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

3

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28 **Abstract**

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

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

51

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

58 stage 1 by 18/43 (42%) participants post Ad26.ZEBOV (dose 1) and 17/43 (40%) post MVA-BN-Filo. In stage
59 2, solicited systemic AEs were reported by 161/298 (54%) adults post Ad26.ZEBOV, 107/246 (43%) post
60 MVA-BN-Filo, 51/102 (50%) post MenACWY and 39/86 (45%) post placebo. Solicited systemic AEs included
61 mostly mild or moderate headache, myalgia, fatigue, and arthralgia. The most frequent unsolicited AE post dose
62 1 and post dose 2 was headache in stage 1 and malaria in stage 2, regardless of vaccine received. Grade 3
63 unsolicited AEs were infrequent, observed in at most 2% of participants regardless of vaccine received. No SAE
64 was considered related to the study vaccine. In stage 1, the post-booster vaccination safety profile was not
65 notably different to that observed post dose 1. Vaccine-induced humoral immune responses were observed in
66 41/42 (98%) stage 1 participants and in 176/179 (98%) stage 2 participants 21 days post dose 2 (geometric mean
67 binding antibody concentration: 4784 EU/ml [95% CI 3736–6125], stage 1 and 3810 EU/ml [95% CI 3312–
68 4383], stage 2). Antibody responses persisted for at least two years.

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

72 **Funding** Innovative Medicines Initiative 2 Joint Undertaking and Janssen Vaccines & Prevention B.V.

73 **Word count: 640**

74

Research in context

Evidence before this study

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

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

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

Added value of this study

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

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

Implications of all the available evidence

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

76 **Introduction**

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

91 A heterologous, two-dose regimen of Ad26.ZEBOV (dose 1) followed by MVA-BN-Filo (dose 2) after 56 days
92 (Ad26.ZEBOV, MVA-BN-Filo vaccine regimen) has ~~recently~~ received marketing authorisation for prophylactic
93 use in adults and children ≥ 1 year old in the EU.¹¹ This vaccine regimen provided protection against Zaire
94 Ebolavirus (EBOV) challenge in macaques and demonstrated a good safety profile with strong and durable
95 immune responses for at least one year in Europeans and healthy Africans, living in areas not affected by Ebola,
96 in three phase 1 studies and one phase 2 study.^{12–16} The study reported herein evaluated the safety, long-term
97 immunogenicity, and humoral immune memory induced by the Ad26.ZEBOV, MVA-BN-Filo vaccine regimen
98 administered with a 56-day interval in healthy adults in Kambia District, an area of Sierra Leone affected by the
99 2014–2016 EVD epidemic and, therefore, at potential risk for future outbreaks.¹⁷

100 **Methods**

101 **Study design**

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

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

111 **Study participants**

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

129 **Randomisation and masking**

130 There was no randomisation in stage 1. Stage 2 participants were centrally randomised using computer-
131 generated block randomisation via an Interactive Web Response System (IWRS), operated by a study
132 pharmacist. Study team personnel (except those with primary responsibility for study vaccine preparation) and
133 participants were blinded to study vaccine allocation until all participants had completed the 6-month post dose
134 2 visit or discontinued the study earlier and the database had been locked. Blinding was achieved using syringes
135 of identical volume taped to conceal the colour of the liquid inside.

136 **Vaccines and vaccination**

137 Ad26.ZEBOV (Janssen Vaccines & Prevention, B.V.) is a monovalent, recombinant, replication-incompetent,
138 Ad26-vectored vaccine encoding the glycoprotein (GP) of the EBOV Mayinga variant. MVA-BN-Filo
139 (Bavarian Nordic) is a recombinant, non-replicating, modified vaccinia Ankara-vectored vaccine encoding the
140 EBOV Mayinga variant GP as well as Sudan virus Gulu variant GP, Marburg virus Musoke variant GP, and Tai
141 Forest virus nucleoprotein. In stage 1, all participants received Ad26.ZEBOV (dose 1) followed by MVA-BN-
142 Filo (dose 2), 56 days later. An Ad26.ZEBOV booster vaccination was also offered to stage 1 participants two
143 years post dose 1 (day 720) (figure 1). Stage 2 participants were randomised 3:1 to receive the same Ebola
144 vaccine regimen, or one dose of a meningococcal polyvalent (serogroups A, C, W135, and Y) conjugate vaccine
145 (MenACWY, Menveo[®], GSK Vaccines, or Nimenrix[®], Pfizer) followed by a saline placebo 56 days later, as an
146 active control (MenACWY, placebo regimen) (figure 1). All vaccines were administered via a single 0.5 mL
147 intramuscular injection into the deltoid muscle at a dose of 5×10^{10} viral particles for Ad26.ZEBOV, 1×10^8
148 Inf.U for MVA-BN-Filo, 0.5 ml reconstituted vaccine solution for MenACWY, and 0.5 mL 0.9% sodium
149 chloride solution for the placebo.

150 **Study outcomes**

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

161 **Safety evaluations**

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

165 (haemoglobin, haematocrit, red blood cell count, platelet count, and white blood cell count with differential),
166 and a serum chemistry panel (ALT, AST and creatinine). Each participant received a 24-hour telephone number
167 to contact in case of medical problems. In stage 1, all AEs were recorded from dose 1 until 56 days after dose 2
168 and then again from the day of the booster vaccination until 28 days post-booster vaccination. In stage 2, AEs
169 were recorded for 28 days following each vaccination. In both stages 1 and 2, SAEs were recorded from dose 1
170 until each participant's last study visit, i.e., three years post dose 1 in stage 1, and two years post dose 1 in stage
171 2.

172 **Immunogenicity assessments**

173 In stage 1, immunological assays were performed on blood samples taken immediately before doses 1 and 2,
174 then at 21 days post dose 2, 155 and 360 days post dose 1 and, subsequently, every six months up to three years
175 post dose 1. In participants who agreed to the booster vaccination, additional immunogenicity samples were
176 collected immediately before the booster vaccination and at four days, seven days, 21 days, six months, and one
177 year post-booster vaccination. In stage 2, immunogenicity samples were collected pre dose 1, 28 days post dose
178 1, pre dose 2, 21 days and six months post dose 2, one and two years post dose 1.

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

187 **Statistical analysis and sample size**

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

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

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

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

214 Participants were considered as responders by ELISA if samples were negative at baseline and positive post
215 baseline with a value that was greater than 2.5 times the lower limit of quantification [LLOQ: 36.11 ELISA
216 units/mL (EU/mL)], or a sample was positive both at baseline and post baseline and there was a greater than
217 2.5-fold increase from baseline. Participants were considered as responders for psVNA if a sample was negative
218 at baseline and positive post baseline and the post-baseline value was greater than two times the LLOQ (120
219 half maximal inhibitory concentration [IC₅₀] titre), or samples were positive both at baseline and post baseline
220 and there was a greater than 2-fold increase from baseline.

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

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

229 All statistical analysis was done using SAS, version 9.2.

230 **Role of the funding source**

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

237

238 **Results**

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

248 Solicited AEs were mostly mild to moderate (grade 1 and 2) (figure 3 and tables S1, S2). At least one solicited
249 local AE was reported in stage 1 by 12/43 (28%) participants post-Ad26.ZEBOV vaccination and by 6/43 (14%)
250 participants post-MVA-BN-Filo vaccination (Figures 3A, 3C and table S1). In stage 2, at least one solicited
251 local AE was reported by 51/298 (17%) adults following Ad26.ZEBOV vaccination and 58/246 (24%)
252 following MVA-BN-Filo vaccination. In the MenACWY, placebo, at least one solicited local AE was reported
253 in 17/102 (17%) adults following MenACWY vaccination and 8/86 (9%) following placebo vaccination (figures
254 3A, 3C and table S1). The most frequent solicited local AE was injection site pain after any vaccination (figures
255 3A, 3C and table S1). One report of grade 3 solicited local AE (injection site pain) was observed post-MVA-
256 BN-Filo vaccination (figure 3C and table S1). Solicited systemic AEs in stage 1 were reported by 18/43 (42%)
257 participants post-Ad26.ZEBOV vaccination and 17/43 (40%) post-MVA-BN-Filo vaccination ([figures 3B, 3D](#)
258 [and](#) table S2). In stage 2, at least one solicited systemic AE was reported by 161/298 (54%) adults following
259 Ad26.ZEBOV vaccination, 107/246 (43%) adults following MVA-BN-Filo vaccination, by 51/102 (50%) adults
260 following MenACWY vaccination, and 39/86 (45%) adults following placebo vaccination (figures 3B, 3D and
261 table S2). Headache, myalgia, fatigue, and arthralgia were the most frequently reported solicited systemic AEs
262 after any vaccination, and grade 3 solicited systemic AEs were infrequently observed (figures 3B, 3D and table
263 S2).

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

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

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

289 Forty-three participants in stage 1 and 259 participants in stage 2 (191 in the Ad26.ZEBOV, MVA-BN-Filo
290 group and 68 in MenACWY, placebo group) fulfilled the criteria for the per-protocol analysis set for
291 immunogenicity. At 56 days post dose 1, EBOV GP-specific binding antibody responses (table 2 and figure 4)
292 were observed in 28/43 (65%) stage 1 and 101/187 (54%) stage 2 participants in the Ad26.ZEBOV, MVA-BN-
293 Filo group, with GMCs of 269 EU/mL (95% CI 208–347) and 236 EU/mL (95% CI 206–270), respectively. At
294 21 days post dose 2, binding antibody responses were observed in 41/42 (98%) stage 1 and in 176/179 (98%)
295 stage 2 participants, with GMCs rising to 4784 EU/mL (95% CI 3736–6125), and 3810 EU/mL (95% CI 3312–
296 4383), respectively.

297 Due to a study pause (for precautionary reasons during the evaluation of two SAEs in a different study),¹⁶ dose 2
298 was delayed in 72 stage 2 participants (time interval between dose 1 and dose 2 ranged from 96 to 147 days).
299 This delayed dose 2 administration did not negatively affect binding antibody responses. At 21 days post-dose 2
300 vaccination, responses were observed in 44/45 (98%) stage 2 participants in the Ad26.ZEBOV, MVA-BN-Filo
301 group who received the delayed dose 2, with a GMC that was similar to the GMC in participants receiving dose
302 2 within the protocol-defined window (dose 2 delayed: 5761 EU/mL, 95% CI 3926–8455 vs dose 2 within
303 protocol-defined window: 3823 EU/mL, 95% CI 3334–4383, table S6).

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

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

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

310 EBOV GP-specific nAb concentrations were measured in a randomly selected stage 2 participant subset (n=74,
311 of which n=55 in the Ebola vaccine arm and n=19 in the control arm) (figure 4 and table S7). At 56 days post
312 dose 1, an EBOV GP-specific nAb response was observed in one participant out of 51 in the Ebola vaccine arm
313 (2%) with a GMT below the LLOQ. At 21 days post dose 2, an EBOV GP-specific nAb response was detected
314 in 52/53 (98%) participants in the Ebola vaccine arm with GMT of 2199 (95% CI 1634–2960). There was a
315 strong positive correlation between Ebola GP-specific binding antibodies and nAbs at 21 days post dose 2 in
316 participants who received Ebola vaccine (Spearman correlation coefficient: 0.751) (figure S1). At day 360, the
317 nAb response persisted in 3/53 (6%) participants in the Ebola vaccine arm. At about two-years post dose 1, nAb
318 responses were observed in 6/51 (12%) participants in the Ebola vaccine arm.

319 Ad26-specific pre-existing nAbs were measured in all participants assigned to stage 1 (n=43), and in a subset of
320 participants assigned to stage 2 [191/298 (64%) in the Ebola vaccine arm; 18/102 (18%) in the control arm].

321 Ad26-specific pre-existing nAbs were detected in 40/43 (93%) stage 1 participants, in 177/191 (93%) stage 2
322 participants in the Ebola vaccine arm and in 17/18 (94%) stage 2 participants in the control arm, with similar
323 GMTs between groups (IC₉₀ GMTs of 111, 124, and 104 in stage 1, stage 2 Ebola vaccine arm and stage 2
324 control arm, respectively) (table S8). There was a negligible correlation between the baseline Ad26-specific nAb
325 titres and the vaccine-induced EBOV GP-specific binding antibody concentrations at 21 days post dose 2
326 (Spearman correlation coefficient: -0.145) (figure S2).

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

332

333

334 **Discussion**

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

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

340 The Ad26.ZEBOV, MVA-BN-Filo vaccine regimen induced EBOV GP-specific binding and nAbs responses
341 observed in 98% of adult participants at 21 days post dose 2 (binding antibody responses: 41/42 [98%] in stage
342 1 and 176/179 [98%] in stage 2; nAb responses: 52/53 [98%] in stage 2). At this time point, a strong positive
343 correlation was observed between binding antibody concentrations and nAb titres. The magnitude of the
344 antibody responses declined over time: at one-year post dose 1, binding antibody responses persisted in 24/31
345 (77%) stage 1 participants and in 82/166 (49%) stage 2 participants; at two-years post dose 1, binding antibody
346 responses persisted in 21/31 (68%) stage 1 participants and 78/155 (50%) stage 2 participants; in a randomly-
347 selected stage 2 subset, nAb responses persisted in 3/53 (6%) participants at one-year post dose 1 and in 6/51
348 (12%) at two years post dose 1.

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

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

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

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

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

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

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

395 The Ad26.ZEBOV, MVA-BN-Filo vaccine regimen with a 56-day interval assessed here has recently received
396 marketing authorisations for prophylactic use in adults and children ≥ 1 year old in the EU.¹¹ This vaccine
397 regimen was previously shown to provide protection in vaccinated non-human primates against an EBOV
398 challenge, which is fully lethal in unvaccinated control animals.¹² In the absence of clinical efficacy data in
399 humans, a statistical approach referred to as immunobridging using data from this and other clinical studies, was
400 used to infer the potential clinical benefit induced by vaccination by correlating the magnitude of vaccine-
401 elicited immune parameters in non-human primates with those observed in vaccinated humans.²³ Although a
402 mechanistic correlate of protection has not yet been identified, the binding antibody GMCs observed 21 days
403 after the second dose in the two-dose regimen, were similar to the GMC of 1262 EU/mL (95% CI 1169–1363)
404 reported 28 days post-rVSV-ZEBOV vaccination using the same assay in the same laboratory.²⁴ rVSV-ZEBOV,
405 which was the first Ebola vaccine to received conditional marketing authorisation in Europe and approval for
406 use in adults in USA and several African countries,^{7–9} is the the only vaccine for which vaccine effectiveness
407 (VE) data are available so far (i.e. estimated VE of 100% from 10 days post vaccination onwards in a phase 3
408 trial in Guinea during the West African outbreak,⁴ and an estimated VE of 97.5% in DRC).⁶

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

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

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

425 ~~Word Count: 4995~~

426 **Contributors**

427 DI and DM drafted the manuscript. DM conducted the literature search for and drafted the research in context
428 section. DI, DM, MOA, FB, KOK, BLo, TM, ES, JF, KEG, MS, GFD, BKe, HL, SL, NG, ML, VB, AG, DH,
429 BC, KL, CR, BG, MD, BLe, DWJ were involved in the study concept and design, study conduct and
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432 B.V. BLe was the clinical trial principal investigator in Sierra Leone. GFD, BR, ASB, and IS contributed to
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434 BKo, GTO, VB, KL, were responsible for laboratory sample analysis, samples management and laboratory
435 results interpretation. TM, ES, LE were responsible for community engagement activities. MJ was the clinical
436 trial pharmacist and was responsible for study vaccine preparation and dispensing. AG and DH conducted the
437 statistical analysis. AG, CR and DM have accessed and verified the data. All the authors reviewed and approved
438 the final manuscript.

439 **Declaration of Interest**

440 Janssen Vaccines & Prevention B.V. was the clinical trial Sponsor and was involved in the design and conduct
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448 study. HL reports grants from GSK and from Merck outside the submitted work. All other authors declare no
449 competing interests.

450 **Data Sharing**

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

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

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

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480

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553

554 **Figures**

555 **Figure 1: Study design diagram**

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

558 Vaccine dosages: 5×10^{10} viral particles for Ad26, 1×10^8 Inf.U for MVA, 0.5 ml reconstituted vaccine
559 solution for MenACWY and 0.5-mL 0.9% sodium chloride solution for the placebo.

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

561

562 **Figure 2: Study flow diagram**

563 **Panel A: Stage 1**

564 **Panel B: Stage 2**

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

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

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

570

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

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

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

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

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

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

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

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

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

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

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

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

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

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

518 Data are labelled at the following time points: Day 1 (pre-vaccination baseline); Day 57 (56 days post dose 1);
519 Day 78 (21 days post dose 2); Day 156 (155 days post dose 1); Day 360 (359 days post dose 1); Day 540 (539
520 days post dose 1); Day 720 (719 days post dose 1); Day 741 (21 days post-Booster vaccination); and Day 1080
521 (359 days post-Booster vaccination). Labels for the following time point tick-marks are omitted: Day 724 (4
522 days post-Booster vaccination), Day 727 (7 days post-booster vaccination).

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

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

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

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

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