Supplementary Material

Supplement to McLean C, Barry H, Kieh M, et al. Immune response of a two-dose heterologous Ebola vaccine regimen: summary of three African clinical trials using a single validated Filovirus Animal Nonclinical Group enzyme-linked immunosorbent assay in a single accredited laboratory.

Contents

Supplementary methods

Trial design and participants

In EBL2004, participants were randomised 2:1:2:1:1 to one of the following study arms: (1) a two-dose regimen of Ad26.ZEBOV on day 0 and MVA-BN-Filo on day 56, (2) matching placebo on days 0 and 56, (3) a single-dose regimen of rVSVDG-ZEBOV-GP on day 0 followed by placebo on day 56, (4) a single-dose regimen of rVSVDG-ZEBOV-GP on day 0 followed by a booster dose of rVSVDG-ZEBOV-GP on day 56, and (5) matching placebo on days 0 and 56.

Two different laboratories performed the Filovirus Animal Nonclinical Group (FANG) enzyme-linked immunosorbent assay (ELISA) in the previously reported results from the EBL2004 study (samples from participants in Guinea and Sierra Leone were processed by the Liberian Institute for Biomedical Research [Charlesville, Margibi County, Liberia] and those from participants in Liberia and Mali were processed by the US National Institute of Allergy and Infectious Diseases Integrated Research Facility [Frederick, MD, USA]).[1] The primary immunologic readout was the percent responders (defined as four-fold increase over baseline or assay developer's lower limit of quantification [LLOQ] and >200 ELISA units [EU]/mL) at month 12.[1]

Regulatory agencies recognise the need for validated surrogate endpoints[2,3] and prefer that a validated assay run in accredited laboratories is used for evaluation of suitability for licensure.[3] The analysis presented here extends that practice using a subset of EBL2004 samples from the Ad26.ZEBOV, MVA-BN-Filo and matching placebo arm, which were sent to Q^2 Solutions laboratory (San Juan Capistrano, CA, USA) for analysis using the US Food and Drug Administration and European Medicines Agency–validated FANG ELISA.

Supplementary immunogenicity results

Ebola virus glycoprotein (EBOV GP)–binding antibody GMCs at baseline in adults

Details of baseline EBOV GP-binding antibody GMCs in EBL2002, EBL2004, and EBL3001 are summarised in supplementary table 3. In EBL2002 and EBL3001, EBOV GP-binding antibody GMCs at baseline in adults were low (ranging from 39 EU/mL in EBL2002 to 69 EU/mL in EBL3001).[4,5] In HIV-infected adults in EBL2002, baseline values were below the LLOQ.

In EBL2004, the GMC at baseline in adults was 39 EU/mL (95% CI: <LLOQ-45) in participants randomised to receive Ad26.ZEBOV, MVA-BN-Filo and <LLOQ (<LLOQ-42) in adults randomised to receive placebo (supplementary table 3). When baseline GMCs in participants randomised to receive active vaccine in EBL2004 were stratified by country, the 95% CI in Sierra Leone was higher than the 95% CIs in Guinea and Mali, but not Liberia. The low baseline binding antibody GMCs observed in EBL2004 are comparable with the baseline GMCs observed in both EBL2002 and EBL3001, although the 95% CI in EBL3001 stage 2 was above the 95% CIs in EBL2002 and EBL2004 (supplementary table 3). When results from EBL2002, EBL2004, and EBL3001 were stratified by country, the 95% CIs in Sierra Leone in EBL2004 and EBL3001 stages 1 and 2 were above the 95% CIs in Uganda, Guinea, and Mali (supplementary table 3). The 95% CIs in Sierra Leone in EBL2004 and EBL3001 stages 1 and 2 overlapped with those in Burkina Faso, Côte d'Ivoire, Kenya, and Liberia.

EBOV GP-binding antibody GMCs at baseline in paediatric participants

EBOV GP-binding antibody GMCs at baseline across all studies, age groups, and countries were low to undetectable. The GMCs at baseline and percent responders for all studies are presented in supplementary table 4. EBOV GP-binding antibody GMCs at baseline in paediatric participants in EBL2002 and EBL3001 stage 2 were low (ranging from 62 EU/mL in older children in EBL3001 to 65 EU/mL in adolescents in EBL3001) or undetectable (both age groups in EBL2002 and younger children in EBL3001).[6,7] The age group- and age-group-and-country–specific baseline GMCs from EBL2002 and EBL3001 are presented in supplementary table 4.

In EBL2004, the GMC at baseline was 49 EU/mL (95% CI: 39-62) in adolescents, <LLOQ (<LLOQ-40) in older children, and <LLOQ (<LLOQ, <LLOQ) in younger children randomised to receive Ad26.ZEBOV, MVA-BN-Filo (supplementary table 4). In participants randomised to receive placebo, the GMC at baseline was 37 EU/mL (<LLOQ-49) in adolescents, <LLOQ (<LLOQ-48) in older children, and <LLOQ (<LLOQ, <LLOQ) in younger

children (supplementary table 4). In the EBL2004 vaccine group, the 95% CI of adolescents was above the 95% CI of younger children. When results from EBL2004 were stratified by both age group and country, the 95% CIs in Guinea and Sierra Leone were above the 95% CI in Mali, but not Liberia.

The low to undetectable baseline binding antibody GMCs observed in EBL2004 are comparable with the baseline GMCs observed in both EBL2002 and EBL3001 stage 2. However, the 95% CI of older children in Sierra Leone in EBL3001 was above the 95% CIs in both EBL2002 and EBL2004.

When results were stratified by both age group and country across studies, the 95% CIs of adolescents in Sierra Leone in EBL2004 and EBL3001 stage 2 were higher than the 95% CIs of adolescents in Burkina Faso and Mali, but not Guinea or Liberia (supplementary table 4). Additionally, the 95% CI of adolescents in Sierra Leone in EBL3001 was above the 95% CI of adolescents in Uganda and Kenya. In older children, the 95% CI in Sierra Leone in EBL3001 stage 2 was above the 95% CIs of older children in Côte d'Ivoire, Kenya, Uganda, and Mali, but not Burkina Faso, Liberia, or Sierra Leone in EBL2004. Stratification by both age group and country did not result in differences in baseline GMCs in younger children.

EBOV GP-binding antibody GMCs in adult and paediatric participants after accounting for between-study variation

As the variation in EBOV GP-binding antibody concentrations in adult and paediatric participants appeared to be largely due to participant characteristics rather than between-study variations, we further investigated the influence of baseline demographics on the EBOV GP-binding antibody concentrations. For adults, we investigated the influence of baseline age, sex, and body-mass index (BMI) by including these effects in the models, as well as the results shown in supplementary table 1. Age and BMI were grand-mean centred before inclusion in the models. Based on the Akaike information criterion (AIC) and the Bayesian information criterion (BIC), the best fitting model (Model 2) was chosen and used to assess the proportion of variance explained by participant characteristics (ie, age and sex). The within-study (residual) variance reduced to 0·190 based on the chosen model (Model 2). Compared with the within-study variance of 0·203 from Model 1, we computed the proportion variance explained when controlling for age and sex as follows: (0·203-0·190)/0·203 = 0·059. This means that controlling for age and sex only reduced the variability in the EBOV GP-binding antibody concentrations by 6%.

For paediatric participants, we also investigated the influence of baseline demographics (age group and sex) by including these effects in the model and the results shown in supplementary table 2. Based on the AIC and BIC, the best fitting model (Model 2) was chosen and used to assess the proportion variance explained when sex was additionally included in the model. The within-study (residual) variance reduced to 0·194 based on the chosen model (Model 2). Compared with the within-study variance of 0·195 from Model 1, we computed the proportion variance explained when controlling for sex as follows: (0·195-0·194)/0·195 = 0·005. This means that controlling for sex only reduced the variability in the EBOV GP-binding antibody concentrations by 0·5%.

References

1 PREVAC Study Team, Kieh M, Richert L, Beavogui AH, Grund B, Leigh B, et al. Randomized trial of vaccines for Zaire Ebola virus disease. *N Engl J Med* 2022; **387**: 2411–424. https://www.nejm.org/doi/full/10.1056/NEJMoa2200072.

2 US Food and Drug Administration. Surrogate endpoint resources for drug and biologic development. https://www.fda.gov/drugs/development-resources/surrogate-endpoint-resources-drug-and-biologic-development (accessed 3/18/22).

3 European Medicines Agency. Guideline on clinical evaluation of vaccines. https://www.ema.europa.eu/en/documents/scientific-guideline/draft-guideline-clinical-evaluation-vaccines-revision-1_en.pdf (accessed 3/18/22).

4 Barry H, Mutua G, Kibuuka H, Anywaine Z, Sirima SB, Meda N, et al. Safety and immunogenicity of 2-dose heterologous Ad26.ZEBOV, MVA-BN-Filo Ebola vaccination in healthy and HIV-infected adults: a randomised,

placebo-controlled phase II clinical trial in Africa. *PLoS Med* 2021; **18:** e1003813. https://doi.org/10.1371/journal.pmed.1003813.

5 Ishola D, Manno D, Afolabi MO, Keshinro B, Bockstal V, Rogers B, et al. Safety and long-term immunogenicity of the two-dose heterologous Ad26.ZEBOV and MVA-BN-Filo Ebola vaccine regimen in adults in Sierra Leone: a combined open-label, non-randomised stage 1, and a randomised, double-blind, controlled stage 2 trial. *Lancet Infect Dis* 2022; **22:** 97–109. https://doi.org/10.1016/S1473-3099(21)00125-0.

6 Anywaine Z, Barry H, Anzal O, Mutua G, Sirima SB, Eholie S, et al. Safety and immunogenicity of 2-dose heterologous Ad26.ZEBOV, MVA-BN-Filo Ebola vaccination in children and adolescents in Africa: a randomised, placebo-controlled, multicentre phase II clinical trial. *PLoS Med* 2022; **19:** e1003865. https://doi.org/10.1371/journal.pmed.1003865.

7 Afolabi MO, Ishola D, Manno D, Keshinro B, Bockstal V, Rogers B, et al. Safety and immunogenicity of the two-dose heterologous Ad26.ZEBOV and MVA-BN-Filo Ebola vaccine regimen in children in Sierra Leone: a randomised, double-blind, controlled trial. *Lancet Infect Dis* 2022; **22:** 110–22. https://doi.org/10.1016/S1473-3099(21)00128-6.

***Supplementary Table 1*: Estimates of linear mixed effects models for adult (≥18 years of age) participants with log10-transformed EBOV GP-binding antibody concentrations as the dependent variable**

	Model 1	**Model 2**	**Model 3**
Fixed effects			
Intercept	3·772 (0·087)*	3·715 (0·072)*	3·717 (0·073)*
Age	–	−0·009 (0·002)*	−0·009 (0·002)*
BMI	–	–	0·002 (0·005)
Sex (female)	–	0·164 (0·041)*	0·156 (0·044)*
Error variance			
Between-study	0·022 (0·023)	0·014 (0·015)	0·014 (0·015)
Residual	0·203 (0·012)*	0·190 (0·011)*	0·191 (0·011)*
Model fit			
AIC	755·6	717·3	719·2
BIC	752·9	712·8	713·8

AIC = Akaike information criterion. BIC = Bayesian information criterion. BMI = body-mass index. EBOV GP = Ebola virus glycoprotein. ML = maximum likelihood. REML = restricted maximum likelihood.
*Statistically significant, $p<0·05$.
Estimates are based on SAS Proc Mixed: parameter estimates, with standard errors in parentheses.
Model 1: only random intercept without predictors; Model 2: random intercept and predictors (age + sex); Model 3: random intercept and predictors (age + sex + BMI).
Estimation method = REML for the reported fixed effects and error variance estimates.
Estimation method = ML for the reported Model fit statistics (AIC and BIC).

***Supplementary Table 2*: Estimates of random intercept models for paediatric (≤17 years of age) participants with log10-transformed EBOV GP-binding antibody concentrations as the dependent variable**

	Model 1	**Model 2**	**Model 3**
Fixed effects			
Intercept	4·072 (0·055)*	4·071 (0·057)*	4·062 (0·059)*
Age group: 1-3 years	0·348 (0·041)*	0·343 (0·053)*	0·342 (0·053)*
Age group: 4-11 years	0·085 (0·035)*	0·086 (0·046)	0·084 (0·047)
Sex (female)	–	–	0·022 (0·031)
Error variance			
Between-study	0·007 (0·008)	0·006 (0·008)	0·006 (0·008)
Residual	0·195 (0·010)*	0·194 (0·010)*	0·194 (0·010)*
Model fit			
AIC	1013·7	1007·7	1009·3
BIC	1011·9	1002·3	1003·0

AIC = Akaike information criterion. BIC = Bayesian information criterion. EBOV GP = Ebola virus glycoprotein. ML = maximum likelihood. REML = restricted maximum likelihood.
*Statistically significant, $p<0·05$.
Estimates are based on SAS Proc Mixed: parameter estimates, with standard errors in parentheses.
The 12-17 years age group was used as the reference age group.
Model 1: random intercept + age group as the only predictor; Model 2: random intercept + age group + random age group (slope) effect; Model 3: random intercept + age group + random age group effect (slope) + sex.
Estimation method = REML for the reported fixed effects and error variance estimates.
Estimation method = ML for the reported Model fit statistics (AIC and BIC).

***Supplementary Table 3*: Anti-EBOV GP-binding antibody responses in adults in EBL2002, EBL2004, and EBL3001 over time**

	Study	N	GMC (95% CI, EU/mL)	% responder (95% CI)
Baseline	EBL2002 (vaccine)	134	39 (<LLOQ; 48)	NA
	Burkina Faso	38	50 (<LLOQ; 77)	NA
	Côte d'Ivoire	18	<LLOQ (<LLOQ; 61)	NA
	Kenya	19	72 (<LLOQ; 173)	NA
	Uganda	59	<LLOQ (<LLOQ; <LLOQ)	NA
	EBL2002 (control)	24	<LLOQ (<LLOQ; 57)	NA
	Burkina Faso	6	38 (<LLOQ; 101)	NA
	Côte d'Ivoire	3	98 (<LLOQ; 11685)	NA
	Kenya	5	<LLOQ (<LLOQ; 57)	NA
	Uganda	10	<LLOQ (<LLOQ; 67)	NA
	EBL2002 HIV-infected (vaccine)	58	<LLOQ (<LLOQ; <LLOQ)	NA
	EBL2002 HIV-infected (control)	12	<LLOQ	NA
	EBL2004 (vaccine)	254	39 (<LLOQ; 45)	NA
	Guinea	86	<LLOQ (<LLOQ; 38)	NA
	Liberia	67	44 (<LLOQ; 62)	NA
	Mali	48	<LLOQ (<LLOQ; 37)	NA
	Sierra Leone	53	65 (43; 98)	NA
	EBL2004 (control)	121	<LLOQ (<LLOQ; 42)	NA
	Guinea	44	<LLOQ (<LLOQ; 41)	NA
	Liberia	33	42 (<LLOQ; 62)	NA
	Mali	25	<LLOQ (<LLOQ; <LLOQ)	NA
	Sierra Leone	19	64 (<LLOQ; 124)	NA
	EBL3001 stage 1 (vaccine)	43	60 (40; 90)	NA
	EBL3001 stage 2 (vaccine)	188	69 (56; 85)	NA
	EBL3001 stage 2 (control)	66	49 (36; 66)	NA
21 days post-dose 2*	EBL2002 (vaccine)	136	7518 (6468; 8740)	99 (96; >99)
	Burkina Faso	40	8496 (6404; 11271)	100 (91; 100)
	Côte d'Ivoire	18	7570 (4948; 11582)	100 (82; 100)
	Kenya	20	8421 (6318; 11225)	95 (74; >99)
	Uganda	58	6631 (5128; 8576)	100 (94; 100)
	EBL2002 (control)	24	<LLOQ (<LLOQ; 53)	4 (0·1; 21)
	Burkina Faso	6	<LLOQ (<LLOQ; 86)	0 (0; 46)
	Côte d'Ivoire	3	106 (<LLOQ; 4594)	0 (0; 71)
	Kenya	5	<LLOQ	0 (0; 52)
	Uganda	10	<LLOQ (<LLOQ; 73)	10 (0·3; 45)
	EBL2002 HIV-infected (vaccine)	59	5283 (4094; 6817)	100 (94; 100)
	EBL2002 HIV-infected (control)	12	<LLOQ (<LLOQ; <LLOQ)	0 (0; 27)
28 days post-dose 2*	EBL2004 (vaccine)	236	6966 (5972; 8127)	>99 (98; 100)
	Guinea	79	7008 (5506; 8918)	100 (95; 100)
	Liberia	66	5120 (3711; 7065)	98 (92; 100)
	Mali	47	13150 (10043; 17217)	100 (92; 100)
	Sierra Leone	44	5550 (3755; 8201)	100 (92; 100)
	EBL2004 (control)	118	<LLOQ (<LLOQ; 45)	4 (1; 10)
	Guinea	44	<LLOQ (<LLOQ; 37)	0 (0; 8)

	Liberia	32	40 (<LLOQ; 60)	0 (0; 11)
	Mali	25	<LLOQ (<LLOQ; <LLOQ)	4 (0; 20)
	Sierra Leone	17	112 (53; 240)	25 (7; 52)
21 days post-dose 2*	EBL3001 stage 1 (vaccine)	42	4784 (3736; 6125)	98 (87; >99)
	EBL3001 stage 2 (vaccine)	182	3810 (3312; 4383)	98 (95; >99)
	EBL3001 stage 2 (control)	62	50 (<LLOQ; 70)	3 (0·4; 12)
Month 12†	EBL2002 (vaccine)	133	342 (291; 401)	78 (70; 85)
	Burkina Faso	39	392 (286; 538)	73 (56; 86)
	Côte d'Ivoire	17	304 (171; 540)	71 (44; 90)
	Kenya	18	532 (376; 754)	82 (57; 96)
	Uganda	59	282 (224; 355)	81 (69; 90)
	EBL2002 (control)	24	<LLOQ (<LLOQ; 45)	0 (0; 14)
	Burkina Faso	6	<LLOQ (<LLOQ; 73)	0 (0; 46)
	Côte d'Ivoire	3	49 (<LLOQ; 3783)	0 (0; 71)
	Kenya	5	<LLOQ	0 (0; 52)
	Uganda	10	<LLOQ (<LLOQ; 74)	0 (0; 31)
	EBL2002 HIV-infected (vaccine)	59	338 (253; 450)	88 (77; 95)
	EBL2002 HIV-infected (control)	12	<LLOQ (<LLOQ; <LLOQ)	0 (0; 27)
	EBL2004 (vaccine)‡	112	437 (352; 542)	80 (72; 87)
	Guinea	51	347 (266; 452)	86 (73; 94)
	Liberia	3	430 (<LLOQ; 142504)	67 (9; 99)
	Mali	9	978 (443; 2161)	100 (66; 100)
	Sierra Leone	49	480 (334; 688)	71 (57; 83)
	EBL3001 stage 1 (vaccine)	31	325 (238; 445)	77 (59; 90)
	EBL3001 stage 2 (vaccine)	168	259 (223; 301)	49 (42; 57)
	EBL3001 stage 2 (control)	62	50 (<LLOQ; 71)	7 (2; 16)

CI = confidence interval. EBOV GP = Ebola virus glycoprotein. EU = enzyme-linked immunosorbent assay unit. GMC = geometric mean concentration. HIV = human immunodeficiency virus. LLOQ = lower limit of quantification. NA = not applicable.

*Binding antibody concentrations were assessed at day 78 ± 3 days in EBL2002, day 84 ± 2 weeks in EBL2004, and day 78 ± 1 week in EBL3001.

†Binding antibody concentrations were assessed at day 365 ± 30 days in EBL2002, day 336 ± 1 month in EBL2004, and day 360 ± 1 month in EBL3001.

‡Data for the EBL2004 placebo group were not available at month 12.

***Supplementary Table 4*: Anti-EBOV GP-binding antibody responses in paediatric participants in EBL2002, EBL2004, and EBL3001 stage 2 over time**

	Study	Adolescents*			Older children†			Younger children‡		
	Country	N	GMC (95% CI, EU/mL)	% responder (95% CI)	N	GMC (95% CI, EU/mL)	% responder (95% CI)	N	GMC (95% CI, EU/mL)	% responder (95% CI)
Baseline	EBL2002 (vaccine)	53	<LLOQ (<LLOQ; 37)	NA	52	<LLOQ (<LLOQ; <LLOQ)	NA	NA	NA	NA
	Burkina Faso	29	<LLOQ (<LLOQ; 41)	NA	22	<LLOQ (<LLOQ; 54)	NA	NA	NA	NA
	Côte d'Ivoire	0	–	NA	11	<LLOQ	NA	NA	NA	NA
	Kenya	12	<LLOQ (<LLOQ; 46)	NA	5	<LLOQ	NA	NA	NA	NA
	Uganda	12	<LLOQ (<LLOQ; 49)	NA	14	<LLOQ (<LLOQ; <LLOQ)	NA	NA	NA	NA
	EBL2002 (control)	10	<LLOQ (<LLOQ; 78)	NA	11	<LLOQ	NA	NA	NA	NA
	Burkina Faso	4	83 (<LLOQ; 975)	NA	4	<LLOQ	NA	NA	NA	NA
	Côte d'Ivoire	0	–	NA	2	<LLOQ	NA	NA	NA	NA
	Kenya	2	<LLOQ	NA	1	<LLOQ	NA	NA	NA	NA
	Uganda	4	<LLOQ	NA	4	<LLOQ	NA	NA	NA	NA
	EBL2004 (vaccine)	127	49 (39; 62)	NA	109	<LLOQ (<LLOQ; 40)	NA	105	<LLOQ (<LLOQ; <LLOQ)	NA
	Guinea	41	59 (38; 92)	NA	52	<LLOQ (<LLOQ; 43)	NA	56	<LLOQ (<LLOQ; <LLOQ)	NA
	Liberia	23	38 (<LLOQ; 67)	NA	14	<LLOQ (<LLOQ; 53)	NA	4	<LLOQ (<LLOQ; <LLOQ)	NA
	Mali	21	<LLOQ (<LLOQ; <LLOQ)	NA	18	<LLOQ (<LLOQ; <LLOQ)	NA	35	<LLOQ (<LLOQ; <LLOQ)	NA
	Sierra Leone	42	71 (46; 110)	NA	25	47 (<LLOQ; 86)	NA	10	<LLOQ (<LLOQ; 46)	NA
	EBL2004 (control)	54	37 (<LLOQ; 49)	NA	54	<LLOQ (<LLOQ; 48)	NA	49	<LLOQ (<LLOQ; <LLOQ)	NA
	Guinea	24	37 (<LLOQ; 56)	NA	20	<LLOQ (<LLOQ; 51)	NA	24	<LLOQ (<LLOQ; <LLOQ)	NA
	Liberia	7	<LLOQ (<LLOQ; <LLOQ)	NA	9	60 (<LLOQ; 216)	NA	1	<LLOQ	NA
	Mali	7	<LLOQ (<LLOQ; <LLOQ)	NA	9	<LLOQ (<LLOQ; 55)	NA	18	<LLOQ (<LLOQ; <LLOQ)	NA
	Sierra Leone	16	65 (38; 111)	NA	16	<LLOQ (<LLOQ; 59)	NA	6	45 (<LLOQ; 488)	NA
	EBL3001 stage 2 (vaccine)	142	65 (52; 81)	NA	130	62 (49; 78)	NA	123	<LLOQ (<LLOQ; <LLOQ)	NA
	EBL3001 stage 2 (control)	46	72 (46; 111)	NA	43	39 (<LLOQ; 54)	NA	41	<LLOQ (<LLOQ; <LLOQ)	NA

21 days post-dose 2[§]	EBL2002 (vaccine)	53	13532 (10732; 17061)	100 (93; 100)	53	17388 (12973; 23306)	100 (93; 100)	NA	NA	NA
	Burkina Faso	29	13487 (9883; 18405)	100 (88; 100)	23	23949 (16739; 34266)	100 (85; 100)	NA	NA	NA
	Côte d'Ivoire	0	–	–	12	16682 (7045; 39504)	100 (72; 100)	NA	NA	NA
	Kenya	12	14875 (9157; 24163)	100 (74; 100)	5	20489 (10806; 38851)	100 (48; 100)	NA	NA	NA
	Uganda	12	12408 (6488; 23730)	100 (74; 100)	13	9627 (4870; 19029)	100 (75; 100)	NA	NA	NA
	EBL2002 (control)	10	37 (<LLOQ; 89)	10 (0·3; 45)	11	<LLOQ (<LLOQ; <LLOQ)	0 (0; 29)	NA	NA	NA
	Burkina Faso	4	68 (<LLOQ; 985)	0 (0; 60)	4	<LLOQ	0 (0; 60)	NA	NA	NA
	Côte d'Ivoire	0	–	–	2	<LLOQ	0 (0; 84)	NA	NA	NA
	Kenya	2	<LLOQ	0 (0; 84)	1	<LLOQ	0	NA	NA	NA
	Uganda	4	<LLOQ (<LLOQ; 119)	25 (0·6; 81)	4	<LLOQ (<LLOQ; 47)	0 (0, 60)	NA	NA	NA
28 days post-dose 2[§]	EBL2004 (vaccine)	125	12279 (10432; 14452)	100 (97; 100)	105	15797 (13289; 18778)	100 (96; 100)	108	25111 (21332; 29559)	100 (96; 100)
	Guinea	42	10073 (7802; 13004)	100 (91; 100)	51	13308 (10575; 16749)	100 (93; 100)	56	28227 (22831; 34898)	100 (94; 100)
	Liberia	23	12809 (7692; 21331)	100 (85; 100)	14	15174 (7117; 32349)	100 (77; 100)	4	16796 (5575; 50599)	100 (29; 100)
	Mali	22	14069 (10382; 19066)	100 (84; 100)	18	17867 (13243; 24106)	100 (80; 100)	38	22308 (16549; 30073)	100 (90; 100)
	Sierra Leone	38	13769 (10025; 18910)	100 (90; 100)	22	21802 (14635; 32479)	100 (85; 100)	10	24016 (11413; 50535)	100 (69; 100)
	EBL2004 (control)	54	<LLOQ (<LLOQ; 44)	0 (0; 7)	50	<LLOQ (<LLOQ; 43)	2 (0; 11)	49	<LLOQ (<LLOQ; 36)	4 (0; 14)
	Guinea	24	<LLOQ (<LLOQ; 52)	0 (0; 14)	19	<LLOQ (<LLOQ; 45)	0 (0; 18)	24	<LLOQ (<LLOQ; <LLOQ)	4 (0; 21)
	Liberia	8	<LLOQ (<LLOQ; 48)	0 (0; 41)	9	60 (<LLOQ; 203)	11 (0; 48)	1	<LLOQ	0 (0; 98)
	Mali	7	<LLOQ (<LLOQ; <LLOQ)	0 (0; 41)	9	<LLOQ (<LLOQ; 47)	0 (0; 34)	18	<LLOQ (<LLOQ; 39)	6 (0; 27)
	Sierra Leone	15	50 (<LLOQ; 87)	0 (0; 22)	13	<LLOQ (<LLOQ; 46)	0 (0; 25)	6	48 (<LLOQ; 571)	0 (0; 46)
21 days post-dose 2[§]	EBL3001 stage 2 (vaccine)	134	9929 (8172; 12064)	98 (94; >99)	124	10212 (8419; 12388)	99 (95; >99)	124	22568 (18426; 27642)	98 (93; >99)
	EBL3001 stage 2 (control)	46	74 (48; 114)	2 (0·1; 12)	43	42 (<LLOQ; 60)	7 (2; 20)	38	<LLOQ (<LLOQ; 38)	3 (0.1; 14)
Month 12[‖]	EBL2002 (vaccine)	52	541 (433; 678)	90 (79; 97)	54	637 (529; 767)	98 (90; >99)	NA	NA	NA
	Burkina Faso	28	453 (359; 571)	93 (77; 99)	23	653 (526; 810)	96 (77; >99)	NA	NA	NA
	Côte d'Ivoire	0	–	–	12	433 (235; 797)	100 (72; 100)	NA	NA	NA
	Kenya	12	716 (371; 1380)	92 (62; >99)	5	1056 (681; 1638)	100 (48; 100)	NA	NA	NA
	Uganda	12	622 (343; 1127)	83 (52; 98)	14	711 (494; 1023)	100 (77; 100)	NA	NA	NA
	EBL2002 (control)	10	<LLOQ (<LLOQ; 90)	10 (0·3; 45)	11	<LLOQ (<LLOQ; <LLOQ)	9 (0.2; 41)	NA	NA	NA
	Burkina Faso	4	60 (<LLOQ; 1117)	0 (0; 60)	4	<LLOQ (<LLOQ; 191)	25 (0·6; 81)	NA	NA	NA
	Côte d'Ivoire	0	–	–	2	<LLOQ	0 (0; 84)	NA	NA	NA
	Kenya	2	<LLOQ	0 (0; 84)	1	<LLOQ	0	NA	NA	NA
	Uganda	4	<LLOQ (<LLOQ; 138)	25 (0·6; 81)	4	<LLOQ	0 (0; 60)	NA	NA	NA
	EBL2004 (vaccine)[¶]	63	731 (589; 907)	77 (64; 87)	33	739 (585; 933)	94 (80; 99)	28	1139 (905; 1432)	100 (87; 100)
	Guinea	18	847 (549; 1307)	82 (57; 96)	8	993 (541; 1821)	100 (63; 100)	11	1005 (705; 1431)	100 (72; 100)

	Liberia	0	NA	NA	0	NA	NA	0	NA	NA
	Mali	3	1034 (344; 3108)	100 (29; 100)	2	641 (<LLOQ; 19030)	100 (16; 100)	9	1400 (965; 2030)	100 (63; 100)
	Sierra Leone	42	669 (510; 878)	73 (56; 85)	23	675 (511; 891)	91 (72; 99)	8	1073 (561; 2052)	100 (63; 100)
	EBL3001 stage 2 (vaccine)	132	386 (326; 457)	70 (61; 77)	123	436 (375; 506)	71 (62; 79)	120	750 (629; 894)	96 (90; 99)
	EBL3001 stage 2 (control)	43	93 (55; 157)	14 (5; 28)	41	46 (<LLOQ; 72)	13 (4; 27)	39	<LLOQ (<LLOQ; 42)	10 (3; 24)

CI = confidence interval. EBOV GP = Ebola virus glycoprotein. EU = enzyme-linked immunosorbent assay unit. GMC = geometric mean concentration. LLOQ = lower limit of quantification. NA = not applicable.

*Participants aged 12-17 years in EBL2002, EBL2004, and EBL3001 stage 2.

†Participants aged 4-11 years in EBL2002 and EBL3001 stage 2 and 5-11 years in EBL2004.

‡Participants aged 1-4 years in EBL2004 and 1-3 years in EBL3001 stage 2.

§Binding antibody concentrations were assessed at day 78 ± 3 days in EBL2002, day 84 ± 2 weeks in EBL2004, and day 78 ± 1 week in EBL3001 stage 2.

‖Binding antibody concentrations were assessed at day 365 ± 30 days in EBL2002, day 336 ± 1 month in EBL2004, and day 360 ± 1 month in EBL3001 stage 2.

¶Data for the EBL2004 placebo group were not available at month 12.

***Supplementary Table 5*: Covariance parameter estimates for adult participants**

Covariance parameter	**Subject**	**Estimate**	**Standard error**	**Z value**	**Pr > Z**
Intercept	studyid	0·02182	0·02285	0·96	0·1697
Residual		0·2032	0·01180	17·22	<0·001
ICC		0·0970	–	–	–

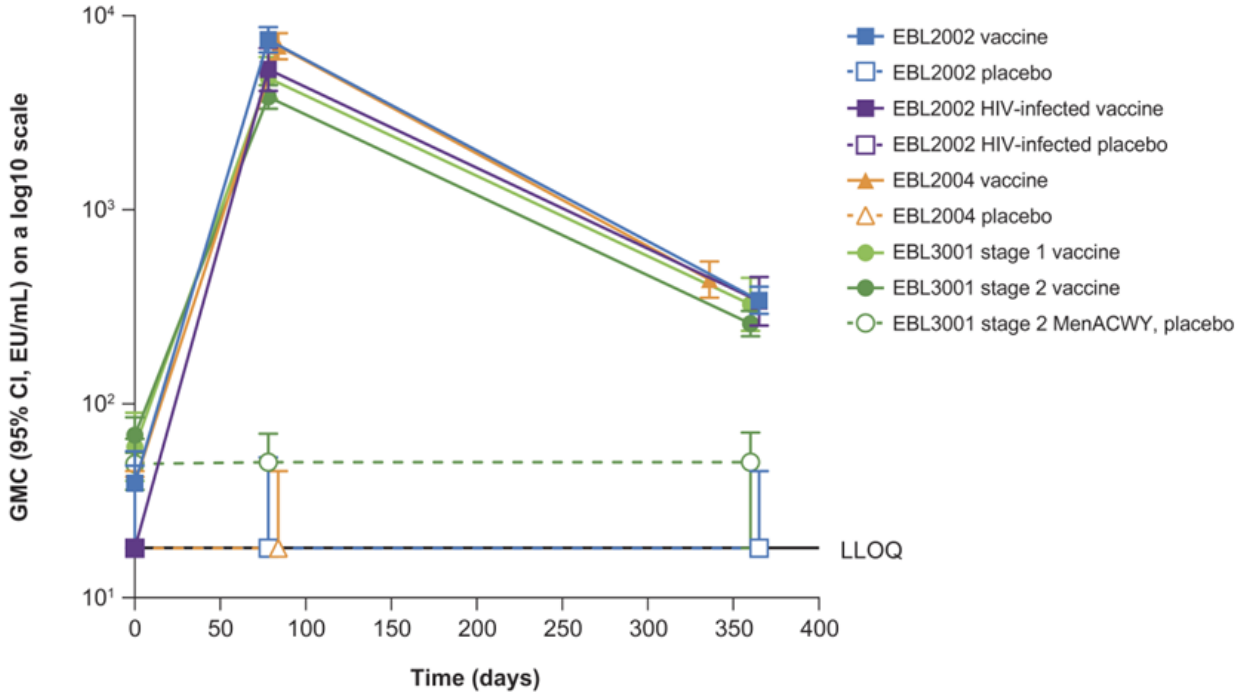
ICC = intraclass correlation coefficient.

***Supplementary Table 6*: Covariance parameter estimates for paediatric participants**

Covariance parameter	**Subject**	**Estimate**	**Standard error**	**Z value**	**Pr > Z**
Intercept	studyid	0·007013	0·007899	0·89	0·1873
Residual		0·1946	0·009605	20·26	<0·001
ICC		0·0348	–	–	–

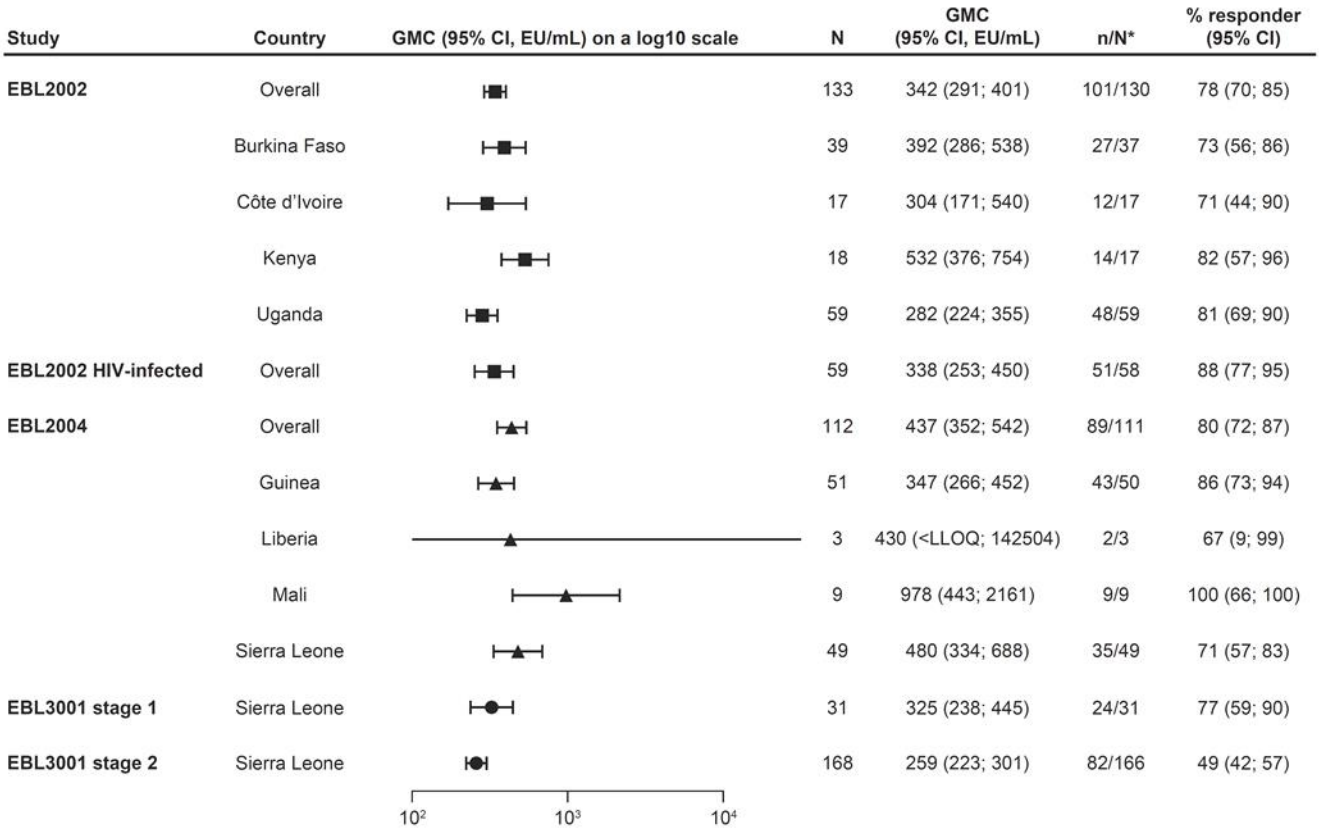
ICC = intraclass correlation coefficient.

***Supplementary Figure 1*: EBOV GP-binding antibody GMCs in adults in EBL2002, EBL2004, and EBL3001 over time.**



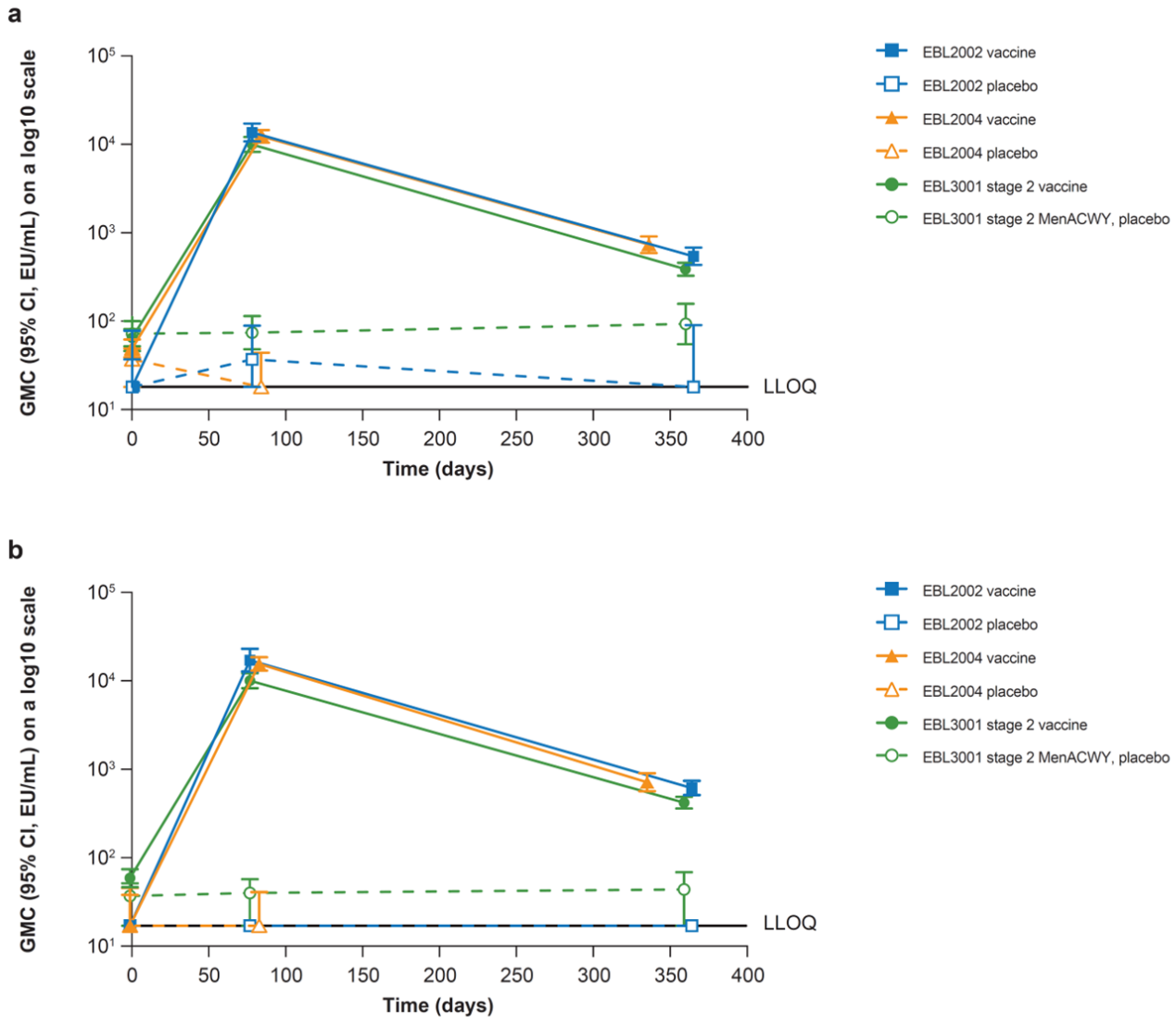
EBOV GP-binding antibody GMCs are presented over time (baseline, 21 or 28 days post-dose 2, and month 12) for the Ebola vaccine and control groups. Samples were analysed according to Q^2 Solution's FANG ELISA standard operating procedure, and a single reportable value for each participant sample at each timepoint was uploaded for statistical analysis. Error bars represent 95% CIs.
CI = confidence interval. EBOV GP = Ebola virus glycoprotein. ELISA = enzyme-linked immunosorbent assay. EU = ELISA unit. FANG = Filovirus Animal Nonclinical Group. GMC = geometric mean concentration. HIV = human immunodeficiency virus. LLOQ = lower limit of quantification. MenACWY = meningococcal quadrivalent conjugate vaccine.

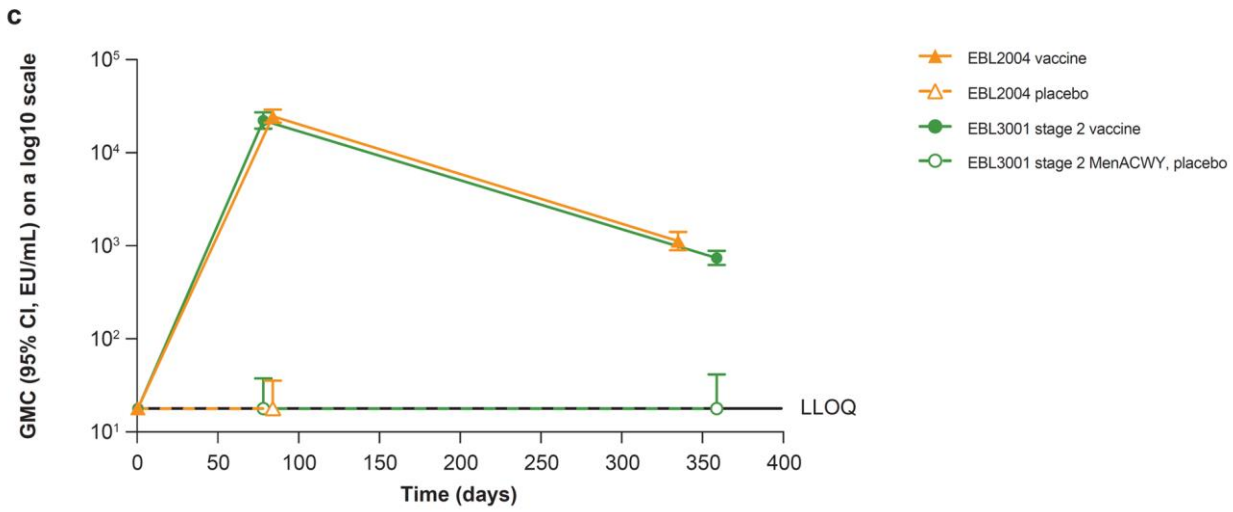
***Supplementary Figure 2*: EBOV GP-binding antibody GMCs in adult participants at month 12, stratified by country.**



EBOV GP-binding antibody GMCs at month 12 are presented overall for adults who received the Ad26.ZEBOV, MVA-BN-Filo vaccine regimen in EBL2002 (n=133), HIV-infected adults in EBL2002 (n=59), adults in EBL2004 (n=112), and adults in EBL 3001 stage 1 (n=31) and stage 2 (n=168), as well as by country in each study. Samples were analysed according to Q^2 Solution's FANG ELISA standard operating procedure, and a single reportable value for each participant sample at each timepoint was uploaded for statistical analysis. Error bars represent 95% CIs.
*****N is the number of participants with data at baseline and at month 12.
CI = confidence interval. EBOV GP = Ebola virus glycoprotein. ELISA = enzyme-linked immunosorbent assay. EU = ELISA unit. FANG = Filovirus Animal Nonclinical Group. GMC = geometric mean concentration. HIV = human immunodeficiency virus. LLOQ = lower limit of quantification.

***Supplementary Figure 3*: EBOV GP-binding antibody GMCs in (a) adolescents, (b) older children, and (c) younger children in EBL2002, EBL2004, and EBL3001 stage 2 over time.**



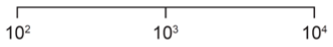


EBOV GP-binding antibody GMCs are presented over time (baseline, 21 or 28 days post-dose 2, and month 12) for the Ebola vaccine and control groups. Samples were analysed according to Q^2 Solution's FANG ELISA standard operating procedure, and a single reportable value for each participant sample at each timepoint was uploaded for statistical analysis. Error bars represent 95% CIs.
CI = confidence interval. EBOV GP = Ebola virus glycoprotein. ELISA = enzyme-linked immunosorbent assay. EU = ELISA unit. FANG = Filovirus Animal Nonclinical Group. GMC = geometric mean concentration. LLOQ = lower limit of quantification. MenACWY = meningococcal quadrivalent conjugate vaccine.

***Supplementary Figure 4*: EBOV GP-binding antibody GMCs in (a) adolescents, (b) older children, and (c) younger children at month 12, stratified by country.**

a

Study	Country	GMC (95% CI, EU/mL) on a log10 scale	N	GMC (95% CI, EU/mL)	n/N*	% responder (95% CI)
EBL2002	Overall		52	541 (433; 678)	47/52	90 (79; 97)
	Burkina Faso		28	453 (359; 571)	26/28	93 (77; 99)
	Côte d'Ivoire		0	–	–	–
	Kenya		12	716 (371; 1380)	11/12	92 (62; >99)
	Uganda		12	622 (343; 1127)	10/12	83 (52; 98)
EBL2004	Overall		63	731 (589; 907)	46/60	77 (64; 87)
	Guinea		18	847 (549; 1307)	14/17	82 (57; 96)
	Liberia		0	–	–	–
	Mali		3	1034 (344; 3108)	3/3	100 (29; 100)
	Sierra Leone		42	669 (510; 878)	29/40	73 (56; 85)
EBL3001 stage 2	Sierra Leone		132	386 (326; 457)	92/132	70 (61; 77)

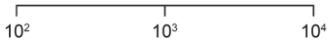


b

Study	Country	GMC (95% CI, EU/mL) on a log10 scale	N	GMC (95% CI, EU/mL)	n/N*	% responder (95% CI)
EBL2002	Overall		54	637 (529; 767)	51/52	98 (90; >99)
	Burkina Faso		23	653 (526; 810)	21/22	96 (77; >99)
	Côte d'Ivoire		12	433 (235; 797)	11/11	100 (72; 100)
	Kenya		5	1056 (681; 1638)	5/5	100 (48; 100)
	Uganda		14	711 (494; 1023)	14/14	100 (77; 100)
EBL2004	Overall		33	739 (585; 933)	31/33	94 (80; 99)
	Guinea		8	993 (541; 1821)	8/8	100 (63; 100)
	Liberia		0	–	–	–
	Mali		2	641 (<LLOQ; 19030)	2/2	100 (16; 100)
	Sierra Leone		23	675 (511; 891)	21/23	91 (72; 99)
EBL3001 stage 2	Sierra Leone		123	436 (375; 506)	85/119	71 (62; 79)

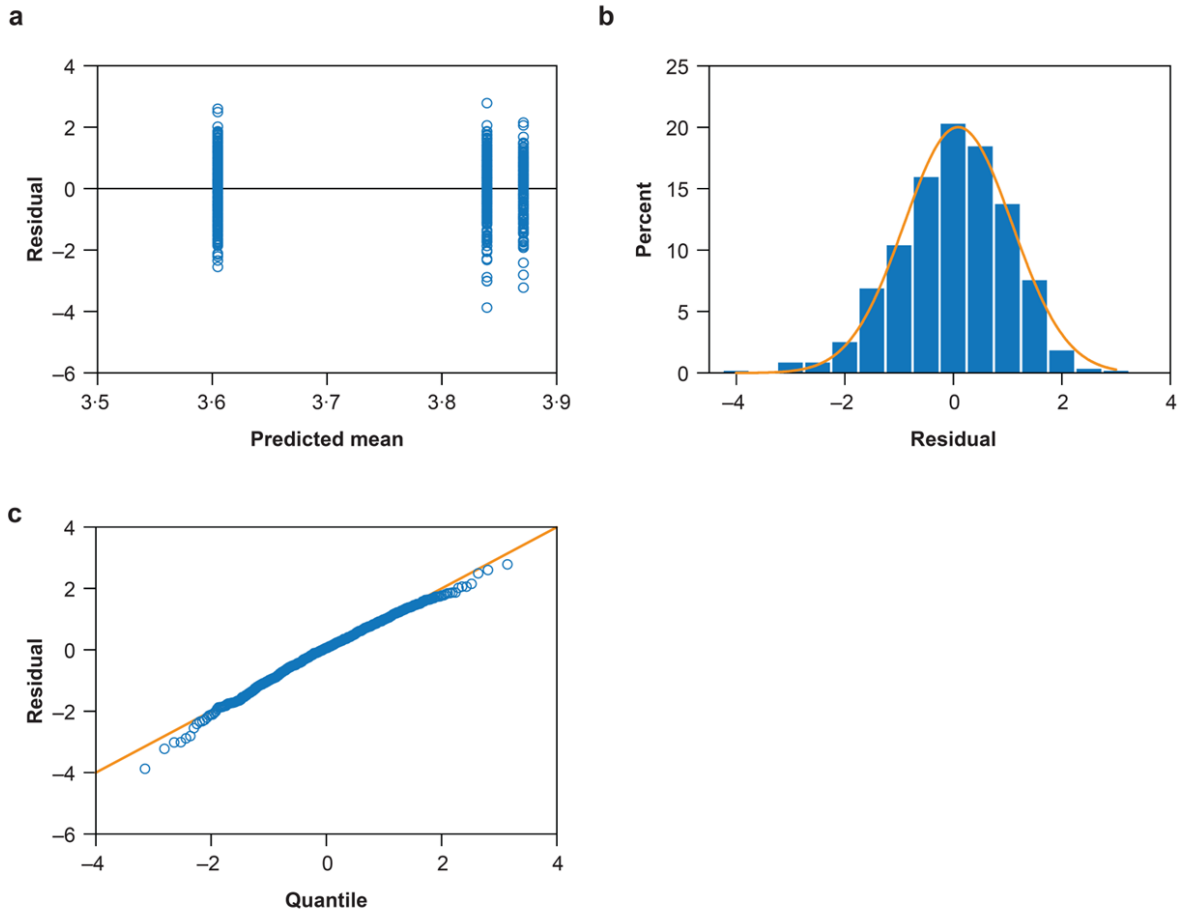
c

Study	Country	GMC (95% CI, EU/mL) on a log10 scale	N	GMC (95% CI, EU/mL)	n/N*	% responder (95% CI)
EBL2004	Overall		28	1139 (905; 1432)	27/27	100 (87; 100)
	Guinea		11	1005 (705; 1431)	11/11	100 (72; 100)
	Liberia		0	–	–	–
	Mali		9	1400 (965; 2030)	8/8	100 (63; 100)
	Sierra Leone		8	1073 (561; 2052)	8/8	100 (63; 100)
EBL3001 stage 2	Sierra Leone		120	750 (629; 894)	112/117	96 (90; 99)



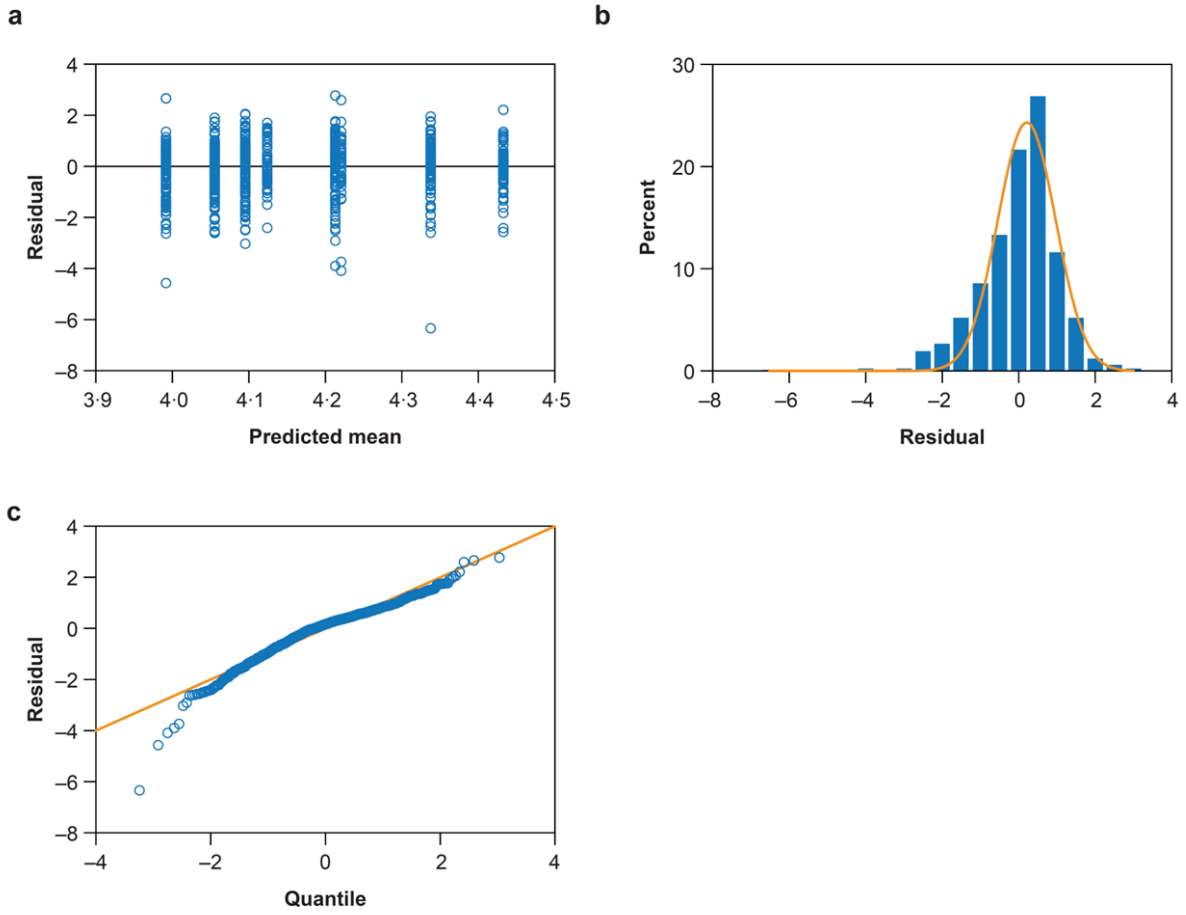
EBOV GP-binding antibody GMCs at month 12 are presented overall for paediatric participants who received the Ad26.ZEBOV, MVA-BN-Filo vaccine regimen, including adolescents aged 12-17 years in EBL2002 (n=52), EBL2004 (n=63), and EBL3001 stage 2 (n=132); older children aged 4-11 years in EBL2002 (n=54) and EBL3001 stage 2 (n=123) and 5-11 years in EBL2004 (n=33); and younger children aged 1-4 years EBL2004 (n=28) and 1-3 years in EBL3001 stage 2 (n=120). GMCs are also shown by country for each study. Samples were analysed according to Q^2 Solution's FANG ELISA standard operating procedure, and a single reportable value for each participant sample at each timepoint was uploaded for statistical analysis. Error bars represent 95% CIs.
*****N is the number of participants with data at baseline and at month 12.
CI = confidence interval. EBOV GP = Ebola virus glycoprotein. ELISA = enzyme-linked immunosorbent assay. EU = ELISA unit. FANG = Filovirus Animal Nonclinical Group. GMC = geometric mean concentration. GMC = geometric mean concentration. LLOQ = lower limit of quantification.

***Supplementary Figure 5*: Conditional studentised residuals for log10-transformed EBOV GP-binding antibody concentrations in adult participants.**



To assess the fit of the data, we used three diagnostic plots: predicted means against residuals, residuals against percent, and Q-Q plots. For a good fit we expected to see (1) random scatter in the plot of predicted means against residuals, (2) normal distribution of the data in the plot of residuals against percent, and (3) data points following the 45° line in the plot of quantiles against residuals.
EBOV GP = Ebola virus glycoprotein. Q-Q = quantile-quantile.

***Supplementary Figure 6*: Conditional studentised residuals for log10-transformed EBOV GP-binding antibody concentrations in paediatric participants.**



To assess the fit of the data, we used three diagnostic plots: predicted means against residuals, residuals against percent, and Q-Q plots. For a good fit we expected to see: (1) random scatter in the plot of predicted means against residuals, (2) normal distribution of the data in the plot of residuals against percent, and (3) data points following the 45° line in the plot of quantiles against residuals.
EBOV GP = Ebola virus glycoprotein. Q-Q = quantile-quantile.