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► **To cite this version:**

Andrew Pollard, Odile Launay, Jean-Daniel Lelievre, Christine Lacabaratz, Sophie Grande, et al.. Safety and immunogenicity of a two-dose heterologous Ad26.ZEBOV and MVA-BN-Filo Ebola vaccine regimen in adults in Europe (EBOVAC2): a randomised, observer-blind, participant-blind, placebo-controlled, phase 2 trial. *The Lancet Infectious Diseases*, 2020, 10.1016/S1473-3099(20)30476-X . hal-03142752

HAL Id: hal-03142752

<https://inria.hal.science/hal-03142752>

Submitted on 24 Apr 2023

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1 **Safety and immunogenicity of a two-dose heterologous Ad26.ZEBOV and MVA-BN®-**
2 **Filo Ebola vaccine regimen: a phase 2 randomised clinical study in Europe (EBOVAC2)**

3

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30 **Abstract = 344 words (limit is 350)**

31 **Words = 4,492 (limit is 4,500)**

32 **References = 27 (limit is 30)**

33

34 **ABSTRACT**

35 **Background** We assessed different regimens of a heterologous two-dose Ebola vaccination
36 strategy using Ad26.ZEBOV, a replication-incompetent adenovirus 26 expressing Zaire
37 Ebola glycoprotein (GP), and MVA-BN-Filo, a non-replicating, recombinant modified
38 vaccinia Ankara virus encoding GPs from Zaire, Sudan, Marburg and nucleoprotein from Tai
39 Forest viruses in adults.

40 **Methods** In this randomised, observer-blind, placebo-controlled phase 2 trial healthy 18–65
41 years-olds in the UK and France received intramuscular injections of Ad26.ZEBOV,
42 followed 28, 56 or 84 days later by MVA-BN-Filo. Controls received two placebo (0.9%
43 saline) injections. Participants recorded solicited adverse events (AEs) for 7 days post-
44 vaccination, unsolicited AEs until 42 days after the second vaccination and serious adverse
45 events (SAEs) throughout the study. Humoral (anti-EBOV GP IgG and neutralising
46 antibodies) and cellular immune responses to EBOV GP were measured at baseline,
47 immediately before and 21 days after MVA-BN-Filo, and one year after Ad26.ZEBOV.

48 **Findings** We enrolled 423 participants from 23 June, 2015, of whom 421 (99.5%) received
49 at least one study injection. All vaccinations were well tolerated with mainly transient
50 mild/moderate adverse events, most frequently injection site pain, fatigue, headache or
51 myalgia. Two neurological SAEs, one considered possibly related to vaccination, resulted in
52 a temporary clinical hold disrupting per protocol vaccination in some participants. Anti-GP
53 IgG was induced in 71 of 77 (92%; 95% CI: 84–97), 66 of 69 (96%; 88–99), and 69 of 69
54 (100%; 93–100) vaccinees 28, 56 and 84 days after Ad26.ZEBOV, respectively. Responder
55 rates increased to 76 of 77 (99%; 95% CI: 93–100), 69 of 69 (100%; 95–100), and 47 of 47
56 (100%; 92–100) 21 days after MVA-BN-Filo. Geometric mean concentrations were higher
57 when the interval was increased from 28 days to 56 or 84 days. Antibodies persisted at the
58 post-dose 1 level up to one year after Ad26.ZEBOV. GP-specific neutralising activity was

59 strongly correlated with these IgG responses. Cellular responses against GP were detected
60 after Ad26.ZEBOV and enhanced by MVA-BN-Filo.

61 **Interpretation** The two-dose Ad26.ZEBOV, MVA-BN-Filo vaccination regimen was safe,
62 well-tolerated and immunogenic; vaccine-induced humoral and cellular immune responses
63 persisted for one year after vaccination.

64 **Funding** IMI and Janssen Vaccines & Prevention B.V.

65

66 INTRODUCTION

67 The Ebola virus was recognised over 40 years ago as the cause of an outbreak of
68 haemorrhagic fever in the northern part of the Democratic Republic of Congo.¹ Ebola disease
69 is caused by a family of Ebola viruses (Zaire, Sudan, Tai Forest, and Bundibugyo) with an
70 animal reservoir. Patients typically present with non-specific viral symptoms from 2 to 21
71 days after exposure to the body fluids of an index case and infection has a case fatality rate of
72 up to 88% in humans.² Since the first documented outbreaks in 1976 numerous short-lived
73 explosive outbreaks (involving 1 to 425 documented cases) have been controlled by
74 enhanced infection control precautions, including isolation.³

75 At the time this study was done no vaccine had been licensed to control this high mortality
76 filovirus infection.⁴ Despite encouraging protection data from development studies in
77 animals, vaccines had not been taken into human clinical trials until a large and complicated
78 outbreak in West Africa in 2014–16 resulting in 11,000 deaths.³ This led to a rapid increase
79 in global vaccine research and development with 13 vaccine candidates now tested in a series
80 of clinical trials.⁵ A recombinant vesicular stomatitis virus (rVSV) vaccine which expresses
81 the glycoprotein (GP) of the Kikwit strain of Zaire Ebola had a high efficacy in a ring-
82 vaccination clinical trial in Guinea during the 2014–16 outbreak.⁶ The WHO Strategic
83 Advisory Group of Experts on Immunization (SAGE) recommended implementation of
84 rVSV as reactive use vaccine for outbreak control and for those at high risk of Ebola
85 exposure such as case contacts and healthcare workers,⁷ and conditional marketing
86 authorisation was recently granted in Europe.

87 In a second large outbreak from August 2018 in the Democratic Republic of Congo (DRC)
88 there have been 2194 deaths reported up to November 2019,⁸ including three exported to
89 Uganda after probable infection in the DRC.⁹ Although the rVSV ring vaccination approach
90 has been proven to be highly effective in the recent outbreak in DRC, the complexities of the

91 political situation in the region and lack of trust of the local population during the outbreak
92 highlight the importance of licensing and deploying effective prophylactic vaccines in times
93 of stability to protect all those at future risk of exposure to Ebola virus, rather than rely on
94 reactive use of the rVSV vaccine. Indeed, the WHO SAGE recommendations include
95 implementation of a prophylactic vaccination regimen in low-risk populations in the DRC.⁷

96 To respond to this urgent need, Janssen Vaccines initiated an accelerated vaccination
97 development programme using their recombinant replication-incompetent adenovirus type 26
98 viral vector platform. In a heterologous two-dose strategy the Ad26.ZEBOV vaccine
99 encoding Mayinga Ebola glycoprotein was paired with the readily available recombinant
100 non-replicating modified vaccinia Ankara viral vector encoding glycoproteins from Ebola
101 Mayinga, Sudan Gulu and Marburg Musoke viruses and nucleoprotein from Tai Forest virus
102 (MVA-BN-Filo) supplied by Bavarian Nordic (Hellerup, Denmark). In non-human primate
103 (NHPs) studies this heterologous Ad26.ZEBOV, MVA-BN-Filo regimen induced protection
104 against lethal Ebola virus challenge with doses given four to eight weeks apart.¹⁰ Phase 1
105 studies of this vaccination strategy in the UK, US, Kenya, Uganda, and Tanzania have
106 demonstrated an excellent safety profile and strong humoral and cellular immune responses
107 in human volunteers,¹¹⁻¹³ that persisted at least 1 year after vaccination.¹⁴

108 These data encouraged further clinical development to develop this promising strategy for
109 disease control and prevention. We present the safety and immunogenicity from a phase 2
110 vaccine study using the heterologous two-dose Ad26.ZEBOV, MVA-BN-Filo immunisation
111 strategy at various intervals in adult volunteers in the UK and France.

112

113 **METHODS**

114 *Study design and participants*

115 This was a randomised, observer-blind, placebo-controlled, phase 2 study in healthy adults to
116 evaluate the safety, tolerability and immunogenicity of a heterologous two-dose Ebola
117 vaccination regimen using Ad26.ZEBOV and MVA-BN-Filo vaccines with three different
118 dosing intervals. We did the study in seven sites in France (Creteil, Lyon, Paris, Rennes,
119 Saint-Etienne, Strasbourg, Tours) and two sites in the UK (London, Oxford). The protocol
120 was approved in France by the French national Ethics Committee “CPP Ile de France III”
121 (recorded under No. 3287) and the French Medicine Agency (ANSM) (recorded under No.
122 150646A-61) and in the UK by the Medicines and Healthcare Products Regulatory Agency
123 (MHRA) and National Research Ethics Service (Reference South Central – Oxford A
124 15/SC/0211). The study was done according to the current Declaration of Helsinki and Good
125 Clinical Practice guidelines. All participants supplied written informed consent before
126 enrolment.

127 The primary objective of the study was to assess the safety and tolerability of the three
128 vaccination schedules with Ad26.ZEBOV administered on Day 1 and MVA-BN-Filo 28, 56
129 or 84 days later. The secondary objective was to assess anti-EBOV glycoprotein (GP) total
130 IgG binding antibody concentrations measured by ELISA at 21 days after the second
131 vaccination. Exploratory objectives included assessment of binding antibody concentrations
132 at other time points, EBOV GP-specific virus neutralising antibody titres measured by
133 pseudovirion neutralisation assay (psVNA), and EBOV GP-specific CD4+ and CD8+ T cell
134 responses at selected timepoints. In addition, exploratory Ad26-vector-shedding was
135 investigated on Days 1, 2, and 8 after vaccination.

136 Participants were adults aged 18–65 years who were healthy in the investigators' judgement
137 based on a physical examination, ECG, laboratory assessments and clinical history at
138 screening. All women had a negative pregnancy test prior to enrollment and each vaccination
139 and were required to use approved effective methods of contraception during the study. Other
140 exclusion criteria included prior exposure to Ebola virus (including travel to West Africa less
141 than one month prior to screening) or previous diagnosis of Ebola virus disease, previous
142 receipt of any Ebola vaccine candidate or any known allergy or history of anaphylaxis or
143 other serious adverse reactions to vaccines or study vaccine constituents (including egg, egg
144 products and aminoglycosides). In case of acute illness or temperature $\geq 38.0^{\circ}\text{C}$ on Day 1,
145 vaccination of the volunteer was delayed until recovery.

146 ***Vaccines***

147 The two vaccines were Ad26.ZEBOV and MVA-BN[®]-Filo used in a heterologous two-dose
148 regimen.^{10–12} Ad26.ZEBOV is a monovalent, recombinant, replication-incompetent
149 adenovirus 26-vectored vaccine expressing Ebola virus Mayinga variant GP. MVA-BN-Filo
150 (Bavarian Nordic) is a recombinant, non-replicating, modified vaccinia Ankara-vectored
151 vaccine expressing Mayinga, Sudan virus Gulu and Marburg virus Musoke variant GPs as
152 well as Taï Forest virus nucleoprotein. The regimen consisted of a first vaccination with $5 \times$
153 10^{10} viral particles of Ad26.ZEBOV, followed by a second vaccination with 1×10^8
154 infectious units of MVA-BN-Filo. Both vaccines and placebo (0.9% saline) were
155 administered by intramuscular injection (0.5 mL) in the deltoid, the second injection being
156 administered in the opposite arm from the first.

157 ***Procedures***

158 After screening, four cohorts (I, II, III and IV) of volunteers were enrolled. Cohorts I, II and
159 III were randomised (1:1:1) using sponsor-supplied randomisation codes into three parallel

160 groups (1, 2 and 3) to receive their two study injections with 28-, 56- or 84-day intervals,
161 respectively (**Figure 1**).

162 All Cohort I participants were enrolled at the Oxford site and randomised to three groups
163 (block size 3) to receive one dose each of Ad26.ZEBOV and MVA-BN-Filo in an open-label
164 fashion at 28-, 56- or 84-day intervals to provide data on safety and timing of anti-EBOV GP
165 antibody-secreting cell responses. Cohort I participants were not included in further safety
166 and immunogenicity analyses.

167 Participants enrolled into Cohorts II and III were randomly allocated to one of the three
168 groups with different vaccination intervals (block size 3) stratified by country and by age
169 (≤ 50 years and > 50 years) and further randomised 14:1 in Cohort II (block size 15), 10:3 in
170 Cohort III (block size 13) to receive active vaccines or placebo. Participants from Cohort II
171 had additional blood sampling for exploratory immunological assessments. Finally, the fourth
172 cohort (Cohort IV) comprising 18 volunteers was to be enrolled in France and randomised
173 (5:1, block size 6) to receive Ad26.ZEBOV or placebo, for Ad26 vector shedding
174 assessments in urine samples and mid-turbinate swabs by quantitative polymerase chain
175 reaction (qPCR).

176 *Safety and tolerability assessments*

177 Participants were assessed at 30 and 60 minutes after each vaccination for any immediate
178 adverse events (AEs). They were supplied with diary cards to report solicited local and
179 systemic AEs and daily body temperature for 7 days after each vaccination, which were
180 graded as mild (Grade 1), moderate (Grade 2) or severe (Grade 3). After 7 days, participants
181 continued to record any other AEs as “unsolicited AEs” until 42 days after the second
182 vaccination in Cohorts I–III and 28 days post vaccination for Cohort IV. Serious AEs (SAEs)
183 were to be reported to the investigators at any time during the study. An independent data

184 monitoring committee (IDMC) was established to assess the safety data on a regular basis
185 during the study.

186 *Immunogenicity assessments*

187 Four serum samples for assessment of immune responses were obtained from all participants
188 in Cohorts II and III: immediately before the first vaccination on Day 1, before the second
189 vaccination on Days 29, 57 or 85, and then 21 days after the second vaccination, and at Day
190 365 in all groups. EBOV GP-specific total IgG binding antibody concentrations were
191 measured using an EBOV GP Filovirus Animal Non-Clinical Group (FANG) ELISA at Q2
192 Solutions (San Juan Capistrano, CA, USA).¹⁵ EBOV GP-specific neutralising antibody
193 (psVNA) titres were measured using a pseudovirion neutralisation assay (**Supplementary**
194 **materials**) at Monogram (San Francisco, CA, USA).

195 Peripheral blood mononuclear cells (PBMC) were collected from a subset of participants and
196 frozen for later analysis to determine the percentage of CD4+ and CD8+ T cells producing
197 interferon (IFN)- γ and/or interleukin (IL)-2 by intracellular cytokine staining (ICS) at the
198 Inserm U955 unit laboratory/VRI. Defrosted PBMC were stimulated by two peptide pools
199 (GP1 and GP2) covering the GP from Mayinga variant of Ebola virus (**Supplementary**
200 **materials**).

201 *Statistical Analyses*

202 This was originally designed as a prospective study with no formal hypothesis testing, for
203 which the planned sample size was 612 participants (pooled across cohorts) to include 492
204 participants randomised to receive Ad26.ZEBOV and MVA-BN-Filo (Cohorts II and III), 30
205 participants in Cohort I to receive Ad26.ZEBOV and MVA-BN-Filo in an open-label
206 fashion, and 90 placebo recipients. Following the occurrence of two SAEs (described in

207 Results) there was a clinical hiatus until the study was restarted following an amended
208 protocol with the agreement of the IDMC and relevant competent authorities.

209 The descriptive safety analysis is presented on the full analysis set consisting of all
210 participants who were randomised and received at least one administration of vaccine or
211 placebo irrespective of protocol deviations. The immunogenicity analysis, based on the per
212 protocol set, includes all randomised and vaccinated participants who were administered both
213 vaccinations within the protocol-defined window (± 1 day), had at least one evaluable
214 immunogenicity sample post-vaccination, and no major protocol violations influencing the
215 immune response. Binding antibodies were expressed as group geometric mean
216 concentrations (GMC) of arbitrary ELISA units with 95% confidence intervals (CI) at each
217 timepoint, and responder rates, i.e. the percentage of each group with post vaccination
218 concentrations >2.5 -fold the Lower Limit of Quantitation (LLOQ), i.e. 36.11 ELISA
219 units/mL, in baseline seronegative individuals, or >2.5 -fold the baseline value in initially
220 seropositive participants. psVNA titres are expressed as geometric mean titres (GMT) with
221 95% confidence intervals (CI) and group proportions of responders at each timepoint. psVNA
222 responders were those negative at baseline and positive post vaccination with a titre two-fold
223 higher than LLOQ (120 IC₅₀ titre), or positive at baseline with greater than two-fold increase
224 post vaccination. Spearman correlation was calculated for EBOV GP-specific binding
225 antibody concentrations (FANG ELISA) and psVNA titres at selected timepoints.

226 For cell-mediated immune responses assessed by ICS, the frequency of responders,
227 calculated on IFN- γ and/or IL-2 separately for the sum of the two EBOV GP peptide pools,
228 was determined at each timepoint. Responders were either: i) negative at baseline and
229 positive post-baseline with a value >2 -fold the LLOQ (0.05%, CD4+ IFN- γ ; 0.08%, CD4+
230 IL-2; 0.06%, CD8+ IFN- γ ; 0.19%, CD8+ IL-2), or ii) positive at baseline with > 2 -fold
231 increase in background adjusted percentage of the combined peptide pools.

232 All statistical analyses were performed by the sponsor with SAS (version 9.2, SAS Institute,
233 Cary, NC) with independent validation completed by Inserm. The study protocol was
234 registered on ClinicalTrials.gov (NCT02416453) and EudraCT (2015-000596-27).

235 ***Role of the funding source***

236 As full-time employees of the legal sponsor, Janssen Vaccines and Prevention, NG, CR, AG,
237 VB, ML, KL and MD took part in the conception, design, and preparation of the protocol,
238 and did some of the laboratory and statistical analyses. Other authors contributed to the
239 design and did the study with no financial interest. All authors had full access to the data,
240 contributed to the manuscript over which academic contributors had full editorial control, and
241 all authors agreed with the decision to submit.

242

243 **RESULTS**

244 The study began in July 2015 but was paused from 27 April 2016 to 9 May 2016 to
245 investigate an SAE and was stopped for a second time by the sponsor on May 20, 2016 until
246 27 Sep 2016 following a second event. After reviewing both cases, including by an
247 independent panel of expert neurologists, the MHRA approved resumption of the study on 27
248 September 2016. Screening for groups 1 and 2 in Cohorts II and III was restarted in the UK
249 but not in France. Randomisation and collection of one-year data for group 3 (84-day
250 regimen) was halted. Following re-consent, participants already vaccinated with dose 1 were
251 offered the second dose outside of the protocol window (**Figure 1**).

252 *Baseline demographics*

253 Of 423 participants enrolled, 421 received at least one dose of study vaccine, including: 30
254 participants in Cohort I, 376 participants in pooled Cohorts II and III (194 in the UK and 182
255 in France) and 15 participants in Cohort IV. Participant demographic data and assignment of
256 pooled Cohorts II and III to groups 1, 2 and 3 are shown in **Table 1**. In total, 363 (86.2%)
257 participants completed the study: 23 (76.7%) of 30 in Cohort I, 325 (86.4%) of 376 in pooled
258 Cohorts II and III, and all 15 (100%) in group 4 (**Figure 1**).

259 *Safety*

260 Solicited local AEs were reported after 206 (62.0%) of 332 Ad26.ZEBOV and 136 (57.6%)
261 of 236 MVA-BN-Filo vaccinations, compared with 11 (15.3%) of 72 placebo injections
262 (**Table 2**). The most frequent local AE was pain at the injection site, reported after 191
263 (57.5%) of 332 Ad26.ZEBOV and 126 (53.4%) of 236 MVA-BN-Filo vaccinations,
264 compared with 9 (12.5%) of 72 placebo injections (**Table 2**). Solicited local AEs were
265 generally described as mild to moderate; six cases (three of pain and two of swelling after
266 Ad26.ZEBOV, and one case of erythema after MVA-BN-Filo) were graded as severe.

267 Median durations of local AEs were two and three days after Ad26.ZEBOV and MVA-BN-
268 Filo vaccinations, respectively, and one day after placebo. One participant reported injection
269 site swelling for 19 days following MVA-BN-Filo vaccination.

270 Solicited systemic AEs were reported after 255 (76.8%) of 332 Ad26.ZEBOV, 116 (49.2%)
271 of 236 MVA-BN-Filo and 33 (45.8%) of 72 placebo administrations. The most frequent
272 systemic AEs were fatigue, headache, myalgia and chills (**Table 2**). Severe solicited systemic
273 AEs were reported after 32 (9.6%) of 332 Ad26.ZEBOV administrations, but lower
274 proportions of MVA-BN-Filo (4 [1.7%] of 236) or placebo (3 [4.2%] of 72) injections. Fever
275 (temperature $\geq 38^{\circ}\text{C}$) was more frequent after receipt of Ad26.ZEBOV (38 [11.5%] of 331)
276 than MVA-BN-Filo (2 [0.9%] of 232) or placebo (3 [4.2%] of 72). There were eight cases of
277 temperature $\geq 39^{\circ}\text{C}$: six after Ad26.ZEBOV, one after MVA-BN-Filo and one after placebo.
278 The median duration of any solicited systemic adverse event was no longer than 3 days.

279 Rates of unsolicited AEs were similar after Ad26.ZEBOV (115 [34.6%] of 332), MVA-BN-
280 Filo (81 [34.3%] of 236), and placebo (24 [33.3%] of 72) and mainly consisted of mild to
281 moderate headache, upper respiratory tract infections and rhinitis (**Table 2**). No clinical
282 laboratory safety signals were observed. The most frequent laboratory abnormalities were
283 decreased haemoglobin from baseline and low segmented neutrophil counts. Both types of
284 abnormality were observed at comparable rates after Ad26.ZEBOV, MVA-BN-Filo and
285 placebo administration. The majority of low segmented neutrophil values were grade 1 or
286 grade 2 in severity; a grade 3 value ($<1.0 \times 10^9/\text{L}$) was reported in 2 participants
287 (**Supplementary Tables 1 and 2**).

288 Thirteen SAEs were reported by 11 (3.3%) of 332 participants who received active vaccines
289 and 2 (4.5%) of 44 participants after a total of 72 doses of placebo (**Table 2**). Two
290 neurological SAEs were initially identified as related to study vaccination and declared as
291 suspected unexpected serious adverse reactions (SUSAR), leading to the study pause. One

292 participant who experienced double vision, pain on moving the eye, and difficulty with
293 balance while walking, was diagnosed with Miller Fisher syndrome. Symptoms onset was
294 about a week after an upper respiratory tract infection (URTI) and one month after MVA-
295 BN-Filo vaccination, and they resolved before the end of the study. After extensive
296 evaluation, including a second neurological evaluation, the investigator and sponsor
297 considered the event was not related to study vaccine and most likely related to the prior
298 URTI. A second participant experienced intermittent episodes of paraesthesia of the palms
299 and soles two weeks after vaccination with Ad26.ZEBOV. The investigator reported the
300 event as an SAE as the participant was unable to continue their work activity. The diagnosis
301 of possible small fibre neuropathy was retained although a definitive diagnosis was not
302 possible as the participant refused further specific diagnostic exploration and no histological
303 examination was performed. The expert panel, investigator and sponsor considered this
304 event, which had not resolved before the end of the study, to be possibly related.

305 *Humoral immunogenicity*

306 The immunogenicity analysis is based on the 223 (59.3%) of 376 participants in Cohorts II
307 and III who received both vaccines according to protocol; 36 participants (29 MVA-BN-Filo
308 and 7 placebo) received their second injections outside of the planned window and 116 did
309 not receive the second injection. Ad26.ZEBOV vaccination on Day 1 induced binding
310 antibody responses (**Figure 2**), with 92.2%, 95.7% and 100% responding to the
311 Ad26.ZEBOV vaccination in the 28-, 56- and 84-day groups, respectively. The magnitude of
312 the response increased with increasing interval; GMCs were 452 EU/mL after 28 days, 880
313 EU/mL after 56 days, and 1156 EU/mL after 84 days (**Table 3**).

314 GMCs increased further 21 days after MVA-BN-Filo vaccination, by 10.4-, 11.6- and 10.0-
315 fold over post Ad26.ZEBOV levels in the 28-, 56- and 84-day groups, when GMCs were
316 4627 EU/mL, 10131 EU/mL, and 11312 EU/mL, respectively. All participants responded to

317 the 2-dose vaccine regimen except for one participant vaccinated with the 28-day interval
318 (**Table 3**). In the 28- and 56-day groups antibodies persisted up to Day 365 when 56 (98.2%)
319 of 57 and all 50 (100%) of these group participants were still responders; the 84-day group
320 was not tested at Day 365. GMCs at Day 365 were similar in both 28- and 56-day groups;
321 1149 EU/mL and 1205 EU/mL, respectively (**Figure 2A**). No placebo recipient demonstrated
322 an increase in antibodies at any point during the study. In a sensitivity analysis of responses
323 to MVA-BN-Filo vaccination administered out of the protocol window, responses were
324 consistent with the per protocol analyses. Increasing the interval >98 days had no negative
325 impact on the response (**Supplementary Figure 1**).

326 The GMT (IC₅₀ titre) of neutralising antibodies 21 days after receiving the vaccinations 28
327 days apart was 988 (95% CI 712–1370), increasing to 4046 (3091–5297) when the interval
328 between vaccinations was 56 days (**Figure 2B** and **Supplementary table 3**). Day 365 GMTs
329 were 354 (257–448) and 341 (268–435), respectively. In the 28-day group responder rates
330 were 43 (87.8%) of 49 participants at Day 50, and 31 (63.3%) of 49 at Day 365. All 52
331 (100%) participants in the 56-day group were responders at Day 78, which persisted in 27
332 (51.9%) of 52 participants at Day 365. There were strong correlations between binding
333 antibody concentrations and neutralising antibody titres measured 21 days after MVA-BN-
334 Filo (Spearman coefficient, $r = 0.825$) and at Day 365 (Spearman coefficient, $r = 0.861$,
335 **Supplementary Figure 2**). No virus neutralising activity was detected in any placebo
336 recipient.

337 *Cell-mediated immune responses*

338 EBOV GP-specific cellular immune responses were assessed in 76 participants from Cohorts
339 II and III (**Figure 3**, **Supplementary Figure 3**, and **Tables 4 and 5**). EBOV GP-specific
340 CD4+ and CD8+ T-cell responses were low after Ad26.ZEBOV dose 1, but EBOV GP-
341 specific CD4+ and CD8+ T-cell responses were observed in substantial proportions of

342 participants 21 days after MVA-BN-Filo vaccination (37–58% for CD4+ T cells, 55–67% for
343 CD8+ T cells). Median CD4+ T-cell responses at 21 days post Dose 2 were similar across the
344 28-, 56-, and 84-day vaccination intervals (median range: 0·12–0·15%). There was a trend
345 for median CD8+ T-cell responses to be higher with the 28-day interval (median 0·29%)
346 compared with the 56- and 84-day intervals (median 0·12% and 0·20%, respectively). No
347 EBOV GP-specific cellular response was detected in placebo recipients (**Supplementary**
348 **Figure 3**). At 21 days post dose 2, across vaccination schedules, the majority of the CD4+ T
349 cell responses were polyfunctional, expressing both IFN- γ and IL-2, while the majority of the
350 CD8+ T cell responses expressed only IFN- γ (**Supplementary Figure 4**).

351 *Ad26.ZEBOV shedding*

352 Shedding of Ad26.ZEBOV was assessed in the 15 participants of group 4 (13 vaccinees and
353 2 placebo) using urine samples and nasal swabs taken on Days 1, 2 and 8 after vaccination.
354 No evidence of shedding was observed in any sample (not shown).

355

356 **DISCUSSION**

357 These first phase 2 results of the two-dose heterologous Ad26.ZEBOV, MVA-BN-Filo
358 vaccination regimen in 421 healthy adults confirm the safety and immunogenicity profiles of
359 this regimen previously observed in phase 1 studies.¹¹⁻¹⁴ The frequency and severity of
360 solicited local reactions, mainly mild to moderate injection site pain, were comparable to
361 those reported in phase 1 studies performed in Oxford,¹¹ and in African participants.^{12,13} The
362 systemic reactogenicity profile comprising mainly headache, myalgia and fatigue was
363 consistent with the phase 1 studies,¹¹⁻¹³ although there were slightly fewer solicited systemic
364 adverse events and laboratory events especially with regards to neutrophil levels than
365 reported in previous studies. More generally, observed local and systemic reactions were also
366 reported with other vectors such as the recombinant adenovirus type-5,¹⁷ the replication
367 defective recombinant chimpanzee adenovirus type 3-vectored ebolavirus vaccine (cAd3-
368 EBO)¹⁸ or the replication-competent, recombinant vesicular stomatitis virus (rVSV).¹⁹ As
369 expected with replication-incompetent vectors, no vector replication was observed following
370 Ad26.ZEBOV when checked in a specific cohort.

371 The study was paused following two neurological SAEs that occurred within weeks of each
372 other. Both events occurred at different phases of the two-dose regimen, a case of Miller
373 Fisher syndrome which most commonly occurs after a recent infection was reported one
374 week after an URTI and one month after receiving MVA-BN-Filo, and the second, which did
375 not have a definitive diagnosis, occurred one month after Ad26.ZEBOV vaccination. The
376 occurrence of these two unsolicited SAEs led to a concurrent and careful assessment of the
377 neurological events, including by an external panel of expert neurologists, but did not raise
378 any specific safety signals. Regulatory authorities in the UK and France confirmed that the
379 development of the Ad26.ZEBOV and MVA-BN-Filo vaccination regimen could continue.

380 Robust EBOV GP-specific binding antibody responses were observed in 99–100% of the
381 participants 21 days after the second dose, and these persisted in 98–100% of the participants
382 tested after 1 year after dose 1. Antibody responses were lower 21 days after the second
383 vaccination when the interval was 28 days rather than 56 or 84 days but levels at one year
384 were similar in 28- and 56-day groups. There was a high degree of correlation between
385 binding and neutralising antibody responses. EBOV-specific CD4+ and CD8+ T cell
386 responses were observed in a substantial portion of participants analysed. CD4+ T cell
387 responses were similar in the three intervals, whereas CD8+ T cell responses tended to be
388 higher in the 28-day interval group. CD4+ and CD8+ T cell responses persisted at least 1
389 year in 11–16% and 42–56% of the participants, respectively.

390 We have shown that a heterologous two-dose vaccination regimen with intervals from 28 to
391 84 days between doses elicits robust humoral and cellular responses. Boosting with MVA
392 vectors has previously been reported after ChAd3 prime immunisation as reported in
393 macaques²⁰ and humans.^{21,22} The Ad26.ZEBOV, MVA-BN-Filo strategy with a 56-day
394 interval provided protection against lethal EBOV challenge in non-human primates (NHP)
395 model,¹⁰ as well as robust humoral and cellular immune responses in humans with binding
396 antibodies induced as early as 14 days after the first vaccination.¹¹

397 In outbreak conditions the single-dose rVSV vaccine had 100% clinical efficacy from 10
398 days after vaccination.⁶ In lethal challenge studies in mice²³ and NHP²⁴ ZEBOV-GP-specific
399 IgG antibodies were found to play a critical role in rVSV-induced protection from 10 days
400 post-vaccination onwards. The specific mechanism of action of Ad26.ZEBOV, MVA-BN-
401 Filo is currently unknown and may differ from that of rVSV, but NHP studies have
402 demonstrated that EBOV GP-specific IgG binding antibodies represent the immune
403 parameter that correlates best with survival for the Ad26.ZEBOV, MVA-BN-Filo regimen.¹⁰
404 Although a mechanistic correlate of protection has not been identified yet, the binding

405 antibody GMCs observed 21 days after the second dose in the two-dose regimens ranged
406 from 4627 to 11312 EU/mL depending on the interval. A GMC of 1262 EU/mL was reported
407 28 days post-rVSV-ZEBOV vaccination of North American and Spanish volunteers using the
408 same assay in the same laboratory.²⁵ In the present study, and consistent with previous
409 reports,¹¹ levels above 1000 EU/mL were maintained up to Day 365, our furthest time-point
410 from vaccination.²⁵ Recent modelling work based on phase 1 data predicts a sustained
411 response over time, which is currently being evaluated in the EBOVAC3 consortium.²⁶

412 Some participants received their MVA-BN-Filo vaccination outside the planned window due
413 to the study pause. Although the number of such individuals was limited, it did provide data
414 on a delayed interval, as may occur in real-life settings. Strong immune responses were
415 observed which were at least as high as those observed in the per protocol population,
416 providing support for flexibility in the timing of dosing that may be needed in real-world use,
417 when dose intervals are not strictly respected.

418 This phase 2 study was done in European volunteers who, except for healthcare and aid
419 workers involved in outbreak response, are unlikely to be targets for vaccination.²⁷ Similar
420 studies assessing different vaccination regimens in different populations of healthy adults and
421 children, and also HIV-infected individuals are ongoing in African populations in Burkina
422 Faso, Cote d'Ivoire, Kenya and Uganda (ClinicalTrials.gov NCT02564523), and Sierra
423 Leone (ClinicalTrials.gov NCT02509494). The ongoing PREVAC study in Sierra Leone and
424 Guinea includes an assessment of the impact of malaria on the immune response
425 (ClinicalTrials.gov NCT02876328).

426 In conclusion, the safety, including the absence of Ad26.ZEBOV shedding, and
427 immunogenicity of the heterologous Ad26.ZEBOV, MVA-BN-Filo regimen with intervals
428 from 28 to 84 days has been demonstrated in this European phase 2 study. Ancillary
429 immunological analyses performed in this study should provide more insight into the immune

430 response established while other phase 2 and 3 studies performed by EBOVAC1 and
431 EBOVAC2 African consortia and PREVAC will provide additional information in target
432 populations at risk of Ebola infection in Africa.

433

434 **Acknowledgements**

435 The authors wish to acknowledge the contribution of all participants who have taken part in
436 the studies; the partners of the EBOVAC2 consortium: the French National Institute for
437 Health and Medical Research (Inserm), the London School of Hygiene and Tropical
438 Medicine, the University of Oxford, the Centre Muraz and Inserm transfert ; the members of
439 the Scientific Advisory Board: David Kraslow, Abdel Babiker, Nigel Klein; the members of
440 Ethical Advisory Board; the members of the DSMB: John Bruce McClain, Marie-Louise
441 Newell, Geert Molenberghs; the members of the neurology panel; the research staff and all
442 members of the Clinical Operations Group at Janssen who worked on the trial (further
443 information in supplement). This work has been supported by the European Commission
444 IMI2 programme under EBOVAC2 (GA 115861), part of the Innovative Medicines Initiative
445 Ebola+ Program and the NIHR Oxford Biomedical Research Centre. AJP is an NIHR Senior
446 Investigator. We thank Keith Veitch (keithveitch communications, Amsterdam, the
447 Netherlands) and Marialuisa Quadri (Janssen Vaccines and Prevention, Leiden, The
448 Netherlands) for medical writing assistance, and Yvonne Salzgeber (Janssen Vaccines AG,
449 Bern, Switzerland) for publication coordination.

450 **Funding**

451 This work has received funding under Grant Agreement EBOVAC2 (No.115861) from the
452 Innovative Medicines Initiative 2 Joint Undertaking which receives support from the
453 European Union's Horizon 2020 research and innovation programme and EFPIA with in-
454 kind contribution from the vaccine developer, Janssen.

455 **Conflicts of interest**

456 AJP chairs the UK Department of Health's (DoH) Joint Committee on Vaccination and
457 Immunisation (JCVI) and the European Medicines Agency Scientific Advisory Group on

458 Vaccines and is a member of the World Health Organization's (WHO) Strategic Advisory
459 Group of Experts. The views expressed in this manuscript are those of the authors and do not
460 necessarily reflect the views of the JCVI, the DoH, or the WHO.

461

462 **Author contributions**

463 VB, MD, AG, MG, NG, CL, JDL, YL, KL, A, LR, CR, MS, RT participated in the
464 conception and design of the work being described in the publication.

465 CB, MG, NG, SG, CL, ML, AP, RT, AW participated in the acquisition/collection of data.

466 CB, VB, EC, MD, AG, NG, CL, OL, JDL, ML, KL, AP, LR, CR, MS, RT, AW participated
467 in the analysis or interpretation of data.

468 CB, VB, EC, MD, AG, MG, SG, NG, CL, OL, JDL, YL, ML, KL, AP, LR, CR, MS, RT,
469 AW drafted or critically revised and reviewed the manuscript for important intellectual
470 content.

471 All authors approve this version of the manuscript and agree to be accountable for all aspects
472 of the work ensuring that questions related to the accuracy or integrity of any part of the work
473 are appropriately investigated and resolved.

474

475

REFERENCES

1. Rosello A, Mossoko M, Flasche S, Van Hoek AJ, Mbala P, Camacho A, et al. Ebola virus disease in the Democratic Republic of the Congo, 1976-2014. *Elife*. 2015; **4**. pii: e09015.
2. Malvy D, McElroy AK, de Clerck H, Günther S, van Griensven J. Ebola virus disease. *Lancet* 2019; **393**(10174): 936–48.
3. Centers for Disease Control and Prevention. Ebola Virus Disease Distribution Map: Cases of Ebola Virus Disease in Africa Since 1976 Atlanta: CDC; 2019 [Available from: https://www.cdc.gov/vhf/ebola/history/distribution-map.html?CDC_AA_refVal=https%3A%2F%2Fwww.cdc.gov%2Fvhf%2Febola%2Foutbreaks%2Fhistory%2Fdistribution-map.html].
4. Piot P, Spencer J. From 1976 to 2018: reflections on early investigations into the Ebola virus. *Trans R Soc Trop Med Hyg* 2018; **112**(12): 527–8.
5. Gross L, Lhomme E, Pasin C, Richert L, Thiebaut R. Ebola vaccine development: Systematic review of pre-clinical and clinical studies, and meta-analysis of determinants of antibody response variability after vaccination. *Int J Infect Dis* 2018; **74**: 83–96.
6. Henao-Restrepo AM, Camacho A, Longini IM, Watson CH, Edmunds WJ, Egger M, et al. Efficacy and effectiveness of an rVSV-vectored vaccine in preventing Ebola virus disease: final results from the Guinea ring vaccination, open-label, cluster-randomised trial (Ebola Ca Suffit!). *Lancet* 2017; **389**(10068): 505–18.
7. WHO. Strategic Advisory Group of Experts (SAGE) on immunization interim recommendations on vaccination against Ebola Virus Disease (EVD) 7 May 2019. Available at: https://www.who.int/immunization/policy/position_papers/interimEbola_recommendations_may_2019.pdf?ua=1 Accessed 21 August 2019.
8. WHO. Ebola in the Democratic Republic of the Congo: Health Emergency Update. Available at: <https://www.who.int/emergencies/diseases/ebola/drc-2019> Accessed 29 October 2019.
9. WHO. Ebola virus disease –Republic of Uganda: Emergencies preparedness, response. Available at: <https://www.who.int/csr/don/13-june-2019-ebola-uganda/en/>

10. Callendret B, Vellinga J, Wunderlich K, Rodriguez A, Steigerwald R, Dirmeier U, et al. A prophylactic multivalent vaccine against different filovirus species is immunogenic and provides protection from lethal infections with Ebolavirus and Marburgvirus species in non-human primates. *PLoS One* 2018; **13**(2): e0192312.
11. Milligan ID, Gibani MM, Sewell R, Clutterbuck EA, Campbell D, Plested E, et al. Safety and immunogenicity of novel Adenovirus type 26- and Modified Vaccinia Ankara-vectored Ebola vaccines: a randomized clinical trial. *JAMA* 2016; **315**(15): 1610–23.
12. Mutua G, Anzala O, Luhn K, Robinson C, Bockstal V, Anumendem D, et al. Safety and immunogenicity of a 2-dose heterologous vaccine regimen with Ad26.ZEBOV and MVA-BN-Filo Ebola vaccines: 12-month data from a phase 1 randomized clinical trial in Nairobi, Kenya. *J Infect Dis* 2019; **220**(1): 57–67.
13. Anywaine Z, Whitworth H, Kaleebu P, Praygod G, Shukarev G, Manno D, et al. Safety and immunogenicity of a 2-dose heterologous vaccination regimen with Ad26.ZEBOV and MVA-BN-Filo Ebola vaccines: 12-month data from a phase 1 randomized clinical trial in Uganda and Tanzania. *J Infect Dis* 2019; **220**(1): 46–56.
14. Winslow RL, Milligan ID, Voysey M, Luhn K, Shukarev G, Douoguih M, et al. Immune responses to novel Adenovirus type 26 and modified Vaccinia virus Ankara-vectored Ebola vaccines at 1 year. *JAMA* 2017; **317**(10): 1075–7.
15. Logue J, Tuznik K, Follmann D, Grandits G, Marchand J, Reilly C, et al. Use of the Filovirus Animal Non-Clinical Group (FANG) Ebola virus immuno-assay requires fewer study participants to power a study than the Alpha Diagnostic International assay. *J Virol Methods* 2018; **255**: 84–90.
16. Horton H, Thomas EP, Stucky JA, Frank I, Moodie Z, Huang Y, et al. Optimization and validation of an 8-color intracellular cytokine staining (ICS) assay to quantify antigen-specific T cells induced by vaccination. *J Immunol Methods* 2007; **323**(1): 39–54.
17. Zhu FC, Hou LH, Li JX, Wu SP, Liu P, Zhang GR et al. Safety and immunogenicity of a novel recombinant adenovirus type-5 vector-based Ebola vaccine in healthy adults in China: preliminary report of a randomised, double-blind, placebo-controlled, phase 1 trial. *Lancet* 2015; **385**: 2272–79.
18. Ledgerwood JE, DeZure AD, Stanley DA, Coates EE, Novik L, Enama ME, et al. Chimpanzee Adenovirus vector Ebola vaccine. *N Engl J Med* 2017; **376**(10): 928–38.

19. Regules JA, Beigel JH, Paolino KM, Voell J, Castellano AR, Hu Z, et al. A recombinant vesicular stomatitis virus Ebola vaccine. *N Engl J Med* 2017; **376**(4): 330–41.
20. Zhu FC, Wurie AH, Hou LH, Liang Q, Li YH, Russell JB, et al. Safety and immunogenicity of a recombinant adenovirus type-5 vector-based Ebola vaccine in healthy adults in Sierra Leone: a single-centre, randomised, double-blind, placebo-controlled, phase 2 trial. *Lancet* 2017; **389**(10069): 621–8.
21. Stanley DA, Honko AN, Asiedu C, Trefry JC, Lau-Kilby AW, Johnson JC, et al. Chimpanzee adenovirus vaccine generates acute and durable protective immunity against ebolavirus challenge. *Nat Med* 2014; **20**(10): 1126–9.
22. Ewer K, Rampling T, Venkatraman N, Bowyer G, Wright D, Lambe T, et al. A monovalent Chimpanzee Adenovirus Ebola vaccine boosted with MVA. *N Engl J Med* 2016; **374**(17): 1635–46.
23. Wong G, Richardson JS, Pillet S, Patel A, Qiu X, Alimonti J, et al. Immune parameters correlate with protection against Ebola virus infection in rodents and nonhuman primates. *Sci Transl Med* 2012; **4**(158): 158ra146.
24. Marzi A, Engelmann F, Feldmann F, Haberthur K, Shupert WL, Brining D, et al. Antibodies are necessary for rVSV/ZEBOV-GP-mediated protection against lethal Ebola virus challenge in nonhuman primates. *Proc Natl Acad Sci USA* 2013; **110**(5): 1893–8.
25. Halperin SA, Das R, Onorato MT, Liu K, Martin J, Grant-Klein RJ, et al. Immunogenicity, lot consistency, and extended safety of rVSVΔG-ZEBOV-GP vaccine: a phase 3 randomized, double-blind, placebo-controlled study in healthy adults. *J Infect Dis* 2019; **220**(7): 1127–35.
26. Pasin C, Balelli I, Van Effelterre T, Bockstal V, Solforosi L, Prague M, et al. Dynamics of the humoral immune response to a prime-boost Ebola vaccine: quantification and sources of variation. *J Virol* 2019 ePub on-line Jun 26. pii: JVI.00579-19.
27. Robert A, Camacho A, Edmunds WJ, Baguelin M, Tamfum JJM, Rosello A, et al. Control of Ebola virus disease outbreaks: Comparison of health care worker-targeted and community vaccination strategies. *Epidemics*. 2019; **27**: 106–14.

Table 1. Demographic characteristics of the study population in all four Cohorts.

	Cohort I	Cohorts II and III						Cohort IV	
	Open label	28-day Interval		56-day Interval		84-day Interval		Ad26 shedding	
	Active vaccines	Active vaccines	Placebo	Active vaccines	Placebo	Active vaccines	Placebo	Ad26	Placebo
Full analysis set, N =	30	112	13	114	13	106	18	13	2
Male, n (%)	13 (43.3)	55 (49.1)	7 (53.8)	52 (45.6)	5 (38.5)	53 (50.0)	8 (44.4)	10 (76.9)	1 (50.0)
Mean age ± SD, years	40.1 ± 15.1	41.0 ± 15.0	39.1 ± 13.9	41.0 ± 14.0	38.2 ± 13.7	38.3 ± 14.3	41.1 ± 15.1	37.9 ± 11.4	47.0 ± 4.2
(range)	(18, 63)	(18, 65)	(22, 60)	(18, 64)	(23, 62)	(18, 65)	(20, 61)	(21, 55)	(44, 50)
Mean BMI ± SD, kg/m²	25.6 ± 5.4	25.2 ± 4.0	23.1 ± 2.7	25.1 ± 4.7	25.5 ± 5.3	24.7 ± 4.0	24.1 ± 2.9	23.8 ± 2.8	22.3 ± 4.2
Country, n (%)									
France	-	54 (48.2)	7 (53.8)	55 (48.2)	7 (53.8)	50 (47.2)	9 (50.0)	13 (100)	2 (100)
UK	30 (100)	58 (51.8)	6 (46.2)	59 (51.8)	6 (46.2)	56 (52.8)	9 (50.0)	-	-
Per Protocol set, n (%) =	-	80 (71.4)	8 (61.5)	70 (61.4)	7 (53.8)	52 (49.1)	6 (33.3)	-	-
Initially seropositive for EBOV GP, n (%) =	-	6 (7.5)	1 (12.5)	6 (8.6)	0 (0)	4 (7.8)	0 (0)	-	-

1 **Table 2.** Incidence rates of the most frequent solicited local and systemic adverse events within 7 days of
2 vaccination or placebo injection, and the most frequent unsolicited adverse events (AEs) and serious
3 adverse events (SAEs) throughout the study in Cohorts II and III.

		Incidence, reports following n doses (%)		
		Ad26.ZEBOV	MVA-BN-Filo	All Placebo
Doses		N = 332	N = 236	N = 72
Local AEs	Any	206 (62.0)	136 (57.6)	11 (15.3)
	<i>Severe</i>	4 (1.2)	1 (0.4)	0
Pain	Any	191 (57.5)	126 (53.4)	9 (12.5)
	<i>Severe</i>	3 (0.9)	0	0
Swelling	Any	36 (10.8)	28 (11.9)	0
	<i>Severe</i>	2 (0.6)	0	0
Pruritis	Any	19 (5.7)	7 (3.0)	3 (4.2)
	<i>Severe</i>	0	0	0
Erythema	Any	5 (1.5)	3 (1.3)	0
	<i>Severe</i>	0	1 (0.4)	0
Systemic AEs	Any	255 (76.8)	116 (49.2)	33 (45.8)
	<i>Severe</i>	32 (9.6)	4 (1.7)	3 (4.2)
Fatigue	Any	203 (61.1)	84 (35.6)	21 (29.2)
	<i>Severe</i>	18 (5.4)	3 (1.3)	2 (2.8)
Headache	Any	162 (48.8)	53 (22.5)	16 (22.2)
	<i>Severe</i>	9 (2.7)	2 (0.8)	1 (1.4)
Myalgia	Any	156 (47.0)	52 (22.0)	15 (20.8)
	<i>Severe</i>	8 (2.4)	1 (0.4)	0
Chills	Any	123 (37.0)	23 (9.7)	6 (8.3)
	<i>Severe</i>	14 (4.2)	3 (1.3)	2 (2.8)
Arthralgia	Any	76 (22.9)	22 (9.3)	5 (6.9)
	<i>Severe</i>	4 (1.2)	1 (0.4)	0
Nausea	Any	45 (13.6)	14 (5.9)	4 (5.6)
	<i>Severe</i>	3 (0.9)	1 (0.4)	0
Fever	≥ 38.0°C	38 (11.5) ^a	2 (0.9) ^b	3 (4.2)
	≥ 39.0°C	6 (1.8)	1 (0.4)	1 (1.4)
Any Unsolicited AE		115 (34.6)	81 (34.3)	24 (33.3)
MEDRA classes of main reported AEs				
Infections and infestations		30 (9.0)	28 (11.9)	7 (9.7)
Upper respiratory tract Infection		4 (1.2)	12 (5.1)	3 (4.2)
Rhinitis		10 (3.0)	7 (3.0)	0
Nervous system disorders		17 (5.1)	12 (5.1)	3 (4.2)
Headache		5 (1.5)	7 (3.0)	2 (2.8)
Investigations		16 (4.8)	13 (5.5)	2 (2.8)
Respiratory, thoracic and mediastinal disorders		17 (5.1)	7 (3.0)	2 (2.8)
Gastrointestinal disorders		9 (2.7)	8 (3.4)	5 (6.9)
Musculoskeletal and connective tissue disorders		12 (3.6)	6 (2.5)	3 (4.2)
General disorders and administration site conditions		10 (3.0)	5 (2.1)	3 (4.2)
SAEs throughout study		Ad26.ZEBOV & MVA-BN-Filo		All placebo
		N = 332		N = 44
Any reported SAE		11 (3.3)		2 (4.5)
SAE related to vaccination		0		0

^a n = 331, ^b n = 232.

Table 3. Geometric mean concentrations (GMC) of EBOV GP binding antibodies and responder rates in Cohorts II and III throughout the study.

Day		28-day interval		56-day interval		84-day interval	
		Ad26, MVA	Placebo	Ad26, MVA	Placebo	Ad26, MVA	Placebo
1	N	80	8	70	7	51	6
	GMC [95% CI]	<LLOQ [<LLOQ; <LLOQ]	<LLOQ [<LLOQ; 58]	<LLOQ [<LLOQ; <LLOQ]	<LLOQ [<LLOQ; <LLOQ]	<LLOQ [<LLOQ; <LLOQ]	<LLOQ [<LLOQ; <LLOQ]
29*	N	77	8	-	-	-	-
	GMC [95% CI] Responders, n (%)	452 [351–581] 71 (92.2)	<LLOQ [<LLOQ; 61] 0 (0)	-	-	-	-
50**	N	77	7	-	-	-	-
	GMC [95% CI] Responders, n (%)	4627 [3649–5867] 76 (98.7)	<LLOQ [<LLOQ; 55] 0 (0)	-	-	-	-
57*	N	-	-	69	7	-	-
	GMC [95% CI] Responders, n (%)	-	-	880 [709–1093] 66 (95.7)	<LLOQ [<LLOQ; <LLOQ] 0 (0)	-	-
78**	N	-	-	69	7	-	-
	GMC [95% CI] Responders, n (%)	-	-	10131 [8554–11999] 69 (100)	<LLOQ [<LLOQ; <LLOQ] 0 (0)	-	-
85*	N	-	-	-	-	52	6
	GMC [95% CI] Responders, n (%)	-	-	-	-	1156 [923–1448] 51/51# (100)	<LLOQ [<LLOQ; <LLOQ] 0 (0)
106**	N	-	-	-	-	48	6
	GMC [95% CI]	-	-	-	-	11312 [9072–14106]	<LLOQ [<LLOQ;

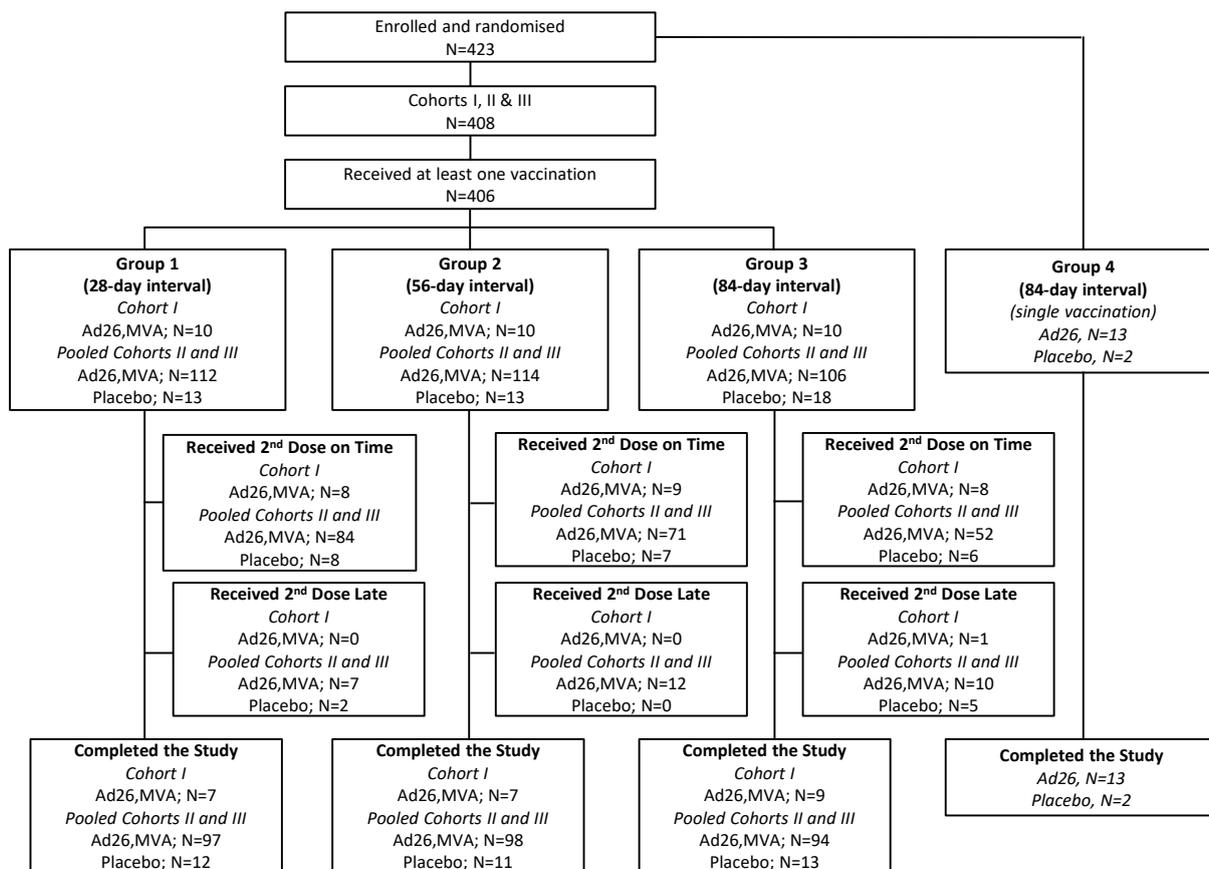
	Responders, n (%)					47/47# (100)	<LLOQ]
							0 (0)
	N	57	6	50	5		
365	GMC [95% CI]	1149 [859–1537]	<LLOQ [<LLOQ; <LLOQ]	1205 [971–1497]	<LLOQ [<LLOQ; <LLOQ]	-	-
	Responders, n (%)	56 (98.2)	0 (0)	50/50 (100)	0 (0)		

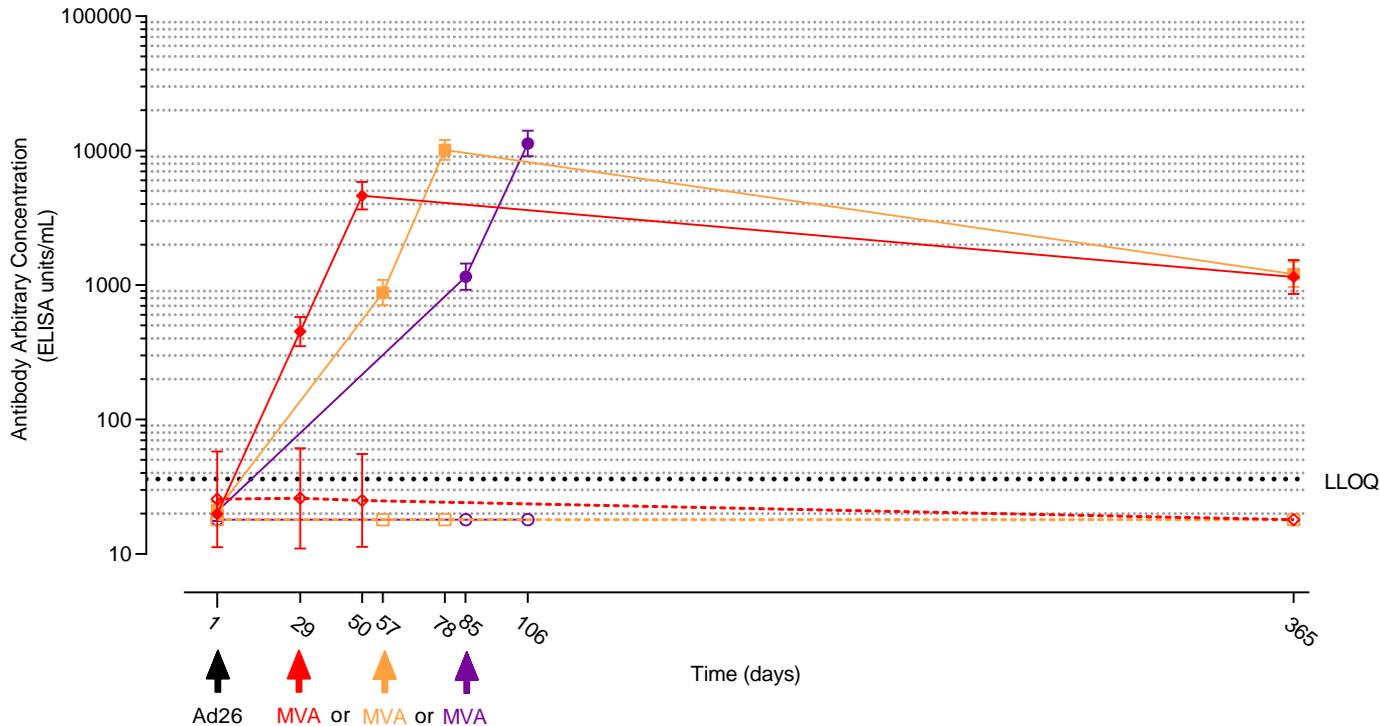
* Pre-dose 2, **21 days post-dose 2. <LLOQ = below the Lower Limit of Quantitation. # Responder rates for those with Day 1 data.

Figure 1. Study flow chart.

Figure 2. Anti-EBOV GP geometric mean binding antibody concentrations (ELISA Units/mL) (A) and virus neutralising geometric mean IC₅₀ titres (B) in the pooled Cohorts II and III (per protocol analysis set).

Figure 3. EBOV GP-specific T cell responses in participants assigned to receive active vaccine as assessed by Intracellular Cytokine Staining.





—◆— Ad26, MVA, 28-Day Interval

—■— Ad26, MVA, 56-Day Interval

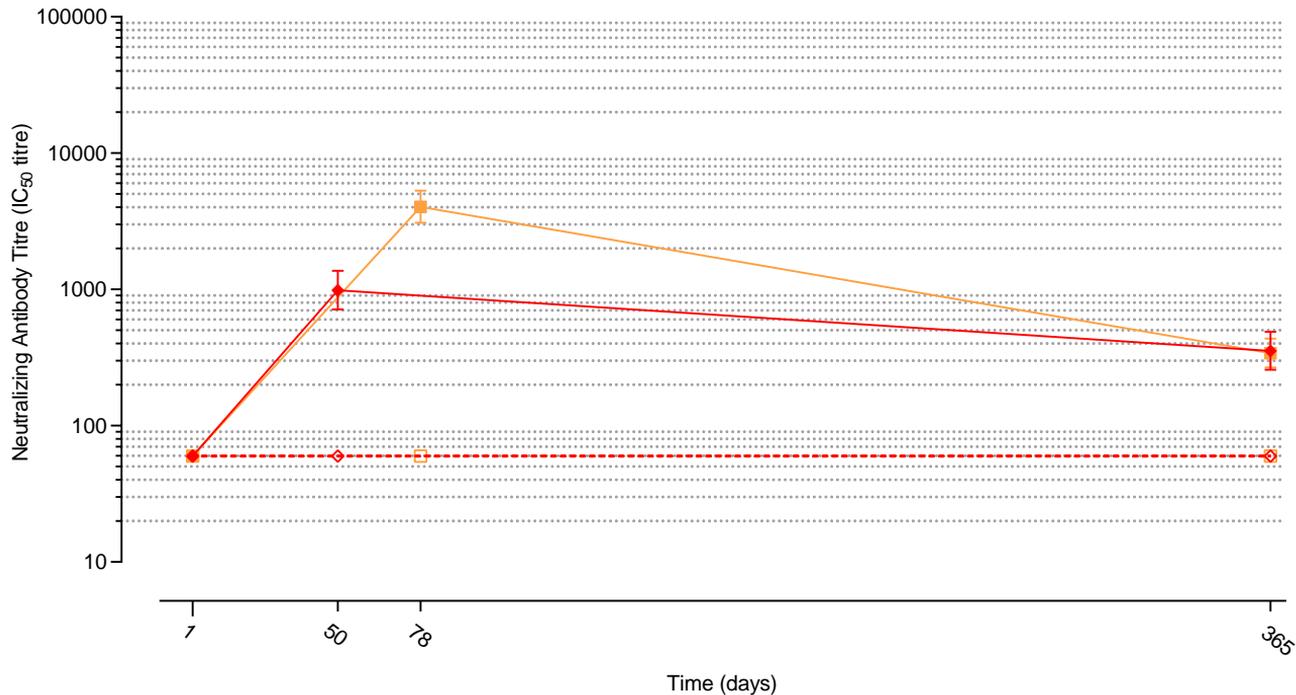
—●— Ad26, MVA, 84-Day Interval

- - -◆- - - Placebo, Placebo, 28-Day Interval

- - -■- - - Placebo, Placebo, 56-Day Interval

- - -●- - - Placebo, Placebo, 84-Day Interval

The error bars represent the geometric mean concentration and its 95% confidence interval



—●— Ad26, MVA, 28-Day Interval

- - -◇- - - Placebo, Placebo, 28-Day Interval

—■— Ad26, MVA, 56-Day Interval

- - -□- - - Placebo, Placebo, 56-Day Interval

The error bars represent the geometric mean titre and its 95% confidence interval

CD4⁺ T Cell Responses

CD8⁺ T Cell Responses

