

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

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25 **Abstract**

26 **Background** Children account for a substantial proportion of cases and deaths from Ebola virus disease (EVD).

27 This study is the first report on the safety and immunogenicity of a two-dose heterologous Ad26.ZEBOV and
28 MVA-BN-Filo Ebola vaccine regimen in a paediatric population.

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

42 **Findings** From 4 April 2017 to 5 July 2018, 576 children (192 in each of the 3 age cohorts) were randomised.
43 Following the first vaccinations (Ad26.ZEBOV vs MenACWY), the most common solicited local AE was pain
44 at injection site in participants aged 12-17 years: 9% (13/143) vs 6% (3/48), 4-11 years: 21% (30/144) vs 4%
45 (2/48) and 1-3 years: 14% (20/144) vs 10% (5/48), respectively. Post-dose 2 vaccinations (MVA- BN-Filo vs
46 placebo), the most common solicited local AE was pain at injection site in participants aged 12-17 years: 15%
47 (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),
48 respectively. The most frequently observed systemic AE post-dose 1 vaccinations (Ad26. ZEBOV vs
49 MenACYW) was headache in participants aged 12-17 years: 29% (41/143) vs 23% (11/48), 4-11 years: 24%
50 (34/144) vs 8 % (4/48) and fever for 1-3 year olds: 11% (16/144) vs 8% (4/48), respectively. Similar trends were
51 observed post-dose 2 vaccinations with MVA.BN-Filo or placebo. Headache was most frequently observed
52 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
53 1-3 year olds: 8% (12/143) vs 15 % (7/48). No Ebola vaccine-related SAEs were reported.

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

58 **Interpretation** The Ad26.ZEBOV, MVA-BN-Filo Ebola vaccine regimen was well tolerated with no safety
59 concerns in children aged 1–17 years and induced robust humoral immune responses, suggesting suitability for
60 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.

66 **Introduction**

67 In the 2014–2016 outbreak of Ebola virus disease (EVD) in West Africa that resulted in 28,652 cases and
68 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
69 Ebola outbreak in the Democratic Republic of Congo (DRC), about 30% of EVD cases were in children aged
70 less than 18 years old.⁵ Children, especially those below five years of age, have a more rapid clinical
71 progression and a relatively high risk of death.³ These features underscore the need for an effective Ebola
72 prevention strategy in paediatric populations.

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

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

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95 **Methods**

96 **Study design**

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

110

111 **Study participants**

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

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

126

127 **Randomisation and masking**

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

134

135 **Investigational vaccines and vaccination**

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

148

149 **Assessment of primary and secondary endpoints**

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

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

159 The secondary endpoint was immunogenicity, measured by EBOV GP-specific binding antibody responses
160 determined by EBOV GP Filovirus Animal Non-Clinical Group (FANG) ELISA measured on day 21 post-dose
161 2. The exploratory outcomes were to assess the humoral immune responses at other relevant time points **and** to
162 assess the neutralising activity of vaccine-induced antibody responses (nAbs) directed against EBOV GP, and
163 against the Ad26 and MVA vectors measured with an Ad26 virus neutralisation assay and a plaque reduction
164 neutralisation test (PRNT), respectively. See supplementary appendix for further details of the procedures
165 involved in the safety and immunogenicity assessments/outcomes.

166

167 **Sample size determination and statistical analyses**

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

171 For the analysis of the EBOV GP-specific nAb response, a subset of participants (28% [54/191] of adolescents
172 aged 12-17 years, 29% [55/192] of children aged 4-11 years, and 29% [56/192] of children aged 1-3 years) were
173 selected at random using a computer software (SAS[®]) in a 3:1 ratio of active to control, to ensure that the
174 distribution of the selected participants was similar to the overall active to control ratio of participants in the
175 study. This was done prior to the analysis of the samples among participants with available samples and no
176 protocol deviations that could have influenced the immune response. The nAb response against the Ad26 vector
177 backbone was measured at baseline (pre-dose 1) in 98% (188/191) of adolescents aged 12-17 years, 93%
178 (179/192) of children aged 4-11 years, and 85% (164/192) of children aged 1-3 years. For the analysis of nAb
179 against the MVA vector (PRNT), a subset of participants (29% [55/191] of participants aged 12-17 years, 29%
180 [55/192] of participants aged 4-11 years, and 29% [56/192] of participants aged 1-3 years) were selected at
181 random as described above for EBOV GP-specific nAb response.

182 Statistical analyses were performed using SAS[®] version 9.2 (SAS Institute Inc., Cary, NC, USA). Descriptive
183 analysis was done without formal hypothesis testing and results were presented by vaccination arms. The full
184 analysis set for safety data comprised all participants who received at least one dose of study vaccine. The
185 analysis set for immunogenicity data (per-protocol) included all vaccinated children, who received both
186 vaccinations within the protocol defined time window, had at least one post vaccination evaluable
187 immunogenicity sample, and had no major protocol deviations that could have influenced the immune response.
188 Responders were defined as either having a negative enzyme-linked immunosorbent assay (ELISA) result at
189 baseline and a positive post-baseline value $> 2.5x$ the lower limit of quantification [LLOQ, ie, 36.11 ELISA
190 units/mL (EU/mL)], or positive at baseline with a post baseline value >2.5 -fold increase from baseline.
191 Participants were considered as responders for the pseudovirion neutralisation assay (psVNA), if negative at
192 baseline and positive post baseline, the latter value being $>2x$ the LLOQ (ie, 120 IC₅₀ titre), or positive at
193 baseline with a post baseline value >2 -fold increase from baseline.
194 Immunoglobulin G binding antibody responses against EBOV GP (by ELISA) and neutralising (nAb) activity
195 (by psVNA) are presented respectively, as geometric mean concentrations (GMCs) and geometric mean titres
196 (GMTs), with 95% confidence intervals (CIs). All values $<LLOQ$ were imputed with half the LLOQ value.
197 Spearman correlation was calculated between EBOV GP-specific binding antibodies (ELISA) and psVNA titres
198 at 21 day post-dose 2.
199 Post-hoc analyses of the immunogenicity by baseline EBOV GP ELISA level were performed to evaluate any
200 potential influence of EBOV GP-specific binding antibodies present at baseline on the vaccine-induced
201 responses (i.e., by a stratification of the 21 day post-dose 2 binding antibody concentrations by baseline ELISA
202 levels and by a correlation analysis).

203

204 **Role of the funding source**

205 This study was funded by the Innovative Medicines Initiative (IMI). IMI had no role in the study design,
206 conduct, or publication of this manuscript. Janssen Vaccines & Prevention B.V sponsored the clinical trial and
207 was involved in the design, conduct of the trial, data analysis, data interpretation, and writing of the report. The
208 corresponding author had full access to all the data in the study and had final responsibility for the decision to

209 submit for publication. There are measures in place to allow all authors to access the study database, should they
210 wish to do so.

211

212 **Results**

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

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

226 In the active control group, solicited local AEs were reported in three (6%) of 48 adolescents following
227 MenACWY vaccination and one (2%) of 46 adolescents following placebo vaccination (figures 2A and 2C). In
228 the 4–11-year-old cohort, two (4%) of 48 children reported at least one solicited local AE following MenACWY
229 vaccination and five (10%) of 48 children reported a local event following placebo vaccination (figures 2E and
230 2G; table S2). In the 1–3 year-old cohort, five (10%) of 48 toddlers were observed to have at least one solicited
231 AE following MenACWY vaccination and no local AEs were reported following placebo vaccination (figures
232 2I and 2K; table S2).

233

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

238 2H; table S3). In the 1–3-year-old cohort, 36 (25%) of 144 toddlers were observed to have at least one solicited
239 systemic AE following Ad26.ZEBOV vaccination and 23 (16%) of 143 toddlers following MVA-BN-Filo
240 vaccination (figures 2J and 2L; table S3). Following MenACWY vaccination, at least one solicited systemic AE
241 was observed in 14 (29%) of 48 adolescents (figure 2B), 15 of 48 children (31 %) (figure 2F), and 12 (15%) of
242 48 toddlers. (figure 2J). Following placebo vaccination, at least one solicited systemic AE was reported in six
243 (13%) of 46 adolescents; eight (17%) of 48 children and 14 (29%) of 48 toddlers (figures 2D, 2H, and 2L
244 respectively; table S3). Headache, fatigue, and chills were the most frequently reported solicited systemic AE
245 after any vaccination in the (12–17 years old) and (4–11 years old) children (figures 2B, 2D, 2F, and 2H,
246 respectively; table S3) while pyrexia (fever), decreased appetite, and decreased activity were the most frequently
247 observed solicited systemic AEs in toddlers (1–3 years old) cohort (figures 2J and 2L; table S3). The frequency
248 of pyrexia was higher in the 1–3-year-old cohort, regardless of the vaccine given, versus other age groups (table
249 S3). Following Ad26.ZEBOV vaccination 16 (11%) of 144 toddlers had fever, and 12/143 (8%) following
250 MVA-BN-Filo vaccination. Similarly, four (8%) of 48 toddlers were observed to have fever following
251 MenACWY vaccination and seven (15%) of 48 toddlers following placebo vaccination (table S3). Grade 3
252 solicited systemic AEs were infrequently observed post vaccination in all age cohorts. The most frequent
253 unsolicited AEs post-dose 1 and post-dose 2 was malaria in all cohorts, irrespective of the types of vaccine
254 given (table S4). None of the AEs were considered related to study vaccine. Grade 3 unsolicited AEs were
255 infrequently observed post vaccination, regardless of vaccine type (table S4).

256

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

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

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

280

281 EBOV GP-specific binding antibody results are summarised in figure 3 and table S7. At 21 days post-dose 2
282 (day 78), binding antibody responses against EBOV GP were observed in 98–99% of participants across all age
283 cohorts. The GMC was 22,568 EU/mL [95% CI 18,426–27,642] in 118/121 (98%) participants aged 1–3 years,
284 10,212 EU/mL [95% CI 8,419–12,388] in 119/120 (99%) participants aged 4–11 years, and 9,929 EU/mL
285 [95% CI 8,172–12,064] in 131/134 (98%) participants aged 12–17 years at 21 days post-dose 2 (figure 3; table
286 S7). Prior to dose 2 vaccination, both the GMC and the responder rate tended to be higher in 115 (94%) of 122
287 participants aged 1–3 years (693 EU/mL; [95% CI 591–812]) than in 91 (64%) of 142 participants aged 12–17
288 years (314 EU/mL; [95% CI 269–366]) or 92 (71%) of 129 participants aged 4–11 years (390 EU/mL; [95% CI
289 334–456]). Compared with the 21 days post-dose 2 time point (day 78), binding antibody concentrations were
290 lower on day 240 (6 months post-dose 2) in all age cohorts, but responses persisted in 99 (73%) of 135
291 participants aged 12–17 years (GMC: 469 EU/mL; [95% CI 397–554]), 90 (74%) of 122 participants aged 4–11
292 years (GMC: 442 EU/mL; [95% CI 377–518]), and 111 (93%) of 119 participants aged 1–3 years ([GMC: 713
293 EU/mL; [95% CI 598–849]) (table S7). The GMCs remained stable between day 240 and day 360 (1-year post-
294 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 IC₅₀ 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 IC₅₀ titre [95% CI
301 1,444–3,111]) and in participants aged 4–11 years (2,483 IC₅₀ 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 IC₅₀ 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 IC₉₀ titre [95% CI 58–101]), in 77% [103/134] aged 4–11 years (GMT: 143 IC₉₀ titre
311 [95% CI 101–201]), and 20% [25/124] aged 1–3 years (GMT: 19 IC₉₀ 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

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

327 Overall, AEs following vaccinations were mild and transient in nature in all age groups. The proportion of study
328 participants with at least one solicited local AE was higher in the Ad26.ZEBOV, MVA-BN-Filo vaccine
329 recipients than in the control group for all age cohorts. Ad26.ZEBOV tended to be more reactogenic than MVA-
330 BN-Filo, especially for solicited local AEs, in the youngest cohort (1–3 years) and for solicited systemic AEs in
331 all age cohorts. Compared with adults, in whom headache, arthralgia, and myalgia were the predominant
332 solicited systemic AEs¹³, the most frequent solicited systemic AEs in participants aged 4–17 years were
333 headache, fatigue, and chills. In participants aged 1–3 years, the most frequently reported solicited systemic AEs
334 were decreased appetite, decreased activity, and pyrexia. These events were of mild intensity (grade 1) and
335 resolved within 24–48 hours in most participants. The observed frequency of pyrexia in participants aged 1–3
336 years was higher compared with the other age groups, regardless of the vaccine given. This agrees with previous
337 findings in similar studies evaluating adenovirus and MVA-vectored vaccines in this age group.^{19,20} Common
338 occurrence of fever in toddlers has also been reported following meningococcal and pneumococcal vaccinations
339 in this age group and is hypothesized to be due to antigen-induced inflammatory responses.^{21–25}

340 A change in haemoglobin concentration from baseline was observed in comparable proportions of study
341 participants in the Ad26.ZEBOV, MVA-BN-Filo and control groups, based on FDA¹⁶ and the Division of
342 Microbiology and Infectious Diseases toxicity grading.²⁶ This development illustrates the challenges commonly
343 faced in AE reporting in paediatric vaccine trials conducted in low-income countries. International laboratory
344 toxicity grading adopted as a ‘gold standard’ does not usually accommodate the epidemiological factors that
345 shape the physiological status of children in low-income countries. The fact that the ‘abnormal’ haemoglobin
346 concentration values were within the acceptable normal ranges of a similar paediatric population in West
347 Africa,¹⁷ underscores the need for context-specific laboratory references for eligibility screening and AE
348 reporting in paediatric vaccine trials in such settings. Nevertheless, given the high prevalence of malaria and
349 other common childhood infectious diseases in the study area, the drop in haemoglobin concentration observed
350 in the children might be as a result of these infections.²⁷

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

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

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

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

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

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

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

393 Word count= 4,629

394 **Author contributions**

395 MOA drafted the manuscript. MOA, DI, DM, KOK, BLo, TM, FB, JF, KG, AG, DH, MS, GFD, BKe, HL, SL,
396 NG, ML, VB, KL, BC, CR, BG, MD, BLe, DWJ were involved in the study concept and design, study conduct,
397 interpretation of results, and revision of the manuscript. DWJ was the lead scientist for the project (EBOVAC1).
398 BLe was the clinical trial principal investigator in Sierra Leone. GFD, BR, ASB, and IS contributed to
399 enrolment and clinical care of participants and data collection. DK was responsible for data management. BLo,
400 BKo, GTO, VB, and KL were responsible for laboratory sample analysis, samples management, and laboratory
401 results interpretation. TM and ES were responsible for community engagement activities. MJ was the clinical
402 trial pharmacist and was responsible for oversight on study vaccine preparation and dispensing. AG and DH
403 conducted the statistical analysis. CR, AG and MOA accessed and verified the data reported in this manuscript.
404 All the authors reviewed and approved the drafts and final manuscript.

405 **Declaration of Interest**

406 Janssen Vaccines & Prevention B.V. was the clinical trial Sponsor and was involved in the design and conduct
407 of the trial, and in the collection and analysis of data. BKe was a full-time employee of Janssen, Pharmaceutical
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414 study. HL reports grants from GSK and from Merck outside the submitted work. All other authors declare no
415 competing interests.

416 **Data sharing statement**

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

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

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

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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 5×10^{10} vp; MVA-BN-Filo at a dose of 1×10^8 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

535

536

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

539

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

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

544 Day 360: 359 days post-dose 1.

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

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

549

Figure 4: EBOV GP-specific neutralising antibody responses (psVNA, IC₅₀ titre) GMT with 95% CI
before and after vaccinations in study participants

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

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

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

LLOQ=lower limit of quantification.