Universiteit Antwerpen

This item is the archived peer-reviewed author-version of:

Ad26.ZEBOV, MVA-BN-Filo Ebola virus disease vaccine regimen plus Ad26.ZEBOV booster at 1 year versus 2 years in health-care and front-line workers in the Democratic Republic of the Congo : secondary and exploratory outcomes of an open-label, randomised, phase 2 trial

Reference:
Larivière Ynke, Zola Matuvanga Trésor, Osangir Bernard, Milolo Solange, Meta Rachel, Kimbulu Primo, Robinson Cynthia, Katwere Michael, McLean Chelsea, Lemey Gwen, ...- Ad26.ZEBOV, MVA-BN-Filo Ebola virus disease vaccine regimen plus Ad26.ZEBOV booster at 1 year versus 2 years in health-care and front-line workers in the Democratic Republic of the Congo : secondary and exploratory outcomes of an open-label, randomised, phase 2 trial
The lancet infectious diseases - ISSN 1473-3099 - (2024), p. 1-14
Full text (Publisher's DOI): https://doi.org/10.1016/S1473-3099(24)00058-6
To cite this reference: https://hdl.handle.net/10067/2046720151162165141

uantwerpen.be
Institutional repository IRUA

Long-term immunogenicity of the Ad26.ZEBOV, MVA-BN-Filo vaccine regimen and safety of and immune memory response to Ad26.ZEBOV booster vaccination in healthcare and frontline workers of the Democratic Republic of the Congo: an open-label, randomised, phase 2 trial

Ynke Larivière (0000-0002-5422-0194)[1,2], Trésor Zola Matuvanga (0000-0002-5830-415X)[1,2,4], Bernard Isekah Osang'ir (0000-0002-5557-3602)[1,2], Solange Milolo (0000-0003-2507-4041)[3], Rachel Meta (0000-0003-3586-459X)[3], Primo Kimbulu (0009-0002-2928-8522)[3], Cynthia Robinson[4], Michael Katwere[4], Chelsea McLean (0000-0002-3548-3482)[4], Gwen Lemey (0000-0002-2879-5330)[1,2], Junior Matangila (0000-0002-9025-3604)[3], Vivi Maketa (0000-0002-9007-1376)[3], Patrick Mitashi (0000-0002-6589-2869)[3], Jean-Pierre Van geertruyden (0000-0001-5006-6364)[2], Pierre Van Damme (0000-0002-8642-1249) [1,¥], Hypolite Muhindo-Mavoko (0000-0002-3307-3324)[3,¥]*

[1] Centre for the Evaluation of Vaccination, Vaccine and Infectious Disease Institute, University of Antwerp, Wilrijk, Belgium (Y Larivière MSc, T Zola MD, Bernard Isekah Osang'ir MSc, Gwen Lemey MA, Prof P Van Damme MD PhD)

[2] Global Health Institute, Department of Family Medicine and Population Health, University of Antwerp, Wilrijk, Belgium (Y Larivière MSc, T Zola MD, Bernard Isekah Osangir MSc, Gwen Lemey MA, Prof JP Van geertruyden MD PhD)

[3] Tropical Medicine Department, University of Kinshasa, Kinshasa, Democratic Republic of the Congo (T Zola MD, Prof V Maketa MD PhD, Prof J Matangila MD PhD, Prof P Mitashi MD PhD, Prof H Muhindo-Mavoko MD PhD)

[4] Janssen Vaccines and Prevention, Leiden, the Netherlands (C Robinson MD, M Katwere MD, C McLean PhD)

*Corresponding author:

Ynke Larivière

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

E-mail : ynke.lariviere@uantwerpen.be; phone : 0032 3 265 9716

¥ Joint last authorship

Word count abstract: 514

Word count (Introduction to end of Discussion): 4935

Summary

Background: Healthcare providers and frontliners are at risk of contracting Ebola virus disease during an Ebola outbreak and consequently of becoming drivers of the disease. We aimed to assess the long-term immunogenicity of the Ad26.ZEBOV, MVA-BN-Filo vaccine regimen and the safety of and the immune memory response to an Ad26.ZEBOV booster vaccination one or two years after the first dose in this at risk population.

Methods: This open-label, single-centre, randomised, phase 2 trial was conducted in Boende, Democratic Republic of the Congo. Adult healthcare providers and frontliners, excluding those with a known history of Ebola virus disease, were vaccinated with a two-dose heterologous regimen administered at a 56-day interval, comprising Ad26.ZEBOV as first dose and MVA-BN-Filo as second dose. After the initial vaccination on Day 1, participants were randomly assigned (1:1) using randomisation envelopes, opened in a sequential order, to receive an Ad26.ZEBOV booster vaccination at one (Arm 1) or two years (Arm 2) after the first dose. Here we present the secondary and exploratory objectives of the trial; results of the primary objective have been published elsewhere. Immunogenicity was measured at 6 timepoints per randomisation arm using Ebola virus glycoprotein-specific immunoglobulin G binding antibody geometric mean concentrations (GMCs), using the Filovirus Animal Non-Clinical Group ELISA. We assessed serious adverse events (SAEs) occurring up to 6 months after the last dose and local and systemic solicited and unsolicited adverse events (AEs) reported for 7 days after booster vaccination. Antibody responses were analysed per protocol, SAEs per full analysis set (FAS), and AEs for all boosted FAS participants. This trial is registered as completed on ClinicalTrials.gov, NCT04186000.

Findings: Between 18 December 2019 and 8 February 2020, 699 healthcare providers and frontliners were enrolled and 698 were randomized (350 to Arm 1, 348 to Arm 2). In both arms,

injection site pain/tenderness (87 [27%] of 319 Arm 1 participants; 90 [28%] of 317 Arm 2 participants) and headache (91 [29%] of 319 Arm 1 participants; 93 [29%] of 317 Arm 2 participants) were the most common local and systemic AE considered related to the investigational product, respectively. One participant experienced a related SAE after booster vaccination (fever of ≥40·0°C). One and two years after the first dose and before booster vaccination, GMCs (95%CI) of 279·9 Elisa Units (EU)/mL (250·6-312·7) and 274·6 EU/mL (242·1-311·5) were observed among Arm 1 and Arm 2 participants, respectively. These values were 5·2 and 4·9 times higher than pre-vaccination on Day 1, respectively. Seven days after booster vaccination, these values increased to 10,781·6 EU/mL (9,354·4-12,426·4) for Arm 1 and 10,746·9 EU/mL (9,208·7-12,542·0) for Arm 2, which was approximately 39 times higher than pre-booster vaccination among both arms. One year after booster vaccination, a 7·6-fold increase in GMC compared to pre-booster vaccination was still observed among Arm 1 participants (2,133·1 EU/mL (95% CI 1,827·7-2,489·7)).

Interpretation: Overall, the vaccine regimen and booster dose were well-tolerated. A similar and robust humoral immune response was reported among participants boosted one and two years after the first dose, supporting the use of the regimen and flexibility of booster dose administration for prophylactic vaccination in at risk populations.

Funding: Innovative Medicines Initiative 2 Joint Undertaking and Coalition for Epidemic Preparedness Innovations.

Research in context

Evidence before this study

The Ad26.ZEBOV, MVA-BN-Filo heterologous two dose regimen, administered at a 56-day interval, was granted marketing authorisation for use under "exceptional circumstances" by the European Medicines Agency in 2020 as prophylactic vaccination against Ebola virus disease

caused by Zaire ebolavirus in children and adults. To assess the safety, immunogenicity and durability of this regimen, we searched the terms *"Ad26.ZEBOV" AND "MVA-BN-Filo" AND ("safety" OR "immunogenicity" OR "durability")* in PubMed on January 3, 2024. No restrictions were placed on the type of article, publication time frame or language. In total, 29 articles were identified. Additionally, to identify existing research on Ad26.ZEBOV booster vaccination, the search string was amended to include *AND "Boost*"* in PubMed on January 3, 2024. Thirteen articles were identified, all of which were also part of the original search output.

Vaccine trials assessing the Ad26.ZEBOV, MVA-BN-Filo vaccine regimen have been conducted in infants (<1 year old), children (1-11 years old), adolescents (12-17 years old), healthy adults, HIV-infected adults with well-controlled infection and on highly active antiretroviral therapy, and children and adults with malaria exposure before and after vaccination. All reported that the vaccine regimen was generally well-tolerated with mostly mild to moderate adverse events reported.

The humoral immune responder rate 21 days after the heterologous two-dose vaccine regimen, administered at a 56-day interval (defined as having a ≥2·5-fold increase in binding antibody concentration over baseline, i.e., before administration of the first dose), ranged between 95% and 100%, depending on the study and population.

In adults, the long-term immunogenicity after primary vaccination has been assessed at 6 months, 8 months, 1 year, 2 years, and 4.5 years. Throughout the articles, a decrease in antibodies is described until 6 months after the first dose, with a stabilisation in geometric mean concentrations (GMCs) thereafter.

Four studies previously reported an Ad26.ZEBOV booster administration, one or more years after the initial dose of the Ad26.ZEBOV, MVA-BN-Filo regimen administered at a 56-day

interval. One was in children and adolescents (N=50), whereby a booster dose was administered more than 3 years after the first dose of the regimen. Among boosted children and adolescents, the responder rate was 100%. Two were in healthy adults; one (N=39) administered a booster dose one year after the first dose and the other (N=29) administered a booster dose two years after the first dose. Overall, the safety profile of the booster dose did not notably differ from the first Ad26.ZEBOV dose and no vaccine-related serious adverse events were reported. Seven days after booster vaccination a 100% responder rate was observed among participants boosted after one year and a 96% responder rate was observed in the participant group boosted after two years. In both studies, responses persisted in 100% of the participants, one year after booster vaccination. Finally, one was in HIV+ adults (N=13) with well-controlled infection and on highly active antiretroviral therapy and administered a booster dose 4.5 years after the first dose. Seven days after booster vaccination a 100% responder rate was observed.

Added value of this study

This study reports the secondary and exploratory outcomes of a trial whose primary endpoint has been reported elsewhere. To the best of our knowledge, this is, to date, the largest published adult (i.e., healthcare providers and frontliners) cohort study assessing the safety, and long-term immunogenicity (up to two years after the first dose) of the Ad26.ZEBOV, MVA-BN-Filo vaccine regimen administered at a 56-day interval. Additionally, this is, to date, the largest trial assessing the safety and humoral immune memory response after an Ad26.ZEBOV booster vaccination, and the first trial to compare, in an exploratory analysis, the humoral immune memory response of an Ad26.ZEBOV booster dose one or two years after the first dose among the same study population.

Implications of all available evidence

Previous studies combined with our study findings show that vaccination with the Ad26.ZEBOV, MVA-BN-Filo vaccine regimen at a 56-day interval is generally well tolerated,

leads to a persistent immune response, and a humoral immune memory response can be elicited with an Ad26.ZEBOV booster vaccination in adults at least as long as two years after initial vaccination. These finding supports the strategy of prophylactic regimen vaccination in HCP and frontliner populations, at higher risk of contracting and spreading Ebola virus disease than the general population, to minimize the impact of the next outbreak in Ebola endemic locations. Booster vaccinations provide a similar and robust humoral immune memory response at one and two years after the first dose, indicating flexibility in booster administration timing (e.g. when an outbreak occurs).

Introduction

Healthcare providers (HCP) are at higher risk of contracting and spreading Ebola virus disease (EVD) than the general population during an Ebola outbreak or epidemic. During the West African Ebola epidemic (2013-2016), a disproportionate amount of EVD deaths was observed between the general population in Guinea, Sierra Leone and Liberia (<0·2%) and the countries' doctors, nurses and midwives (1·5%-8·1%, depending on the country).[1] Such a decrease in the HCP-population can have a considerable impact on healthcare provision and consequently on population health in low and middle income countries (LMICs) already struggling with shortages in skilled HCP.[1,2] Preventing devastating consequences of future Ebola outbreaks and preparing Ebola endemic areas against the next outbreak is therefore crucial.

The two-dose heterologous Ad26.ZEBOV, MVA-BN-Filo Ebola virus vaccine regimen consists of a monovalent, recombinant, replication-incompetent, adenovirus type 26 (Ad26) vector-based vaccine, encoding the EBOV glycoprotein (GP) of the Mayinga variant (Ad26.ZEBOV), and a multivalent, recombinant, replication-incompetent, modified vaccinia Ankara (MVA) vector-based vaccine, encoding GPs from the EBOV Mayinga variant, Sudan

virus Gulu variant, and Marburg Musoke variant and the nucleoprotein from the Tai Forest virus (MVA-BN-Filo). The regimen has shown to be safe and immunogenic.[3-8]

Though determining clinical vaccine efficacy of the regimen has not been possible through vaccine trials in humans, the heterologous vaccine regimen was protective in challenged non-human primates.[9] Through immunobridging, researchers inferred the likelihood of the vaccine regimen's protective effect in humans from its protective effect in animals during animal challenge studies, and found that the vaccine regimen will likely provide protection against EBOV disease in humans.[10,11] Modelling studies have shown that prophylactic vaccination of HCP and frontliners would be the most effective way to reduce EVD and its related morbidity and mortality, even at a 30% vaccine coverage and 50-60% vaccine efficacy.[12,13]

At the time of this writing, the Democratic Republic of the Congo (DRC) has been victim to fifteen EVD outbreaks since its discovery in 1976.[14] We previously reported safety and immunogenicity results following the primary vaccine regimen in HCP and frontliners living and working in the Boende health zone, DRC.[8] However, limited information has been published on the persistence of antibodies after vaccination with the primary regimen and whether the timing of the booster dose has an influence on the immune memory response.[4,5] Here, we present the secondary and exploratory objectives of this trial which consist of the long-term persistence of vaccine-induced antibodies after the primary regimen, and the safety and (long-term – only for Arm 1) immunogenicity of an Ad26.ZEBOV booster dose one or two years after the first dose in the same population.

Methods

Study design and participants

This open-label, randomised, phase 2 trial was performed at one study site in Boende, DRC, and recruited registered HCP and frontliners as participants. HCP were considered

professionals who work in a healthcare facility and are potentially exposed to EBOV within this facility (e.g., doctors, nurses, midwives, lab technicians, health facility cleaners, etc.). Professions who are potentially exposed to EBOV in the community were considered frontliners (e.g., community health care workers, first aid workers, stretcher bearers, etc.). Moving forward, HCP and frontliners will collectively be referred to as HCP.

During the recruitment period, HCP living and working in the Boende health zone were invited to attend a workshop explaining the objectives of the trial, the intended trial procedures and the informed consent procedure. If HCP were still willing to participate in the trial after the workshop, they were asked to return to the trial site on the next day for a screening visit. The trial site consisted of a wing of the general reference hospital of Boende that was refurbished (in the context of capacity building) and rented by the study team for the duration of the trial.[15] This wing or trial site was locked off from the rest of the hospital.

Participants were eligible when they were older than 18 years, did not have a known history of EVD, had a good understanding of the trial and its consenting process as determined by a test of understanding (consisting of 10 true or false questions; a score of ≥9/10 was required to pass; three attempts were allowed), were apparently healthy as judged by the study physician (based on vital signs and physical examination), were not pregnant (negative pregnancy test required), breastfeeding, or planning to become pregnant within 3 months after the first vaccine dose, were available for the entire study duration, willing to provide contact information and have means to be contacted. Additionally, participants were not allowed to have had an organ and/or stem cell transplant, have a history of chronic urticaria, be vaccinated with any experimental Ebola vaccines within 3 months prior to enrolment, or any Ad26-based vaccine in the past.

The trial was conducted according to the most recent Declaration of Helsinki and Good Clinical Practice guidelines,[16,17] and obtained approval from the National Ethics Committee of the Ministry of Health of the DRC (n°121/CNES/BN/PMMF/2019) and the Ethics Committee of

the University Hospital of Antwerp/University of Antwerp (19/14/177). All participants provided written informed consent prior to enrolment. Further information on the clinical trial itself is available on ClinicalTrials.gov, NCT04186000, and the study protocol was published in Larivière et al. (2021).[18]

Study procedures and randomisation

The timing and duration of study procedures are shown in Figure 1. Participants were vaccinated with the heterologous 2-dose Ad26.ZEBOV ($5x10^{10}$ viral particles), MVA-BN-Filo ($1x10^{8}$ infectious units) vaccine regimen at a 56-day interval, followed by an Ad26.ZEBOV ($5x10^{10}$ viral particles) booster dose one or two years after the first dose. Vaccinations were administered via a 0·5mL intramuscular injection in the deltoid muscle, changing arms for each vaccination. If participants presented with an acute illness, such as fever above 38·0°C on the day of vaccine administration, vaccination was postponed until the illness was resolved.

Randomisation was performed by the data management team using a software algorithm that randomly assigned a booster time point (1:1) to a sequential number. These sequential numbers were compiled in a randomisation list that was used to create sealed envelopes, which were assembled and checked by delegated PI staff. On Day 1, after vaccination with the first dose, delegated site staff opened the envelopes in a sequential order, randomly assigning participants to receive an Ad26.ZEBOV booster vaccination either one year or two years after the initial dose. The opening of the envelopes in sequential order was monitored by external clinical research associates. No blinding or masking of participants, study staff or laboratory staff took place.

Participants remained in observation for 30 minutes after vaccination to record any immediate serious adverse events (SAEs). For 7 days after booster vaccination, participants recorded local and systemic solicited adverse events (AEs) and unsolicited AEs in a participant journal. On

Day 8 after booster vaccination, these recorded symptoms were discussed with a study physician during a reactogenicity assessment and relatedness and severity were collected. For this trial, relatedness to the investigational product (IP) was collected using a binary Related (definitely, probably and possibly)/Not related (unlikely, unrelated) scale. Severity was assessed using the Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials.[19] Grade 3 AEs were events that led to the inability to work or perform usual activities and/or when taking a narcotic pain reliever was required.[19] Grade 4 AEs were events that led to an emergency room visit or hospitalisation.[19] If any AE was still ongoing on Day 8 after booster vaccination, participants were asked to return to the site when the symptom had resolved to report an end date. SAEs, as defined by the ICH E2A clinical safety data management scientific guideline,[20] were collected from enrolment until 6 months after the last received dose. Therefore, SAE collection was generally longer after the primary vaccine regimen than after the booster dose; and overall, SAE collection was longer for Arm 2 than Arm 1 participants. For SAE reporting, tollfree numbers were made available to participants and each scheduled visit participants were asked if they had experienced any medical event that could be considered a SAE. Finally, six months after the second dose and after the booster dose, participants were contacted via telephone (or visited at home by study staff when living outside the mobile phone network range around Boende)[21] to ask if any SAE had occurred since last contact.

For immunogenicity assessments, blood samples were collected prior to each primary regimen vaccination, at 21 days after the second dose, at one year after the first dose for Arm 1, at one and two years after the first dose for Arm 2, at 7 days post-booster vaccination for both arms, and at one year after booster vaccination for Arm 1. EBOV GP-specific IgG binding antibodies were analysed using the validated EBOV GP (Kikwit) Filovirus Animal Non-clinical Group enzyme-linked immunosorbent assay (FANG ELISA).[22] The laboratory analysis was conducted

by Q² Solutions who changed laboratory location from San Juan Capistrano, CA, USA (vaccine regimen immunogenicity analysis) to Research Triangle Park, NC, USA (booster immunogenicity analysis) through the course of the trial. As endorsed by the FDA, the assay was shown to be equivalent between the two laboratory locations, across the entire assay range (data not shown).

Outcomes

Here we describe the results from secondary objectives of the trial, assessing the safety and immunogenicity of an Ad26.ZEBOV booster vaccination administered one or two years after the initial dose, and the exploratory objectives: 1) assessing the long-term persistence of vaccine-induced antibodies after the primary vaccine regimen and booster vaccination (for Arm 1 only), and 2) comparing binding antibody responses after booster vaccination given one or two years after the first dose. Other endpoints of this trial, including the primary endpoint, were reported elsewhere.[8,23]

Immunogenicity was assessed using Ebola virus GP-specific IgG antibody geometric mean concentrations (GMC) and responder rates (defined as the proportion of participants with a ≥2·5-fold increase in the lower limit of quantification (LLOQ) after vaccination when the participant had antibodies below or equal to the LLOQ (≤36·11 ELISA Units (EU)/mL) at baseline on Day 1 or a ≥2·5-fold increase in antibody value after vaccination when the participant already had a value above the LLOQ at baseline on Day 1). The safety of the regimen and booster vaccination was measured as the number of participants with (related) SAEs occurring up to 6 months after the last received vaccination and the number of participants with (related) local and systemic solicited and unsolicited AEs reported for 7 days after booster vaccination.

Statistical analysis

The sample size was a convenience sample based on the number of HCPs living and working in the Boende health zone, and was not based on formal hypothesis testing considerations. To assess whether the comparison of the humoral immune memory response between those boosted at 1 year or 2 years was possible with this convenience sample, a power calculation was performed after the study had started, which showed a power of 99% to detect a difference between the two arms.[18]

Demographics, baseline characteristics and SAEs are summarised for the full analysis set (FAS), which included all enrolled participants that received at least one dose of the Ebola vaccine regimen. AEs are summarised for all participants in the FAS that received a booster dose. Immunogenicity analysis was performed per protocol set 1 (PPS1) to assess the humoral immune response after vaccination with the primary Ebola vaccine regimen and per protocol set 2 (PPS2) to assess the humoral immune response after booster vaccination. Per protocol set analyses included all participants among whom blood sample collection and vaccination occurred per protocol, at least one evaluable immunogenicity serum sample after vaccination (after dose 1 or dose 2 for PPS1 and after the booster for PPS2) was available, and no major protocol deviations with an impact on the immune response were reported.

As a post-hoc analysis, and to identify differences in the number of Arm 1 and Arm 2 participants reporting AEs 7 days after booster vaccination, risk ratio's with 95% CI were calculated.

Ebola virus GP-specific IgG antibody responses are reported as GMCs with 95% confidence intervals. All values below or equal to the LLOQ were imputed with half the LLOQ (18·055 EU/mL) and values above the upper limit of quantification (ULOQ; 194,938·88 EU/mL) were imputed with the ULOQ. For calculation of the responder rate, values below or equal to the

LLOQ were imputed to the LLOQ (36·11 EU/mL). Cohen's d statistics were used to compare the post-booster antibody concentrations between Arm 1 and Arm 2. Finally, a post-hoc analysis was performed to assess whether the GMCs after vaccination differed for participants with or without baseline binding antibody concentrations above the LLOQ.

R version 4.3.1 was used to perform all statistical analysis.

Role of the funding source

The funders of the trial played no role in the study design, data collection, analysis, or interpretation, nor in the writing of the article or the decision to submit the article for publication.

Results

HCP were recruited between 18 December 2019 and 8 February 2020. In total 699 participants were enrolled, one of which withdrew consent prior to any study activity being performed. The FAS therefore consisted of 698 participants, 350 in arm 1 and 348 in arm 2 (Figure 2). Demographic and baseline characteristics for these participants are presented in Table 1. Overall, 691 participants (346 in arm 1 and 345 in arm 2) were vaccinated with both doses of the primary vaccine regimen and 636 participants (319 in arm 1 and 317 in arm 2) received the booster dose. Of these, 685 could be included in PPS1 and 624 in PPS2. Overall, 643 participants (92%) completed the study with the last participant visit taking place on 12 October 2022.

Local and systemic solicited AEs post-booster vaccination in Arm 1 and Arm 2 were mostly mild (Table 2). At least one local solicited AE was reported by 95 (30%) of 319 Arm 1 participants and 95 (30%) of 317 Arm 2 participants. The most commonly reported local solicited AE among both arms was pain/tenderness of the injection site (87 [27%] of 319 Arm 1 participants and 90 [28%] of 317 Arm 2 participants). One participant in Arm 2 reported a

severe pain/tenderness event. All local AEs were considered related to the IP. At least one systemic solicited AE was reported by 133 (42%) of 319 Arm 1 participants and 127 (40%) of 317 Arm 2 participants. Of these, 128 (40%) Arm 1 participants and 123 (39%) Arm 2 participants were considered to have had systemic AEs related to the IP. Headache was the most commonly reported related systemic solicited AE among both arms, followed by myalgia, fatigue and nausea. Severe vaccine-related headache (n=4), fatigue (n=2) and myalgia (n=3) were infrequently reported by participants in both arms. The median time in duration for local and systemic solicited AEs was 2 days (IQR = 3 days and 5 days, respectively).

Fever related to vaccination was reported by 21 participants within seven days after booster vaccination, including nine (3%) in Arm 1 and 12 (4%) in Arm 2. One Arm 2 participant experienced fever above 40·0°C at three days after booster vaccination, which was reported as a related SAE by the Principal Investigator (PI) and categorised as an "other medically important event". No hospitalisation was required to treat this SAE and it resolved without sequelae the day after onset. No other related SAEs were reported during the trial. In total, 47 participants experienced one or more SAE(s) (27 following the primary vaccination regimen and 19 after booster vaccination, six in Arm 1 and 13 in Arm 2; one Arm 2 participant experienced two simultaneous SAE after primary vaccination and one after booster vaccination), with 64 SAEs reported in total (42 occurring between the primary regimen and the booster vaccination and 22 events following booster vaccination, seven in Arm 1 and 15 in Arm 2) (Supplementary Table 1). The majority of SAEs were considered resolved without sequelae. Five participants reported an SAE that was considered resolved with sequelae and three participants had fatal SAEs (Supplementary Table 1).

Unsolicited AEs post-booster vaccination were reported among 143 (22%) of 636 participants, 64 (20%) of 319 Arm 1 participants and 79 (25%) of 317 Arm 2 participants (Table 2). For 59 (9%) participants these were considered related to the IP. One unsolicited AE of Grade 4 in

346 severity (potentially life-threatening), considered unrelated to the IP, was reported for a
347 participant in Arm 2, i.e., an AE of abdominal pain with onset one day after booster vaccination
348 that resolved after five days. This Grade 4 unsolicited AE was associated with an AE of typhoid
349 fever, which was considered serious by the investigator and reported as an SAE. The most
350 frequent unsolicited AEs related to the IP when classified per system organ class were
351 gastrointestinal disorders (3%, n=18; diarrhoea and abdominal pain), nervous system disorders
352 (2%, n=14; dizziness and drowsiness), musculoskeletal and connective tissue disorders (1%,
353 n=8; muscle pain or weakness and joint pain), and general disorders and administration site
354 conditions (1%, n=7; chills and itchiness of the injection site).

355 In a post-hoc analysis, no statistically significant differences were observed in the number of
356 Arm 1 and Arm 2 participants reporting solicited and unsolicited AEs after booster vaccination
357 (Table 2).

358 Participant GMCs of binding antibodies against the Ebola virus GP are presented in Table 3
359 and Figure 3. At baseline before the first dose of the primary regimen, Arm 1 participants had
360 a GMC of 53·7 ELISA units (EU)/mL (95% CI 46·5-62·1) and Arm 2 participants 56·2 EU/mL
361 (95% CI 48·4-65·2). Ad26.ZEBOV vaccination on Day 1 resulted in 204 (60%) of 342 Arm 1
362 participants and 231 (68%) of 342 Arm 2 participants being classified as responders 56 days
363 later. Twenty-one days after MVA-BN-Filo vaccination, 328 (96%) of 342 Arm 1 participants
364 and 327 (96%) of 341 Arm 2 participants were classified as responders. One year after the first
365 dose for Arm 1 and Arm 2 and before booster vaccination for Arm 1, the GMC was 305·7
366 EU/mL (95% CI 281·5-332·1) overall, 279·9 EU/mL (95% CI 250·6-312·7) for Arm 1
367 participants and 334·8 EU/mL (95% CI 296·0-378·7) for Arm 2 participants. When compared
368 to the binding antibody GMC against EBOV GP at baseline on Day 1 before vaccination, a 5.6-
369 fold increase in GMC was observed one year later, 5·2-fold increase for Arm 1 participants and
370 6·0-fold increase for Arm 2 participants. Two years after the first dose and before booster

vaccination, Arm 2 participants had a GMC of 274·6 EU/mL (95% CI 242·1-311·5), which was 4·9 times higher than at baseline on Day 1 before vaccination.

Eight participants had antibody values below the LLOQ before booster vaccination. Of these, seven had a rapid (i.e., within 7 days after vaccination) and strong immune memory response (>15-fold increase in LLOQ) after booster vaccination. For one participant (Arm 2) no antibody response was observed after booster vaccination. However, for this participant no response was observed after either of the Ad26.ZEBOV vaccinations, only after MVA-BN-Filo vaccination.

Within seven days after booster vaccination, Arm 1 and Arm 2 participants' GMCs had both increased approximately 39-fold (compared to pre-booster vaccination) to 10,781·6 EU/mL (95% CI 9,354·4-12,426·4) and 10,746·9 EU/mL (95% CI 9,208·7-12,542·0), respectively, and out of 314 Arm 1 participants 307 (98%) were responders, compared to 303 out of 310 (98%) Arm 2 participants (Table 3). The difference in average antibody level after booster vaccination between Arm 1 and Arm 2 was negligible (Cohen's d = -0.065 [-0.222-0.092]). One year after booster vaccination, a 39·7-fold increase in GMC compared to baseline on Day 1 and a 7·6-fold increase in GMC compared to pre-booster vaccination was observed for Arm 1 participants (GMC of 2,133·1 EU/mL (95% CI 1,827·7-2,489·7)).

At baseline on Day 1, binding antibody concentrations above the LLOQ were observed in 50% (344 of 684) of participants (Table 3). After the first Ad26.ZEBOV vaccination, participants with antibody concentrations below the LLOQ at baseline had a steeper increase in antibody response than participants with baseline antibody concentrations above the LLOQ (Supplementary Figure 1). Before the second dose (MVA-BN-Filo), participants with antibody concentrations below the LLOQ at baseline had a lower numerical binding antibody GMC than participants with antibody concentrations above the LLOQ at baseline. Nevertheless, this difference in antibody levels was no longer present 21 days after full vaccination with the heterologous-two dose vaccine regimen and a similar peak in antibody response after

vaccination with the 2-dose regimen was observed in both groups. Among participants with antibody concentrations below the LLOQ at baseline, more waning of antibodies was observed one year (Arm 1 and Arm 2) and two years (Arm 2) after the initial dose. Even so, the humoral immune response 7 days after booster vaccination led to similar GMC values in participants with antibody concentrations below and above LLOQ at baseline on Day 1, independent of the timing of the booster dose.

Discussion

This was the first large cohort trial in adults to assess the persistence of binding antibodies after the Ad26.ZEBOV, MVA-BN-Filo vaccine regimen and a booster dose, and the safety and immune memory response, after an Ad26.ZEBOV booster dose administered one or two years after the first dose. Overall, the vaccine regimen and booster dose were well-tolerated, which corresponds to findings from previous trials assessing the safety of the Ad26.ZEBOV, MVA-BN-Filo vaccine regimen, followed by an Ad26.ZEBOV booster vaccination in a healthy adult population.[4,5] One SAE, i.e., fever of more than 40·0°C, was considered related to booster vaccination by the PI. No other SAEs were considered related to vaccination.

When comparing the AE safety profile after an Ad26.ZEBOV booster dose among our HCP population with safety profiles reported after Ad26.ZEBOV vaccination as first dose of the primary vaccine regimen in adults from several African countries and the United Kingdom, similar findings were observed.[3-7] In these studies, like for our participants, AEs after Ad26.ZEBOV vaccination were mostly mild to moderate and transient, with injection-site pain as the most frequently reported local solicited AE.[3-7] Likewise, for systemic AEs, headache, fatigue and myalgia were most commonly reported.[3-7] A comparison in AE reporting between the Ad26.ZEBOV initial dose and booster dose was unfortunately not possible within this study. Solicited and unsolicited adverse events were only collected after booster vaccination and not

after vaccination with the primary vaccine regimen. There were two reasons for this decision. First, many study participants had to travel long distances on foot, by bike, motorbike, dug-out canoes, etc. to reach the trial site. Therefore, scheduled study visits were limited to a minimum during protocol development. Second, the safety profile of the Ad26.ZEBOV, MVA-BN-Filo vaccine regimen had been studied in several phase 1 and 2 trials before the start of this trial and was considered safe.[3-7]

As previously reported, we observed a strong immune response 21 days after the primary vaccination with the heterologous two-dose vaccine regimen, at which point 95% (652 of 679) of HCP participants (in this article updated to 96% (655 of 683) with four results of back-up Day 1 samples included) could be considered responders.[8] Though this percentage is slightly lower than the 97·0-100·0% responder rates observed in previous trials assessing the immunogenicity of the Ad26.ZEBOV, MVA-BN-Filo regimen administered at a 56-day interval,[3-7,24] this could be due to the high number of HCP participants in our study that already had EBOV-specific binding antibodies above the LLOQ at baseline (344 [50%] of 684 participants). As a 2·5-fold increase of antibodies was required to be considered a responder (if the participant's antibodies were already above the LLOQ at baseline) instead of 2·5×LLOQ, a higher number of participants with detectable antibodies at baseline could lead to a slightly lower percentage considered responders. Additionally, it was found that participants with antibodies above the LLOQ at baseline on Day 1 had a smaller fold-increase in binding antibodies at 56 days after the first dose than participants with antibodies below the LLOQ. However, this difference was no longer observed after the full regimen was administered. Furthermore, though the waning of antibodies after vaccination seemed steeper for the group that did not have detectable antibodies at baseline, the GMC after booster vaccination was similar for both groups, indicating no notable effect on immune memory response.

444 We observed a decrease in the presence of vaccine-induced antibodies between Day 78 and
445 Day 365/Day 730 with a stabilisation between Day 365 and 730, at a GMC similar to that of
446 the Day 57 visit. This was consistent with studies observing a decline between Day 78 and six
447 months after the first dose (time point not assessed in our trial), after which the circulating
448 antibody concentration stabilised at a level similar to Day 57 values.[3,25] When assessed and as
449 observed in our trial, the stabilisation persisted up to two years after the first dose.[5,25] However,
450 As no human vaccine efficacy data is available for the Ad26.ZEBOV, MVA-BN-Filo vaccine
451 regimen, it is not possible to say which level of antibodies can lead to protection. For this reason,
452 immunobridging has been used to infer the likelihood of protection in humans from NHP
453 models.[10,11] Unfortunately, the current immunobridging model does not provide information on
454 how the persistence of the vaccine-induced immune response relates to the durability of
455 protection in humans, nor has a new model been performed to assess the effect of booster
456 doses.[25] Only one approved Ebola vaccine (rVSV△G-ZEBOV) has been able to show 97.5-
457 100% clinical efficacy in humans through ring vaccination in the DRC and Guinea.[26,27] For this
458 vaccine, a seroresponse of 200 ELISA units per mL or higher after vaccination that is at least
459 twice the baseline value (pre-vaccination) or more, has been described as a possible correlate
460 of protection.[28] As the same FANG ELISA was used to measure the EBOV GP-binding
461 antibody response after vaccination for both the rVSV△G-ZEBOV vaccine and the
462 Ad26.ZEBOV, MVA-BN-Filo vaccine regimen, these results may be extrapolatable to the
463 Ad26.ZEBOV, MVA-BN-Filo vaccine regimen. If this is possible, then at each time point the
464 geometric mean concentration in our trial was above this threshold, including pre-booster
465 vaccination at the Year 1 and Year 2 visits. However, while a similar “waning followed by a
466 stabilization”-pattern of antibodies has been observed among NHP after vaccination with the
467 regimen, an Ebola virus challenge administered 1.6 years after initial vaccination with the
468 Ad26.ZEBOV, MVA-BN-Filo vaccine regimen was fully lethal in NHP that did not receive a

booster dose prior to the challenge, but fully protective (with minimal morbidity and absence of viremia) in boosted animals,[25] even when the Ebola virus challenge occurred as soon as 3 days after booster vaccination.[25] Therefore, booster doses may also be indicated in humans prior to exposure to Ebola virus in case of an outbreak.

Independent of the antibody persistence prior to the booster vaccination and the timing of the booster, a humoral immune memory response was observed seven days after Ad26.ZEBOV booster vaccination in both arms, leading to a 98% responder rate in both Arm 1 (307 of 314 participants) and Arm 2 (303 of 310 participants) participants. Additionally, in all but one participant a rapid (within 7 days) and strong immune memory response (>15-fold) was observed after booster vaccination when antibody levels had declined below the LLOQ prior to booster vaccination. This responder rate at 7 days after booster vaccination (98%) was similar to the responder rate reported in two smaller studies administering a booster dose after one year (100%) or after two (96%) years in healthy adults.[4,5]

There were some limitations of this trial. First, taking into account the time to recruit 700 participants, the trial was planned to last approximately three years within a four-year project. For this reason, a blood collection time point one year after booster vaccination in Arm 2 was not planned as this would prolong the trial with an additional six months and not enough time to analyse the samples, clean the database and analyse the data. At this moment, it was therefore not possible to compare the long-term persistence of antibodies after booster vaccination between both study arms. Second, neutralising anti-EBOV GP antibodies were not measured. Assessing both binding and neutralising antibodies was not within the financial possibilities of the project in this large cohort of participants. The focus on binding antibodies was supported by NHP challenge models that had shown a strong correlation between binding antibody responses and survival.[9]

As a huge accomplishment, a 92% (643 out of 699) participant retention rate was achieved in Boende, a remote area of DRC, over the more than 2·5-year trial duration. In 2014, 69 suspected, probable or confirmed EBOV cases were reported in the vicinity of Boende.[29] By performing this trial and vaccinating approximately 20% of the HCP living and working in the Boende health zone, who may be exposed to, and therefore become drivers of EVD in the event of a future outbreak, we aimed to improve readiness for future Ebola outbreaks in this Ebola endemic area of DRC.

In conclusion, the vaccine regimen and booster dose were well-tolerated and the primary vaccine regimen led to persisting EBOV GP binding antibodies up to two years after the first dose. Additionally, a rapid and similar immune memory response was recalled by an Ad26.ZEBOV booster vaccination one and two years after the vaccine regimen, illustrating flexibility in booster administration timing. Combined with modelling research that has estimated a considerable decrease in Ebola cases, hospitalisations and deaths when preventative vaccination strategies targets a limited percentage of HCP in Ebola endemic areas,[13] our data suggests that an Ad26.ZEBOV booster vaccination could be considered for previously vaccinated individuals at risk of Ebola infection (such as at risk HCP, in emergency situations, or during an outbreak) up to at least two years after vaccination with the vaccine regimen.

Declarations

Author contributions

YL performed all analyses included in the article and wrote the article. BIO performed data management and data analysis for the trial at sponsor level and verified analyses in the article with those in the clinical study report. PK performed data management for the trial at principal investigator level. HMM was principal investigator of the trial, with PM and JM as co-principal investigators. The University of Antwerp (PVD, JPVG) was sponsor of the trial. YL and GL

were project managers at sponsor level. VM was project manager at investigator level. TZM was the study site coordinator. SM and RM were assistant site coordinators. SM was in charge of safety and cold chain and investigational product management. CR, MK, CM were involved in trial discussions at investigational product level. YL, BIO, GL, JPVG, PVD, TZM, PK, HMM, PM, and JM have access to the raw study data. The data was queried by BIO, YL, and an external data manager from DFNet (in charge of database build and maintenance). The data was verified by monitors from an external clinical research organization. All authors reviewed and contributed to the final manuscript. PVD and HMM were responsible for the decision to submit the manuscript for publication.

Funding

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

Data sharing

Individual deidentified participants' data and a data dictionary will be made available to others in the scientific community upon request after publication of this study. Standard criteria for making data available for valid research projects will be used, following application by suitably qualified researchers and upon presentation of a defined analysis plan. For data access requests, please contact cev@uantwerpen.be. The clinical study protocol has been published.

Acknowledgements

We acknowledge the hard work and dedication of the local nurses, medical doctors and laboratory technicians, working in sync with medical doctors, laboratory technicians, and data managers from the University of Kinshasa. We acknowledge this study would not have been possible without the entire EBL2007 study group (Supplementary material). A special thank you to Dr. Emmanuel Esanga, Mme Maguy, Pitchou Kasongo, Dr. Michael Bojabwa and Dr. Danoff Engbu for their investment in this trial. We also acknowledge, with gratitude, the support provided by the Africa Contract Research Organization (CRO), with a special thank you to Victorine Owira, and by DFNet Research through the development and maintenance of the study database, with a special thank you to Karolyn Scott. We also thank all consortium partners for their collaboration and inspiring discussions. Finally and most importantly, we thank all the healthcare providers and frontliners that took time out of their busy schedules to take part in this trial as participants.

Declaration of interests

CR, MK and CM were full-time employees of Janssen, Companies of Johnson & Johnson, at the time of the study and report stock or stock options in Janssen, Pharmaceutical Companies of Johnson & Johnson. HM was appointed member of the Advisory Board of Janssen Global Services during the COVID-19 pandemic. All other authors declare that they had no competing interests at the time of the study.

Reference List

1. Evans DK, Goldstein M, Popova A. Health-care worker mortality and the legacy of the Ebola epidemic. Lancet Glob Health 2015; 3(8): e439-e40.

2. Elston JW, Cartwright C, Ndumbi P, Wright J. The health impact of the 2014-15 Ebola outbreak. Public Health 2017; 143: 60-70.

3. Anywaine Z, Whitworth H, Kaleebu P, et al. Safety and Immunogenicity of a 2-Dose Heterologous Vaccination Regimen With Ad26.ZEBOV and MVA-BN-Filo Ebola Vaccines: 12-Month Data From a Phase 1 Randomized Clinical Trial in Uganda and Tanzania. J Infect Dis 2019; 220(1): 46-56.

4. Barry H, Mutua G, Kibuuka H, et al. Safety and immunogenicity of 2-dose heterologous Ad26.ZEBOV, MVA-BN-Filo Ebola vaccination in healthy and HIV-infected adults: A randomised, placebo-controlled Phase II clinical trial in Africa. PLoS Med 2021; 18(10): e1003813.

5. Ishola D, Manno D, Afolabi MO, et al. Safety and long-term immunogenicity of the two-dose heterologous Ad26.ZEBOV and MVA-BN-Filo Ebola vaccine regimen in adults in Sierra Leone: a combined open-label, non-randomised stage 1, and a randomised, double-blind, controlled stage 2 trial. Lancet Infect Dis 2022; 22(1): 97-109.

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

7. Mutua G, Anzala O, Luhn K, et al. Safety and Immunogenicity of a 2-Dose Heterologous Vaccine Regimen With Ad26.ZEBOV and MVA-BN-Filo Ebola Vaccines: 12-Month Data From a Phase 1 Randomized Clinical Trial in Nairobi, Kenya. J Infect Dis 2019; 220(1): 57-67.

8. Larivière Y, Garcia-Fogeda I, Zola Matuvanga T, et al. Safety and Immunogenicity of the Heterologous 2-Dose Ad26.ZEBOV, MVA-BN-Filo Vaccine Regimen in Health Care Providers and Frontliners of the Democratic Republic of the Congo. J Infect Dis 2023.

9. Callendret B, Vellinga J, Wunderlich K, et al. A prophylactic multivalent vaccine against different filovirus species is immunogenic and provides protection from lethal

infections with Ebolavirus and Marburgvirus species in non-human primates. PLoS One 2018; 13(2): e0192312.

10. Bockstal V, Leyssen M, Heerwegh D, et al. Non-human primate to human immunobridging demonstrates a protective effect of Ad26.ZEBOV, MVA-BN-Filo vaccine against Ebola. NPJ Vaccines 2022; 7(1): 156.

11. Roozendaal R, Hendriks J, van Effelterre T, et al. Nonhuman primate to human immunobridging to infer the protective effect of an Ebola virus vaccine candidate. NPJ Vaccines 2020; 5(1): 112.

12. Potluri R, Kumar A, Oriol-Mathieu V, Van Effelterre T, Metz L, Bhandari H. Model-based evaluation of the impact of prophylactic vaccination applied to Ebola epidemics in Sierra Leone and Democratic Republic of Congo. BMC Infect Dis 2022; 22(1): 769.

13. Bisanzio D, Davis AE, Talbird SE, et al. Targeted preventive vaccination campaigns to reduce Ebola outbreaks: An individual-based modeling study. Vaccine 2023; 41(3): 684-93.

14. Center for Disease Control and Prevention. History of Ebola Virus Disease (EVD) Outbreaks. 2023. https://www.cdc.gov/vhf/ebola/history/chronology.html (accessed 4 December 2023.

15. Zola Matuvanga T, Larivière Y, Lemey G, et al. Setting-up an Ebola vaccine trial in a remote area of the Democratic Republic of the Congo: Challenges, mitigations, and lessons learned. Vaccine 2022; 40(25): 3470-80.

16. Association WM. World Medical Association Declaration of Helsinki: Ethical Principles for Medical Research Involving Human Subjects. JAMA 2013; 310(20): 2191-4.

17. Agency EM. ICH: E6 (R2) Good Clinical Practice–Scientific Guideline. 2016.

611 18. Larivière Y, Zola T, Stoppie E, et al. Open-label, randomised, clinical trial to evaluate
612 the immunogenicity and safety of a prophylactic vaccination of healthcare providers by
613 administration of a heterologous vaccine regimen against Ebola in the Democratic Republic of
614 the Congo: the study protocol. BMJ Open 2021; 11(9): e046835.

615 19. US Food Drug Administration. Toxicity grading scale for healthy adult and adolescent
616 volunteers enrolled in preventive vaccine clinical trials. Rockville, MD: US Food and Drug
617 Administration 2007.

618 20. European medicines Agency. Clinical Safety Data Management: Definitions and
619 Standards for Expedited Reporting, 1995.

620 21. Larivière Y, Matuvanga TZ, Lemey G, et al. Conducting an Ebola vaccine trial in a
621 remote area of the Democratic Republic of the Congo: Challenges, mitigations, and lessons
622 learned. Vaccine 2023; 41(51): 7587-97.

623 22. Rudge TL, Jr., Sankovich KA, Niemuth NA, et al. Development, qualification, and
624 validation of the Filovirus Animal Nonclinical Group anti-Ebola virus glycoprotein
625 immunoglobulin G enzyme-linked immunosorbent assay for human serum samples. PLoS One
626 2019; 14(4): e0215457.23.

627 23. Zola Matuvanga T, Mariën J, Larivière Y, et al. Low seroprevalence of Ebola virus in
628 health care providers in an endemic region (Tshuapa province) of the Democratic Republic of
629 the Congo. PLoS One 2023; 18(9): e0286479.

630 24. Pollard AJ, Launay O, Lelievre JD, et al. Safety and immunogenicity of a two-dose
631 heterologous Ad26.ZEBOV and MVA-BN-Filo Ebola vaccine regimen in adults in Europe
632 (EBOVAC2): a randomised, observer-blind, participant-blind, placebo-controlled, phase 2 trial.
633 Lancet Infect Dis 2021; 21(4): 493-506.

634 25. McLean C, Dijkman K, Gaddah A, et al. Persistence of immunological memory as a
635 potential correlate of long-term, vaccine-induced protection against Ebola virus disease in
636 humans. Frontiers in Immunology 2023; 14.

637 26. Henao-Restrepo AM, Camacho A, Longini IM, et al. Efficacy and effectiveness of an
638 rVSV-vectored vaccine in preventing Ebola virus disease: final results from the Guinea ring
639 vaccination, open-label, cluster-randomised trial (Ebola Ça Suffit!). Lancet 2017; 389(10068):
640 505-18.

641 27. World health Organization. Preliminary results on the efficacy of rVSV-ZEBOV-GP
642 Ebola vaccine using the ring vaccination strategy in the control of an Ebola outbreak in the
643 Democratic Republic of the Congo: an example of integration of research into epidemic
644 response. An example of integration of research into epidemic response 2019.

645 28. Grais RF, Kennedy SB, Mahon BE, et al. Estimation of the correlates of protection of
646 the rVSVΔG-ZEBOV-GP Zaire ebolavirus vaccine: a post-hoc analysis of data from phase 2/3
647 clinical trials. Lancet Microbe 2021; 2(2): e70-e8.

648 29. Maganga GD, Kapetshi J, Berthet N, et al. Ebola virus disease in the Democratic
649 Republic of Congo. N Engl J Med 2014; 371(22): 2083-91.

Figures and tables

Figures

Figure 1. Study design

: Blood sample collection Arm 1 and Arm 2; : Blood sample collection Arm 1 only; : Blood sample collection Arm 2 only; *Adverse events (AE) were collected using a participant diary; SAE : Serious adverse events; PB : post-booster.

Figure 2. Study flow chart

[$]These participants became pregnant within the protocol prohibited window and were discontinued from the trial as they did not receive both doses of the two-dose heterologous regimen; *Participants for whom treatment was stopped but other trial activities (e.g., safety follow-up) continued; PPS1: per protocol set 1; PPS2: per protocol set 2; LTFU: Lost to follow-up.

Figure 3. Geometric mean concentrations with 95% confidence intervals of EBOV-specific binding antibodies for Per Protocol Set 2 participants

LLOQ = Lower limit of quantification (36.11 ELISA Units/mL); the colour of the vaccination determines whether both arms (black), only Arm 1 (blue), or only Arm 2 (green) received the vaccine.

Table 1. Baseline sociodemographic characteristics of healthcare providers and frontliners (full analysis set) enrolled in the Ebola vaccine trial in Boende, the Democratic Republic of the Congo. Demographics were collected at the start of the trial between December 2019 and February 2020 and are reported per arm and overall.

Characteristic	**Arm 1 (N = 350)**	**Arm 2 (N = 348)**	**Overall (N = 698)**
Sex as assigned at birth, n (%)			
Male	260 (74)	274 (79)	534 (77)
Female	90 (26)	74 (21)	164 (23)
Black, n (%)	350 (100)	348 (100)	698 (100)
Age, years			
Mean (SD)	45·4 (11·5)	44·6 (12·5)	45·0 (12·0)
Median (range)	46 (20-75)	46 (19-74)	46 (19-75)
Profession, n (%)			
Community health worker	117 (33)	119 (34)	236 (34)
Nurse	87 (25)	94 (27)	181 (26)
First aid worker	91 (26)	86 (25)	177 (25)
Hygienist	22 (6)	15 (4)	37 (5)
Midwife	19 (5)	11 (3)	30 (4)
Doctor	6 (2)	7 (2)	13 (2)
Health facility cleaner	2 (1)	8 (2)	10 (1)
Care giver	2 (1)	5 (1)	7 (1)
Lab technician	2 (1)	0 (0)	2 (0)
Pharmacist aid	0 (0)	2 (1)	2 (0)
Other	2 (1)	1 (0)	3 (0)
Work establishment, n (%)			
Health centre	182 (52)	189 (54)	371 (53)
Red cross	91 (26)	86 (25)	177 (25)
Hospital	44 (13)	40 (12)	84 (12)
Health post	21 (6)	16 (5)	37 (5)
Health area	4 (1)	6 (2)	10 (1)
Provincial health department	6 (2)	3 (1)	9 (1)
Health zone	1 (0)	7 (2)	8 (1)
Health inspection	0 (0)	1 (0)	1 (0)
Staff member of the expanded programme on immunisation	1 (0)	0 (0)	1 (0)
Medical history*, n (%)			
Yes	71 (20)	64 (18)	135 (19)
No	279 (80)	284 (82)	563 (81)
Smallpox vaccination against MPOX, n (%)			
Yes	61 (17)	68 (20)	129 (19)
No	289 (83)	280 (80)	569 (81)

N, all participants who received at least one study vaccine dose; n, number of participants in indicated category; SD, standard deviation; * During the medical history inquiry the study doctor asked about any current or past medical problems and treatments that the participant has or had. "Yes" indicates that the participant reported current or past medical problems. "No" indicates that the participant did not report any current or past medical problems.

Table 2. Overview of solicited and unsolicited adverse events, considered related to the investigational product, after booster vaccination observed in Arm 1, Arm 2 and overall and of serious adverse events reported up to six months after the last received dose.

	Overall (N[1] = 636)	**Arm 1 (N[1] = 319)**	**Arm 2 (N[1] = 317)**	**RR (95% CI)[Δ]**
Solicited adverse events[#], n (%) n*				
Any local adverse event[§§]	190 (30) 230	95 (30) 104	95 (30) 126	0·997 (0·901-1·104)
Mild	169 (27) 200	85 (27) 92	84 (26) 108	1·002 (0·913-1·100)
Moderate	28 (4) 29	12 (4) 12	16 (5) 17	0·987 (0·954-1·020)
Severe	1 (0) 1	0 (0) 0	1 (0) 1	0·997 (0·991-1·003)
Potentially life-threatening	0 (0) 0	0 (0) 0	0 (0) 0	1·000 (1·000-1·000)
Erythema				
Any	24 (4) 24	10 (3) 10	14 (4) 14	0·987 (0·957-1·018)
Severe	0 (0) 0	0 (0) 0	0 (0) 0	1·000 (1·000-1·000)
Swelling				
Any	15 (2) 15	7 (2) 7	8 (3) 8	0·997 (0·973-1·021)
Severe	0 (0) 0	0 (0) 0	0 (0) 0	1·000 (1·000-1·000)
Pain/Tenderness				
Any	177 (28) 191	87 (27) 87	90 (28) 104	0·985 (0·894-1·084)
Severe	1 (0) 1	0 (0) 0	1 (0) 1	0·997 (0·991-1·003)
Any systemic adverse events	260 (41) 565	133 (42) 252	127 (40) 313	1·028 (0·903-1·170)
Mild	232 (36) 424	115 (36) 186	117 (37) 238	0·987 (0·877-1·110)
Moderate	77 (12) 125	38 (12) 57	39 (12) 68	0·996 (0·940-1·055)
Severe	12 (2) 15	7 (2) 9	5 (2) 6	1·006 (0·985-1·028)
Potentially life-threatening	1 (0) 1	0 (0) 0	1 (0) 1	0·997 (0·991-1·003)
Any related systemic adverse events	251 (39) 543	128 (40) 242	123 (39) 301	1·022 (0·916-1·159)
Mild	225 (35) 411	111 (35) 179	114 (36) 232	0·982 (0·875-1·102)
Moderate	75 (12) 118	37 (12) 55	38 (12) 63	0·996 (0·941-1·054)
Severe	10 (2) 13	6 (2) 8	4 (1) 5	1·006 (0·987-1·026)
Potentially life-threatening	1 (0) 1	0 (0) 0	1 (0) 1	0·997 (0·991-1·003)
Fatigue				
Any	121 (19) 137	60 (19) 60	61 (19) 77	0·995 (0·922-1·072)
Severe	2 (0) 2	0 (0) 0	2 (1) 2	0·994 (0·985-1·002)
Any related	120 (19) 136	59 (18) 59	61 (19) 77	0·991 (0·920-1·068)

Severe related	2 (0) 2	0 (0) 0	2 (1) 2	0·994 (0·985-1·002)
Headache				
Any	194 (31) 224	97 (30) 101	97 (30) 123	0·997 (0·900-1·105)
Severe	5 (1) 5	4 (1) 4	1 (0) 1	1·010 (0·996-1·024)
Any related	184 (29) 211	91 (29) 94	93 (29) 117	0·989 (0·895-1·092)
Severe related	4 (1) 4	3 (1) 3	1 (0) 1	1·006 (0·994-1·019)
Nausea				
Any	39 (6) 44	17 (5) 18	22 (7) 26	0·983 (0·945-1·023)
Severe	0 (0) 0	0 (0) 0	0 (0) 0	1·000 (1·000-1·000)
Any related	38 (6) 42	16 (5) 16	22 (7) 26	0·980 (0·942-1·019)
Severe related	0 (0) 0	0 (0) 0	0 (0) 0	1·000 (1·000-1·000)
Myalgia				
Any	124 (19) 133	63 (20) 64	61 (19) 69	1·006 (0·932-1·086)
Severe	3 (0) 3	2 (1) 2	1 (0) 1	1·003 (0·992-1·014)
Any related	122 (19) 131	63 (20) 64	59 (18) 67	1·014 (0·940-1·094)
Severe related	3 (0) 3	2 (1) 2	1 (0) 1	1·003 (0·992-1·014)
Fever				
Any	24 (4) 27	9 (3) 9	15 (5) 18	0·980 (0·951-1·011)
≥ 38.0°C	19 (3) 21	6 (2) 6	13 (4) 15	0·977 (0·951-1·004)
≥ 39.0°C	5 (1) 5	3 (1) 3	2 (1) 2	1·003 (0·989-1·017)
≥ 40.0°C	1 (0) 1	0 (0) 0	1 (0) 1	0·997 (0·991-1·003)
Any related	21 (3) 23	9 (3) 9	12 (4) 14	0·990 (0·962-1·019)
≥ 38.0°C related	16 (3) 18	6 (2) 6	10 (3) 12	0·987 (0·963-1·012)
≥ 39.0°C related	4 (1) 4	3 (1) 3	1 (0) 1	1·006 (0·994-1·019)
≥ 40.0°C related	1 (0) 1	0 (0) 0	1 (0) 1	0·997 (0·991-1·003)
Unsolicited adverse events[#], n (%) n*				
Any unsolicited adverse event	143 (22) 226	64 (20) 94	79 (25) 132	0·939 (0·864-1·021)
Mild	113 (18) 162	53 (17) 71	60 (19) 91	0·972 (0·904-1·045)
Moderate	44 (7) 60	17 (5) 21	27 (8) 39	0·966 (0·926-1·008)
Severe	3 (0) 3	2 (1) 2	1 (0) 1	1·003 (0·992-1·014)
Potentially life-threatening	1 (0) 1	0 (0) 0	1 (0) 1	0·997 (0·991-1·003)
Any related unsolicited adverse event	59 (9) 73	31 (10) 38	28 (9) 35	1·010 (0·961-1·061)
Mild	43 (7) 52	22 (7) 26	21 (7) 26	1·003 (0·962-1·046)
Moderate	16 (3) 18	8 (3) 10	8 (3) 8	1·000 (0·975-1·025)
Severe	3 (0) 3	2 (1) 2	1 (0) 1	1·003 (0·992-1·014)

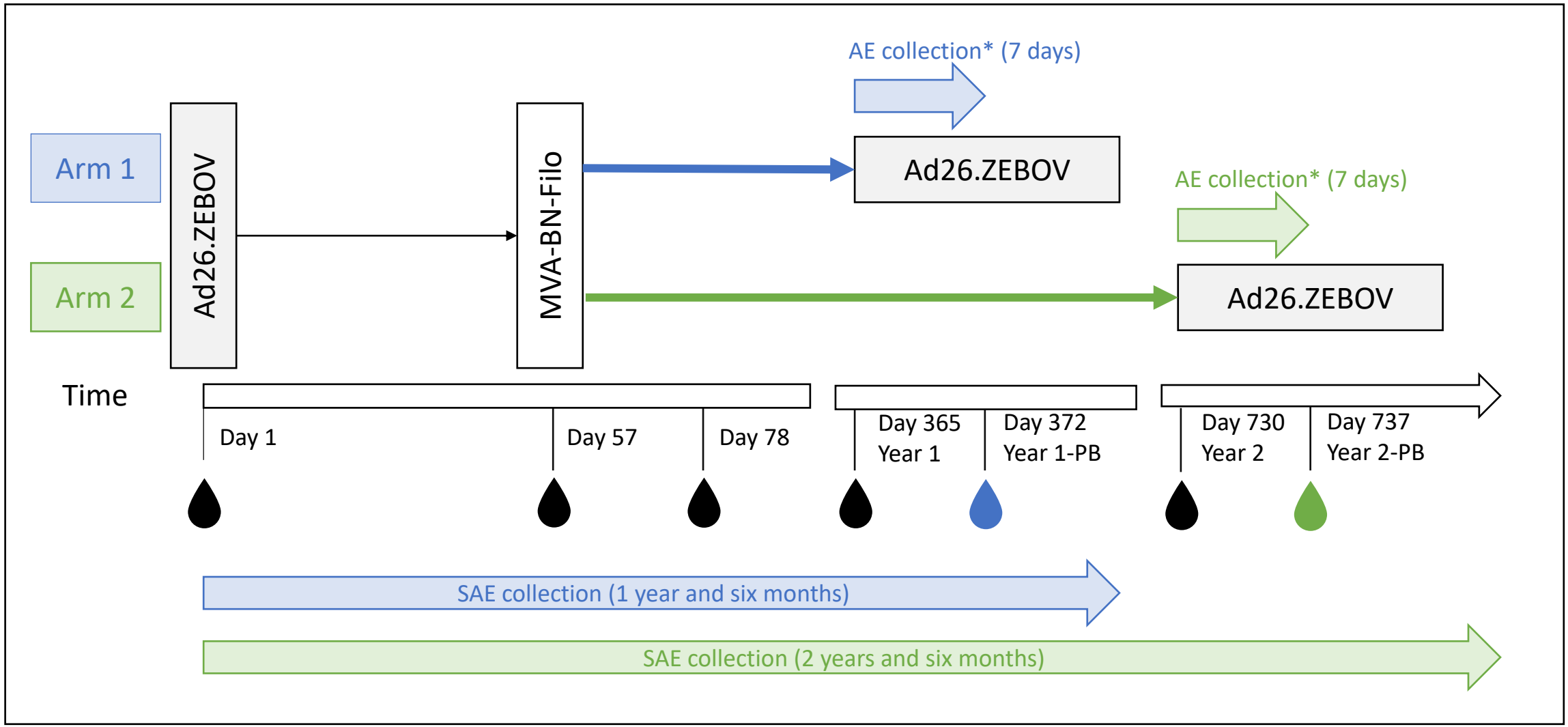
Potentially life-threatening	0 (0) 0	0 (0) 0	0 (0) 0	1·000 (1·000-1·000)
Serious adverse events[§], n/N (%) n*				
Any reported serious adverse event	47/698 (7) 64	15/350 (4) 22	32/348 (9) 42	-
Serious adverse event related to vaccination	1/698 (0) 1	0/350 (0) 0	1/348 (0) 1	-
SAE Outcome				-
Fatal	3/698 (0) 4	1/350 (0) 1	2/348 (1) 3	-
Recovered/resolved	40/698 (6) 55	15/350 (4) 21	25/348 (7) 34	-
Recovered/resolved With Sequelae	5/698 (1) 5	0/350 (0) 0	5/348 (1) 5	-

This table reports the number of participants that experienced a solicited, unsolicited or serious adverse event. Some participants experienced more than one event. N^1 = number of participants in FAS1; N = number of participants in FAS; n = number of participants experiencing the event; n*=number of events; n (%) is the number (percentage) of the participants who had at least 1 event; % = n/N^1*100 or n/N*100; [$]All local solicited adverse events were considered related to the investigational product; [#]Occurring within 7 days after Ad26.ZEBOV booster vaccination; [§]Full Analysis Set (FAS) database; [△]RR: Risk Ratios were calculated to determine whether there was a difference in the number of participants reporting AEs between Arm 1 and Arm 2; a value of 1 within the confidence intervals indicates no significant differences.

Table 3. Geometric mean concentration of Ebola virus glycoprotein binding antibodies induced by the Ad26.ZEBOV. MVA-BN-Filo vaccine regimen and by an Ad26.ZEBOV booster vaccination after one year (Arm 1) or after two years (Arm 2) and the number of responders at each time point.

	N	Responders[†] n (%)	N	GMC EU/mL (95% CI)	Fold increase in GMC compared to baseline on Day 1
Baseline Day 1*					
Arm 1	342[$]	166 (49)	342[$]	53·7 (46·5-62·1)	NA
Arm 2	342	178 (52)	342	56·2 (48·4-65·2)	NA
Overall	684[$]	344 (50)	684[$]	54·9 (49·5-60·9)	NA
Day 57*					
Arm 1	342[$]	204 (60)	343[$]	248·2 (223·4-275·8)	4·6
Arm 2	342	231 (68)	342	303·1 (270·5-339·7)	5·4
Overall	684[$]	435 (64)	685[$]	274·3 (253·7-296·4)	5·0
Day 78*					
Arm 1	342[$]	328 (96)	343[$]	3,854·6 (3,340·7-4,447·5)	71·8
Arm 2	341[#]	327 (96)	341[#]	4,505·2 (3,903·8-5,199·3)	80·2
Overall	683[$,#]	655 (96)	684[$,#]	4,166·3 (3,765·5-4,609·8)	75·9
Day 365/Year 1[§]					
Arm 1	314	188 (60)	314	279·9 (250·6-312·7)	5·2
Arm 2	305[£]	208 (68)	305[£]	334·8 (296·0-378·7)	6·0
Overall	619	396 (64)	619	305·7 (281·5-332·1)	5·6
Day 372/Year 1 post-booster[§]					
Arm 1	314	307 (98)	314	10,781·6 (9,354·4-12,426·4)	200·8
Arm 2	NA	NA	NA	NA	NA
Overall	NA	NA	NA	NA	NA
Day 730/Year 2[§]					
Arm 1	299[Δ]	269 (90)	299[Δ]	2,133·1 (1,827·7-2,489·7)	39·7
Arm 2	310	185 (60)	310	274·6 (242·1-311·5)	4·9
Overall	NA	NA	NA	NA	NA
Day 737/Year 2 post-booster[§]					
Arm 1	NA	NA	NA	NA	NA
Arm 2	310	303 (98)	310	10,746·9 (9,208·7-12,542·0)	191·2
Overall	NA	NA	NA	NA	NA

Where applicable blood samples were collected pre-vaccination; N = number of participants; n (%) is the number (percentage) of participants considered responders; [†]Responders were defined as participants with at least a 2.5-fold increase in binding antibody concentration from their baseline value (for participants with values above the lower limit of quantification (LLOQ) at baseline) or LLOQ (for participants with values below the LLOQ at baseline); GMC = Geometric mean concentration; EU = ELISA Units; NA = not applicable;*Analyses were conducted on per protocol set 1; [$]Results from one Day 1 sample could not be obtained as the sample exceeded the refrigerator storage stability of 30 days in the analysing laboratory before a result could be obtained. Blood samples collected during later visits from this participant are included to determine GMC with 95% confidence intervals. As the reference baseline sample was missing, the participant could at no time point be included to determine the responder rate; [#]One participant missed the Day 78 visit; [§]Analyses were conducted on per protocol set 2; [£]Five Arm 2 participants missed the Year 1 visit but returned for their Year 2 booster vaccination and were bled and vaccinated per protocol; [Δ] For 15 Arm 1 participants vaccinated and bled per protocol at Year 1, a blood sample could not be collected at the Year 2 visit for various reasons.


AE collection* (7 days)
Arm 1
Ad26.ZEBOV
MVA-BN-Filo
Ad26.ZEBOV
AE collection* (7 days)
Arm 2
Ad26.ZEBOV
Time
Day 1
Day 57
Day 78
Day 365
Year 1
Day 372
Year 1-PB
Day 730
Year 2
Day 737
Year 2-PB
SAE collection (1 year and six months)
SAE collection (2 years and six months)

720 healthcare providers and frontliners assessed for eligibility

21 excluded

20 did not meet inclusion criteria or had exclusion criteria
1 LTFU

699 enrolled

1 withdrew consent between enrolment and vaccination

698 received Ad26.ZEBOV on Day 1 (Full analysis set)

698 randomly assigned to a study arm (ratio 1:1)

350 to Arm 1

4 did not receive the MVA-BN-Filo vaccine

2 moved
1 withdrew consent
1 pregnant[$]

346 received MVA-BN-Filo on Day 57

343 bled and vaccinated per protocol (PPS1)
3 bled and/or vaccinated out of window (part of full analysis set)

27 did not receive booster vaccine

9 moved
7 pregnant*
5 medical decision*
2 LTFU
2 breastfeeding*
2 withdrew consent

319 received Ad26.ZEBOV booster on Day 365

314 bled and vaccinated per protocol (PPS2)
2 bled out of window (part of full analysis set)
3 not bled per protocol on Day 57 (part of full analysis set)

29 did not complete the study

12 moved
10 LTFU
4 withdrew consent
2 died
1 pregnant within protocol prohibited window

321 completed the study

348 to Arm 2

3 did not receive the MVA-BN-Filo vaccine

1 moved
2 pregnant[$]

345 received MVA-BN-Filo on Day 57

342 bled and vaccinated per protocol (PPS1)
3 bled and/or vaccinated out of window (part of full analysis set)

28 did not receive booster vaccine

8 LTFU
5 breastfeeding*
5 moved
4 pregnant*
4 medical decision*
1 withdrew consent
1 died

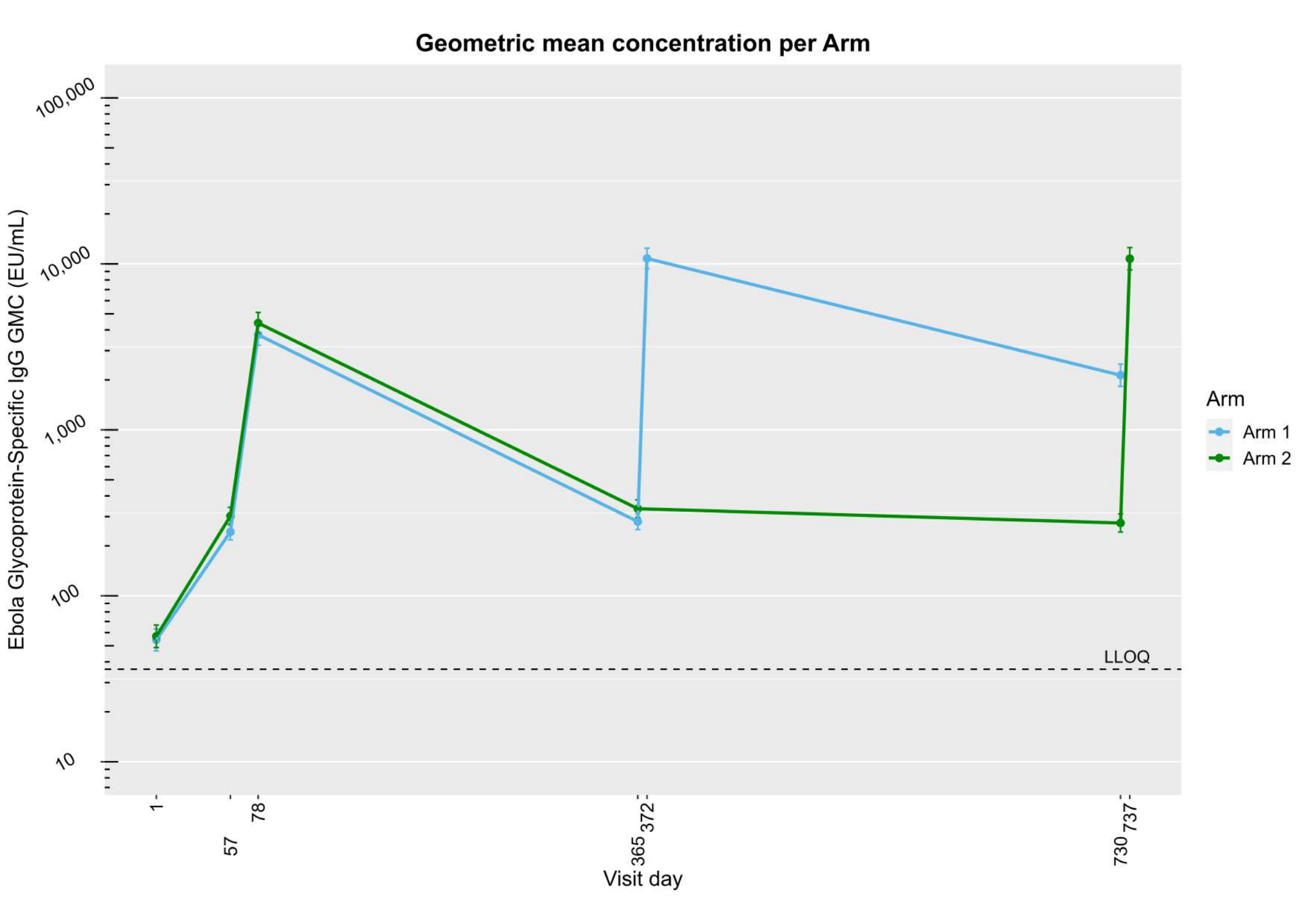
317 received Ad26.ZEBOV booster on Day 730

310 bled and vaccinated per protocol (PPS2)
5 bled out of window (part of full analysis set)
1 not bled after booster
1 not bled per protocol on Day 57 (part of full analysis set)

26 did not complete the study

12 LTFU
6 moved
4 medical decision
2 pregnant within protocol prohibited window
1 withdrew consent
1 died

322 completed the study


Geometric mean concentration per Arm
Ebola Glycoprotein-Specific IgG GMC (EU/mL)
100,000
10,000
1,000
100
10
LLOQ
1
57
78
365
372
730
737
Visit day
Arm
Arm 1
Arm 2

Supplementary material

EBL2007 study group

List of contributors to the study over the four-year study duration period (2018-2022):

Local study team in Boende

Study managers: Hypolite Muhindo Mavoko. Junior Matangila Rika. Patrick Mitashi Mulopo. Vivi Maketa; Site coordinator: Emmanuel Esanga Longomo. Trésor Zola Matuvanga; Assistant site coordinators: Solange Milolo Tshilumba. Rachel Meta; Data managers: Pitchou Kasongo Bile. Daniel Kipasa Mambu. Primo Kimbulu Lumba; Study medical doctors: Rachel Meta. Michael Bojabwa Mondjo. Danoff Endbu Elunzi. Lazare Bakongo Isofefu. Lucien Nkoyi. Yves Tchuma Bisimwa. Jimmy Mpato Manga. Bienvenue Bolingo; Study nurses: Benedicte Liuba Balao. Rebecca Asieli Malaza. Jeanette Likinda. Guylain Mondje Bakongo. Sandra Mpia Bienga. Junior Mputu Ikomoli. Clarisse Ikuma Bampunga. Kanza Baye Nsase. Amba Boongo. Marguerite Mbenga Lolu; Study laboratory technicians and biologists: Elisabeth Mukundi Madinda. Patience Masinga Mbuku. Rodin Mukele Lungaba. Trésor Lipetsi Loyenga. Blandine Bokomo Belenge. Claudine Bakambo Luende; Cold chain and IP management: Solange Milolo Tshilumba. Emannual Esanga. Michael Bobjabwa Mondjo; Pharmacist: Francis Ngoy Kankienza; Safety management team: Rachel Meta. Yves Tchuma Bisimwa. Trésor Zola Matuvanga; Site financial coordinator: Maguy Issekitolo Fatuma Mpona; Electrician: Likali Bofuke; Study facility cleaners: Sorros. Lokuli Lokwa. Bofete Liweli; Study facility guards: Nicolas Boya Likuwa. Jean Bakalo Mpeti. Bokongola Ifambe. Daudin Lokuli

Principal investigator team (University of Kinshasa)

Principal investigator and co-principal investigators: Hypolite Muhindo Mavoko. Junior Matangila Rika. Patrick Mitashi Mulopo; Study coordinator: Vivi Maketa; Site coordinators: Tresor Zola Matuvanga. Solange Milolo Tshilumba; Data managers: Pitchou Kasongo Bile. Daniel Kipasa Mambu. Primo Kimbulu Lumba; Logistics coordinators: Rody Loshinga Masudi; Financial officer: Feza Gisèle Nabazungu; Principal administrator: Phanuel Katembo; Social scientists: Freddy Bikioli; assistant social scientist: Henri Kimina; Cold chain team Kinshasa: Japhet Kabalu Tshiongo. Daddy Mangungulu Bambi; safety team: Michael Bobjabwa Mondjo. Danoff Endbu Elunzi.

Sponsor team (University of Antwerp):

Sponsor and co-sponsor: Pierre Van Damme. Jean-Pierre Van geertruyden; Personal assistant sponsor: Nele Brusselaers; Project managers: Bonome Nturo. Elke Stoppie. Ynke Larivière. Jessie De Bie. Gwen Lemey; Administrative and financial coordinators: Jan Vervoort. Gwen Lemey. Peter Vermeiren; Data managers: Swabra Nakato. Alfred Dusabimana. Ynke Larivière. Bernard Isekah Osang'ir; Statistician: Bernard Isekah Osang'ir; Medical reviewers: Kanchana Withanage. Katie Steenackers. Ilse De Coster. Marie-Annick Götze; Lead social scientists: Séverine Thys. Antea Paviotti; Social scientist: Maha Salloum; Modelling lead: Niel Hens; Modeller: Irene Garcia-Fogeda

Janssen team

Global program leader: Kim Offergeld. Annick Bessems; Global Trial Leader: Helga Pissens. Ines Martinez Vazquez. Adriana Hollestein; Global clinical trial assistant: Agnieszka Kwasniak. Kinga Mojzes;Disease management program leader: Paula Mc Kenna; Senior trial supply management specialist: Ellen Teunissen. Katrien Aerts; Trial supply manager: Megan Seels; Regulatory CTA submission manager: Jade Yee; Lead associate-submissions manager: Tomeka Harris. Guusje Hoogeveen; Medical leader: Cynthia Robinson; Global data manager: Regina In't Veld. Ernesto Fernandez. Julia Chiang; Data delivery senior manager: Tinne De Cnodder; Project manager J&J global public health: Anneleen Vuchelen; Study responsible physician; Joachim Doua. Katwere Michael; Regional trial manager: Joel Nawatsi; Trial supply team lead: Nanou Van Gils; Local safety officer contact: Amani Ghadban. Damelya Medetbekova; Project lead clinical immunology: Griet Van Roey. Maaike Ligthart; Clinical supplies integrator: Tom Reijns. Max Grafe. Sohandra Randrasana; Statistical programming lead: Lee Armishaw. Meenakshi Behl; Statistical leader: Auguste Gaddah; Biomarker leader: Vicki Bockstal. Chelsea McLean; Regulatory medical writer: Marleen Van Looveren; Quality assurance representative: Carolyn Artis

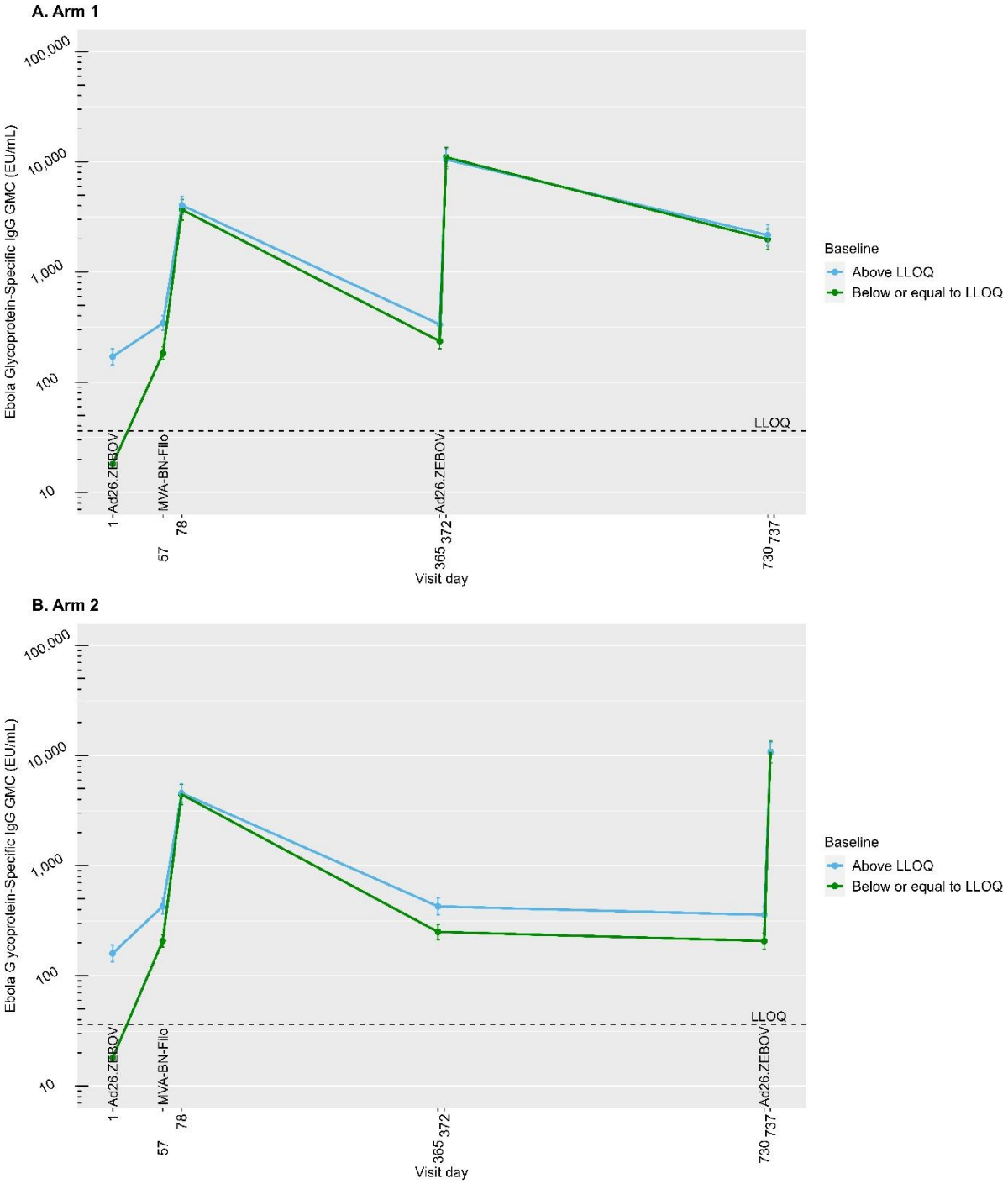
Clinical Research Organization team (ACE Research)

CEO ACE research: Odika Apollo. Victoria Tifft; Project Manager: Victorine Owira; Project manager: Sue Chase. Leslie Shupenko; In-country project manager: Andy Numbi. Dacquin Kasumba; Regulatory affairs: Amos Ndhere; Safety manager: Lucas Tina; Project specialist: Oliver Kipkemei; Clinical monitors: Jerry Liwono. Trésor Bodjick. Zakaria Gansane. James Okwach. Willy Mutangala. Simon Pierre Kisisa. Ken Awuondo; Quality assurance: Gonzaga Onyuka. Lillian Nambuchi. Penina Apudo

Data management team (DFNet)

President DFNet: Lisa Ondrejcek; Director Biometrics: Gavin Robertson; Clinical coding supervisor; Brian Postle; Data managers: Brian Postle. Yvonne Hong. Karolyn Scott; Statistical programmer: Jerad Post; Clinical coding lead: Khris Kline

Supplementary Figures



Supplementary Figure 1. Geometric mean concentration among both study arms according to the baseline antibody detectability

Binding antibody geometric mean concentrations (GMC) for participants with a baseline GMC below or equal to the lower limit of quantification (LLOQ = 36.11 ELISA Units/mL) in green versus for participants with a baseline GMC above the LLOQ in blue among (A) Arm 1 participants and (B) Arm 2 participants.

Supplementary tables

Supplementary Table 1. Overview of serious adverse events

SAE nr.	Timing	System Organ Class (SOC)	MeDra Preferred Term	Toxicity grade	Relatedness	Outcome	Arm
1	Vaccine regimen	Congenital, familial and genetic disorders	Hydrocele	Moderate	Not related to IP	Recovered/resolved	Arm 2
2	Vaccine regimen	Gastrointestinal disorders	Abdominal adhesions	Severe	Not related to IP	Recovered/resolved	Arm 2
3	Vaccine regimen	Gastrointestinal disorders	Inguinal hernia	Moderate	Not related to IP	Recovered/resolved	Arm 2
4	Vaccine regimen	Gastrointestinal disorders	Inguinal hernia	Moderate	Not related to IP	Recovered/resolved	Arm 2
5	Vaccine regimen	Gastrointestinal disorders	Dyspepsia	Mild	Not related to IP	Recovered/resolved	Arm 1
6	Vaccine regimen	Gastrointestinal disorders	Enteritis	Moderate	Not related to IP	Recovered/resolved	Arm 2
7	Vaccine regimen	Gastrointestinal disorders	Inguinal hernia	Severe	Not related to IP	Recovered/resolved	Arm 2
8	Vaccine regimen	Gastrointestinal disorders	Abdominal strangulated hernia	Moderate	Not related to IP	Recovered/resolved	Arm 1
9	Vaccine regimen	General disorders and administration site conditions	Asthenia	Severe	Not related to IP	Recovered/resolved	Arm 2
10	Vaccine regimen	General disorders and administration site conditions	Pyrexia	Mild	Not related to IP	Recovered/resolved	Arm 2
11	Vaccine regimen	Infections and infestations	Malaria	Severe	Not related to IP	Recovered/resolved	Arm 1
12	Vaccine regimen	Infections and infestations	Typhoid fever	Moderate	Not related to IP	Recovered/resolved	Arm 1
13	Vaccine regimen	Infections and infestations	Malaria	Moderate	Not related to IP	Recovered/resolved	Arm 1
14	Vaccine regimen	Infections and infestations	Malaria	Severe	Not related to IP	Recovered/resolved	Arm 2
15	Vaccine regimen	Infections and infestations	Typhoid fever	Severe	Not related to IP	Recovered/resolved	Arm 2
16	Vaccine regimen	Infections and infestations	Dermo-hypodermitis	Severe	Not related to IP	Fatal	Arm 2
17	Vaccine regimen	Infections and infestations	Malaria	Severe	Not related to IP	Recovered/resolved	Arm 1
18	Vaccine regimen	Infections and infestations	Malaria	Severe	Not related to IP	Recovered/resolved	Arm 2
19	Vaccine regimen	Infections and infestations	Typhoid fever	Moderate	Not related to IP	Recovered/resolved	Arm 2
20	Vaccine regimen	Infections and infestations	HIV infection	Severe	Not related to IP	Fatal	Arm 1
21	Vaccine regimen	Infections and infestations	Malaria	Severe	Not related to IP	Recovered/resolved	Arm 2
22	Vaccine regimen	Infections and infestations	Malaria	Severe	Not related to IP	Recovered/resolved	Arm 1
23	Vaccine regimen	Infections and infestations	Pneumonia	Moderate	Not related to IP	Recovered/resolved	Arm 1
24	Vaccine regimen	Injury, poisoning and procedural complications	Lower limb fracture	Moderate	Not related to IP	Recovered/resolved with sequelae	Arm 2
25	Vaccine regimen	Injury, poisoning and procedural complications	Wound haemorrhage	Moderate	Not related to IP	Recovered/resolved	Arm 2
26	Vaccine regimen	Metabolism and nutrition disorders	Dehydration	Severe	Not related to IP	Recovered/resolved	Arm 2

27	Vaccine regimen	Metabolism and nutrition disorders	Dehydration	Moderate	Not related to IP	Recovered/resolved	Arm 1
28	Vaccine regimen	Metabolism and nutrition disorders	Dehydration	Severe	Not related to IP	Recovered/resolved	Arm 2
29	Vaccine regimen	Neoplasms benign, malignant and unspecified (incl cysts and polyps)	Uterine leiomyoma	Moderate	Not related to IP	Recovered/resolved	Arm 2
30	Vaccine regimen	Nervous system disorders	Cerebrovascular accident	Severe	Not related to IP	Recovered/resolved with sequelae	Arm 2
31	Vaccine regimen	Nervous system disorders	Cerebrovascular accident	Severe	Not related to IP	Recovered/resolved with sequelae	Arm 2
32	Vaccine regimen	Pregnancy, puerperium and perinatal conditions	Foetal distress syndrome	Severe	Not related to IP	Recovered/resolved	Arm 1
33	Vaccine regimen	Pregnancy, puerperium and perinatal conditions	Abortion spontaneous	Moderate	Not related to IP	Recovered/resolved	Arm 1
34	Vaccine regimen	Pregnancy, puerperium and perinatal conditions	Placenta praevia haemorrhage	Severe	Not related to IP	Recovered/resolved	Arm 1
35	Vaccine regimen	Pregnancy, puerperium and perinatal conditions	Foetal distress syndrome	Severe	Not related to IP	Recovered/resolved	Arm 1
36	Vaccine regimen	Pregnancy, puerperium and perinatal conditions	Abortion spontaneous	Moderate	Not related to IP	Recovered/resolved	Arm 2
37	Vaccine regimen	Renal and urinary disorders	Ureterolithiasis	Severe	Not related to IP	Fatal*	Arm 2
38	Vaccine regimen	Renal and urinary disorders	Calculus bladder	Severe	Not related to IP	Fatal*	Arm 2
39	Vaccine regimen	Renal and urinary disorders	Urinary retention	Moderate	Not related to IP	Recovered/resolved	Arm 2
40	Vaccine regimen	Renal and urinary disorders	Calculus bladder	Severe	Not related to IP	Recovered/resolved	Arm 1
41	Vaccine regimen	Reproductive system and breast disorders	Ovarian cyst	Moderate	Not related to IP	Recovered/resolved	Arm 2
42	Vaccine regimen	Skin and subcutaneous tissue disorders	Skin ulcer	Severe	Not related to IP	Recovered/resolved	Arm 2
43	After booster dose	Gastrointestinal disorders	Inguinal hernia	Moderate	Not related to IP	Recovered/resolved	Arm 2
44	After booster dose	Gastrointestinal disorders	Umbilical hernia	Severe	Not related to IP	Recovered/resolved	Arm 1
45	After booster dose	Gastrointestinal disorders	Inguinal hernia	Moderate	Not related to IP	Recovered/resolved	Arm 2
46	After booster dose	Gastrointestinal disorders	Inguinal hernia	Moderate	Not related to IP	Recovered/resolved	Arm 2
47	After booster dose	Gastrointestinal disorders	Strangulated umbilical hernia	Severe	Not related to IP	Recovered/resolved	Arm 1
48	After booster dose	Gastrointestinal disorders	Inguinal hernia	Moderate	Not related to IP	Recovered/resolved	Arm 2
49	After booster dose	General disorders and administration site conditions	Pyrexia	Severe	Related to IP	Recovered/resolved	Arm 2
50	After booster dose	Infections and infestations	Typhoid fever	Moderate	Not related to IP	Recovered/resolved	Arm 2
51	After booster dose	Infections and infestations	Appendicitis	Moderate	Not related to IP	Recovered/resolved	Arm 2
52	After booster dose	Infections and infestations	Postoperative wound infection	Moderate	Not related to IP	Recovered/resolved	Arm 2
53	After booster dose	Infections and infestations	Appendicitis	Moderate	Not related to IP	Recovered/resolved	Arm 2
54	After booster dose	Infections and infestations	Appendicitis	Moderate	Not related to IP	Recovered/resolved	Arm 2
55	After booster dose	Infections and infestations	Typhoid fever	Moderate	Not related to IP	Recovered/resolved	Arm 2

56	After booster dose	Infections and infestations	Appendicitis	Moderate	Not related to IP	Recovered/resolved	Arm 2
57	After booster dose	Infections and infestations	Appendicitis	Severe	Not related to IP	Recovered/resolved	Arm 1
58	After booster dose	Injury, poisoning and procedural complications	Clavicle fracture	Moderate	Not related to IP	Recovered/resolved with sequelae	Arm 2
59	After booster dose	Injury, poisoning and procedural complications	Head injury	Moderate	Not related to IP	Recovered/resolved	Arm 1
60	After booster dose	Injury, poisoning and procedural complications	Uterine rupture	Potentially life threatening	Not related to IP	Recovered/resolved	Arm 1
61	After booster dose	Nervous system disorders	Ischaemic stroke	Moderate	Not related to IP	Recovered/resolved with sequelae	Arm 2
62	After booster dose	Pregnancy, puerperium and perinatal conditions	Stillbirth	Severe	Not related to IP	Recovered/resolved	Arm 1
63	After booster dose	Renal and urinary disorders	Urinary retention	Severe	Not related to IP	Recovered/resolved	Arm 1
64	After booster dose	Renal and urinary disorders	Calculus bladder	Moderate	Not related to IP	Recovered/resolved	Arm 2

IP = Investigational product; * These are two events reported simultaneously for one participant, leading to a fatal outcome in the participant.