Safety and long-term immunogenicity of a two-dose Ad26.ZEBOV, MVA-BN-Filo Ebola vaccine regimen in adults: a randomised, double-blind, controlled trial in Sierra Leone

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Abstract

Background The West African and Democratic Republic of Congo Ebola epidemics highlight an urgent need for safe and effective vaccines to prevent Ebola virus disease. This study assesses safety, long-term immunogenicity and immune responses of a two-dose heterologous vaccination regimen of Ad26.ZEBOV and MVA-BN-Filo in Sierra Leone, a country previously affected by Ebola.

Methods The study comprised an open-label stage 1 and a randomised, double-blind, controlled stage 2 (ClinicalTrials.gov NCT02509494). In stage 1, healthy adults received Ad26.ZEBOV ($5x10^{10}$ viral particles; dose 1) followed by MVA-BN-Filo ($1x10^{8}$ infectious units; dose 2) 56 days later. An Ad26.ZEBOV booster vaccination was offered two years post dose 1. In stage 2, participants were randomised 3:1 to receive Ad26.ZEBOV, MVA-BN-Filo vaccine regimen or meningococcal conjugate vaccine (MenACWY) followed by placebo, 56 days later. Stage 2 participants were randomised using block randomisation via an Interactive Web Response System. Study team personnel (except those with primary responsibility for study vaccine preparation) and participants were blinded to the study vaccine allocation. The evaluation of safety and tolerability of the Ad26.ZEBOV, MVA-BN-Filo vaccine regimen was the study primary outcome and was assessed in all participants by collecting solicited local and systemic adverse events (AEs) in the first seven days after each vaccination, unsolicited AEs in the first 28 days after each vaccination and serious AEs (SAEs) until each participant's last study visit. The secondary outcomes were to assess binding antibody responses at 21-day post dose 2 in a per-protocol set of participants and to assess the safety and tolerability of the Ad26.ZEBOV booster vaccination in Stage 1 participants. The primary analysis set for safety comprised all participants who received at least one dose of study vaccine while the primary analysis set for immunogenicity included all participants who received both vaccinations within the protocol defined time window, had at least one post-vaccination evaluable immunogenicity sample and had no major protocol deviations that could have influenced the immune response.

Findings Overall, 443 adults (stage 1: n=43; stage 2: n=400) were vaccinated with Ad26.ZEBOV, MVA-BN-Filo (n=341), or MenACWY, placebo (n=102). Both regimens were well tolerated with no safety concerns. Solicited local AEs (mostly mild or moderate injection site pain) were reported in stage 1 by 12/43 (28%) participants post Ad26.ZEBOV (dose 1) and by 6/43 (14%) participants post MVA-BN-Filo. In stage 2, solicited local AEs were reported by 51/298 (17%) adults post Ad26.ZEBOV, 58/246 (24%) post MVA-BN-Filo, 17/102 (17%) post MenACWY and eight 8/86 (9%) post placebo. Solicited systemic AEs were reported in

stage 1 by 18/43 (42%) participants post Ad26.ZEBOV (dose 1) and 17/43 (40%) post MVA-BN-Filo. In stage 2, solicited systemic AEs were reported by 161/298 (54%) adults post Ad26.ZEBOV, 107/246 (43%) post MVA-BN-Filo, 51/102 (50%) post MenACWY and 39/86 (45%) post placebo. Solicited systemic AEs included mostly mild or moderate headache, myalgia, fatigue, and arthralgia. The most frequent unsolicited AE post dose 1 and post dose 2 was headache in stage 1 and malaria in stage 2, regardless of vaccine received. Grade 3 unsolicited AEs were infrequent, observed in at most 2% of participants regardless of vaccine received. No SAE was considered related to the study vaccine. In stage 1, the post-booster vaccination safety profile was not notably different to that observed post dose 1. Vaccine-induced humoral immune responses were observed in 41/42 (98%) stage 1 participants and in 176/179 (98%) stage 2 participants 21 days post dose 2 (geometric mean binding antibody concentration: 4784 EU/ml [95% CI 3736–6125], stage 1 and 3810 EU/ml [95% CI 3312–4383], stage 2). Antibody responses persisted for at least two years.

Interpretation The Ad26.ZEBOV, MVA-BN-Filo vaccine regimen was well tolerated and immunogenic, with humoral immune responses persisting for at least 2 years after vaccination. These data support the use of this vaccine regimen for Ebola virus disease prophylaxis in adults.

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Research in context

Evidence before this study

A PubMed search on 20 February 2020 [ebola AND (vaccin* OR immunis* OR immuniz*) AND (trial* OR study), no language restrictions] identified 733 citations. Following title/abstract screening, we found 40 publications reporting immunogenicity and/or safety results from 34 clinical trials of Ebola vaccine candidates and we consulted a WHO overview of candidate vaccines dated 19 August 2019.

Several vaccine candidates have been tested in Phase I and II clinical trials (rVSV-ZEBOV, ChAd3-EBO-Z, Ad5-EBOV, GamEvac-Combi, etc.) with an acceptable safety profile and promising immunogenicity results. Data on effectiveness against Ebola virus disease (EVD) were available only for one vaccine, rVSV-ZEBOV (estimated effectiveness: 100% in a ring vaccination trial conducted in Guinea during the 2014–2016 outbreak and 97·5% in a ring vaccination strategy to control the 2018-2020 EVD outbreak in Democratic Republic of Congo).

The two-dose Ad26.ZEBOV, MVA-BN-Filo Ebola vaccine regimen has demonstrated a good safety profile in Europeans and healthy Africans living in areas not affected by Ebola, in three phase 1 studies. The most common AEs were injection site pain and headache. No vaccine-related SAEs were reported. This vaccine regimen induced durable immune responses for at least one year in healthy adults.

Added value of this study

This is the first large-scale study that provides data on the safety, long-term immunogenicity, and humoral immune memory response induced by the Ad26.ZEBOV, MVA-BN-Filo Ebola vaccine regimen in healthy adults from a population that was severely affected by the 2014-2016 EVD epidemic in West Africa.

The vaccine regimen was well tolerated and induced humoral immune responses persisting for at least two years. Booster vaccination with Ad26.ZEBOV, given two years after initial vaccination, induced a strong anamnestic response within seven days. These findings will inform the future use of this vaccine regimen, for example, they could justify the strategy of boosting previously immunised individuals at the start of an EVD outbreak. These findings also supported the decision of the European Commission to grant marketing authorisations for the Ad26.ZEBOV, MVA-BN-Filo Ebola vaccine regimen in the European Union.

Implications of all the available evidence

Several vaccines against EVD have been shown to be safe and immunogenic in clinical trials. One vaccine, rVSV-ZEBOV, has also been proven to be highly effective in preventing EVD. Vaccine research must continue in order to determine the long-term immunogenicity of these vaccines and assess different options for prophylactic vaccination in populations at potential risk of EVD or for reactive vaccination during EVD outbreaks.

Introduction

The magnitude of the Ebola outbreak in 2014–2016 in West Africa was unprecedented, with more than 28,600 cases reported and 11,300 subsequent deaths.[1] The second largest outbreak began in 2018 in the Democratic Republic of Congo (DRC) and lasted for nearly two years with more than 3400 cases and 2200 deaths reported.[2] Recurrent Ebola virus disease (EVD) outbreaks are anticipated and have occurred with increasing frequency in some African countries.[3] Finding safe and effective vaccines against EVD that can be used along with other outbreak control measures, therefore, remains a priority. The recombinant vesicular stomatitis virus Ebola vaccine, rVSV-ZEBOV-GP, which showed effectiveness in a ring-vaccination trial conducted in Guinea during the 2014–2016 outbreak,[4] was recommended by the World Health Organisation (WHO) in emergency situations and ~~has been~~was deployed widely as part of the outbreak control response in DRC. [5,6] This vaccine received conditional marketing authorisation in the European Union (EU) and approval for use in adults in USA and several African countries.[7–9] However, as part of the preparedness measures for future outbreaks, the Strategic Advisory Group of Experts on Immunization recommended to the WHO that urgent consideration should be given to the development of additional vaccines against Ebola, focussing on safety and induction of appropriate immune responses.[10]

A heterologous, two-dose regimen of Ad26.ZEBOV (dose 1) followed by MVA-BN-Filo (dose 2) after 56 days (Ad26.ZEBOV, MVA-BN-Filo vaccine regimen) has ~~recently~~ received marketing authorisation for prophylactic use in adults and children ≥1 year old in the EU. [11] This vaccine regimen provided protection against Zaire Ebolavirus (EBOV) challenge in macaques and demonstrated a good safety profile with strong and durable immune responses for at least one year in Europeans and healthy Africans, living in areas not affected by Ebola, in three phase 1 studies and one phase 2 study.[12–16] The study reported herein evaluated the safety, long-term immunogenicity, and humoral immune memory induced by the Ad26.ZEBOV, MVA-BN-Filo vaccine regimen administered with a 56-day interval in healthy adults in Kambia District, an area of Sierra Leone affected by the 2014–2016 EVD epidemic and, therefore, at potential risk for future outbreaks.[17]

Methods

Study design

This study (VAC52150EBL3001) included an open-label stage 1 and a randomised, double-blind, controlled stage 2 component. The rationale for an open-label stage 1 was to obtain initial safety data, as it was the first time the experimental Ad26.ZEBOV, MVA-BN-Filo vaccine regimen was used in Sierra Leone and the national health authority requested the inclusion of this initial stage in the study design. Enrolment of stage 1 participants

was followed by initiation of stage 2 after review of stage 1 safety data by an Independent Data Monitoring Committee. The study was approved by the Sierra Leone Ethics and Scientific Review Committee, the Pharmacy Board of Sierra Leone, and the London School of Hygiene and Tropical Medicine Ethics Committee. This study was registered with Clinicaltrials.gov, NCT02509494. The protocol is available in the supplementary material.

Study participants

Eligible stage 1 participants were healthy adults aged ≥18 years old residing in or near Kambia town, Sierra Leone, with no intention to move from the area within the next 5 months and who were considered healthy based on physical examination and absence of laboratory abnormalities at screening. Women of childbearing age were required to adopt adequate birth control measures (i.e., contraceptive injection, oral contraception, barrier methods) from at least 14 days before dose 1, and to have a negative urine β-human chorionic gonadotrophin pregnancy test at screening and immediately prior to each vaccination. Male participants who were sexually active were requested to use condoms, starting prior to enrolment. Exclusion criteria included (but not limited to): breast feeding or pregnancy; prior EVD or vaccination with a candidate Ebola vaccine; prior vaccination with a live-attenuated vaccine within 30 days before each dose, or with an inactivated vaccine within 15 days before each dose; or a previous severe adverse reaction to a vaccine. Extensive social science research was conducted prior to the trial start to ensure effective community engagement and appropriate recruitment strategies.[18,19] Documented informed consent from a community leader was required before the study start. Participants provided informed consent after passing a test of understanding. If the participant was unable to read or write, the procedures were explained, and informed consent was witnessed by a literate third person not involved in the study. Stage 2 inclusion and exclusion criteria were consistent with stage 1 criteria. Stage 2 also enrolled children 1–17 years old and data from these paediatric cohorts is presented in a separate publication. The full list of inclusion and exclusion criteria is provided in the protocol (supplementary material).

Randomisation and masking

There was no randomisation in stage 1. Stage 2 participants were centrally randomised using computer-generated block randomisation via an Interactive Web Response System (IWRS), operated by a study pharmacist. Study team personnel (except those with primary responsibility for study vaccine preparation) and participants were blinded to study vaccine allocation until all participants had completed the 6-month post dose 2 visit or discontinued the study earlier and the database had been locked. Blinding was achieved using syringes of identical volume taped to conceal the colour of the liquid inside.

Vaccines and vaccination

Ad26.ZEBOV (Janssen Vaccines & Prevention, B.V.) is a monovalent, recombinant, replication-incompetent, Ad26-vectored vaccine encoding the glycoprotein (GP) of the EBOV Mayinga variant. MVA-BN-Filo (Bavarian Nordic) is a recombinant, non-replicating, modified vaccinia Ankara-vectored vaccine encoding the EBOV Mayinga variant GP as well as Sudan virus Gulu variant GP, Marburg virus Musoke variant GP, and Taï Forest virus nucleoprotein. In stage 1, all participants received Ad26.ZEBOV (dose 1) followed by MVA-BN-Filo (dose 2), 56 days later. An Ad26.ZEBOV booster vaccination was also offered to stage 1 participants two years post dose 1 (day 720) (figure 1). Stage 2 participants were randomised 3:1 to receive the same Ebola vaccine regimen, or one dose of a meningococcal polyvalent (serogroups A, C, W135, and Y) conjugate vaccine (MenACWY, Menveo®, GSK Vaccines, or Nimenrix®, Pfizer) followed by a saline placebo 56 days later, as an active control (MenACWY, placebo regimen) (figure 1). All vaccines were administered via a single 0·5 mL intramuscular injection into the deltoid muscle at a dose of 5×10^{10} viral particles for Ad26.ZEBOV, 1×10^{8} Inf.U for MVA-BN-Filo, 0·5 ml reconstituted vaccine solution for MenACWY, and 0·5 mL 0·9% sodium chloride solution for the placebo.

Study outcomes

For stage 1 and 2, the primary study outcome was to assess the safety and tolerability of the Ad26.ZEBOV, MVA-BN-Filo vaccine regimen, as expressed by the number of participants with solicited local and systemic adverse events (AEs) in the first seven days after each vaccination, unsolicited AEs in the first 28 days after each vaccination and serious adverse events (SAEs) until each participant's last study visit. The secondary outcomes were to assess binding antibody responses as measured by EBOV GP Enzyme-Linked Immunosorbent Assay (ELISA) at 21 days post dose 2 and, for stage 1 alone, to assess the safety and tolerability of an Ad26.ZEBOV booster vaccination administered at least two years post dose 1. The exploratory outcomes were to assess the humoral immune responses at other relevant time points and to assess the neutralising activity of vaccine-induced antibody responses (nAbs) directed against EBOV GP, and against the Ad26 and MVA vectors.

Safety evaluations

To record any immediate AEs, participants were observed for at least 30 minutes after vaccination. Local and systemic solicited AEs were recorded by diary cards for seven days following each vaccination. Clinical laboratory tests were performed seven days after each vaccination, comprising a haematology panel

(haemoglobin, haematocrit, red blood cell count, platelet count, and white blood cell count with differential), and a serum chemistry panel (ALT, AST and creatinine). Each participant received a 24-hour telephone number to contact in case of medical problems. In stage 1, all AEs were recorded from dose 1 until 56 days after dose 2 and then again from the day of the booster vaccination until 28 days post-booster vaccination. In stage 2, AEs were recorded for 28 days following each vaccination. In both stages 1 and 2, SAEs were recorded from dose 1 until each participant's last study visit, i.e., three years post dose 1 in stage 1, and two years post dose 1 in stage 2.

Immunogenicity assessments

In stage 1, immunological assays were performed on blood samples taken immediately before doses 1 and 2, then at 21 days post dose 2, 155 and 360 days post dose 1 and, subsequently, every six months up to three years post dose 1. In participants who agreed to the booster vaccination, additional immunogenicity samples were collected immediately before the booster vaccination and at four days, seven days, 21 days, six months, and one year post-booster vaccination. In stage 2, immunogenicity samples were collected pre dose 1, 28 days post dose 1, pre dose 2, 21 days and six months post dose 2, one and two years post dose 1.

Immunoglobulin G responses against EBOV GP were analysed using the EBOV GP (Kikwit) Filovirus Animal Non-Clinical Group (FANG) ELISA, as in previous studies.[13–16] The analysis was conducted at Q2 Solutions – Vaccine Testing Laboratory, San Juan Capistrano, CA. In a randomly selected subset of stage 2 participants, the nAb response was assessed using an EBOV GP (Makona) pseudovirion neutralisation Assay (psVNA) at Monogram Biosciences, San Francisco, CA (supplementary material). nAbs against the Ad26 and MVA vector backbones were measured at baseline using an Ad26-specific virus neutralisation assay (Ad26 VNA) performed by Janssen and a plaque reduction neutralisation test (PRNT), performed by Bavarian Nordic, Munich, Germany, respectively.

Statistical analysis and sample size

The planned sample size for stage 1 (n=40) and stage 2 (n=400; 300 receiving Ad26.ZEBOV, MVA-BN-Filo and 100 receiving MenACWY, placebo) were calculated to provide, when combined, a ≥99% probability of observing at least one SAE occurring at a rate of 1/10 or more in each group. The probability of observing at least one SAE occurring at a rate of 1/100 was 95% with 300 participants.

For the analysis of the EBOV GP-specific nAb response, a subset of 74 out of 260 (28%) adult stage 2 participants were selected at random using a computer software (SAS) in a 3:1 ratio of active to control to

ensure that the distribution of the selected participants was similar to the overall active to control ratio in stage 2 of the study. This was done prior to the analysis of the samples, among a number of stage 2 participants with available samples and no protocol deviations that could have influenced the immune response. No stage 1 participants were analysed for EBOV GP-specific nAb response. This subset selection was made, not based on a separate sample size calculation, but on the number of samples that could be analysed in a reasonable amount of time and was considered large enough to provide a representative characterisation of the neutralising antibody response. For the analysis of nAb against the Ad26 (VNA) and MVA (PRNT) vectors, all stage 1 participants and the same subset of 74 stage 2 participants as described above, were analysed. Subsequently, it was decided to analyse for nAb against the Ad26 vector also all the remaining stage 2 participants in the per-protocol analysis set who received the active vaccine regimen.

The primary analysis in stage 1 and stage 2 was performed when all adult participants completed the study or discontinued early. The primary analysis set for safety (full analysis set) comprised all participants who received at least 1 dose of study vaccine. Data are shown by vaccination group (as treated). The primary analysis set for immunogenicity (per-protocol) included all vaccinated participants (i.e., stage 1 non-randomised and stage 2 randomised participants), who received both dose 1 and dose 2 within the protocol-defined window, and who had at least 1 post-vaccination evaluable immunogenicity sample, and who had no major protocol deviations that could have influenced the immune response. A sensitivity analysis was performed in participants who received dose 2 outside the protocol-defined window. Since the main purpose of stages 1 and 2 of this study was to provide preliminary evaluation of safety and immunogenicity without formal hypothesis testing, all data were analysed using descriptive statistics.

Participants were considered as responders by ELISA if samples were negative at baseline and positive post baseline with a value that was greater than 2·5 times the lower limit of quantification [LLOQ: 36·11 ELISA units/mL (EU/mL)], or a sample was positive both at baseline and post baseline and there was a greater than 2·5-fold increase from baseline. Participants were considered as responders for psVNA if a sample was negative at baseline and positive post baseline and the post-baseline value was greater than two times the LLOQ (120 half maximal inhibitory concentration [IC_{50}] titre), or samples were positive both at baseline and post baseline and there was a greater than 2-fold increase from baseline.

Immunoglobulin G responses against EBOV GP (ELISA) and nAb activity (psVNA) are shown as geometric mean concentrations (GMCs) and geometric mean titres (GMTs), respectively, with 95% confidence intervals

(CIs). All values below the LLOQ were imputed with half the LLOQ value. Spearman correlation coefficient was calculated between EBOV GP-specific binding antibodies (ELISA) and psVNA titres at 21 days post dose 2. A post-hoc correlation analysis between Ad26 neutralising antibody titres prior to vaccination and EBOV GP binding antibody responses 21 days post dose 2 was conducted. In addition, a post-hoc correlation analysis between EBOV GP binding antibody concentrations measured at baseline and EBOV GP binding antibody responses 21 days post dose 2 was performed (Supplementary Material).

All statistical analysis was done using SAS, version 9.2.

Role of the funding source

This study received funding from the Innovative Medicines Initiative (IMI) and from Janssen Vaccines & Prevention B.V. IMI had no role in the study design, conduct, or publication of this manuscript. Janssen had a role in study design, data collection, data analysis, data interpretation, and writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication. There are measures in place to allow all authors to access the study database, should they wish to do so.

Results

Adult participants were recruited between 30 September 2015 and 19 October 2016, and follow-up was completed on 28 November 2018. In stage 1, 43 adults received the Ad26.ZEBOV, MVA-BN-Filo vaccine regimen. In stage 2, 400 participants were randomised and received dose 1 of either Ad26.ZEBOV, MVA-BN-Filo, or MenACWY followed by placebo at day 56. The safety analysis includes all 43 stage 1 adults who received Ad26.ZEBOV, MVA-BN-Filo, and for stage 2 includes 298 adults who received Ad26.ZEBOV as dose 1, 246 who received MVA-BN-Filo as dose 2, 102 adults who received MenACWY, and 86 who received placebo (figure 2). Baseline characteristics of the participants are shown in table 1. In stage 2, the demographic characteristics of the Ad26.ZEBOV, MVA-BN-Filo, and MenACWY, placebo groups were similar. Twenty-nine stage 1 participants received a booster dose of Ad26.ZEBOV two years post dose 1.

Solicited AEs were mostly mild to moderate (grade 1 and 2) (figure 3 and tables S1, S2). At least one solicited local AE was reported in stage 1 by 12/43 (28%) participants post-Ad26.ZEBOV vaccination and by 6/43 (14%) participants post-MVA-BN-Filo vaccination (Figures 3A, 3C and table S1). In stage 2, at least one solicited local AE was reported by 51/298 (17%) adults following Ad26.ZEBOV vaccination and 58/246 (24%) following MVA-BN-Filo vaccination. In the MenACWY, placebo, at least one solicited local AE was reported in 17/102 (17%) adults following MenACWY vaccination and 8/86 (9%) following placebo vaccination (figures 3A, 3C and table S1). The most frequent solicited local AE was injection site pain after any vaccination (figures 3A, 3C and table S1). One report of grade 3 solicited local AE (injection site pain) was observed post-MVA-BN-Filo vaccination (figure 3C and table S1). Solicited systemic AEs in stage 1 were reported by 18/43 (42%) participants post-Ad26.ZEBOV vaccination and 17/43 (40%) post-MVA-BN-Filo vaccination (figures 3B, 3D and table S2). In stage 2, at least one solicited systemic AE was reported by 161/298 (54%) adults following Ad26.ZEBOV vaccination, 107/246 (43%) adults following MVA-BN-Filo vaccination, by 51/102 (50%) adults following MenACWY vaccination, and 39/86 (45%) adults following placebo vaccination (figures 3B, 3D and table S2). Headache, myalgia, fatigue, and arthralgia were the most frequently reported solicited systemic AEs after any vaccination, and grade 3 solicited systemic AEs were infrequently observed (figures 3B, 3D and table S2).

The most frequent unsolicited AE post dose 1 and post dose 2 was headache in stage 1 and malaria in stage 2, regardless of vaccine received (table S3). Grade 3 unsolicited AEs were infrequent, observed in at most 2% of participants regardless of vaccine received (table S4).

Twenty-three (5%) participants reported at least one SAE throughout the study (table S5); some participants had more than one SAE. Most of the SAEs occurred outside of the 28-day window for analysis of unsolicited AEs. In the 28-day period following dose 1, no stage 1 participant and 2/298 (1%) stage 2 participants in the Ebola vaccine arm reported at least one SAE following Ad26.ZEBOV and 1/102 (1%) stage 2 participants in the control arm reported at least one SAE following MenACWY. In the 28-day period following dose 2, no stage 1 and no stage 2 participants reported any SAE. In the 28-day period following the booster dose, no stage 1 participant reported any SAE. No SAE was considered related to the study vaccine. One death occurred in the Ad26.ZEBOV, MVA-BN-Filo group during the long-term follow-up period at day 198 post dose 2, due to severe dehydration as a result of severe vomiting in a participant with a history of heavy alcohol usage and use of unidentified traditional herbs.

The post-booster vaccination AE profile was not notably different to that observed post dose 1 (tables S1–S5) in the participants that received the Ad26.ZEBOV booster vaccination two years post dose 1.

Forty-three participants in stage 1 and 259 participants in stage 2 (191 in the Ad26.ZEBOV, MVA-BN-Filo group and 68 in MenACWY, placebo group) fulfilled the criteria for the per-protocol analysis set for immunogenicity. At 56 days post dose 1, EBOV GP-specific binding antibody responses (table 2 and figure 4) were observed in 28/43 (65%) stage 1 and 101/187 (54%) stage 2 participants in the Ad26.ZEBOV, MVA-BN-Filo group, with GMCs of 269 EU/mL (95% CI 208–347) and 236 EU/mL (95% CI 206–270), respectively. At 21 days post dose 2, binding antibody responses were observed in 41/42 (98%) stage 1 and in 176/179 (98%) stage 2 participants, with GMCs rising to 4784 EU/mL (95% CI 3736–6125), and 3810 EU/mL (95% CI 3312–4383), respectively.

Due to a study pause (for precautionary reasons during the evaluation of two SAEs in a different study),[16] dose 2 was delayed in 72 stage 2 participants (time interval between dose 1 and dose 2 ranged from 96 to 147 days). This delayed dose 2 administration did not negatively affect binding antibody responses. At 21 days post-dose 2 vaccination, responses were observed in 44/45 (98%) stage 2 participants in the Ad26.ZEBOV, MVA-BN-Filo group who received the delayed dose 2, with a GMC that was similar to the GMC in participants receiving dose 2 within the protocol-defined window (dose 2 delayed: 5761 EU/mL, 95% CI 3926–8455 *vs* dose 2 within protocol-defined window: 3823 EU/mL, 95% CI 3334–4383, table S6).

At day 156 (three months post dose 2, only measured in stage 1), the magnitude of binding antibody responses had decreased, with GMC of 544 EU/mL (95% CI 422–701), then remained approximately stable until day 720 (table 2 and figure 4). At day 360, persistent binding antibody responses were observed in 24/31 (77%) stage 1

297 participants, and in 82/166 (49%) stage 2 participants, with GMCs of 325 EU/mL (95% CI 238–445) and 259
298 EU/mL (95% CI 223–301), respectively. At day 720, a persistent response was observed in 21/31 (68%) stage 1
299 participants and in 78/155 (50%) stage 2 participants, with GMCs of 279 EU/mL (95% CI 201–386) and 255
300 EU/mL (95% CI 212–306) respectively.

301 Following the Ad26.ZEBOV booster vaccination given to a subset of stage 1 participants two years post dose 1,
302 24/25 (96%) displayed a strong increase in binding antibody responses seven days post-booster vaccination with
303 a GMC of 11166 EU/mL (95% CI 5881–21201), a 40-fold increase in GMC versus pre-booster vaccination time
304 point. At 21 days post-booster vaccination, all 29 participants had a response with a GMC of 30411 EU/mL
305 (95% CI 21972–42091), an approximate 110-fold increase versus pre-booster vaccination (table 2 and figure 4)
306 and 6-fold greater than 21 days post dose 2 levels. Binding antibody concentrations decreased at one-year post
307 booster, with GMC of 3237 EU/mL (95% CI 2305–4547), however, persistent responses were observed in all 26
308 participants still on follow-up, at a level approximately 10-fold higher than that observed at one and two years
309 post dose 1.

310 EBOV GP-specific nAb concentrations were measured in a randomly selected stage 2 participant subset (n=74,
311 of which n=55 in the Ebola vaccine arm and n=19 in the control arm) (figure 4 and table S7). At 56 days post
312 dose 1, an EBOV GP-specific nAb response was observed in one participant out of 51 in the Ebola vaccine arm
313 (2%) with a GMT below the LLOQ. At 21 days post dose 2, an EBOV GP-specific nAb response was detected
314 in 52/53 (98%) participants in the Ebola vaccine arm with GMT of 2199 (95% CI 1634–2960). There was a
315 strong positive correlation between Ebola GP-specific binding antibodies and nAbs at 21 days post dose 2 in
316 participants who received Ebola vaccine (Spearman correlation coefficient: 0·751) (figure S1). At day 360, the
317 nAb response persisted in 3/53 (6%) participants in the Ebola vaccine arm. At about two-years post dose 1, nAb
318 responses were observed in 6/51 (12%) participants in the Ebola vaccine arm.
319 Ad26-specific pre-existing nAbs were measured in all participants assigned to stage 1 (n=43), and in a subset of
320 participants assigned to stage 2 [191/298 (64%) in the Ebola vaccine arm; 18/102 (18%) in the control arm].
321 Ad26-specific pre-existing nAbs were detected in 40/43 (93%) stage 1 participants, in 177/191 (93%) stage 2
322 participants in the Ebola vaccine arm and in 17/18 (94%) stage 2 participants in the control arm, with similar
323 GMTs between groups (IC_{90} GMTs of 111, 124, and 104 in stage 1, stage 2 Ebola vaccine arm and stage 2
324 control arm, respectively) (table S8). There was a negligible correlation between the baseline Ad26-specific nAb
325 titres and the vaccine-induced EBOV GP-specific binding antibody concentrations at 21 days post dose 2
326 (Spearman correlation coefficient: –0·145) (figure S2).

Prior to vaccination, MVA-specific neutralising antibodies were analysed in almost all [42/43 (98%)] stage 1 participants and a subset [56/298 (19%) in the Ebola vaccine arm; 18/102 (18%) in the control arm] of stage 2 participants. Neutralising antibodies against the MVA vector were observed in only 2/42 (5%) stage 1 participants and 3/56 (5%) of stage 2 in the Ebola vaccine arm, and in 3/18 (17%) stage 2 participants in the control arm. The GMT values at baseline were all below the LLOQ (table S9).

Discussion

This is the first clinical study of the Ad26.ZEBOV, MVA-BN-Filo vaccine regimen undertaken in an area that was affected by the West African Ebola outbreak in 2014–2016.

The regimen was well tolerated, with injection site pain the most frequent solicited local AE, and headache, myalgia, fatigue, and arthralgia the commonest solicited systemic AEs. No SAEs were considered related to the study vaccine.

The Ad26.ZEBOV, MVA-BN-Filo vaccine regimen induced EBOV GP-specific binding and nAbs responses observed in 98% of adult participants at 21 days post dose 2 (binding antibody responses: 41/42 [98%] in stage 1 and 176/179 [98%] in stage 2; nAb responses: 52/53 [98%] in stage 2). At this time point, a strong positive correlation was observed between binding antibody concentrations and nAb titres. The magnitude of the antibody responses declined over time: at one-year post dose 1, binding antibody responses persisted in 24/31 (77%) stage 1 participants and in 82/166 (49%) stage 2 participants; at two-years post dose 1, binding antibody responses persisted in 21/31 (68%) stage 1 participants and 78/155 (50%) stage 2 participants; in a randomly-selected stage 2 subset, nAb responses persisted in 3/53 (6%) participants at one-year post dose 1 and in 6/51 (12%) at two years post dose 1.

Although more than 90% of the adult participants had pre-existing nAbs specific for the Ad26 vector, statistical correlation analyses indicated that there was no association between Ad26-specific pre-existing immunity and the vaccine-induced EBOV GP-specific binding antibody responses. Hence, irrespective of whether there is low or high Ad26 seroprevalence where the vaccine is deployed, pre-existing immunity for the Ad26 vector should not have an impact on the immunogenicity of the vaccine.

The immunogenicity findings described here are consistent with data observed in previous studies, which have shown the safety and immunogenicity of this vaccine regimen in a European population,[14,16,20] and in East African populations that were not affected by the 2014–2016 outbreak.[13,15] The kinetics of the humoral responses observed in phase 1 and 2 clinical studies were confirmed in this study.[13–16,20]

A limited number of stage 2 participants received their dose 2 outside the protocol-defined window. A sensitivity analysis showed that the extension of the time interval between Ad26.ZEBOV and MVA-BN-Filo had no adverse effect on vaccine-induced immune responses at 21 days post dose 2, as 44/~~46~~ 45 (98%) participants who received the delayed dose 2 showed EBOV GP-specific binding antibody responses with a GMC that was similar to that observed in the group receiving dose 2 within the protocol-defined window. Our

study also shows that a booster vaccination with Ad26.ZEBOV given two years post initial vaccination was well tolerated and induced a strong anamnestic response evidenced by an approximately 40-fold and 110-fold increase in binding antibody concentrations at seven- and 21-days post-booster vaccination, respectively (compared with pre-booster levels). Binding antibody concentrations decreased at one-year post booster; yet, responses were observed in all the participants at a level about 6-fold higher than the level observed at one- and two-years post dose 1. This finding demonstrates that the Ad26.ZEBOV, MVA-BN-Filo vaccine regimen had induced humoral immune memory, which we believe can be triggered by future natural infections and is a significant finding in relation to future considerations of the deployment of this vaccine. Prophylactic vaccination with the Ad26.ZEBOV, MVA-BN-Filo vaccine regimen could be considered for a medium-to-long-term strategy. In addition, as a precautionary measure, a booster vaccination with Ad26.ZEBOV could be considered in anticipation of an imminent exposure to Ebola virus.

This study has some limitations including gender imbalance of the study population, as most participants were male due to local socioeconomic and cultural factors; exclusion of pregnant women as is generally conventional during new investigational product trials (with the related requirement for contraception in those of child bearing potential);[21] the measurements of neutralising antibody titres in only a subset of participants and the booster dose offered only to Stage 1 participants. The study was initially planned as a large cluster randomised trial with vaccine effectiveness as primary endpoint; however, the study design and outcomes were changed following the decline of the EVD outbreak in Sierra Leone (i.e. the cluster randomised trial component was removed, the follow-up was extended and the booster dose in stage 1 participants was included). The addition of the booster dose for Stage 1 participants was an attempt to get some data on how long the anamnestic response would last in participants previously vaccinated with the Ad26.ZEBOV, MVA-BN-Filo vaccine regimen. Since Stage 1 participants were the first to be vaccinated in the study, they were also the first group to reach the two years post-dose 1 timepoint, when the booster dose was due to be administered, and they were the only group, for which we had enough time to conduct the one-year follow-up after the booster dose.

Notwithstanding these limitations, the study had many strengths, including the enrolment of participants in a previously Ebola-affected country and a two-year follow-up period, which provided the opportunity to assess the durability of immune responses, and booster vaccination given two years after the initial vaccination. The study commencing during the Ebola outbreak, in a largely rural setting with a research-naïve population, has provided valuable lessons regarding clinical trial set-up and conduct under difficult conditions.[19] Participant retention was challenging, especially in the outbreak aftermath as some individuals relocated outside the study

area for work, business or study. Despite this challenge, reasonable long-term retention rates were achieved due to concerted community trust-building and participant follow-up arrangements.[18,19,22]

The Ad26.ZEBOV, MVA-BN-Filo vaccine regimen with a 56-day interval assessed here has recently received marketing authorisations for prophylactic use in adults and children ≥1 year old in the EU. [11] This vaccine regimen was previously shown to provide protection in vaccinated non-human primates against an EBOV challenge, which is fully lethal in unvaccinated control animals.[12] In the absence of clinical efficacy data in humans, a statistical approach referred to as immunobridging using data from this and other clinical studies, was used to infer the potential clinical benefit induced by vaccination by correlating the magnitude of vaccine-elicited immune parameters in non-human primates with those observed in vaccinated humans.[23] Although a mechanistic correlate of protection has not yet been identified, the binding antibody GMCs observed 21 days after the second dose in the two-dose regimen, were similar to the GMC of 1262 EU/mL (95% CI 1169–1363) reported 28 days post-rVSV-ZEBOV vaccination using the same assay in the same laboratory.[24] rVSV-ZEBOV, which was the first Ebola vaccine to received conditional marketing authorisation in Europe and approval for use in adults in USA and several African countries,[7–9] is the the only vaccine for which vaccine effectiveness (VE) data are available so far (i.e. estimated VE of 100% from 10 days post vaccination onwards in a phase 3 trial in Guinea during the West African outbreak,[4] and an estimated VE of 97·5% in DRC).[6]

Recognising the threat of unpredictable future Ebola outbreaks, further vaccine development work is vital to strengthen international health security by diversifying vaccination strategy options. Additional studies are in progress, such as PREVAC, a randomised trial (ClinicalTrials.gov NCT02876328) currently underway in Sierra Leone, Guinea, Liberia, and Mali, which is assessing three vaccine strategies in adults and children, including the Ad26.ZEBOV, MVA-BN-Filo regimen, the rVSV-ZEBOV-GP single-dose vaccine, and a rVSV-ZEBOV-GP two-dose regimen.[25] Another study, DRC-EB-001 (ClinicalTrials.gov Identifier: NCT04152486), is ongoing in North Kivu, DRC, to provide population-level vaccination with the Ad26.ZEBOV, MVA-BN-Filo two-dose regimen. VAC52150EBL2007 and VAC52150EBL2009 (ClinicalTrials.gov Identifier: NCT04186000 and NCT04028349) are two open-label studies that will provide additional information on the immunogenicity and safety of the vaccine regimen and are ongoing in DRC and Uganda, respectively.

In conclusion, our findings show that in healthy, African adult volunteers living in a region that was previously affected by EVD, the Ad26.ZEBOV, MVA-BN-Filo vaccine regimen administered in a 56-day interval is well tolerated and induces humoral immune responses that persist for at least two years, as well as humoral immune

memory. Booster vaccination with Ad26.ZEBOV given two years after initial vaccination induces a strong anamnestic response within seven days, which could be valuable for populations at imminent risk of exposure to Ebola virus, such as health workers in Ebola-endemic settings.

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Contributors

DI and DM drafted the manuscript. DM conducted the literature search for and drafted the research in context section. DI, DM, MOA, FB, KOK, BLo, TM, ES, JF, KEG, MS, GFD, BKe, HL, SL, NG, ML, VB, AG, DH, BC, KL, CR, BG, MD, BLe, DWJ were involved in the study concept and design, study conduct and interpretation of results. DWJ was the lead scientist for the program (EBOVAC1) at the London School of Hygiene and Tropical Medicine. MD was the lead scientist for the program at Janssen Vaccines & Prevention B.V. BLe was the clinical trial principal investigator in Sierra Leone. GFD, BR, ASB, and IS contributed to enrolment and clinical care of participants and data collection. DK was responsible for data management. BLo, BKo, GTO, VB, KL, were responsible for laboratory sample analysis, samples management and laboratory results interpretation. TM, ES, LE were responsible for community engagement activities. MJ was the clinical trial pharmacist and was responsible for study vaccine preparation and dispensing. AG and DH conducted the statistical analysis. AG, CR and DM have accessed and verified the data. All the authors reviewed and approved the final manuscript.

Declaration of Interest

Janssen Vaccines & Prevention B.V. was the clinical trial Sponsor and was involved in the design and conduct of the trial, and in the collection and analysis of data. BKe was a full-time employee of Janssen, Pharmaceutical Companies of Johnson & Johnson at the time of the study. NG, ML, AG, DH, VB, KL, BC, CR and MD were full-time employees of Janssen, Pharmaceutical Companies of Johnson & Johnson at the time of the study, and declared ownership of shares in Janssen, Pharmaceutical Companies of Johnson & Johnson. DM and JF reports grants from Innovative Medicines Initiative (IMI), non-financial support and other from Janssen Vaccines & Prevention B.V during the conduct of the study; grants and non-financial support from Janssen Vaccines & Prevention B.V outside the submitted work. TM and KG reports grants from IMI during the conduct of the study. HL reports grants from GSK and from Merck outside the submitted work. All other authors declare no competing interests.

Data Sharing

Janssen has an agreement with the Yale Open Data Access (YODA) Project to serve as the independent review panel for evaluation of requests for CSRs and participant level data from investigators and physicians for scientific research that will advance medical knowledge and public health. Data will be made available following publication and approval by YODA of any formal requests with a defined analysis plan. For more information on this process or to make a request, please visit The Yoda Project site at http://yoda.yale.edu. The

data sharing policy of Janssen Pharmaceutical Companies of Johnson & Johnson is available at https://www.janssen.com/clinical-trials/transparency

We believe that the study methods and results in adult participants are clearly documented in this article. Study methods for enrolment of children and their results will be presented in a separate publication. The clinical study protocol is available in the supplementary materials.

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Figures

***Figure 1*: Study design diagram**

Vaccines: Ad26.ZEBOV (Ad26); MVA-BN-Filo (MVA); Meningococcal quadrivalent (serogroups A, C, W135 and Y) conjugate vaccine (MenACWY).

Vaccine dosages: 5×10^{10} viral particles for Ad26, 1×10^{8} Inf.U for MVA, 0·5 ml reconstituted vaccine solution for MenACWY and 0·5-mL 0·9% sodium chloride solution for the placebo.

Inf.U=infectious units; Pbo=placebo; vp=viral particles

***Figure 2*: Study flow diagram**

***Panel A*: Stage 1**

***Panel B*: Stage 2**

*Treatment discontinuation: did not receive dose 2 (irrespective of whether follow-up continued to study completion).

[†]Study discontinuation: follow-up did not continue to the end of the study (irrespective of the number of doses received).

[‡]Properly screened and eligible, but by protocol deviation received vaccination before randomisation.

***Figure 3*: Solicited adverse events after vaccination in Stage 1 participants (Ad26.ZEBOV, MVA-BN-Filo only) and Stage 2 participants**

(Ad26.ZEBOV, MVA-BN-Filo or MenACWY, Placebo).

Solicited adverse events were observed during the period of seven days post vaccination.

***Panel A*: Solicited local AE, post dose 1**

***Panel B*: Solicited systemic AE, post dose 1**

***Panel C*: Solicited local AE, post dose 2**

***Panel D*: Solicited systemic AE, post dose 2**

Grade 3 solicited AEs - severe AEs which required medical attention but are not immediately life threatening.

***Figure 4*: EBOV GP-specific antibody responses**

Vaccines: Ad26.ZEBOV (Ad26); MVA-BN-Filo (MVA). Control: Meningococcal quadrivalent (serogroups A, C, W135, and Y) conjugate vaccine (MenACWY; dose 1), Placebo (dose 2).

***Panel A*: EBOV GP-specific binding antibody responses (ELISA units/mL)**

The response profile of each study group is shown as geometric mean concentrations of anti-EBOV GP IgG in EU/mL.

The error bars represent the geometric mean concentration and its 95% confidence interval at each time point.

Vaccination: Stage 1 (non-randomised open-label study): Ebola vaccine only (Ad26.ZEBOV, MVA-BN-Filo regimen); with Ad26.ZEBOV booster vaccination at two years (Day 720) post dose 1. Stage 2 (randomised controlled study): Ebola vaccine (Ad26.ZEBOV, MVA-BN-Filo regimen) or MenACWY, Placebo control regimen.

Data are labelled at the following time points: Day 1 (pre-vaccination baseline); Day 57 (56 days post dose 1); Day 78 (21 days post dose 2); Day 156 (155 days post dose 1); Day 360 (359 days post dose 1); Day 540 (539 days post dose 1); Day 720 (719 days post dose 1); Day 741 (21 days post-Booster vaccination); and Day 1080 (359 days post-Booster vaccination). Labels for the following time point tick-marks are omitted: Day 724 (4 days post-Booster vaccination), Day 727 (7 days post-booster vaccination).

***Panel B:* EBOV GP-specific neutralising antibody responses (psVNA, IC50 Titre)**

The response profile of each study group is shown as geometric mean titres.

The error bars represent the geometric mean concentration and its 95% confidence interval at each time point.

Data are labelled at the following time points: Day 1 (pre-vaccination baseline); Day 57 (56 days post dose 1);

Day 78 (21 days post dose 2); Day 360 (359 days post dose 1); Day 720 (719 days post dose 1).