods of birth control to be highly effective: established  
48 use of oral, injected or implanted hormonal methods of contraception; placement  
49 of an intrauterine device or intrauterine system; barrier methods: condom or  
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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  $\beta$ -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 contraception 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  $\geq 38.0^{\circ}\text{C}$  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).

#### 143 **Randomization procedure**

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

1  
2  
3 157 Delegated site staff will assign and open booklets and envelopes in sequential order during study visits.  
4  
5 158 Each envelope will contain two stickers. The first will contain space for writing the subject ID and  
6  
7 159 subject's initials, the second will contain the randomization number and treatment description (pre-  
8  
9 160 printed based on the study randomization list). Upon opening the sealed envelope, the subject ID and  
11  
12 161 initials must be written in the space provided on the first sticker and the subject ID sticker must be  
13  
14 162 placed on the outside of the envelope. To ensure proper source documentation, the sticker with the  
15  
16 163 treatment information must be placed on the corresponding *Randomization worksheet*. Thereafter,  
17  
18 164 the empty envelope, with the subject ID sticker on the outside, must be placed back in the booklet.  
19  
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21 165 These booklets are to be stored by the principal investigator.  
22  
23  
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### 25 166 **Study procedures (Figure 1)**

26  
27 167 At Day 1, interested participants are informed about the study and are required to pass a test of  
28  
29 168 understanding before providing written consent. No study activities are performed before the  
30  
31 169 participant has signed the informed consent form. Afterwards, the study medical doctor evaluates  
32  
33 170 his/her general health based on the inclusion criteria, vital signs (blood pressure, pulse/heart rate  
34  
35 171 [both at rest] and body temperature) are collected and a urine pregnancy test for women of  
36  
37 172 childbearing potential is performed. Further during this first visit, a blood sample is taken for baseline  
38  
39 173 testing of binding antibody level (i.e. humoral immune response) against EBOV glycoprotein (GP) using  
40  
41 174 Filovirus Animal Non-Clinical Group (FANG) Ebola virus enzyme-linked immunosorbent assay (ELISA)  
42  
43 175 and the presence of pre-existing Human anti-EBOV GP IgG and anti-EBOV nucleoprotein (NP) IgG using  
44  
45 176 LUMINEX assay. For Day 1 samples, both FANG ELISA and LUMINEX assay will be carried out. FANG  
46  
47 177 ELISA is performed for all EBOVAC trials in the same laboratory (for consistency and comparability) and  
48  
49 178 LUMINEX assay will provide a more detailed array of IgG (and IgM) antibodies that are not obtained  
50  
51 179 via FANG ELISA. For the first 100 enrolled participants an additional test on the collected serum is  
52  
53 180 performed to measure the neutralizing antibody level against Ad26 and MVA vectors using respectively  
54  
55 181 Ad26 Virus Neutralizing Assay (VNA) and MVA Plaque Reduction Immunogenicity Test (PRNT).  
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3 182 Subsequently, a blood sample for baseline safety assessment is collected to test haemoglobin,  
4  
5 183 haematocrit, blood cell count (white and red), platelet count, urea, creatinine and transaminases.  
6  
7 184 Then, participants are vaccinated with the first dose (Ad26.ZEBOV) and they are given instructions to  
8  
9  
10 185 contact the study team for any occurring serious adverse events (SAEs), or in case of pregnancy of a  
11  
12 186 participant during the study. After vaccination, participants remain at the study site for an observation  
13  
14 187 period of 30 minutes to make sure no SAEs occur. SAEs are collected from first dose vaccination until  
15  
16 188 6 months post booster (PB). Lastly on Day 1, randomization is performed to determine the timing of  
17  
18 189 the booster vaccine at either 1 year or 2 years after the first dose. Contact information is verified, an  
19  
20 190 appointment for the second dose on Day 57 is arranged and a participant card is printed. Innovatively,  
21  
22 191 next to a participant card, a biometric identification tool via iris scanning is foreseen to ensure correct  
23  
24 192 identification of the participants during all study related visits.  
25  
26  
27

28 193 At Day 57, participants return to the study site for urine pregnancy testing (for women of childbearing  
29  
30 194 potential), vital signs measurement, assessment of safety (SAEs), a blood sample for immunogenicity  
31  
32 195 assessment (the binding antibody levels against EBOV GP using FANG ELISA) and afterwards  
33  
34 196 administration of the second vaccine (MVA-BN-Filo). After an observation period of 30 minutes,  
35  
36 197 participants are reminded to contact the study team for any SAEs that occurs, or in case of pregnancy  
37  
38 198 of a participant during the study. Contact information is verified and an appointment for the 21-day  
39  
40 199 post-dose 2 visit (Day 78) is arranged.  
41  
42  
43

44 200 At 21 days post-dose 2 (Day 78), all participants return to the study site for a safety assessment (SAEs)  
45  
46 201 and for the collection of a blood sample for immunogenicity assessment. Contact information is re-  
47  
48 202 verified and they are reminded to contact the study team in case of SAE occurrence, or in case of  
49  
50 203 pregnancy of a participant.  
51  
52  
53

54 204 To make sure no valuable information is missed, participants are contacted by phone to inquire about  
55  
56 205 any occurrence of pregnancies (female participants) and SAEs at approximately 6 months post-dose 2  
57  
58 206 vaccination. At 1 year after the first vaccine dose, when all participants return to the site, the clinical  
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2  
3 207 trial staff inquires after the occurrence of SAEs and a blood sample is collected for immunogenicity  
4  
5 208 assessment of all participants (where applicable before administration of the booster dose).  
6  
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8 209 At 1 year or 2 years post first dose, depending on the study arm, a booster vaccination with  
9  
10 210 Ad26.ZEBOV is given. Prior to vaccination, the general well-being of the participant will be evaluated  
11  
12 211 and urine pregnancy testing (for women of childbearing potential), as well as a vital signs measurement  
13  
14 212 will be performed. After vaccination, participants remain at the study site for a 30 minute observation  
15  
16 213 period. Participants are asked to collect solicited and unsolicited adverse events (AEs) in a participant  
17  
18 214 diary starting on the day of the vaccination and continuing for the subsequent 7 days. At Day 8 PB the  
19  
20 215 safety data including solicited and unsolicited AEs is reviewed and a blood sample for immunogenicity  
21  
22 216 assessment is taken to document the immune response.  
23  
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25  
26 217 At 6 months PB, all participants are contacted by phone and questioned about any SAEs or pregnancies  
27  
28 218 (female participants) that have occurred since the last vaccination. For all participants at 2 years after  
29  
30 219 first dose, a sample is collected for immunogenicity assessment (where applicable before  
31  
32 220 administration of the booster dose) and a safety assessment (SAEs) is performed for those returning  
33  
34 221 for their booster vaccination.  
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37  
38 222 The total duration of the study is 2 years and 6 months post-first dose. The study is considered  
39  
40 223 completed when the last participant has been contacted for the 6 months PB phone call or has left the  
41  
42 224 study.  
43  
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45

#### 46 225 **Study intervention**

47  
48  
49 226 According to the predefined schedule (Figure 1), participants receive a 0.5 mL intramuscular injection  
50  
51 227 into the deltoid muscle of the upper arm with Ad26.ZEBOV or MVA-BN-Filo. The injection site should  
52  
53 228 be free from any injury, local skin conditions, or other issues that might interfere with the evaluation  
54  
55 229 of local reactions. Every vaccination is given in the opposite arm of the previous vaccination (unless  
56  
57 230 the opposite arm has a condition that prevents evaluating the arm after injection). No local or topical  
58  
59 231 anaesthetic is used prior to the injection.  
60

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3 232 The second or booster vaccination is not administered if any of the following events occur at any time  
4  
5 233 after the first dose vaccination:

- 6  
7  
8 234 • A participant experiences anaphylaxis clearly attributable to vaccination with the study  
9  
10 235 vaccine; OR  
11  
12 236 • A participant experiences generalized urticaria within 72 hours of vaccination considered  
13  
14 237 to be related to study vaccine; OR  
15  
16  
17 238 • A participant experiences a serious adverse event considered to be related to the study  
18  
19 239 vaccine; OR  
20  
21  
22 240 • A participant experiences injection site ulceration, abscess or necrosis considered to be  
23  
24 241 related to the study vaccine; OR  
25  
26 242 • A participant has confirmed EVD; OR  
27  
28 243 • A female participant of childbearing potential has a positive urine  $\beta$ -human chorionic  
29  
30 244 gonadotropin pregnancy test before vaccination (on Day 57, Year 1 or Year 2 [depending  
31  
32 245 on the randomization group]); OR  
33  
34  
35 246 • A female participant of childbearing potential has a positive urine  $\beta$ -human chorionic  
36  
37 247 gonadotropin pregnancy test between Dose 2 and the booster dose and is still pregnant  
38  
39 248 or breastfeeding at the time of the booster dose; OR  
40  
41  
42 249 • A participant takes a concomitant treatment with drugs that may alter the immune  
43  
44 250 response; OR  
45  
46  
47 251 • The principal investigator believes that for safety reasons it is in the best interest of a  
48  
49 252 participant to discontinue the study intervention.

50  
51  
52 253 Participants experiencing any of the events described above are still followed up for safety and  
53  
54 254 immunogenicity according to the protocol. The decision to discontinue the study intervention is at the  
55  
56 255 discretion of the principal investigator (University of Kinshasa) and after consultation with the sponsor  
57  
58 256 (University of Antwerp) for any of the events described above.

## 257 **Patient and public involvement**

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

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

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

## 276 **Data management**

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

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2  
3 281 database. Additionally, the sponsor performs quality checks of the entered data in the database and  
4  
5 282 during monitoring visits source data verification is performed.  
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### 8 283 **Statistical analysis**

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11 284 A differentiation in analysis will be made according to: 1) the *Full Analysis Set* (FAS; all participants who  
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13 285 received at least one dose, regardless of the occurrence of protocol deviations), 2) *Per Protocol Set for*  
14  
15 286 *primary vaccination series* (all vaccinated subjects, who received both dose 1 and dose 2 [administered  
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17 287 within the protocol-defined visit window] vaccinations, have at least 1 post-vaccination [i.e. after the  
18  
19 288 date of dose 1] evaluable immunogenicity sample, and have no major protocol deviations influencing  
20  
21 289 the immune response) and 3) *Per Protocol Set for the Booster/Dose 3 vaccination* (includes all subjects  
22  
23 290 in the per protocol set for the primary vaccination series who received Dose 3 and have at least 1 post-  
24  
25 291 dose 3 [i.e. after the date of booster vaccination] evaluable immunogenicity sample, and have no major  
26  
27 292 protocol deviations influencing the immune response).  
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30  
31 293 Subject information (i.e. demographics and baseline characteristics, disposition information,  
32  
33 294 treatment compliance, extent of exposure, protocol deviations and concomitant medications) is  
34  
35 295 planned to be tabulated and summarized with descriptive statistics for all subjects. For continuous  
36  
37 296 data such as age, the mean and standard deviation will be provided if applicable, otherwise the  
38  
39 297 geometric means, related standard deviations or median and interquartile ranges will be used.  
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43 298 For the immunogenicity analysis, two *Per-Protocol Sets* will be used, i.e., the *Per-Protocol Set for*  
44  
45 299 *primary vaccination series* and the *Per-Protocol Set for the booster*. If more than 10% of participants  
46  
47 300 from the FAS are excluded from the per protocol immunogenicity set, the immunogenicity analysis will  
48  
49 301 be repeated on the FAS to evaluate the robustness of the analysis results. A subgroup analysis of the  
50  
51 302 immune response at different time points will be performed stratified by age (18-40, 40-60 and >60),  
52  
53 303 gender, prior vaccinia/smallpox vaccination, pre-existing Ebola antibodies (positive or negative for pre-  
54  
55 304 existing Human anti-EBOV GP IgG and anti-EBOV NP IgG, and for both), baseline immunogenicity  
56  
57 305 (positivity versus negativity for antibody levels against EBOV GP using FANG ELISA) and the presence  
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3 306 of neutralizing antibody levels against Ad26 and MVA vectors using Ad26 VNA and MVA PRNT assay  
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5 307 (only the first 100 enrolled participants). For these planned subgroup analyses, N (%), Geometric Mean  
6  
7 308 Concentrations and 95% confidence intervals (CI) will be provided as appropriate.  
8  
9

10 309 Safety analyses include SAEs collected during the whole study and solicited and unsolicited AEs for 1  
11  
12 310 week post booster vaccination. The analysis of SAEs will be performed using the FAS and the solicited  
13  
14 311 and unsolicited AEs will be analysed for the participants who received the booster vaccination.  
15  
16 312 Continuous variables will be summarized using the following statistics: number of observations;  
17  
18 313 arithmetic or geometric mean/median (if applicable) with their related measures of dispersion (95%  
19  
20 314 CI for the mean, standard deviation or inter quartile range (Q1-Q3)). Minimum and maximum  
21  
22 315 frequencies and percentages (one decimal place) will be generated for categorical variables.  
23  
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25

26 316 The primary endpoint analysis is planned to be performed when all participants have completed the  
27  
28 317 21-day post dose 2 visit (Day 78) or discontinued earlier. This analysis includes all available  
29  
30 318 immunogenicity and safety data up to this point (date cut-off). Additional interim analyses may be  
31  
32 319 performed during the study for the purpose of informing future vaccine-related decisions in a timely  
33  
34 320 manner.  
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38 321 The final analysis will be performed when all participants have completed the last study-related phone  
39  
40 322 call (6 months PB) or left the study.  
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## 44 323 **DISCUSSION**

45  
46 324 The aim of this phase 2 trial is to obtain further safety and immunogenicity data on the 2-dose  
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48 325 prophylactic heterologous Ebola vaccine regimen and to assess the safety and immunogenicity of a  
49  
50 326 booster dose with Ad26.ZEBOV administered either 1 or 2 years post first dose in a larger group. By  
51  
52 327 doing so, this study will feed the immunogenicity and safety databases of the Ad26.ZEBOV and MVA-  
53  
54 328 BN-Filo vaccines.  
55  
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57  
58 329 Boende (the capital city of Tshuapa province) was selected as the trial site for several reasons. First,  
59  
60 330 the Tshuapa province recovered from an EBOV outbreak that occurred in the Boende Health District

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2  
3 331 in 2014[21]. Following this outbreak, a study (n=565) conducted in the Tshuapa region in 2015 found  
4  
5 332 that 41.4% of the tested HCP were seroreactive to at least one EBOV protein and 2.8% of the HCP  
6  
7 333 showed a neutralizing capacity while never having developed EVD symptoms[20]. This observation  
8  
9 334 suggests a possible endemic EBOV exposure in the Tshuapa province of DRC. These are interesting  
10  
11 335 observations for future ecologic research as the ecology and reservoir(s) of EBOV and other filoviruses  
12  
13 336 remain largely unknown[25, 26]. Second, in addition to the previous outbreak of EVD, Boende was  
14  
15 337 chosen to perform the current clinical trial as there was expertise available after carrying out a phase  
16  
17 338 3 monkey pox vaccine trial that took place in 2017[24].

20  
21 339 The initial protocol aimed to provide descriptive information on immunogenicity and safety of the  
22  
23 340 heterologous 2-dose vaccine regimen contributing to licensure of the 2 dose heterologous vaccine  
24  
25 341 regimen. The sample size was determined based on the feasibility assessment obtained from the  
26  
27 342 ongoing monkeypox vaccine trial[24]. Approximately 50% of the total HCP and front-liners in the  
28  
29 343 Boende health district would be included in the Ebola vaccine trial. After finalizing the protocol, a  
30  
31 344 secondary objective comparing two booster arms was added. For this objective a power a power of  
32  
33 345 0.99 was calculated based on the following parameters: two-sided t-test equal samples of 350  
34  
35 346 participants, significance level of 0.05, an effect size of 0.35 in antibody response and a retention rate  
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37 347 of 90%. The effect size was based on former documented trial data. It is important to note that a  
38  
39 348 varying antibody response after booster vaccination is not directly correlated with protective vaccine  
40  
41 349 efficacy.

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46  
47 350 In conclusion, because EVD remains a very deadly disease, effective and safe preventive measures will  
48  
49 351 play a crucial role to protect vulnerable communities. While the prophylactic heterologous 2-dose  
50  
51 352 regimen was recently granted market authorisation by the European Commission, further research  
52  
53 353 into the safety and immunogenicity of the 2-dose regimen is still required to obtain worldwide  
54  
55 354 licensure of the regimen. Furthermore, limited data has previously been collected on the safety and

1  
2  
3 355 immunogenicity of a booster dose with Ad26.ZEBOV. This is the first randomized vaccine trial that looks  
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5 356 into the safety and immunogenicity of two different booster arms in a large cohort.  
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## 8 357 **ETHICS AND DISSEMINATION**

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11 358 This protocol was submitted and approved by the National Ethics Committee of the Ministry of Health  
12  
13 359 of the Democratic Republic of Congo (approval number: n°121/CNES/BN/PMMF/2019). Prior to being  
14  
15 360 enrolled in the trial, all participants are required to provide written informed consent by signing the  
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17 361 informed consent form after having performed a test of understanding. If the participant is unable to  
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19 362 read or write, an impartial witness should be present for the entire informed consent process (which  
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21 363 includes reading and explaining all written information) and should personally date and sign the  
22  
23 364 informed consent form after the oral consent of the participant is obtained. No study-related  
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25 365 procedures are performed until the participant has signed the informed consent form.  
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29 366 The trial was registered on Clinicaltrial.gov on December 4<sup>th</sup>, 2019 (NCT04186000) and recruitment  
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31 367 started on December 18<sup>th</sup>, 2019. All participants were recruited by the 8<sup>th</sup> of February 2020 and the  
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33 368 study is planned to finish in July, 2022. Results of the trial will be entered on Clinicaltrial.gov, published  
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35 369 in peer-reviewed journals and presented at international conferences.  
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## 39 370 **DECLARATIONS**

### 40 371 **Author contributions**

41  
42 372 YL wrote the manuscript. TZ, ES, YL, VM, JM, PM, HMM, JPVG and PVD wrote the initial English protocol  
43  
44 373 on which this manuscript is based. TZ, VM, PM, JM and HMM translated it into French for submission  
45  
46 374 to the National Ethics Committee and the “Direction de la Pharmacie et des Médicaments” of the  
47  
48 375 Ministry of Health of the Democratic Republic of Congo as well as the National Scientific committee  
49  
50 376 against Ebola. JDB, TZ, ES, VM, JM, PM, HMM, JPVG and PVD reviewed and contributed to the final  
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52 377 manuscript.  
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382 Associations (EFPIA) and the Coalition for Epidemic Preparedness Innovations (CEPI). For this trial, the  
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**393 Competing interests**

394 The authors declare that they have no competing interests.

**395 REFERENCES**

- 396 1. Baseler L, Chertow DS, Johnson KM, et al. The Pathogenesis of Ebola Virus Disease. *Annu Rev*  
397 *Pathol.* 2017;12:387-418.
- 398 2. Muyembe-Tamfum JJ, Mulangu S, Masumu J, et al. Ebola virus outbreaks in Africa: past and  
399 present. *Onderstepoort J Vet Res.* 2012;79(2):451.
- 400 3. Rewar S, Mirdha D. Transmission of ebola virus disease: an overview. *Ann Glob Health.*  
401 2014;80(6):444-51.



- 1  
2  
3 402 4. Rouquet P, Froment JM, Bermejo M, et al. Wild animal mortality monitoring and human Ebola  
4  
5 403 outbreaks, Gabon and Republic of Congo, 2001-2003. *Emerg Infect Dis*. 2005;11(2):283-90.  
6  
7 404 5. World Health Organization. Ebola virus disease. *Fact sheet N 103*. Updated September 2014.  
8  
9 405 6. Report of an International Commission. Ebola haemorrhagic fever in Zaire, 1976. *Bull World*  
10  
11 406 *Health Organ*. 1978;56(2):271-93.  
12  
13 407 7. Malvy D, McElroy AK, de Clerck H, et al. Ebola virus disease. *The Lancet*. 2019;393(10174):936-  
14  
15 408 48.  
16  
17 409 8. European Centre for Disease Prevention and Control. Outbreak of Ebola virus disease in North  
18  
19 410 Kivu – Democratic Republic of the Congo – 2021 [Available from:  
20  
21 411 [https://www.ecdc.europa.eu/en/news-events/outbreak-ebola-virus-disease-north-kivu-democratic-](https://www.ecdc.europa.eu/en/news-events/outbreak-ebola-virus-disease-north-kivu-democratic-republic-congo-2021)  
22  
23 412 [republic-congo-2021](https://www.ecdc.europa.eu/en/news-events/outbreak-ebola-virus-disease-north-kivu-democratic-republic-congo-2021)].  
24  
25 413 9. International Committee on Taxonomy. *Virus Metadata Repository: version May 1,*  
26  
27 414 *2020;MSL35*.  
28  
29 415 10. World Health Organization. *Situation Report Ebola Virus Disease*.  
30  
31 416 <http://apps.who.int/ebola/ebola-situation-reports>; 10 June 2016.  
32  
33 417 11. Ebovac. EBOVAC3 2020 [Available from: <https://www.ebovac.org/ebovac-3/>].  
34  
35 418 12. European Commission. Vaccine against Ebola: Commission grants new market authorisation  
36  
37 419 July 2020 [Available from: [https://ec.europa.eu/commission/presscorner/detail/en/ip\\_20\\_1248](https://ec.europa.eu/commission/presscorner/detail/en/ip_20_1248)].  
38  
39 420 13. Anywaine Z, Whitworth H, Kaleebu P, et al. Safety and Immunogenicity of a 2-Dose  
40  
41 421 Heterologous Vaccination Regimen With Ad26.ZEBOV and MVA-BN-Filo Ebola Vaccines: 12-Month  
42  
43 422 Data From a Phase 1 Randomized Clinical Trial in Uganda and Tanzania. *J Infect Dis*. 2019;220(1):46-  
44  
45 423 56.  
46  
47 424 14. Milligan ID, Gibani MM, Sewell R, et al. Safety and Immunogenicity of Novel Adenovirus Type  
48  
49 425 26- and Modified Vaccinia Ankara-Vectored Ebola Vaccines: A Randomized Clinical Trial. *Jama*.  
50  
51 426 2016;315(15):1610-23.  
52  
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- 1  
2  
3 427 15. Mutua G, Anzala O, Luhn K, et al. Safety and Immunogenicity of a 2-Dose Heterologous Vaccine  
4  
5 428 Regimen With Ad26.ZEBOV and MVA-BN-Filo Ebola Vaccines: 12-Month Data From a Phase 1  
6  
7 429 Randomized Clinical Trial in Nairobi, Kenya. *J Infect Dis.* 2019;220(1):57-67.
- 9  
10 430 16. Shukarev G, Callendret B, Luhn K, et al. A two-dose heterologous prime-boost vaccine regimen  
11  
12 431 eliciting sustained immune responses to Ebola Zaire could support a preventive strategy for future  
13  
14 432 outbreaks. *Hum Vaccin Immunother.* 2017;13(2):266-70.
- 16  
17 433 17. Safety and immunogenicity of a two-dose heterologous Ad26.ZEBOV and MVA-BN®-Filo Ebola  
18  
19 434 vaccine regimen: a phase 2 randomised clinical study in Europe (EBOVAC2). 29th ECCMID; 2019;  
20  
21 435 Amsterdam, Netherlands.
- 23  
24 436 18. Goldstein N, Bockstal V, Bart S, et al. Safety and Immunogenicity of Heterologous and  
25  
26 437 Homologous 2-Dose Regimens of Adenovirus Serotype 26—and Modified Vaccinia Ankara—Vectored  
27  
28 438 Ebola Vaccines: A Randomized, Controlled Phase 1 Study. *J Infect Dis.* 2020.
- 29  
30 439 19. Evans DK, Goldstein M, Popova A. Health-care worker mortality and the legacy of the Ebola  
31  
32 440 epidemic. *Lancet Glob Health.* 2015;3(8):e439-e40.
- 34  
35 441 20. Hoff NA, Mukadi P, Doshi RH, et al. Serologic Markers for Ebolavirus Among Healthcare  
36  
37 442 Workers in the Democratic Republic of the Congo. *J Infect Dis.* 2019;219(4):517-25.
- 38  
39 443 21. Maganga GD, Kapetshi J, Berthet N, et al. Ebola virus disease in the Democratic Republic of  
40  
41 444 Congo. *N Engl J Med.* 2014;371(22):2083-91.
- 43  
44 445 22. Nanclares C, Kapetshi J, Lionetto F, et al. Ebola Virus Disease, Democratic Republic of the  
45  
46 446 Congo, 2014. *Emerg Infect Dis.* 2016;22(9):1579-86.
- 47  
48 447 23. World Health Organization. Meeting of the Strategic Advisory Group of Experts on  
49  
50 448 Immunization, October 2018—Conclusions and recommendations. *Weekly Epidemiological Record.*  
51  
52 449 2018;93(49):661-79.
- 54  
55 450 24. Petersen BW, Kabamba J, McCollum AM, et al. Vaccinating against monkeypox in the  
56  
57 451 Democratic Republic of the Congo. *Antiviral Res.* 2019;162:171-7.
- 58  
59  
60

- 1  
2  
3 452 25. Gryseels S, Mbala-Kingebeni P, Akonda I, et al. Role of Wildlife in Emergence of Ebola Virus in  
4  
5 453 Kaigbono (Likati), Democratic Republic of the Congo, 2017. *Emerg Infect Dis.* 2020;26(9):2205-9.  
6  
7 454 26. Marí Saéz A, Weiss S, Nowak K, et al. Investigating the zoonotic origin of the West African Ebola  
8  
9 455 epidemic. *EMBO Mol Med.* 2015;7(1):17-23.  
10  
11  
12 456 27. d-maps.com, cartographer Map DR of the Congo: boundaries, provinces. [https://d-](https://d-maps.com/carte.php?num_car=4886&lang=en2020)  
13  
14 457 [maps.com/carte.php?num\\_car=4886&lang=en2020](https://d-maps.com/carte.php?num_car=4886&lang=en2020).  
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## 458 **FIGURE TITLES AND LEGENDS**

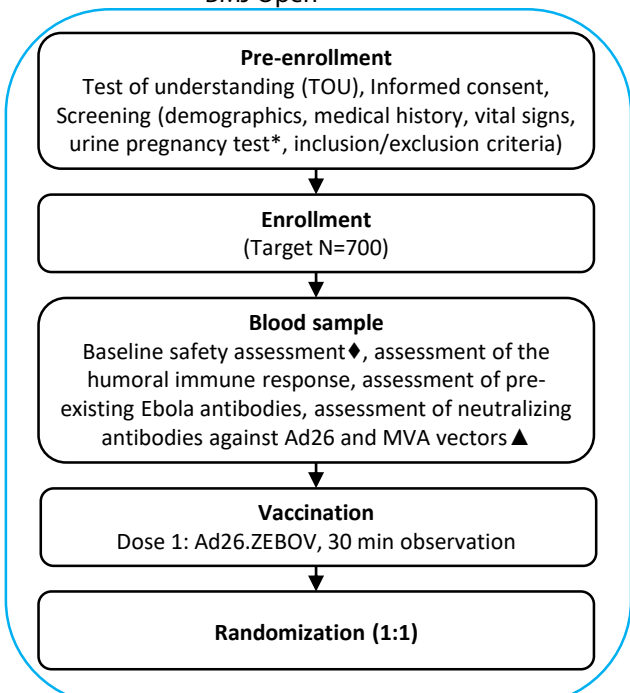
### 459 **Figure 1. Study time and events overview**

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

### 470 **Figure 2. Study site location**

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

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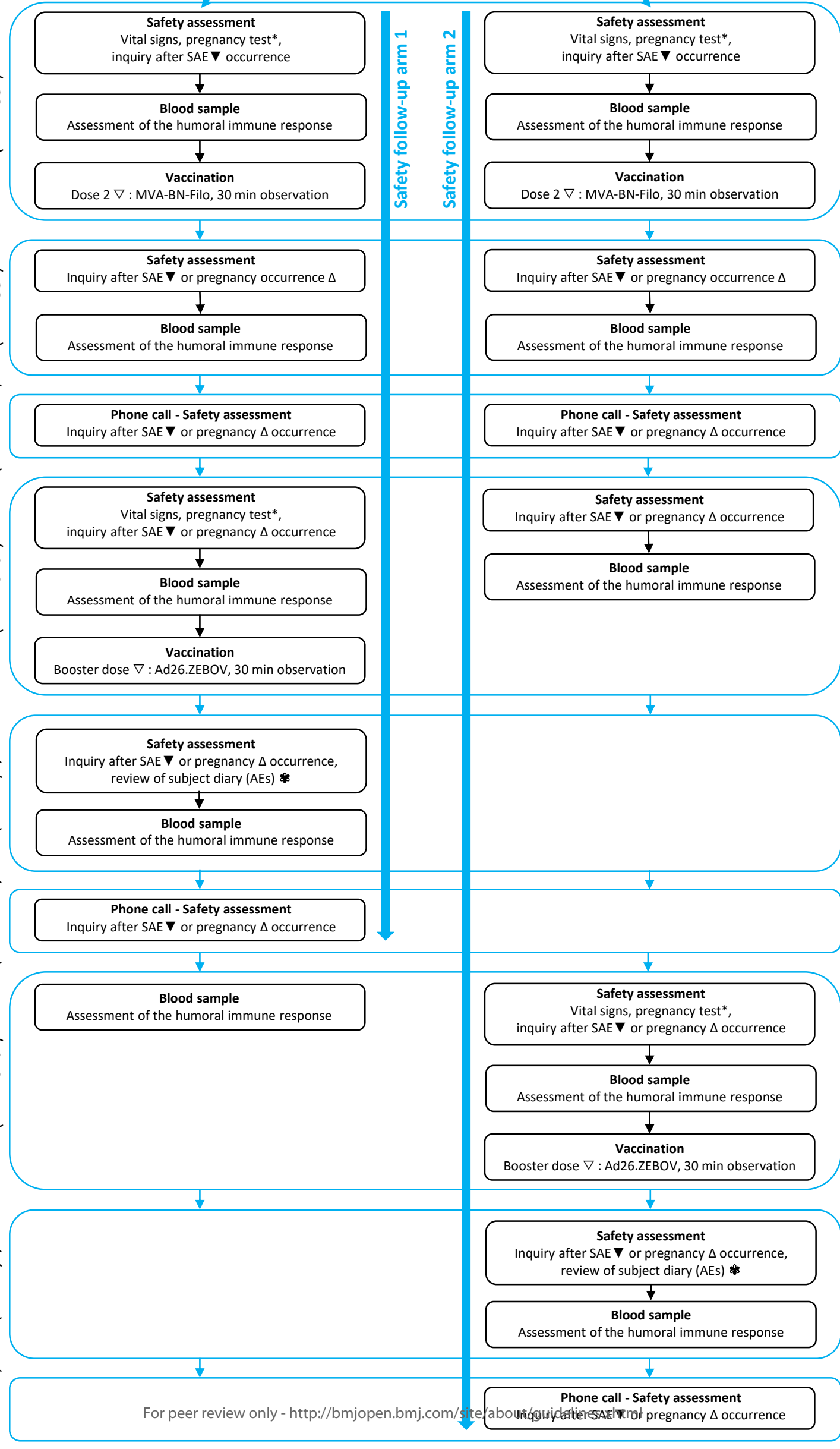


**Study arm 1 (N=350)**

**Study arm 2 (N=350)**

Safety follow-up arm 1

Safety follow-up arm 2



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

Section/item	ItemNo	Description	Location in manuscript
<b>Administrative information</b>			
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
<b>Introduction</b>			
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
<b>Methods: Participants, interventions, and outcomes</b>			
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
<b>Methods: Assignment of interventions (for controlled trials)</b>			
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
<b>Methods: Data collection, management, and analysis</b>			
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
<b>Methods: Monitoring</b>			
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
<b>Ethics and dissemination</b>			
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
<b>Appendices</b>			
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:	<i>BMJ Open</i>
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
<b>Primary Subject Heading</b>:	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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1 **Open-label, Randomized, Clinical Trial to Evaluate the Immunogenicity and Safety of a**  
2 **Prophylactic Vaccination of Health Care Providers by Administration of a Heterologous**  
3 **Vaccine Regimen Against Ebola in the Democratic Republic of the Congo: the Study protocol**

4 *Ynke Larivière (0000-0002-5422-0194)<sup>1,2\*</sup>, Trésor Zola (0000-0002-5830-415X)<sup>3</sup>, Elke Stoppie<sup>1,2</sup>, Vivi*  
5 *Maketa (0000-0002-9007-1376)<sup>3</sup>, Junior Matangila (0000-0002-9025-3604)<sup>3</sup>, Patrick Mitashi (0000-*  
6 *0002-6589-2869)<sup>3</sup>, Jessie De Bie (0000-0001-9035-1549)<sup>1,2</sup>, Hypolite Muhindo-Mavoko (0000-0002-*  
7 *3307-3324)<sup>3</sup>, Jean-Pierre Van Geertruyden (0000-0001-5006-6364)<sup>2</sup>, Pierre Van Damme (0000-0002-*  
8 *8642-1249)<sup>1</sup>*

9 <sup>1</sup> *Centre for the Evaluation of Vaccination, Vaccine and Infectious Disease Institute, University of*  
10 *Antwerp, Wilrijk, Belgium (Y Larivière MSc, J De Bie PhD, P Van Damme MD PhD)*

11 <sup>2</sup> *Global Health Institute, Department of Family Medicine and Population Health, University of Antwerp,*  
12 *Wilrijk, Belgium (Y Larivière MSc, J De Bie PhD, JP Van geertruyden MD PhD)*

13 <sup>3</sup> *Tropical Medicine Department, University of Kinshasa, Kinshasa, Democratic Republic of the Congo (T*  
14 *Zola MD, V Maketa MD PhD, J Matangila MD PhD, P Mitashi MD PhD, H Muhindo-Mavoko MD PhD)*

15 Corresponding author:

16 \*Ynke Larivière

17 Centre for the Evaluation of Vaccination, Vaccine and Infectious Disease Institute, University  
18 of Antwerp, Campus Drie Eiken, Universiteitsplein 1, Building S2, 2610 Wilrijk, Belgium

19 [ynke.lariviere@uantwerpen.be](mailto:ynke.lariviere@uantwerpen.be)

20 0032 3 265 9716

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**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 4<sup>th</sup> 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 *Zaire 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]).

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3 72 One of the initiatives to develop such a vaccine came from an international consortium, funded by the  
4  
5 73 Innovative Medicines Initiative 2 (IMI2) Joint Undertaking, dedicated to evaluate a prophylactic Ebola  
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7 74 vaccine regimen comprised of 2 candidate Ebola vaccines (Ad26.ZEBOV and MVA-BN-Filo) and aiming  
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9  
10 75 to bring this prophylactic Ebola vaccine to licensure[11]. Ad26.ZEBOV is a monovalent vaccine  
11  
12 76 developed to provide active EBOV-specific immunity. MVA-BN-Filo, which is administered 56 days after  
13  
14 77 the Ad26.ZEBOV vaccine, is a multivalent vaccine developed to establish active immunity to EBOV,  
15  
16 78 *Sudan Ebolavirus*, *Tai Forest Ebolavirus* and the Marburg virus (also part of the *Filoviridae* family). In  
17  
18 79 July 2020, the 2-dose prophylactic vaccine regimen was granted market authorisation by the European  
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21 80 Commission[12].  
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23  
24 81 Several projects (PREVAC, EBOVAC1, EBOVAC2 and EBOVAC3) within the IMI2 Joint Undertaking, of  
25  
26 82 which the first in-human clinical trials started in 2014, were at the basis of this successful  
27  
28 83 authorisation[11]. Within these projects, multiple clinical trials assessed or are assessing the timing,  
29  
30 84 tolerability, safety and immunogenicity of different Ad26.ZEBOV and MVA-BN-Filo vaccine regimen in  
31  
32 85 healthy adults ( $\geq 18$  years old) and children/adolescents (1-17 years old) via phase 1, 2, 2B and 3  
33  
34 86 studies. Initial trials showed that healthy adult participants had higher geometric mean concentrations  
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36 87 of binding antibodies for the regimen where Ad26.ZEBOV vaccination was followed by an MVA-BN-Filo  
37  
38 88 vaccination 56 days later. Moreover, 100% of participants had detectable Ebola glycoprotein-specific  
39  
40 89 Immunoglobulin G (IgG) antibodies up to at least 240 days after vaccination[13-15]. Even though some  
41  
42 90 local (erythema, swelling and pain at injection site) and systemic (headache, nausea, pyrexia, myalgia  
43  
44 91 and fatigue) solicited adverse events were recorded, the vaccine regimen was generally well tolerated  
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46 92 across studies[13-17].  
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51 93 While it is of utmost importance that the 2-dose prophylactic vaccine regimen is safe and leads to an  
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53 94 immune response, it is also crucial to find out whether or not this regimen can lead to induced immune  
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55 95 memory at the time of imminent risk (i.e. an outbreak) through a booster vaccination. To evaluate this  
56  
57 96 induced immune memory response, three previous studies within EBOVAC projects have administered  
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3 97 a booster vaccine with Ad26.ZEBOV at either 1 year (NCT02325050; NCT02564523) or 2 years  
4  
5 98 (NCT02509494) post-dose 1. Results from the NCT02325050 trial have already shown that an  
6  
7 99 immunological memory was rapidly induced via booster vaccination with Ad26.ZEBOV, indicating that  
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9  
10 100 booster vaccination can be considered for at risk individuals (e.g. when an outbreak occurs) that were  
11  
12 101 previously vaccinated with the 2-dose heterologous prophylactic regimen[18]. However, these trials  
13  
14 102 only evaluated booster vaccination in a small amount of participants ( $n \leq 39$ ) and it still has to be  
15  
16 103 explored whether the induced immune memory response differs depending on the timing of the  
17  
18 104 booster dose (i.e. 1 or 2 years after dose 1).

20  
21 105 Healthcare settings play an important role in the control of EVD and therefore health care providers  
22  
23 106 (HCP) and front-liners, due to occupational exposure, are not only more at risk of disease acquisition  
24  
25 107 but also facilitate the spread of the virus[19-22]. Knowing that outbreaks of EVD often occur in regions  
26  
27 108 where there is already a shortage of HCP and front-liners, this further depletes a weak health care  
28  
29 109 system and the quality of care. Consequently, the Strategic Advisory Group of Experts stated in 2018  
30  
31 110 that for preventive strategies, vaccination of HCP as part of an emergency preparedness plan has  
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33 111 significant potential of reducing the scale and duration of outbreaks[23].

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38 112 This phase 2 randomized clinical trial aims to determine the safety and immunogenicity of the 2-dose  
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40 113 heterologous vaccine regimen with Ad26.ZEBOV followed by MVA-BN-Filo 56 days later. Additionally,  
41  
42 114 this trial aims to assess the safety and immunogenicity of a booster Ad26.ZEBOV vaccine administered  
43  
44 115 either 1 or 2 years post first dose and to compare the induced immune memory response between  
45  
46 116 both booster arms. The trial is conducted in a cohort of HCP and front-liners from the Boende health  
47  
48 117 district in DRC, a well-known population at risk from clinical and epidemiological perspective.

## 51 52 118 **METHODS**

### 53 54 119 **Study design and setting**

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56  
57 120 This study is an open-label, monocentric, phase 2, randomized trial to evaluate the immunogenicity  
58  
59 121 and safety of Ad26.ZEBOV ( $5 \times 10^{10}$  viral particles) as first dose and MVA-BN-Filo ( $1 \times 10^8$  infectious units)

122 as second dose vaccination at a 56-day interval in HCP and front-liners who may be exposed to Ebola  
 123 in the event of a future Ebola outbreak in DRC. Additionally, after randomization (1:1) a booster of  
 124 Ad26.ZEBOV ( $5 \times 10^{10}$  viral particles) will be offered at either 1 year or 2 years after the first dose (Figure  
 125 1).

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

## 128 Objectives

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

131 **Table 1. Objectives and endpoints**

Objectives	Endpoints
<b>Primary</b>	
<ul style="list-style-type: none"> <li>To assess binding antibody responses post-dose 2 vaccination with MVA-BN-Filo.</li> </ul>	<ul style="list-style-type: none"> <li>Binding antibody levels against the EBOV GP using FANG ELISA at 21 days post-dose 2 (Day 78) vaccination with MVA-BN-Filo.</li> </ul>
<b>Secondary</b>	
<ul style="list-style-type: none"> <li>To assess binding antibody responses after booster vaccination with Ad26.ZEBOV given at 1 or 2 years after first dose.</li> </ul>	<ul style="list-style-type: none"> <li>Binding antibody levels against the EBOV GP using FANG ELISA at 7 days (excluding the day of vaccination) post booster.</li> </ul>
<ul style="list-style-type: none"> <li>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.</li> </ul>	<ul style="list-style-type: none"> <li>Serious adverse events from first dose vaccination until 6 months post booster.</li> <li>Solicited and unsolicited local and systemic adverse events until 7 days post booster vaccination (day of vaccination and subsequent 7 days) with Ad26.ZEBOV.</li> </ul>
<b>Exploratory</b>	
<ul style="list-style-type: none"> <li>To assess binding antibody responses at different time points as indicated in the Study time and events overview (Figure 1).</li> </ul>	<ul style="list-style-type: none"> <li>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).</li> </ul>

<ul style="list-style-type: none"> <li>To assess neutralizing antibody response directed against the Adenovirus vector prior to vaccination.</li> </ul>	<ul style="list-style-type: none"> <li>Neutralizing antibody levels against Ad26 using Ad26 VNA at the first visit.</li> </ul>
<ul style="list-style-type: none"> <li>To assess neutralizing antibody response directed against the MVA vector prior to vaccination.</li> </ul>	<ul style="list-style-type: none"> <li>Neutralizing antibody levels against MVA-BN-Filo using MVA PRNT assay at the first visit.</li> </ul>
<ul style="list-style-type: none"> <li>To assess seroprevalence of Ebola virus disease prior to vaccination.</li> </ul>	<ul style="list-style-type: none"> <li>Presence of pre-existing Human anti-EBOV GP IgG and anti-EBOV NP IgG using LUMINEX assay.</li> </ul>
<p><i>ELISA: enzyme-linked immunosorbent assay; EU/mL: ELISA units/mL; FANG: Filovirus Animal Nonclinical Group. VNA: Virus Neutralization Assay; PRNT: Plaque Reduction Neutralization Test</i></p>	

### 132 Participant population and sample size

133 A total of 700 Registered HCP and front-liners in DRC (working in the Boende General Reference  
 134 Hospital, Health Centres or Health Posts in the Boende health district) are planned to be recruited from  
 135 the Tshuapa province. This assessment was based on information obtained from an ongoing  
 136 (monkeypox) vaccine trial in the same area at the time the protocol was being written[24]. From  
 137 discussions with the monkeypox research group, it became clear that a high enrollment rate and  
 138 retention rate (>90% after two years) could be expected among HCP and front-liners in the Boende  
 139 health district. Based on this ongoing monkeypox trial, it was estimated that enrolling approximately  
 140 50% of the HCP and front-liners working in the Boende health district would be feasible. The participant  
 141 population is thus a convenience sample and the sample size is defined upon the feasibility of  
 142 recruitment of HCP and front-liners in the region.

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

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3 149 the first dose[25]. To obtain the effect size, the difference in geometric mean concentrations (log scale)  
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5 150 of the EBOV GP-specific antibody responses between the two groups was divided by the pooled  
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7 151 standard deviations[26]. With a power of 0.99 it will thus be possible to perform a formal comparative  
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9 152 analysis of the induced immune memory response of the two booster arms.

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12 153 Unfortunately no power analysis could be performed to determine whether the sample size is  
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14 154 sufficiently large to perform a formal statistical comparison of safety response (AEs and SAEs) from  
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16 155 both arms. In the current combined Investigator's Brochure of the vaccines[25], safety information is  
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18 156 pooled for all booster doses independent of the timing of its administration (1 year or 2 years post-  
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20 157 dose 1) and thus no effect size can be calculated until the unpooled data from the different trials is  
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22 158 obtained.

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27 159 Inclusion and exclusion criteria that determine the eligibility of participants are reported in Table 2.

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30 160 **Table 2. Inclusion and exclusion criteria**

<b>Inclusion criteria</b>
<p data-bbox="193 1120 1401 1164">Each potential participant must satisfy all of the following criteria to be enrolled in the study:</p> <ol data-bbox="193 1164 1401 2038" style="list-style-type: none"> <li data-bbox="193 1164 1401 1254">1. The participant must pass the Test of Understanding.  <i data-bbox="193 1254 1401 1388">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.</i></li> <li data-bbox="193 1388 1401 1881">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.</li> <li data-bbox="193 1881 1401 1926">3. The participant must be a man or women aged 18 years or older.</li> <li data-bbox="193 1926 1401 2038">4. The participant must be a documented HCP in DRC.</li> </ol>

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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  $\beta$ -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 contraception 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.

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3 5. The participant has an acute illness (this does not include minor illnesses such as mild  
4 diarrhea or mild upper respiratory tract infection) or temperature  $\geq 38.0^{\circ}\text{C}$  on Day 1.  
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6 Participants with such symptoms will be excluded from enrollment at that time, but may be  
7  
8 rescheduled for enrollment at a later date if feasible.  
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12 6. The participant is a pregnant or breastfeeding women, or women planning to become  
13 pregnant while enrolled in this study until at least 3 months after the Ad26.ZEBOV  
14 vaccination or 1 month after MVA-BM-Filo.  
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18 7. The participant has significant conditions or clinically significant findings at screening or vital  
19 signs for which, in the opinion of the investigator, participation would not be in the best  
20 interest of the participant (e.g. compromise the safety or well-being) or that could prevent,  
21 limit, or confound the protocol-specified assessments.  
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24  
25 *Note: Participants who have recently received treatment for acute, uncomplicated malaria*  
26 *are eligible for participation if at least 3 days have elapsed from the conclusion of a standard,*  
27 *recommended course of therapy for malaria; participants who are acutely ill with malaria at*  
28 *the time of screening should complete therapy and wait an additional 3 days after*  
29 *completion before screening for the study.*  
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33 *Note: Participants with sickle cell trait can be included.*  
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37 8. The participant had major surgery (per the investigator's judgment) within the 4 weeks prior  
38 to screening, or has planned major surgery during the study (from the start of screening  
39 onwards).  
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43 9. The participant had a post-organ and/or stem cell transplant whether or not with chronic  
44 immunosuppressive therapy.  
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48 10. The participant received an investigational drug or investigational vaccines or used an  
49 invasive investigational medical device within 3 months prior to screening, or current or  
50 planned participation in another clinical study during the study.  
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54 *Note: Participation in an observational clinical study is allowed.*  
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11. The participant has a history of chronic urticaria (recurrent hives).

161 **Randomization procedure**

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

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



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3 184 **Study procedures (Figure 1)**  
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6 185 At Day 1, interested participants are informed about the study and are required to pass a test of  
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8 186 understanding before providing written consent. No study activities are performed before the  
9  
10 187 participant has signed the informed consent form. Afterwards, the study medical doctor evaluates  
11  
12 188 his/her general health based on the inclusion criteria, vital signs (blood pressure, pulse/heart rate  
13  
14 189 [both at rest] and body temperature) are collected and a urine pregnancy test for women of  
15  
16 190 childbearing potential is performed. Further during this first visit, a blood sample is taken for baseline  
17  
18 191 testing of binding antibody level (i.e. humoral immune response) against EBOV glycoprotein (GP) using  
19  
20 192 Filovirus Animal Non-Clinical Group (FANG) Ebola virus enzyme-linked immunosorbent assay (ELISA)  
21  
22 193 and the presence of pre-existing Human anti-EBOV GP IgG and anti-EBOV nucleoprotein (NP) IgG using  
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24 194 LUMINEX assay. For Day 1 samples, both FANG ELISA and LUMINEX assay will be carried out. FANG  
25  
26 195 ELISA is performed for all EBOVAC trials in the same laboratory (for consistency and comparability) and  
27  
28 196 LUMINEX assay will provide a more detailed array of IgG antibodies that are not obtained via FANG  
29  
30 197 ELISA. For the first 100 enrolled participants an additional test on the collected serum is performed to  
31  
32 198 measure the neutralizing antibody level against Ad26 and MVA vectors using the Ad26 Virus  
33  
34 199 Neutralizing Assay (VNA) and MVA Plaque Reduction Immunogenicity Test (PRNT), respectively.  
35  
36 200 Subsequently, a blood sample for baseline safety assessment is collected to test haemoglobin,  
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38 201 haematocrit, blood cell count (white and red), platelet count, urea, creatinine and transaminases.  
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40 202 Then, participants are vaccinated with the first dose (Ad26.ZEBOV) and they are given instructions to  
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42 203 contact the study team for any occurring serious adverse events (SAEs), or in case of pregnancy of a  
43  
44 204 participant during the study. After vaccination, participants remain at the study site for an observation  
45  
46 205 period of 30 minutes to make sure no SAEs occur. SAEs are collected from first dose vaccination until  
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48 206 6 months post booster. Lastly on Day 1, randomization is performed to determine the timing of the  
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50 207 booster vaccine at either 1 year or 2 years after the first dose. Contact information is verified, an  
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52 208 appointment for the second dose on Day 57 is arranged and a participant card is printed. Innovatively,  
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3 209 next to a participant card, a biometric identification tool via iris scanning is foreseen to ensure correct  
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5 210 identification of the participants during all study related visits.  
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8 211 At Day 57, participants return to the study site for urine pregnancy testing (for women of childbearing  
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10 212 potential), vital signs measurement, assessment of safety (SAEs), a blood sample for immunogenicity  
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12 213 assessment (the binding antibody levels against EBOV GP using FANG ELISA) and afterwards  
13  
14 214 administration of the second vaccine (MVA-BN-Filo). After an observation period of 30 minutes,  
15  
16 215 participants are reminded to contact the study team for any SAEs that occurs, or in case of pregnancy  
17  
18 216 of a participant during the study. Contact information is verified and an appointment for the 21-day  
19  
20 217 post-dose 2 visit (Day 78) is arranged.  
21  
22

23  
24 218 At 21 days post-dose 2 (Day 78), all participants return to the study site for a safety assessment (SAEs)  
25  
26 219 and for the collection of a blood sample for immunogenicity assessment. Contact information is re-  
27  
28 220 verified and they are reminded to contact the study team in case of SAE occurrence, or in case of  
29  
30 221 pregnancy of a participant.  
31  
32

33  
34 222 To make sure no valuable information is missed, participants are contacted by phone to inquire about  
35  
36 223 any occurrence of pregnancies (female participants) and SAEs at approximately 6 months post-dose 2  
37  
38 224 vaccination.  
39  
40

41 225 At 1 year and 2 years after the first vaccine, when all participants return to the site, the clinical trial  
42  
43 226 staff inquires after the occurrence of SAEs and a blood sample is collected for immunogenicity  
44  
45 227 assessment of all participants (where applicable before administration of the booster dose).  
46  
47 228 Depending on the study arm, a booster vaccination with Ad26.ZEBOV is given either 1 or 2 years after  
48  
49 229 the first dose. Prior to vaccination, the general well-being of the participant is evaluated and urine  
50  
51 230 pregnancy testing (for women of childbearing potential), as well as a vital signs measurement are  
52  
53 231 performed. After vaccination, participants remain at the study site for a 30 minute observation period.  
54  
55 232 Participants are asked to collect solicited and unsolicited adverse events (AEs) in a participant diary  
56  
57 233 starting on the day of the vaccination and continuing for the subsequent 7 days.  
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3 234 At Day 8 post booster the safety data including solicited and unsolicited AEs is reviewed and a blood  
4  
5 235 sample for immunogenicity assessment is taken to document the immune response. Should any  
6  
7 236 solicited AEs persist at Day 8 post booster, participants are asked to continue monitoring these in their  
8  
9  
10 237 participant diary. Once the solicited AEs have resolved, they are asked to make an unscheduled visit at  
11  
12 238 the site so this information can be reported.

13  
14  
15 239 At 6 months post booster, all participants are contacted by phone and questioned about any SAEs or  
16  
17 240 pregnancies (female participants) that have occurred since the last vaccination.

18  
19  
20 241 The total duration of the study is 2 years and 6 months post-first dose. The study is considered  
21  
22 242 completed when the last participant has been contacted for the 6 months post booster phone call or  
23  
24 243 has left the study.

#### 25 26 27 28 244 **Study intervention**

29  
30 245 According to the predefined schedule (Figure 1), participants receive a 0.5 mL intramuscular injection  
31  
32 246 into the deltoid muscle of the upper arm with Ad26.ZEBOV or MVA-BN-Filo. The injection site should  
33  
34 247 be free from any injury, local skin conditions, or other issues that might interfere with the evaluation  
35  
36 248 of local reactions. Every vaccination is given in the opposite arm of the previous vaccination (unless  
37  
38 249 the opposite arm has a condition that prevents evaluating the arm after injection). No local or topical  
39  
40 250 anaesthetic is used prior to the injection.

41  
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44  
45 251 The second or booster vaccination is not administered if any of the following events occur at any time  
46  
47 252 after the first dose vaccination:

- 48  
49  
50 253 • A participant experiences anaphylaxis clearly attributable to vaccination with the study  
51  
52 254 vaccine; OR  
53  
54 255 • A participant experiences generalized urticaria within 72 hours of vaccination considered  
55  
56 256 to be related to study vaccine; OR

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3 257 • A participant experiences a serious adverse event considered to be related to the study  
4  
5 258 vaccine; OR  
6  
7  
8 259 • A participant experiences injection site ulceration, abscess or necrosis considered to be  
9  
10 260 related to the study vaccine; OR  
11  
12 261 • A participant has confirmed EVD; OR  
13  
14 262 • A female participant of childbearing potential has a positive urine  $\beta$ -human chorionic  
15  
16 263 gonadotropin ( $\beta$ -HCG) pregnancy test before vaccination (on Day 57, Year 1 or Year 2  
17  
18 [depending on the randomization group]); OR  
19 264  
20  
21 265 • A female participant of childbearing potential has a positive urine  $\beta$ -HCG pregnancy test  
22  
23 266 between dose 2 and the booster dose and is still pregnant or breastfeeding at the time of  
24  
25 267 the booster dose; OR  
26  
27  
28 268 • A participant takes a concomitant treatment with drugs that may alter the immune  
29  
30 269 response; OR  
31  
32  
33 270 • The principal investigator believes that for safety reasons it is in the best interest of a  
34  
35 271 participant to discontinue the study intervention.

36  
37  
38 272 Participants experiencing any of the events described above are still followed up for safety and  
39  
40 273 immunogenicity according to the protocol. The decision to discontinue the study intervention is at the  
41  
42 274 discretion of the principal investigator (University of Kinshasa) and after consultation with the sponsor  
43  
44 275 (University of Antwerp) for any of the events described above.

#### 46 47 276 **Patient and public involvement**

48  
49  
50 277 Difficulties were expected when setting up a clinical trial in Boende, a remote and resource-limited  
51  
52 278 area of DRC. However, to avoid and anticipate some of these challenges and in order to support  
53  
54 279 vaccination compliance, a collaboration is established between the study team and the Provincial  
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56 280 Division of Health. Throughout the trial, workshops are organized for HCP in the health district of  
57  
58  
59 281 Boende to sensitize and inform about EVD and other relevant medical topics. These gatherings should  
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2  
3 282 not only facilitate enrollment in the trial but also increase the engagement of participants by enhancing  
4  
5 283 their understanding of the clinical trial and the importance of adherence. During these workshops time  
6  
7 284 is available for questions and discussions. In addition to these gatherings for trial participants,  
8  
9  
10 285 community engagement activities and the training and capacity building of the local clinical trial team  
11  
12 286 that is executing the trial (under supervision of the University of Kinshasa as principal investigator) are  
13  
14 287 organised for the duration of the trial.

15  
16  
17 288 Each participant receives an individual visit schedule upon enrollment in the trial and when  
18  
19 289 participants miss a planned study visit, community health workers of the Ministry of Health trace the  
20  
21 290 individual participant. Consent is asked in the informed consent form for this mode of contact.

22  
23  
24 291 Furthermore, prior to the start of the clinical trial, a pilot study was performed whereby potential  
25  
26 292 participants were interviewed on the feasibility and acceptability of the use of a biometric iris scanning  
27  
28 293 tool for participant identification during the trial and the use of telephone messaging with visit  
29  
30 294 reminders for participant adherence[27].

### 31 32 33 34 295 **Data management**

35  
36  
37 296 All information is collected during study visits on source documents by study staff. These source  
38  
39 297 documents with confidential information are transcribed into the electronic clinical database by site  
40  
41 298 data managers. To make sure that all entered data (collected in DFExplore version 5.2.1) is correct, the  
42  
43 299 principal investigator reviews each source document and confirms its correct transcription in the  
44  
45 300 database. Additionally, the sponsor performs quality checks of the entered data in the database and,  
46  
47 301 during monitoring visits, source data verification is performed.

### 48 49 50 51 302 **Statistical analysis**

52  
53  
54 303 A differentiation in analysis will be made according to: 1) the *Full Analysis Set* (FAS; all participants who  
55  
56 304 received at least one dose, regardless of the occurrence of protocol deviations), 2) *Per Protocol Set for*  
57  
58 305 *primary vaccination series* (all vaccinated participants, who received both dose 1 and dose 2  
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3 306 [administered within the protocol-defined visit window] vaccinations, have at least 1 post-vaccination  
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5 307 [i.e. after the date of dose 1] evaluable immunogenicity sample, and have no major protocol deviations  
6  
7 308 influencing the immune response) and 3) *Per Protocol Set for the Booster vaccination* (includes all  
8  
9 309 participants in the per protocol set for the primary vaccination series who received a booster dose and  
10  
11 310 have at least 1 post booster vaccination evaluable immunogenicity sample, and have no major protocol  
12  
13 311 deviations influencing the immune response).

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16  
17 312 Participant information (i.e. demographics and baseline characteristics, disposition information,  
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19 313 treatment compliance, extent of exposure, protocol deviations and concomitant medications) is  
20  
21 314 planned to be tabulated and summarized with descriptive statistics for all participants. For continuous  
22  
23 315 data, such as age, the mean and standard deviation will be provided if applicable, otherwise the  
24  
25 316 geometric means, related standard deviations or median and interquartile ranges will be used.

26  
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28  
29 317 For the immunogenicity analysis, two *Per-Protocol Sets* will be used, i.e., the *Per-Protocol Set for*  
30  
31 318 *primary vaccination series* and the *Per-Protocol Set for the booster*. If more than 10% of participants  
32  
33 319 from the FAS are excluded from the per protocol immunogenicity set, the immunogenicity analysis will  
34  
35 320 be repeated on the FAS to evaluate the robustness of the analysis results. A subgroup analysis of the  
36  
37 321 immune response at different time points will be performed stratified by age (18-40, 40-60 and >60),  
38  
39 322 gender, prior vaccinia/smallpox vaccination, pre-existing Ebola antibodies (positive or negative for pre-  
40  
41 323 existing Human anti-EBOV GP IgG and anti-EBOV NP IgG, and for both), baseline immunogenicity  
42  
43 324 (positivity versus negativity for antibody levels against EBOV GP using FANG ELISA) and the presence  
44  
45 325 of neutralizing antibody levels against Ad26 and MVA vectors using Ad26 VNA and MVA PRNT assays  
46  
47 326 (only the first 100 enrolled participants), respectively. For these planned subgroup analyses, N (%),  
48  
49 327 Geometric Mean Concentrations and 95% confidence intervals (CI) will be provided as appropriate.  
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51 328 Finally, a formal comparative analysis of the induced immune memory response between the two  
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53 329 booster arms will be performed.  
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3 330 Safety analyses include SAEs collected during the whole study and solicited and unsolicited AEs for 1  
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5 331 week post booster vaccination. The analysis of SAEs will be performed using the FAS and the solicited  
6  
7 332 and unsolicited AEs will be analysed for the participants who received the booster vaccination.  
8  
9  
10 333 Continuous variables will be summarized using the following statistics: number of observations;  
11  
12 334 arithmetic or geometric mean/median (if applicable) with their related measures of dispersion (95%  
13  
14 335 CI for the mean, standard deviation or inter quartile range (Q1-Q3)). Minimum and maximum  
15  
16 336 frequencies and percentages (one decimal place) will be generated for categorical variables. If the  
17  
18 337 unpooled safety data from the NCT02564523 and NCT02509494 studies can be obtained, a power  
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20 338 analysis will be performed to assess whether the safety data of the two booster arms can be compared  
21  
22 339 through formal statistical analysis.

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26 340 The primary endpoint analysis is planned to be performed when all participants have completed the  
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28 341 21-day post-dose 2 visit (Day 78) or discontinued earlier. This analysis includes all available  
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30 342 immunogenicity and safety data up to this point (date cut-off). Additional interim analyses may be  
31  
32 343 performed during the study for the purpose of informing future vaccine-related decisions in a timely  
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34 344 manner.

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38 345 The final analysis will be performed when all participants have completed the last study-related phone  
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40 346 call (6 months post booster) or left the study.

## 41 42 43 347 **DISCUSSION**

44  
45 348 The aim of this phase 2 trial is to obtain further safety and immunogenicity data on the 2-dose  
46  
47 349 prophylactic heterologous Ebola vaccine regimen and to assess the safety and immunogenicity of a  
48  
49 350 booster dose with Ad26.ZEBOV administered either 1 or 2 years post first dose in a large cohort of HCP  
50  
51 351 and front-liners. By doing so, this study will feed the immunogenicity and safety databases of the  
52  
53 352 Ad26.ZEBOV and MVA-BN-Filo vaccines. It will also be the first study to compare the induced immune  
54  
55 353 memory response between two different booster arms in a large cohort of adults.  
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3 354 Boende (the capital city of Tshuapa province) was selected as the trial site for several reasons. First,  
4  
5 355 the Tshuapa province recovered from an EBOV outbreak that occurred in the Boende Health District  
6  
7 356 in 2014[21]. Following this outbreak, a study (n=565) conducted in the Tshuapa region in 2015 found  
8  
9 357 that 41.4% of the tested HCP were seroreactive to at least one EBOV protein and 2.8% of the HCP  
10  
11 358 showed a neutralizing capacity while never having developed EVD symptoms[20]. This observation  
12  
13 359 suggests a possible endemic EBOV exposure in the Tshuapa province of DRC. These are interesting  
14  
15 360 observations for future ecologic research as the ecology and reservoir(s) of EBOV and other filoviruses  
16  
17 361 remain largely unknown[28, 29]. Second, in addition to the previous outbreak of EVD, Boende was  
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19 362 chosen to perform the current clinical trial as there was expertise available after carrying out a phase  
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21 363 3 monkey pox vaccine trial that took place in 2017[24].

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26 364 Some limitations are present in the current set-up of the trial. First, by focussing on occupation  
27  
28 365 (registered HCP and front-liners) rather than age and gender, in the inclusion and exclusion criteria,  
29  
30 366 the aim is to easily reach the target of 700 participants. However, a recent review by Flanagan et al.  
31  
32 367 (2017) has shown that immune responses to vaccination can differ based on gender and age[30]. To  
33  
34 368 take this limitation into account, stratification for age and gender has been foreseen during statistical  
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36 369 analysis. Second, while HIV-positive participants can participate in this trial if their general condition is  
37  
38 370 good, it is not possible to be certain of the HIV-status of all participants as no routine checks prior to  
39  
40 371 enrollment or during the course of the trial are foreseen. It is possible that some participants either  
41  
42 372 are unwilling to share their HIV-positive status as a consequence of the stigma that is often linked to  
43  
44 373 it[31] or are simply unaware of their positive status (e.g. during an asymptomatic phase of the  
45  
46 374 disease[32]). However, due to the low prevalence (0.6%) of HIV-positive people in the province of the  
47  
48 375 trial[31], it was chosen not to perform routine checks and to trust the willingness of a participant to  
49  
50 376 share his/her status as it is not considered an exclusion criterium for the trial. Finally, at the start of  
51  
52 377 the project the protocol initially only included a vaccination strategy with the 2-dose heterologous  
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54 378 vaccine regimen (Ad26.ZEBOV followed by MVA-BN-Filo 56 days later) and was later adapted to include  
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56 379 a booster vaccination at the request of the vaccine producer. The purpose of the initial observational  
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3 380 trial was, next to obtaining additional immunogenicity data, a way to see if performing a vaccination  
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5 381 trial in a remote area of DRC was feasible and accepted by the population. While writing the protocol  
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7 382 however, administering a booster dose in this large cohort was added as a novel aspect and thus this  
8  
9 383 was entered as a secondary objective/endpoint. Currently it is unknown whether this booster dose will  
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11 384 be required or not at the moment of an outbreak and what its protective effect would be. However,  
12  
13 385 to explore its safety and immunogenicity, this study protocol was transformed and became a  
14  
15 386 randomized clinical trial. Unfortunately, as the comparison of the two booster arm induced immune  
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17 387 responses is not required for approval of the licensure of the 2-dose heterologous vaccine regimen  
18  
19 388 and the booster dose was added as a second stage to the study design, no sample size calculations  
20  
21 389 were initially performed for this trial and sample size was selected based on available information from  
22  
23 390 a previous monkeypox vaccine trial in the same area. While this trial thus mainly has a descriptive set-  
24  
25 391 up, scientifically it is interesting to learn if there is a significant difference in the induced immune  
26  
27 392 memory response of the two booster arms. For this reason, a power analysis was retrospectively  
28  
29 393 performed to determine whether it would be possible to compare the induced immune memory  
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31 394 response of the two arms. Fortunately this will be possible as a power of 0.99 was calculated and a  
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33 395 formal statistical comparison induced immune memory response of the two booster arms has now  
34  
35 396 been foreseen in the Statistical Analysis Plan. It is however important to take into account that a  
36  
37 397 varying antibody response after booster vaccination is not necessarily directly correlated with  
38  
39 398 protective vaccine efficacy[33] and that a high power (99% for this study) can lead to significant  
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41 399 differences, even if the difference between both groups is small. Prudent and careful interpretation of  
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43 400 the results will thus be crucial[34].  
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50 401 In conclusion, because EVD remains a very deadly disease, effective and safe preventive measures will  
51  
52 402 play a crucial role to protect vulnerable communities. While the prophylactic heterologous 2-dose  
53  
54 403 regimen was recently granted market authorisation by the European Commission, further research  
55  
56 404 into the safety and immunogenicity of the 2-dose regimen is still required to obtain worldwide  
57  
58 405 licensure of the regimen. Furthermore, limited data has previously been collected on the safety and  
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3 406 immunogenicity of a booster dose with Ad26.ZEBOV. This is the first large, randomized vaccine trial  
4  
5 407 that assesses and compares the safety and immunogenicity of two different booster arms in a large  
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7 408 cohort.  
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## 10 409 **ETHICS AND DISSEMINATION**

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12  
13 410 This protocol was submitted and approved by the National Ethics Committee of the Ministry of Health  
14  
15 411 of the Democratic Republic of Congo (approval number: n°121/CNES/BN/PMMF/2019). Prior to being  
16  
17 412 enrolled in the trial, all participants are required to provide written informed consent by signing the  
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19 413 informed consent form after having performed a test of understanding. If the participant is unable to  
20  
21 414 read or write, an impartial witness should be present for the entire informed consent process (which  
22  
23 415 includes reading and explaining all written information) and should personally date and sign the  
24  
25 416 informed consent form after the oral consent of the participant is obtained. No study-related  
26  
27 417 procedures are performed until the participant has signed the informed consent form.  
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32 418 The trial was registered on Clinicaltrial.gov on December 4<sup>th</sup>, 2019 (NCT04186000) and recruitment  
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34 419 started on December 18<sup>th</sup>, 2019. All participants were recruited by the 8<sup>th</sup> of February 2020 and the  
35  
36 420 study is planned to finish in October, 2022. Results of the trial will be entered on Clinicaltrial.gov,  
37  
38 421 published in peer-reviewed journals and presented at international conferences.  
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40

## 41 422 **DECLARATIONS**

### 42 423 **Author contributions**

43  
44  
45 424 YL wrote the manuscript. TZ, ES, YL, VM, JM, PM, HMM, JPVG and PVD wrote the initial English protocol  
46  
47 425 on which this manuscript is based. TZ, VM, PM, JM and HMM translated the English protocol into  
48  
49 426 French for submission to the National Ethics Committee and the “Direction de la Pharmacie et des  
50  
51 427 Médicaments” of the Ministry of Health of the Democratic Republic of Congo as well as the National  
52  
53 428 Scientific committee against Ebola. All authors (YL, TZ, ES, JDB, VM, JM, PM, HMM, JPVG and PVD)  
54  
55 429 reviewed and contributed to the final manuscript.  
56  
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**445 Competing interests**

446 The authors declare that they have no competing interests.

**447 REFERENCES**

- 448 1. Baseler L, Chertow DS, Johnson KM, Feldmann H, Morens DM. The Pathogenesis of Ebola Virus  
449 Disease. *Annu Rev Pathol.* 2017;12:387-418.
- 450 2. Muyembe-Tamfum JJ, Mulangu S, Masumu J, Kayembe JM, Kemp A, Paweska JT. Ebola virus  
451 outbreaks in Africa: past and present. *Onderstepoort J Vet Res.* 2012;79(2):451.
- 452 3. Rewar S, Mirdha D. Transmission of ebola virus disease: an overview. *Ann Glob Health.*  
453 2014;80(6):444-51.

- 1  
2  
3 454 4. Rouquet P, Froment JM, Bermejo M, Kilbourn A, Karesh W, Reed P, et al. Wild animal mortality  
4  
5 455 monitoring and human Ebola outbreaks, Gabon and Republic of Congo, 2001-2003. *Emerg Infect Dis.*  
6  
7 456 2005;11(2):283-90.  
8  
9  
10 457 5. World Health Organization. Ebola virus disease. Fact sheet N 103. Updated September 2014.  
11  
12 458 6. Report of an International Commission. Ebola haemorrhagic fever in Zaire, 1976. *Bull World*  
13  
14 459 *Health Organ.* 1978;56(2):271-93.  
15  
16 460 7. Malvy D, McElroy AK, de Clerck H, Günther S, van Griensven J. Ebola virus disease. *The Lancet.*  
17  
18 461 2019;393(10174):936-48.  
19  
20  
21 462 8. World health Organization. Ebola - North Kivu, Democratic Republic of the Congo, 2021 2021  
22  
23 463 [Available from: <https://www.who.int/emergencies/situations/ebola-2021-north-kivu>.  
24  
25 464 9. International Committee on Taxonomy. Virus Metadata Repository: version May 1, 2020;  
26  
27 465 MSL35.  
28  
29  
30 466 10. World Health Organization. Situation Report Ebola Virus Disease.  
31  
32 467 <http://apps.who.int/ebola/ebola-situation-reports>; 10 June 2016.  
33  
34 468 11. Ebovac. EBOVAC3 2020 [Available from: <https://www.ebovac.org/ebovac-3/>.  
35  
36 469 12. European Commission. Vaccine against Ebola: Commission grants new market authorisation  
37  
38 470 July 2020 [Available from: [https://ec.europa.eu/commission/presscorner/detail/en/ip\\_20\\_1248](https://ec.europa.eu/commission/presscorner/detail/en/ip_20_1248).  
39  
40  
41 471 13. Anywaine Z, Whitworth H, Kaleebu P, Praygod G, Shukarev G, Manno D, et al. Safety and  
42  
43 472 Immunogenicity of a 2-Dose Heterologous Vaccination Regimen With Ad26.ZEBOV and MVA-BN-Filo  
44  
45 473 Ebola Vaccines: 12-Month Data From a Phase 1 Randomized Clinical Trial in Uganda and Tanzania. *J*  
46  
47 474 *Infect Dis.* 2019;220(1):46-56.  
48  
49  
50 475 14. Milligan ID, Gibani MM, Sewell R, Clutterbuck EA, Campbell D, Plested E, et al. Safety and  
51  
52 476 Immunogenicity of Novel Adenovirus Type 26- and Modified Vaccinia Ankara-Vectored Ebola Vaccines:  
53  
54 477 A Randomized Clinical Trial. *Jama.* 2016;315(15):1610-23.  
55  
56  
57 478 15. Mutua G, Anzala O, Luhn K, Robinson C, Bockstal V, Anumendem D, et al. Safety and  
58  
59 479 Immunogenicity of a 2-Dose Heterologous Vaccine Regimen With Ad26.ZEBOV and MVA-BN-Filo Ebola  
60

- 1  
2  
3 480 Vaccines: 12-Month Data From a Phase 1 Randomized Clinical Trial in Nairobi, Kenya. *J Infect Dis.*  
4  
5 481 2019;220(1):57-67.  
6  
7 482 16. Shukarev G, Callendret B, Luhn K, Douoguih M. A two-dose heterologous prime-boost vaccine  
8  
9 483 regimen eliciting sustained immune responses to Ebola Zaire could support a preventive strategy for  
10  
11 484 future outbreaks. *Hum Vaccin Immunother.* 2017;13(2):266-70.  
12  
13  
14 485 17. Safety and immunogenicity of a two-dose heterologous Ad26.ZEBOV and MVA-BN<sup>®</sup>-Filo Ebola  
15  
16 486 vaccine regimen: a phase 2 randomised clinical study in Europe (EBOVAC2). 29th ECCMID; 2019;  
17  
18 487 Amsterdam, Netherlands.  
19  
20  
21 488 18. Goldstein N, Bockstal V, Bart S, Luhn K, Robinson C, Gaddah A, et al. Safety and Immunogenicity  
22  
23 489 of Heterologous and Homologous 2-Dose Regimens of Adenovirus Serotype 26—and Modified Vaccinia  
24  
25 490 Ankara—Vectored Ebola Vaccines: A Randomized, Controlled Phase 1 Study. *The Journal of Infectious*  
26  
27 491 *Diseases.* 2020.  
28  
29  
30 492 19. Evans DK, Goldstein M, Popova A. Health-care worker mortality and the legacy of the Ebola  
31  
32 493 epidemic. *Lancet Glob Health.* 2015;3(8):e439-e40.  
33  
34 494 20. Hoff NA, Mukadi P, Doshi RH, Bramble MS, Lu K, Gadoth A, et al. Serologic Markers for  
35  
36 495 Ebolavirus Among Healthcare Workers in the Democratic Republic of the Congo. *J Infect Dis.*  
37  
38 496 2019;219(4):517-25.  
39  
40  
41 497 21. Maganga GD, Kapetshi J, Berthet N, Kebela Ilunga B, Kabange F, Mbala Kingebeni P, et al. Ebola  
42  
43 498 virus disease in the Democratic Republic of Congo. *N Engl J Med.* 2014;371(22):2083-91.  
44  
45  
46 499 22. Nanclares C, Kapetshi J, Lionetto F, de la Rosa O, Tamfun JJ, Alia M, et al. Ebola Virus Disease,  
47  
48 500 Democratic Republic of the Congo, 2014. *Emerg Infect Dis.* 2016;22(9):1579-86.  
49  
50  
51 501 23. World Health Organization. Meeting of the Strategic Advisory Group of Experts on  
52  
53 502 Immunization, October 2018—Conclusions and recommendations. *Weekly Epidemiological Record.*  
54  
55 503 2018;93(49):661-79.  
56  
57  
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2  
3 504 24. Petersen BW, Kabamba J, McCollum AM, Lushima RS, Wemakoy EO, Muyembe Tamfum JJ, et  
4  
5 505 al. Vaccinating against monkeypox in the Democratic Republic of the Congo. *Antiviral Res.*  
6  
7 506 2019;162:171-7.  
8  
9  
10 507 25. Janssen Vaccines & Prevention B.V. Investigator's Brochure VAC52150 (Ad26.ZEBOV, MVA-BN-  
11  
12 508 Filo [MVA-mBN226B]). 1 ed 18 December 2020. 163 p.  
13  
14 509 26. Robert I. Kabacoff. *Power Analysis 2017* [Available from:  
15  
16 510 <https://www.statmethods.net/stats/power.html>.  
17  
18  
19 511 27. Zola Matuvanga T, Johnson G, Larivière Y, Esanga Longomo E, Matangila J, Maketa V, et al. Use  
20  
21 512 of Iris Scanning for Biometric Recognition of Healthy Adults Participating in an Ebola Vaccine Trial in  
22  
23 513 the Democratic Republic of the Congo: Mixed Methods Study. *J Med Internet Res.* 2021;23(8):e28573.  
24  
25 514 28. Gryseels S, Mbala-Kingebeni P, Akonda I, Angoyo R, Ayoub A, Baelo P, et al. Role of Wildlife  
26  
27 515 in Emergence of Ebola Virus in Kaigbono (Likati), Democratic Republic of the Congo, 2017. *Emerg Infect*  
28  
29 516 *Dis.* 2020;26(9):2205-9.  
30  
31  
32 517 29. Marí Saéz A, Weiss S, Nowak K, Lapeyre V, Zimmermann F, Dux A, et al. Investigating the  
33  
34 518 zoonotic origin of the West African Ebola epidemic. *EMBO Mol Med.* 2015;7(1):17-23.  
35  
36  
37 519 30. Flanagan KL, Fink AL, Plebanski M, Klein SL. Sex and Gender Differences in the Outcomes of  
38  
39 520 Vaccination over the Life Course. *Annu Rev Cell Dev Biol.* 2017;33:577-99.  
40  
41 521 31. UNICEF. DEUXIÈME ENQUÊTE DÉMOGRAPHIQUE ET DE SANTÉ (EDS-RDC II 2013-2014). 2014.  
42  
43 522 32. Moir S, Chun TW, Fauci AS. Pathogenic mechanisms of HIV disease. *Annu Rev Pathol.*  
44  
45 523 2011;6:223-48.  
46  
47  
48 524 33. Meyer M, Malherbe DC, Bukreyev A. Can Ebola Virus Vaccines Have Universal Immune  
49  
50 525 Correlates of protection? *Trends Microbiol.* 2019;27(1):8-16.  
51  
52  
53 526 34. Colquhoun D. The reproducibility of research and the misinterpretation of p-values. *R Soc*  
54  
55 527 *Open Sci.* 2017;4(12):171085.  
56  
57 528 35. d-maps.com, cartographer Map DR of the Congo: boundaries, provinces. [https://d-](https://d-maps.com/carte.php?num_car=4886&lang=en2020)  
58  
59 529 [maps.com/carte.php?num\\_car=4886&lang=en2020](https://d-maps.com/carte.php?num_car=4886&lang=en2020).  
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3 530 **FIGURE TITLES AND LEGENDS**  
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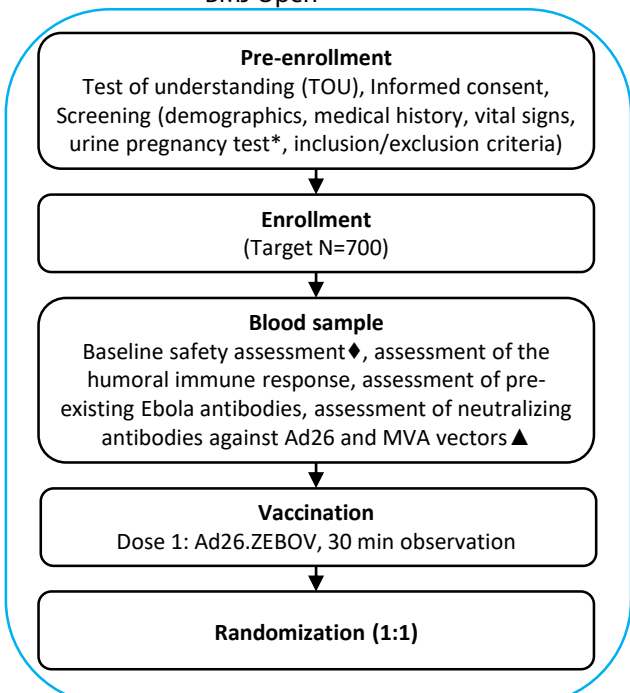
5 531 **Figure 1. Study time and events overview**  
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8 532 SAE: Serious Adverse Event; \* Only for female participants of childbearing potential; ♦ Abnormal  
9 533 results will not exclude a participant, as results will not be reviewed prior to enrollment; ▲ Only the  
10 534 first 100 participants enrolled will be tested for neutralizing antibody response against ad26 VNA and  
11 535 MVA vectors. Other blood analyses are for all 700 participants; ▼ Concomitant therapies given in  
12 536 conjunction with a serious adverse event (SAE) should be recorded from signing of the ICF onwards  
13 537 until 6 months post booster; ▽ The Investigator may withhold the second vaccine or booster dose if a  
14 538 participant's clinical status changes prior to vaccination. The participant should continue to be followed  
15 539 for safety and immunogenicity according to the protocol; Δ Only for female participants; ✱ Solicited  
16 540 and unsolicited Adverse Events (AEs) will be collected in a participant diary during 1 week post booster  
17 541 vaccination.  
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32 542 **Figure 2. Study site location**  
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35 543 On the left, the Democratic Republic of Congo (DRC) is highlighted on a map of the African continent.  
36 544 On the right, the study site location (Boende, Tshuapa province) is marked on a map of DRC indicating  
37 545 its provinces[35].  
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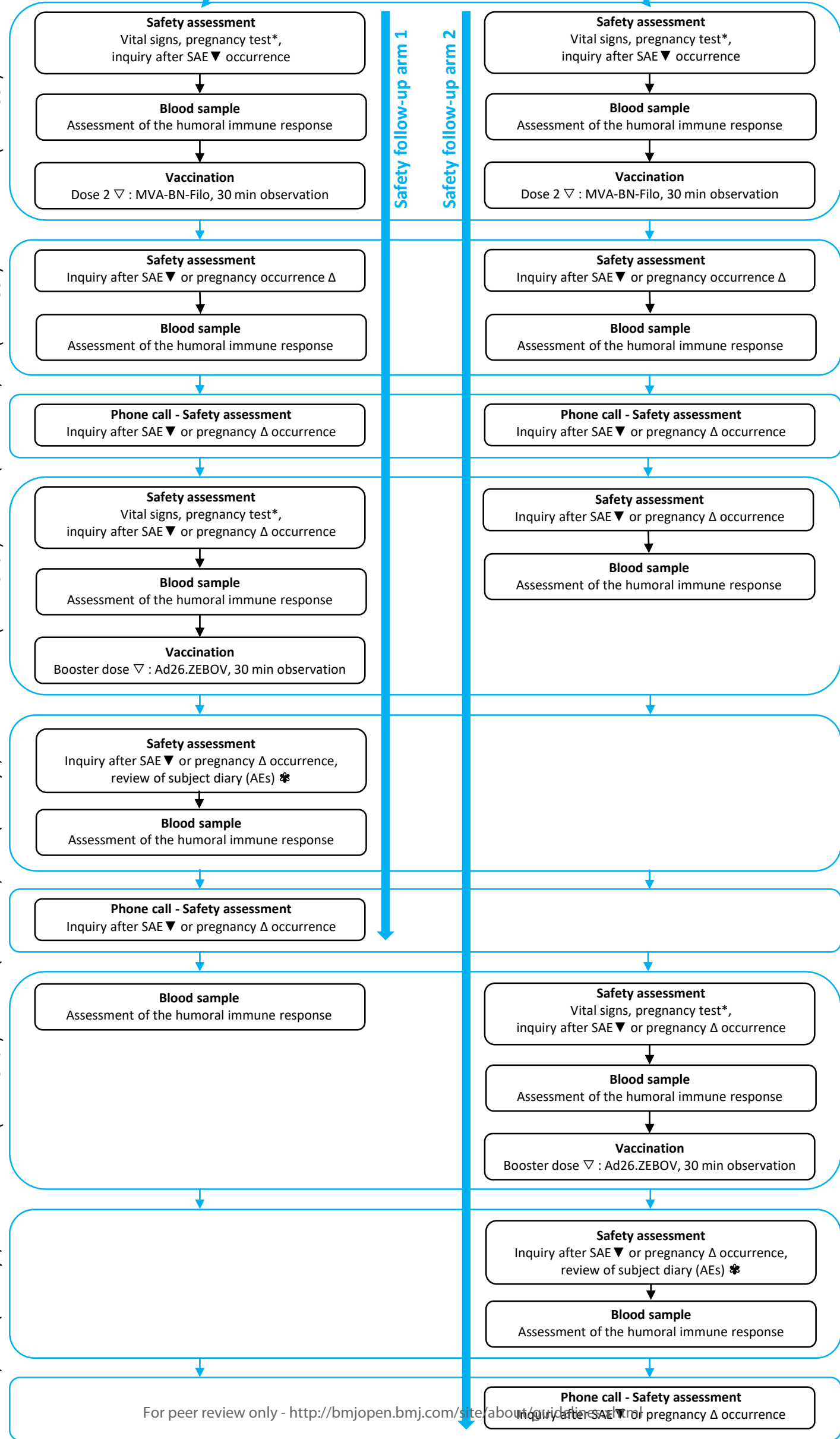


**Study arm 1 (N=350)**

**Study arm 2 (N=350)**

**Safety follow-up arm 1**

**Safety follow-up arm 2**





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

Section/item	ItemNo	Description	Location in manuscript
<b>Administrative information</b>			
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
<b>Introduction</b>			
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
<b>Methods: Participants, interventions, and outcomes</b>			
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
<b>Methods: Assignment of interventions (for controlled trials)</b>			
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
<b>Methods: Data collection, management, and analysis</b>			
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
<b>Methods: Monitoring</b>			
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
<b>Ethics and dissemination</b>			
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
<b>Appendices</b>			
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