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

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Abstract

Background Children account for a substantial proportion of cases and deaths from Ebola virus disease (EVD). This study is the first report on the safety and immunogenicity of a two-dose heterologous Ad26.ZEBOV and MVA-BN-Filo Ebola vaccine regimen in a paediatric population.

Methods This randomised, double-blind, controlled trial was conducted in Sierra Leone (clinicaltrials.gov NCT02509494). Healthy children were enrolled in three age cohorts (12–17, 4–11, 1–3 years) and randomised to receive vaccination with Ad26.ZEBOV (dose 1) followed by MVA-BN-Filo (dose 2), or one dose of polyvalent meningococcal conjugate vaccine (MenACWY) followed by a placebo, given 56 days later. The participants were randomised using the interactive web response system (IWRS). Study team personnel (except those with primary responsibility for study vaccine preparation) and participants were blinded to study vaccine allocation. Safety was the primary outcome and was assessed by adverse events (AEs) in the first 28 days after each vaccination and serious AEs (SAE) until the end of the study. The secondary outcome was humoral immune response, measured by binding antibody responses (FANG ELISA) at 21-day post-dose 2. The primary analysis set for safety comprised all participants who received at least one dose of study vaccine while the primary analysis for immunogenicity data included all children, who received both vaccinations within the protocol defined time window and had no major protocol deviations that could have influenced the immune response.

Findings From 4 April 2017 to 5 July 2018, 576 children (192 in each of the 3 age cohorts) were randomised. Following the first vaccinations (Ad26.ZEBOV vs MenACWY), the most common solicited local AE was pain at injection site in participants aged 12-17 years: 9% (13/143) vs 6% (3/48), 4-11 years: 21% (30/144) vs 4% (2/48) and 1-3 years: 14% (20/144) vs 10% (5/48), respectively. Post-dose 2 vaccinations (MVA- BN-Filo vs placebo), the most common solicited local AE was pain at injection site in participants aged 12-17 years: 15% (21/142) vs 2% (1/46), 4-11 years: 14% (20/143) vs 10% (5/48) and 1-3 years: 5% (7/143) vs 0 (0/48), respectively. The most frequently observed systemic AE post-dose 1 vaccinations (Ad26. ZEBOV vs MenACYW) was headache in participants aged 12-17 years: 29% (41/143) vs 23% (11/48), 4-11 years: 24% (34/144) vs 8 % (4/48) and fever for 1-3 year olds: 11% (16/144) vs 8% (4/48), respectively. Similar trends were observed post-dose 2 vaccinations with MVA.BN-Filo or placebo. Headache was most frequently observed among 12-17 year olds: 11% (15/142) vs 7% (3/46), 4-11 year olds: 15% (21/143) vs 17 % (8/48) and fever for 1-3 year olds: 8% (12/143) vs 15 % (7/48). No Ebola vaccine-related SAEs were reported.

Binding antibody responses were observed in all cohorts at 21 days post-dose 2: 9,929 EU/mL (8,172–12,064) for 12–17 years (131/134 [98%] responders), 10,212 EU/mL (8,419–12,388) for 4–11 years (119/120 [99%] responders) and 22,568 EU/mL (18,426–27,642) for 1–3 years (118/121 [98%] responders), with antibody levels still detectable up to 12 months post-dose 1 in nearly all participants.

Interpretation The Ad26.ZEBOV, MVA-BN-Filo Ebola vaccine regimen was well tolerated with no safety concerns in children aged 1–17 years and induced robust humoral immune responses, suggesting suitability for Ebola prophylaxis in children.

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Research in context

Evidence before this study

Ebola virus disease (EVD) is a highly contagious infection. Children accounted for ~20% of infected people during the 2014–2016 Ebola outbreak in West Africa, and for ~30% in the 2018-2020 outbreak in the Democratic Republic of Congo. No Ebola vaccine studies were conducted in children before the 2014–2016 outbreak. We searched Medline and Embase for peer-reviewed articles reporting Ebola vaccine trials in children and adolescents from the database inception until 3 April 2020, using the search terms [Ebola AND (vaccin* OR immunis* OR immuniz*) AND (trial* OR study) AND (child* OR infant* OR pediatr* OR paediatr* OR adolescen*)]. No language restrictions were applied. Public clinical trials registries and a WHO report on overview of Ebola candidate vaccines as of 19 August 2019, were also searched. The database searches yielded 190 citations. After screening of titles/abstracts and de-duplication, four relevant publications were identified, as summarised below:

A randomised, open label phase I trial in Gabon evaluated a recombinant vesicular stomatitis virus-vectored vaccine expressing a Zaire Ebola virus surface glycoprotein (rVSVΔG-ZEBOV) in 20 children aged 6–12 years and 20 adolescents aged 13–17 years. The vaccine had an acceptable safety and immunogenicity profile, but the children and adolescents in this study had a higher vaccine replication than that observed in adults, which led to shedding of the vaccine in the saliva and urine. To address this concern, a low dose vaccine was recommended by the authors for children and adolescents.

Given the promising safety findings obtained from the first trial, two additional studies evaluated a ring vaccination approach in 303 children and adolescents in Guinea in 2015 and 2016. Adverse events data indicated no safety concerns in children/adolescents. This vaccine (Ervebo®) has been granted conditional authorisation by European Medicine Agency (EMA) and licenced by the US Food and Drug Administration for use in adults who are at risk of EVD.

A phase II randomised, observer-blind, placebo-controlled trial evaluated the safety, reactogenicity, and immunogenicity of a chimpanzee adenovirus vectored Ebola vaccine (ChAd3-EBO-Z) in children in Mali and Senegal. A total of 600 children (200 each in 1–5, 6–12, and 13–17-year-old cohorts) were randomised 1:1 to receive ChAd3-EBO-Z at day 0 and meningococcal sero-groups A, C, W-135, Y tetanus toxoid conjugate vaccine (MenACWY-TT) given at 6 months, or MenACWY-TT given at day 0, and ChAd3-EBO-Z given at 6 months. The vaccine was tolerable and immunogenic. However, increased reactogenicity and stronger immune responses to the vaccine were observed in the youngest age group (1–5 year-olds). The authors reported that this could be because the youngest age group received a higher dosage of the vaccine, relative to their body mass.

Added value of this study

This is the first study evaluating a two-dose vaccine regimen, Ad26.ZEBOV, MVA-BN-Filo, in a randomised, double-blind, controlled trial in paediatric age groups. Overall, 576 children/adolescents aged 1–17 years were enrolled in an age de-escalating fashion in three age cohorts (12–17, 4–11, 1–3 years) and randomised 3:1 to receive vaccination with Ad26.ZEBOV (dose 1) followed by MVA-BN-Filo (dose 2) or one dose of polyvalent meningococcal conjugate vaccine (MenACWY) followed by a placebo, given 56 days later. We found that this two-dose Ebola vaccine regimen, was well tolerated by the study participants with no safety concerns identified. The vaccine regimen also induced strong humoral immune responses that persisted at least up to 12 months after the first vaccination.

Implications of all the available evidence

To date, three candidate vaccines against EVD (rVSV-ZEBOV; ChAd3-EBO-Z and Ad26.ZEBOV, MVA-BN-Filo) have been evaluated in children and adolescents and were all found to have acceptable safety and immunogenicity profile. To our knowledge, we report the first study evaluating a two-dose vaccine regimen, Ad26.ZEBOV, MVA-BN-Filo, in a paediatric population. Before July 2020, no licenced vaccine was available against EVD for this vulnerable age group. Data from our study contributed to the recent approval and marketing authorisations granted by the EMA Committee for Medicinal Products for Human Use for the two-dose Ad26.ZEBOV, MVA-BN-Filo vaccine regimen. This manuscript reports on a significant progress in the EVD vaccine development for use in the paediatric age group, as a mark of public health preparedness and response.

Introduction

In the 2014–2016 outbreak of Ebola virus disease (EVD) in West Africa that resulted in 28,652 cases and 11,325 deaths,[1,2] about 20% of cases were in children aged less than 15 years old.[3,4] Similarly, in the 2018–2020 Ebola outbreak in the Democratic Republic of Congo (DRC), about 30% of EVD cases were in children aged less than 18 years old.[5] Children, especially those below five years of age, have a more rapid clinical progression and a relatively high risk of death.[3] These features underscore the need for an effective Ebola prevention strategy in paediatric populations.

The clinical evaluation of several candidate vaccines was accelerated because of both outbreaks.[6] A live-attenuated, single-dose, recombinant vesicular stomatitis virus vaccine expressing the glycoprotein (GP) of Zaire Ebola virus (rVSV-ZEBOV), was shown to provide protection against EVD during the 2014–2016 Ebola outbreak in Guinea using a ring vaccination approach.[7] This vaccine was also used during the 2018–2020 outbreak in the DRC as part of the outbreak response in adults and in children aged 1–17 years, under expanded access.[8] The vaccine has received conditional approval by European Medicines Agency (EMA)[9] and US Food and Drug Administration approval for use in adults.[10] Following recommendations by the Strategic Advisory Group of Experts on vaccination against EVD,[11] a two-dose Ebola vaccine regimen, Ad26.ZEBOV, MVA-BN-Filo, has also been used to vaccinate adults and children aged 1–17 years in the DRC and Rwanda. Recently, the European Commission granted approval under exceptional circumstances of the two-dose heterologous vaccine regimen for use in children and adults.[12]

A heterologous two-dose vaccination regimen with adenovirus 26 (Ad26) and modified vaccinia virus Ankara (MVA) expressing the glycoprotein of Zaire Ebola virus (Ad26.ZEBOV and MVA-BN-Filo) was evaluated in a randomised controlled trial in a community affected by Ebola during the West Africa epidemic. This vaccine regimen has been shown to have an acceptable safety profile and to induce robust humoral immune responses in adults.[13] Here, we report the safety and immunogenicity of this vaccine regimen in children, aged 1–17 years from the same community. Data presented in this manuscript contributed to the recent approval and marketing authorisations granted by the EMA Committee for Medicinal Products for Human Use for the two-dose Ad26.ZEBOV, MVA-BN-Filo vaccine regimen in adults and children.[12]

Methods

Study design

This study (VAC52150EBL3001) was a randomised, double-blind, controlled trial with three paediatric age cohorts. Enrolment commenced with older children followed by the younger age cohorts initiated based on safety data from the preceding older one: adolescents aged 12–17 years, children aged 4–11 years, and finally, toddlers aged 1–3 years (figure 1a–c). The children were enrolled from 21 March 2017 to 1 July 2019 at three trial clinics located within Kambia District, North-Western province of Sierra Leone, an area severely affected by the 2014–16 Ebola outbreak. An Independent Data Monitoring Committee assessed the safety results of each age cohort before proceeding with the enrolment of the first 96 participants in the next cohort. The trial protocol was approved by the Sierra Leone Ethics and Scientific Review Committee, the London School of Hygiene & Tropical Medicine Ethics Committee, and the Pharmacy Board of Sierra Leone. The trial was conducted according to the International Council for Harmonisation of Technical Requirements for Pharmaceuticals for Human Use (ICH) Good Clinical Practice guidelines and was monitored by an external contract research organisation, ICON Government and Public Health Solutions. This study is registered with Clinicaltrials.gov, NCT02509494.

Study participants

Community engagement activities, including meetings, radio discussion programmes, and a drama, were held with the local community to provide information about the trial and discuss any questions or concerns that parents or guardians had. Parents or guardians of healthy children aged 1–17 years who expressed an interest in the trial were subsequently invited, along with their children/wards, to the trial clinics for eligibility assessments. Prior to enrolment, parents/guardians were given information about the trial in a language they understood and after passing a test of understanding, they provided written, informed consent for their child/ward to join the trial. Children aged seven years and above also gave written assent. A trial physician obtained a detailed medical history from the parents/guardians of a potentially eligible child and conducted a physical examination to ascertain that the child was well. A blood sample was then collected for measurement of baseline haematological and biochemical parameters. Urinary β-hCG tests were conducted for potential female participants considered to be of childbearing potential, in order to exclude pregnancy. A child was eligible for enrolment if they were within the correct age group for the trial, and in good health as determined by

clinical examination and measurement of haematological and biochemical variables. The full list of inclusion and exclusion criteria is presented in the protocol provided in the supplementary material.

Randomisation and masking

Study participants were randomised to either Ad26.ZEBOV, MVA-BN-Filo vaccine, or active control arm using a computer-generated 3:1 block randomisation schedule via an Interactive Web Response System (IWRS) operated by a study pharmacist. Study participants, their parents/guardians, and all study team members (except study pharmacists who operated the IWRS were blind to the study vaccine allocation. Masking tape was used to cover the dispensing syringes containing the treatment allocated to each study child. This process guaranteed treatment concealment until after completion of study follow-up visits by all participants.

Investigational vaccines and vaccination

As previously described,[14,15] Ad26.ZEBOV and MVA-BN-Filo were manufactured by Janssen Vaccines & Prevention B.V, Leiden, Netherlands and Bavarian Nordic A/S, Kvistgaard, Denmark, respectively under Good Manufacturing Practice conditions. A polyvalent conjugate vaccine against meningococcal serogroups A, C, W135, and Y (MenACWY) was chosen as a comparator vaccine to provide some benefits to children in the control group. This is because bacterial meningitis is endemic in the study area and meningitis vaccine is not included in the Sierra Leonean routine childhood immunisation schedule. The study vaccines were administered intramuscularly into the deltoid area of the arm in the adolescent and 4–11 year old children while the 1–3 year-olds received the vaccines in the anterolateral thigh. Participants in the Ebola vaccine arm received Ad26.ZEBOV (5×10^{10} vp) (dose 1) followed eight weeks later by MVA-BN-Filo (1×10^{8} inf U) (dose 2), and those randomised to the active control arm received MenACWY (dose 1), followed eight weeks later by saline (dose 2). Study participants aged less than two years at enrolment also received a third vaccination of Men ACWY at 3 months post dose 2.

Assessment of primary and secondary endpoints

The primary endpoints were safety, measured by the occurrence of (i) solicited local and systemic adverse symptoms during a 7-day follow-up period after each vaccination (day 1 for dose 1; day 57 for dose 2); (ii) unsolicited systemic symptoms during a 28-day follow-up after each vaccination; (iii) abnormal laboratory

results during the study period; (iv) serious adverse events (SAE) or immediate reportable events (IRE) throughout the study period. The neuro-inflammatory disorders categorised as IRE (listed in supplementary material) were reported to the sponsor within 24 hours. After each vaccination, study participants were directly observed in the trial clinics for 30 minutes and then followed up at home. Trained field assistants visited the study children at home daily for seven days after each vaccination to administer a standardised, purpose-designed reactogenicity diary card to the study participant and/or their parent/guardian.

The secondary endpoint was immunogenicity, measured by EBOV GP-specific binding antibody responses determined by EBOV GP Filovirus Animal Non-Clinical Group (FANG) ELISA measured on day 21 post-dose 2. 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 measured with an Ad26 virus neutralisation assay and a plaque reduction neutralisation test (PRNT), respectively. See supplementary appendix for further details of the procedures involved in the safety and immunogenicity assessments/outcomes.

Sample size determination and statistical analyses

A sample size of 192 children (n=144 in the Ad26.ZEBOV, MVA-BN-Filo arm; n=48 in the active control arm) for each age cohort was to provide ≥99% probability of observing at least one SAE occurring in the Ad26.ZEBOV, MVA-BN-Filo arm, if the true rate is 1/10 or more in each age group.

For the analysis of the EBOV GP-specific nAb response, a subset of participants (28% [54/191] of adolescents aged 12-17 years, 29% [55/192] of children aged 4-11 years, and 29% [56/192] of children aged 1-3 years) 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 of participants in the study. This was done prior to the analysis of the samples among participants with available samples and no protocol deviations that could have influenced the immune response. The nAb response against the Ad26 vector backbone was measured at baseline (pre-dose 1) in 98% (188/191) of adolescents aged 12-17 years, 93% (179/192) of children aged 4-11 years, and 85% (164/192) of children aged 1-3 years. For the analysis of nAb against the MVA vector (PRNT), a subset of participants (29% [55/191] of participants aged 12-17 years, 29% [55/192] of participants aged 4-11 years, and 29% [56/192] of participants aged 1-3 years) were selected at random as described above for EBOV GP-specific nAb response.

Statistical analyses were performed using SAS® version 9.2 (SAS Institute Inc., Cary, NC, USA). Descriptive analysis was done without formal hypothesis testing and results were presented by vaccination arms. The full analysis set for safety data comprised all participants who received at least one dose of study vaccine. The analysis set for immunogenicity data (per-protocol) included all vaccinated children, 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.

Responders were defined as either having a negative enzyme-linked immunosorbent assay (ELISA) result at baseline and a positive post-baseline value > 2·5x the lower limit of quantification [LLOQ, ie, 36·11 ELISA units/mL (EU/mL)], or positive at baseline with a post baseline value >2·5-fold increase from baseline. Participants were considered as responders for the pseudovirion neutralisation assay (psVNA), if negative at baseline and positive post baseline, the latter value being >2x the LLOQ (ie, 120 IC_{50} titre), or positive at baseline with a post baseline value >2-fold increase from baseline.

Immunoglobulin G binding antibody responses against EBOV GP (by ELISA) and neutralising (nAb) activity (by psVNA) are presented respectively, as geometric mean concentrations (GMCs) and geometric mean titres (GMTs), with 95% confidence intervals (CIs). All values <LLOQ were imputed with half the LLOQ value. Spearman correlation was calculated between EBOV GP-specific binding antibodies (ELISA) and psVNA titres at 21 day post-dose 2.

Post-hoc analyses of the immunogenicity by baseline EBOV GP ELISA level were performed to evaluate any potential influence of EBOV GP-specific binding antibodies present at baseline on the vaccine-induced responses (i.e., by a stratification of the 21 day post-dose 2 binding antibody concentrations by baseline ELISA levels and by a correlation analysis).

Role of the funding source

This study was funded by the Innovative Medicines Initiative (IMI). IMI had no role in the study design, conduct, or publication of this manuscript. Janssen Vaccines & Prevention B.V sponsored the clinical trial and was involved in the design, conduct of the trial, 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

Overall, 756 children were screened across the three age groups, of whom 192 were enrolled in each age cohort (total enrolled = 576). The number of children screened in each age cohort, the number who failed screening, or who were excluded for other reasons, and the reasons for exclusions are shown in figure 1a–c. Baseline demographic parameters are summarised in table 1.

Overall, solicited AEs were mostly mild-to-moderate (grade 1 and 2) (figure 2, table S1). In all cohorts, the most frequent solicited local AE was injection site pain after any vaccination (figures 2A, 2C, 2E, 2G, 2I, and 2K; table S2). No grade 3 solicited local AEs were observed after any vaccination in any age group. Solicited local AEs were reported in the 12–17-year-old cohort by 14 (10%) of 143 participants post-Ad26.ZEBOV vaccination and by 21 (15%) of 142 participants post-MVA-BN-Filo vaccination (figures 2A and 2C). In the 4–11-year-old cohort, at least one solicited local AE was reported by 30 (21%) of 144 children following Ad26.ZEBOV vaccination and 22/144 (15%) following MVA-BN-Filo vaccination (figures 2E and 2G; table S2). In the 1–3-year-old cohort, at least one solicited local AE was observed in 21 (15%) of 144 toddlers following Ad26.ZEBOV vaccination and 7/143 (5%) following MVA-BN-Filo vaccination (figures 2I and 2K; table S2).

In the active control group, solicited local AEs were reported in three (6%) of 48 adolescents following MenACWY vaccination and one (2%) of 46 adolescents following placebo vaccination (figures 2A and 2C). In the 4–11-year-old cohort, two (4%) of 48 children reported at least one solicited local AE following MenACWY vaccination and five (10%) of 48 children reported a local event following placebo vaccination (figures 2E and 2G; table S2). In the 1–3 year-old cohort, five (10%) of 48 toddlers were observed to have at least one solicited AE following MenACWY vaccination and no local AEs were reported following placebo vaccination (figures 2I and 2K; table S2).

Solicited systemic AEs in 12–17-year-old cohort were reported by 52 (36%) of 143 participants post-Ad26.ZEBOV vaccination and 26 (18%) of 142 adolescents, post MVA-BN-Filo (figures 2B and 2D; table S3). In the 4–11-year-old cohort, at least one solicited systemic AE was reported by 45 (31%) of 144 children following Ad26.ZEBOV vaccination and in 27 (19%) of 143 children following MVA-BN-Filo (figures 2F and

2H; table S3). In the 1–3-year-old cohort, 36 (25%) of 144 toddlers were observed to have at least one solicited systemic AE following Ad26.ZEBOV vaccination and 23 (16%) of 143 toddlers following MVA-BN-Filo vaccination (figures 2J and 2L; table S3). Following MenACWY vaccination, at least one solicited systemic AE was observed in 14 (29%) of 48 adolescents (figure 2B), 15 of 48 children (31 %) (figure 2F), and 12 (15%) of 48 toddlers. (figure 2J). Following placebo vaccination, at least one solicited systemic AE was reported in six (13%) of 46 adolescents; eight (17%) of 48 children and 14 (29%) of 48 toddlers (figures 2D, 2H, and 2L respectively; table S3). Headache, fatigue, and chills were the most frequently reported solicited systemic AE after any vaccination in the (12–17 years old) and (4–11 years old) children (figures 2B, 2D, 2F, and 2H, respectively; table S3) while pyrexia (fever), decreased appetite, and decreased activity were the most frequently observed solicited systemic AEs in toddlers (1–3 years old) cohort (figures 2J and 2L; table S3). The frequency of pyrexia was higher in the 1–3-year-old cohort, regardless of the vaccine given, versus other age groups (table S3). Following Ad26.ZEBOV vaccination 16 (11%) of 144 toddlers had fever, and 12/143 (8%) following MVA-BN-Filo vaccination. Similarly, four (8%) of 48 toddlers were observed to have fever following MenACWY vaccination and seven (15%) of 48 toddlers following placebo vaccination (table S3). Grade 3 solicited systemic AEs were infrequently observed post vaccination in all age cohorts. The most frequent unsolicited AEs post-dose 1 and post-dose 2 was malaria in all cohorts, irrespective of the types of vaccine given (table S4). None of the AEs were considered related to study vaccine. Grade 3 unsolicited AEs were infrequently observed post vaccination, regardless of vaccine type (table S4).

No SAEs or deaths related to the Ebola vaccine regimen were observed during the study period. Forty-nine SAEs were reported in 24 participants (table S5). Apart from one case of acute severe asthma, all SAEs in the two younger age cohorts were related to infectious diseases (malaria, respiratory tract infections including pneumonia and bronchiolitis, peritonitis, postoperative wound infection, subcutaneous abscesses, sepsis, bacterial meningitis, gastroenteritis, chronic osteomyelitis) and complications of malaria (anaemia, iron deficiency anaemia, thrombocytopenia, and febrile convulsion). Approximately 67% of SAEs were observed after the first 28 days following any vaccination. One SAE (severe thrombocytopenia) observed in a 3-year-old child about 50 days after receiving MenACWY was considered to be possibly related to dose 1 vaccination and was reported as a suspected unexpected serious adverse reaction. Two fatal SAEs were recorded: a 17-year-old participant in the control group died of severe typhoid fever on day 319, and a 3-year-old in the Ad26.ZEBOV,

MVA-BN-Filo group died of severe malaria and severe anaemia on day 74, 22 days after MVA-BN-Filo vaccination. Both deaths were considered unrelated to study vaccine.

Using the FDA Toxicity Grading Scale,[16] a grade 3 'haemoglobin change from baseline' was observed in two adolescents after Ad26.ZEBOV vaccination and eight after MVA-BN-Filo vaccination, two after MenACWY vaccination, and three after placebo administration. No other grade 3 abnormalities were observed in this age cohort (table S6). All adolescents meeting the FDA criteria for a grade 3 'haemoglobin change from baseline' had a haemoglobin value within the adapted laboratory normal ranges in the region.[17] The change from baseline grading scale parameter for haemoglobin only applied to the adolescent cohort while the two younger age groups grading was based on the absolute value. In children aged 4–11 years, all grade 3 laboratory abnormalities were observed in at most one participant. Amongst the 1–3-year-old cohort, all grade 3 laboratory abnormalities were observed in at most three participants, except for grade 3 haemoglobin values, which were observed in one participant after Ad26.ZEBOV vaccination, six toddlers after MVA-BN-Filo vaccination and none after MenACWY or placebo vaccination (table S6).

EBOV GP-specific binding antibody results are summarised in figure 3 and table S7. At 21 days post-dose 2 (day 78), binding antibody responses against EBOV GP were observed in 98–99% of participants across all age cohorts. The GMC was 22,568 EU/mL [95% CI 18,426–27,642]) in 118/121 (98%) participants aged 1–3 years, 10,212 EU/mL [95% CI 8,419–12,388]) in 119/120 (99%) participants aged 4–11 years, and 9,929 EU/mL [95% CI 8,172–12,064]) in 131/134 (98%) participants aged 12–17 years at 21 days post-dose 2 (figure 3; table S7). Prior to dose 2 vaccination, both the GMC and the responder rate tended to be higher in 115 (94%) of 122 participants aged 1–3 years (693 EU/mL; [95% CI 591–812]) than in 91 (64%) of 142 participants aged 12–17 years (314 EU/mL; [95% CI 269–366]) or 92 (71%) of 129 participants aged 4–11 years (390 EU/mL; [95% CI 334–456]). Compared with the 21 days post-dose 2 time point (day 78), binding antibody concentrations were lower on day 240 (6 months post-dose 2) in all age cohorts, but responses persisted in 99 (73%) of 135 participants aged 12–17 years (GMC: 469 EU/mL; [95% CI 397–554]), 90 (74%) of 122 participants aged 4–11 years (GMC: 442 EU/mL; [95% CI 377–518]), and 111 (93%) of 119 participants aged 1–3 years ([GMC: 713 EU/mL; [95% CI 598–849]) (table S7). The GMCs remained stable between day 240 and day 360 (1-year post-dose 1). On day 360, persistent responses were still observed in 92 (70%) of 132 participants aged 12–17 years

295 (GMC: 386 EU/mL; 326–457), 85 (71%) of 119 children aged 4–11 years (GMC: 436 EU/mL; 375–506), and in
296 112 (96%) of 117 toddlers aged 1–3 years, (GMC: 750 EU/mL; 629–894), respectively (table S7).

297

298 At 21 days post-dose 2 (day 78), EBOV GP-specific nAb responses were detected in 94–95% of participants
299 across age cohorts (figure 4; table S8). The GMT in participants aged 1–3 years (8,142 IC_{50} titre [95% CI
300 4,869–13,615]) was 3- to 4-fold higher than the GMT in participants aged 12–17 years (2,120 IC_{50} titre [95% CI
301 1,444–3,111]) and in participants aged 4–11 years (2,483 IC_{50} titre [95% CI 1,719–3,587]) (figure 4). There was
302 a strong positive correlation (corrected for age cohort) between EBOV GP-specific nAb and EBOV GP-specific
303 binding antibody concentrations at this time point [partial Spearman correlation coefficient: 0·881] (figure S1).
304 On day 360 (1-year post-dose 1), nAb responses were observed in 8% (3/40) of participants aged 12–17 years,
305 15% (6/40) aged 4–11 years, and 49% (18/37) aged 1–3 years. The GMT value was either low (252 IC_{50} titre
306 [95% CI 189–336] for children aged 1–3 years) or <LLOQ (age 4–17 years).

307 Neutralising antibodies against the Ad26 vector backbone were assessed at baseline in 98% (188/191) of
308 participants aged 12–17 years, 93% (179/192) of 4 –11 years, and 85% (164/192) of 1–3 years. At baseline,
309 neutralising antibodies against the Ad26 vector backbone were observed in 78% (111/142) of participants aged
310 12–17 years (GMT: 77 IC_{90} titre [95% CI 58–101]), in 77% [103/134] aged 4–11 years (GMT: 143 IC_{90} titre
311 [95% CI 101–201]), and 20% [25/124] aged 1–3 years (GMT: 19 IC_{90} titre [95% CI: <LLOQ; 26]). Similar
312 results were observed in the control group (table S9). In post-hoc analyses corrected for age group effect, a
313 negligible negative correlation was observed between baseline Ad26 VNA titres and vaccine-induced EBOV
314 GP-specific binding antibody levels (ELISA) at 21 days post-dose 2 (partial Spearman correlation coefficient:
315 0.204) (figure S2).

316 None of the participants tested for neutralising antibodies against the MVA vector backbone showed pre-
317 existing MVA neutralising antibodies.

318

319 **Discussion**

320 This is the first clinical trial reporting safety and immunogenicity data of a two-dose heterologous Ebola vaccine
321 regimen with Ad26.ZEBOV, MVA-BN-Filo in a paediatric population from an area affected by Ebola during
322 the 2014–2016 outbreak in West Africa. Consistent with previous studies in adults,[14,15,18] this vaccine regimen

was well tolerated in paediatric participants and no safety concerns were identified. No deaths or SAEs attributed to the Ebola vaccines were observed, and there were no AEs warranting discontinuation of study vaccinations. Immune responses were observed in the study participants, as assessed by both an anti-GP binding assay and by a viral neutralisation assay.

Overall, AEs following vaccinations were mild and transient in nature in all age groups. The proportion of study participants with at least one solicited local AE was higher in the Ad26.ZEBOV, MVA-BN-Filo vaccine recipients than in the control group for all age cohorts. Ad26.ZEBOV tended to be more reactogenic than MVA-BN-Filo, especially for solicited local AEs, in the youngest cohort (1–3 years) and for solicited systemic AEs in all age cohorts. Compared with adults, in whom headache, arthralgia, and myalgia were the predominant solicited systemic AEs[13], the most frequent solicited systemic AEs in participants aged 4–17 years were headache, fatigue, and chills. In participants aged 1–3 years, the most frequently reported solicited systemic AEs were decreased appetite, decreased activity, and pyrexia. These events were of mild intensity (grade 1) and resolved within 24–48 hours in most participants. The observed frequency of pyrexia in participants aged 1–3 years was higher compared with the other age groups, regardless of the vaccine given. This agrees with previous findings in similar studies evaluating adenovirus and MVA-vectored vaccines in this age group.[19,20] Common occurrence of fever in toddlers has also been reported following meningococcal and pneumococcal vaccinations in this age group and is hypothesized to be due to antigen-induced inflammatory responses.[21–25]

A change in haemoglobin concentration from baseline was observed in comparable proportions of study participants in the Ad26.ZEBOV, MVA-BN-Filo and control groups, based on FDA[16] and the Division of Microbiology and Infectious Diseases toxicity grading.[26] This development illustrates the challenges commonly faced in AE reporting in paediatric vaccine trials conducted in low-income countries. International laboratory toxicity grading adopted as a 'gold standard' does not usually accommodate the epidemiological factors that shape the physiological status of children in low-income countries. The fact that the 'abnormal' haemoglobin concentration values were within the acceptable normal ranges of a similar paediatric population in West Africa,[17] underscores the need for context-specific laboratory references for eligibility screening and AE reporting in paediatric vaccine trials in such settings. Nevertheless, given the high prevalence of malaria and other common childhood infectious diseases in the study area, the drop in haemoglobin concentration observed in the children might be as a result of these infections.[27]

There were no Ebola vaccine-related SAEs in this study. One toddler developed severe thrombocytopenia following receipt of MenACWY at first vaccination. Most episodes of vaccine-associated thrombocytopenia are

asymptomatic, rare and of limited duration, nevertheless, there are some reports of severe thrombocytopenia associated with bleeding following administration of measles-mumps-rubella vaccine and, sometimes, with other routine childhood vaccines.[28] Although some monovalent, non-replicating vaccines also have the potential to cause symptomatic thrombocytopenia,[29] this event was not observed in any of the children who received the Ad26.ZEBOV, MVA-BN-Filo vaccine regimen.

EBOV GP-specific binding antibody responses were observed at 21 days post-dose 2 in at least 98% of the study participants in each age cohort and persisted at least up to one-year post-dose 1. The overall trend of higher immune responses in the children aged 1–3 years (relative to the older children and adults in the study) is consistent with similar findings reported in a study of a ChAd3-ZEBOV vaccine in children,[20] and with an adenovirus-based malaria vaccine in Gambia.[19,30] While a clear-cut reason for this phenomenon has yet to be established, possible suppression of immune responses by recurrent multiple and/or chronic infections, such as malaria and/or helminth infections, which are prevalent in the West African setting and are known to impact humoral immune responses in older children,[31] is a plausible explanation.

Although the percentage of children with pre-existing neutralising antibodies against the Ad26 vector was the lowest in the youngest age group, the correlation analysis between pre-existing Ad26 seropositivity and the EBOV GP-specific antibody responses post vaccination at an individual level indicates that pre-existing immunity against the Ad26 vector had no impact on the vaccine-induced antibody responses. Hence, Ad26 pre-existing immunity had a negligible impact on the observed difference in EBOV GP binding antibody concentrations between the youngest children and the older paediatric cohorts. The two-dose vaccination regimen evaluated in this study induced robust EBOV GP-specific neutralising antibody responses. At 21 days post-dose 2, there was a strong positive correlation between the binding antibody concentrations and neutralising antibody titres, suggesting that the majority of the vaccine-induced EBOV GP-specific binding antibodies also have a neutralising function.

A limitation of this study is that it focused only on safety and immunogenicity, despite the need to rapidly develop and roll out an efficacious, prophylactic Ebola vaccine for paediatric age groups. Since the study took place at a time when the West Africa Ebola outbreak had been brought under control, it was not possible to demonstrate efficacy of the vaccine regimen. Consequently, it was necessary to use a statistical modelling (immunobridging) approach to infer the potential clinical benefits induced by Ad26.ZEBOV and MVA-BN-Filo in the study participants. This modelling involved correlating the magnitude of vaccine-elicited immune responses associated with protection in non-human primates with those observed in vaccinated human

participants, including a pooled analysis specific for the paediatric population[32,33] following an approach similar to that employed for establishing the efficacy of a vaccine against anthrax.[34]

In conclusion, this study has demonstrated that Ad26.ZEBOV, MVA-BN-Filo Ebola vaccine regimen is safe, well-tolerated, and induces strong and durable anti-GP binding immune responses and is likely to be protective against EVD in adolescents and children. These findings contributed to the recent approval provided by the EMA Committee for Medicinal Products for Human Use for the two-dose Ad26.ZEBOV, MVA-BN-Filo vaccine regimen in children,[12] marking a significant milestone in public health preparedness and EVD response for this vulnerable age group. Given that EVD affects a substantial proportion of children during outbreaks, the prophylactic Ebola vaccine would be beneficial in offering protection against EVD and mitigates the challenges of diagnostic dilemma, reduced chances of survival, and persistence of long-time sequelae in children.[3]

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Author contributions

MOA drafted the manuscript. MOA, DI, DM, KOK, BLo, TM, FB, JF, KG, AG, DH, MS, GFD, BKe, HL, SL, NG, ML, VB, KL, BC, CR, BG, MD, BLe, DWJ were involved in the study concept and design, study conduct, interpretation of results, and revision of the manuscript. DWJ was the lead scientist for the project (EBOVAC1). 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, and KL were responsible for laboratory sample analysis, samples management, and laboratory results interpretation. TM and ES were responsible for community engagement activities. MJ was the clinical trial pharmacist and was responsible for oversight on study vaccine preparation and dispensing. AG and DH conducted the statistical analysis. CR, AG and MOA accessed and verified the data reported in this manuscript. All the authors reviewed and approved the drafts and 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 statement

Janssen has an agreement with the Yale Open Data Access (YODA) Project to serve as the independent review panel for evaluation of requests for clinical study reports 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.

The clinical study protocol for this study is available in the supplementary materials. We have also reported clearly the participant data (in text, tables, figures, and appendices) in the methods, results and supplementary sections of this manuscript**.** Individual participant data, including data dictionaries, will not be shared.

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References

1 2014-2016 Ebola Outbreak in West Africa. Retrieved from https://www.cdc.gov/vhf/ebola/history/2014-2016-outbreak/index.html. Accessed 23 June 2020.

2 Lo TQ, Marston BJ, Dahl BA, De Cock KM. Ebola: Anatomy of an epidemic. *Annu Rev Med* 2017; **68**: 359–70.

3 Agua-Agum J, Ariyarajah A, Blake IM, et al. Ebola virus disease among children in West Africa. *N Engl J Med* 2015; **372**: 1274–7.

4 Cost of the Ebola Epidemic. Retrieved from https://www.cdc.gov/vhf/ebola/history/2014-2016-outbreak/cost-of-ebola.html. Accessed 23 June 2020.

5 Ebola virus disease – Democratic Republic of the Congo. Retrieved from https://www.who.int/csr/don/09-April-2020-ebola-drc/en/. Accessed 23 June 2020.

6 Matz KM, Marzi A, Feldmann H. Ebola vaccine trials: progress in vaccine safety and immunogenicity. *Expert Rev Vaccines* 2019; **18**: 1229–42.

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

8 Shears P, Garavan C.The 2018/19 Ebola epidemic the Democratic Republic of the Congo (DRC): epidemiology, outbreak control, and conflict. *Infection Prevention in Practice* 2020; **2**:10038 https://doi.org/10.1016/j.infpip.2020.100038

9 European Commission. Vaccine against Ebola: Commission grants first-ever market authorisation. Retrieved from https://ec.europa.eu/commission/presscorner/detail/en/IP_19_6246. Accessed 23 June 2020

10 US FDA. First FDA-approved vaccine for the prevention of Ebola virus disease, marking a critical milestone in public health preparedness and response. Retrieved from https://www.fda.gov/news-events/press-announcements/first-fda-approved-vaccine-prevention-ebola-virus-disease-marking-critical-milestone-public-health. Accessed 15 December 2020.

11 Strategic Advisory Group of Experts (SAGE) on Immunization Interim Recommendations on Vaccination against Ebola Virus Disease (EVD)_7 May 2019. Retrieved from

https://www.who.int/immunization/policy/position_papers/interim_ebola_recommendations_may_2019.pdf?ua=1. Accessed 23 June 2020

12 European Commission. Vaccine against Ebola: Commission grants new market authorisations. Retrieved from https://ec.europa.eu/commission/presscorner/detail/en/IP_20_1248. Accessed 09 July 2020

13 Ishola D, Manno D, Afolabi MO, et al. 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 initiated during the Ebola outbreak in Sierra Leone. [Submitted to Lancet ID in parallel to this submission].

14 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**: 57–67.

15 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**: 46–56.

16 US FDA. Guidance for Industry: Toxicity Grading Scale for Healthy Adult and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials. Revised September 2007. Retrieved from https://www.fda.gov/regulatory-information/search-fda-guidance-documents/toxicity-grading-scale-healthy-adult-and-adolescent-volunteers-enrolled-preventive-vaccine-clinical. Accessed 23 June 2020

17 Dosoo DK, Asante KP, Kayan K, et al. Biochemical and hematologic parameters for children in the middle belt of Ghana. *Am J Trop Med Hyg* 2014; **90**: 767–73.

18 Pollard AJ, Launay O, Lelievre JD, et al. Safety and immunogenicity of a two-dose heterologous Ad26.ZEBOV and MVA-BN-Filo Ebola vaccine regimen in adults in Europe (EBOVAC2): a randomised, observer-blind, participant-blind, placebo-controlled, phase 2 trial. *Lancet Infect Dis* 2020; S1473-3099(20)30476-X.

19 Afolabi MO, Tiono AB, Adetifa UJ, et al. Safety and immunogenicity of ChAd63 and MVA ME-TRAP in West African children and infants. *Mol Ther* 2016; **24**: 1470–7.

20 Tapia MD, Sow SO, Mbaye KD, et al. Safety, reactogenicity, and immunogenicity of a chimpanzee adenovirus vectored Ebola vaccine in children in Africa: a randomised, observer-blind, placebo-controlled, phase 2 trial. *Lancet Infect Dis* 2020: **20**: 719–30.

21 BLA Clinical Review Memorandum. Retrieved from https://www.fda.gov/media/134270/download. Accessed 23 June 2020.

22 MENVEO [Meningococcal (Groups A, C, Y, and W-135) Oligosaccharide Diphtheria CRM197 Conjugate Vaccine]. Retrieved from https://www.gsksource.com/pharma/content/dam/GlaxoSmithKline/US/en/Prescribing_Information/Menveo/pdf/MENVEO.PDF. Accessed 18 May 2020.

23 Mendoza YG, Garric E, Leach A, et al. Safety profile of the RTS,S/AS01 malaria vaccine in infants and children: additional data from a phase III randomized controlled trial in sub-Saharan Africa. *Human Vacc Immunother* 2019;**15**: 2386–98.

24 Odusanya OO, Kuyinu YA, Kehinde OA, et al. Safety and immunogenicity of 10-valent pneumococcal non typeable *Haemophilus influenzae* protein D conjugate vaccine (PHiD-CV) in Nigerian children. *Hum Vaccin Immunother* 2014; **10**: 757–66.

25 Thompson A, Gurtman A, Patterson S, et al. Safety of 13-valent pneumococcal conjugate vaccine in infants and children: Meta-analysis of 13 clinical trials in 9 countries, *Vaccine* 2013; **31**: 5289–95.

26 Division of Microbiology and Infectious Diseases (DMID) Pediatric Toxicity Tables November 2007 draft. Retrieved from https://www.niaid.nih.gov/sites/default/files/dmidpedtox.pdf. Accessed 23 June 2020

27 Wirth JP, Rohner F, Woodruff BA, et al. Anemia, micronutrient deficiencies, and malaria in children and women in Sierra Leone prior to the Ebola outbreak -findings of a cross-sectional study. *PLoS One* 2016; **11**: e0155031-e.

28 Mantadakis E, Farmaki E, Buchanan GR. Thrombocytopenic purpura after measles-mumps-rubella vaccination: a systematic review of the literature and guidance for management. *J Pediatr* 2010; **156**: 623–8.

29 Cecinati V, Principi N, Brescia L, Giordano P, Esposito S. Vaccine administration and the development of immune thrombocytopenic purpura in children. *Hum Vaccin Immunother* 2013; **9**: 1158–62.

519 30 Bliss CM, Drammeh A, Bowyer G, et al. Viral vector malaria vaccines induce high-level T cell and
520 antibody responses in West African children and infants. *Mol Ther* 2017; **25**: 547–59.

521 31 Mwangi TW, Bethony JM, Brooker S. Malaria and helminth interactions in humans: an epidemiological
522 viewpoint. *Ann Trop Med Parasitol* 2006; **100**: 551–70.

523 32 Bockstal V, Roozendaal R, Effelterre TV, et al. Immunobridging approach to assess clinical benefit as the
524 basis for licensure of the monovalent Ebola vaccine. Retrieved from
525 https://www.imi.europa.eu/sites/default/files/events/2018/ScientificSymposium/37-
526 %20Viki%20Bockstal.pdf. Accessed 23 June 2020.

527 33 Roozendaal R, Hendriks J, van Effelterre T, Spiessens B, Dekking L, Solforosi L. Immunological bridging
528 to infer efficacy of a prophylactic Ebola vaccine candidate using non-human primate challenge model. *npj*
529 *Vaccines 2020*; **5**: 112.

530 34 Schiffer JM, Chen L, Dalton S, Niemuth NA, Sabourin CL, Quinn CP. Bridging non-human primate
531 correlates of protection to reassess the Anthrax Vaccine Adsorbed booster schedule in humans. *Vaccine*
532 2015; **33**: 3709–16.

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534 ***Figure 1a*: CONSORT flow diagram for 12–17-year-old cohort**

***Figure 1b*: CONSORT flow diagram for 4–11-year-old cohort**

***Figure 1c*: CONSORT flow diagram for 1–3-year-old cohort**

***Figure 2*: Solicited local and systemic adverse events in the paediatric cohorts**

Vaccines: Ad26.ZEBOV at a dose of $5x10^{10}$ vp; MVA-BN-Filo at a dose of $1x10^{8}$ Inf.U. Control: Meningococcal quadrivalent (serogroups A, C, W135 and Y) conjugate vaccine (MenACWY; dose 1), Placebo (dose 2). n=number of participants with data.

***Figure 2A*: Solicited local AEs, reported during a 7-day follow-up period after dose 1 vaccination (day 1); 12–17-year-old cohort**

***Figure 2B*: Solicited systemic AEs, reported during a 7-day follow-up period after dose 1 vaccination (day 1); 12–17-year-old cohort**

***Figure 2C*: Solicited local AEs, reported during a 7-day follow-up period after dose 2 vaccination (on day 57); 12–17-year-old cohort**

***Figure 2D*: Solicited systemic AEs, reported during a 7-day follow-up period after dose 2 vaccination (on day 57); 12–17-year-old cohort**

***Figure 2E*: Solicited local AEs, reported during a 7-day follow-up period after dose 1 vaccination (day 1); 4–11-year-old cohort**

***Figure 2F*: Solicited systemic AEs, reported during a 7-day follow-up period after dose 1 vaccination (day 1); 4–11-year-old cohort**

***Figure 2G*: Solicited local AEs, reported during a 7-day follow-up period after dose 2 vaccination (on day 57); 4–11-year-old cohort**

***Figure 2H*: Solicited systemic AEs, reported during a 7-day follow-up period after dose 2 vaccination (on day 57); 4–11-year-old cohort**

***Figure 2I*: Solicited local AEs, reported during a 7-day follow-up period after dose 1 vaccination (day 1); 1–3-year-old cohort**

***Figure 2J*: Solicited systemic AEs, reported during a 7-day follow-up period after dose 1 vaccination (day 1); 1–3-year-old cohort**

***Figure 2K*: Solicited local AEs, reported during a 7-day follow-up period after dose 2 vaccination (on day 57); 1–3-year-old cohort**

***Figure 2L*: Solicited systemic AEs, reported during a 7-day follow-up period after dose 2 vaccination (on day 57); 1–3-year-old cohort**

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536

***Figure 3*: Geometric mean concentration (with 95% CI) of EBOV GP-specific binding antibody before and after vaccinations in study participants**

Participants administered either Ad26.ZEBOV (dose 1; day 1) and MVA-BN-Filo (dose 2; day 57) or MenACWY (dose 1; day 1) and Placebo (dose 2; day 57). Responses are expressed as geometric mean concentrations (ELISA units/mL, 95% CI). Grey dotted line represents the LLOQ.

Day 1: Baseline; Day 57: 56 days post-dose 1; Day 78: 21 days post-dose 2; Day 240: 179 days post-dose 2;

Day 360: 359 days post-dose 1.

Vaccines: Ad26=Ad26.ZEBOV at a dose of $5x10^{10}$ vp; MVA=MVA-BN-Filo at a dose of $1x10^{8}$ Inf.U;
Control: Meningococcal quadrivalent (serogroups A, C, W135 and Y) conjugate vaccine (MenACWY; dose 1), Placebo (dose 2).

ELISA=enzyme-linked immunosorbent assay; LLOQ=lower limit of quantification.

***Figure 4*: EBOV GP-specific neutralising antibody responses (psVNA, IC_{50} titre) GMT with 95% CI) before and after vaccinations in study participants**

Participants administered either Ad26.ZEBOV (dose 1; day 1) and MVA-BN-Filo (dose 2; day 57) or MenACWY (dose 1; day 1) and Placebo (dose 2; day 57). The error bars represent the geometric mean titre and its 95% confidence interval. Grey dotted line represents the LLOQ.

Day 1: Baseline; Day 57: 56 days post-dose 1; Day 78: 21 days post-dose 2; Day 360: 359 days post-dose 1.

Vaccines: Ad26=Ad26.ZEBOV at a dose of $5x10^{10}$ vp; MVA=MVA-BN-Filo at a dose of $1x10^{8}$ Inf.U;
Control: Meningococcal quadrivalent (serogroups A, C, W135 and Y) conjugate vaccine (MenACWY; dose 1), Placebo (dose 2).

LLOQ=lower limit of quantification.