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1 **Prediction of long-term humoral response induced**
2 **by the two-dose heterologous Ad26.ZEBOV,**
3 **MVA-BN-Filo vaccine against Ebola**

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14 **ABSTRACT** The persistence of the long-term immune response induced by the heterologous Ad26.ZEBOV, MVA-
15 BN-Filo two-dose vaccination regimen against Ebola has been investigated in several clinical trials. Longitudinal
16 data on IgG-binding antibody concentrations were analyzed from 487 participants enrolled in six Phase I and
17 Phase II clinical trials conducted by the EBOVAC1 and EBOVAC2 consortia. A model based on ordinary differential
18 equations describing the dynamics of antibodies and short- and long-lived antibody-secreting cells (ASCs) was
19 used to model the humoral response from 7 days after the second vaccination to a follow-up period of 2 years.
20 Using a population-based approach, we first assessed the robustness of the model, which was originally estimated
21 based on Phase I data, against all data. Then we assessed the longevity of the humoral response and identified
22 factors that influence these dynamics. We estimated a half-life of the long-lived ASC of at least 15 years and
23 found an influence of geographic region, sex, and age on the humoral response dynamics, with longer antibody
24 persistence in Europeans and women and higher production of antibodies in younger participants.

25 **KEYWORDS:** Antibody response longevity, Ebola, mechanistic modeling, vaccine

26 INTRODUCTION

27 The 2014-2016 Ebola virus disease (EBOV) outbreak in West Africa and the current SARS-CoV-2 pandemic have led
28 to accelerated development of vaccines to control the spread of infection and reduce the severity of disease in
29 infected individuals. As a result, effective vaccines were developed and became available quickly after the start
30 of these two epidemics. In the case of Ebola, the recombinant replication-competent vesicular stomatitis viral
31 vectored vaccine (Ervebo) was approved by the FDA in December 2019 (1) and used during epidemics in a ring
32 vaccination strategy. The two-dose heterologous strategy, combining immunizations with Ad26.ZEBOV (Zabdeno)
33 and MVA-BN-Filo (Mvabea), was approved by the European Commission in July 2020 (2) under exceptional circum-
34 stances for use in children and adults. An important question for those who have already been vaccinated, and for
35 using the vaccines for a preventive strategy to control the occurrence of outbreaks, is the duration of protection
36 conferred by vaccination.

37 In the context of rapid vaccine development, long-term follow-up in large populations of vaccinated persons,
38 as with older vaccines, is not possible (3, 4). When data are sparse, mathematical modelling is helpful because
39 it can provide estimates of the duration of response by using additional information from biological knowledge

40 about the vaccine mechanism and biological parameters. It is also helpful in quantifying the effect of factors
41 that influence the response to the vaccine. This type of work is performed by modelling the dynamics of one or
42 several markers that could be considered as good correlates of protection (5). Vaccine efficacy and mechanisms
43 of action, or optimal immunogenic vaccine doses, have been evaluated for various infectious diseases, such as
44 influenza (6, 7, 8), yellow fever (9, 10), Zika (11), tuberculosis (12), and more recently SARS-CoV-2 (13). In the case of
45 the Ad26.ZEBOV and MVA-BN-Filo vaccine strategy, the concentration of binding antibodies is considered a good
46 correlate of protection based on work performed in non-human primates (14). It was agreed with the FDA to be
47 suitable for use in a Biological Licensing Application under the Animal Rule (15) and it was the basis for marketing
48 authorization in the EU.

49 In a previous publication (16), we used a mathematical model for antibody-secreting cell (ASC) dynamics that
50 distinguishes between short-lived and long-lived cells (SL and LL, respectively), and we estimated the model pa-
51 rameters using the data from the available first Phase I studies. We found that antibody production is maintained
52 by the population of long-lived cells with an estimated half-life of at least 5 years. New data from three Phase II
53 studies (17, 18, 19) conducted in two international consortia (EBOVAC1 and EBOVAC2) provided an opportunity to
54 validate the model and better characterise factors associated with the variation of the antibody response.

55 RESULTS

56 **Descriptive analysis of the data** The baseline and demographic characteristics of the 487 participants in-
57 cluded in the study are shown in Table 1. In all results hereafter, Benjamini and Hochberg correction (20) for
58 multiple testing has been used (see section Methods for more details). Comparable baseline characteristic in
59 terms of age, body mass index (BMI) and weight are observed in European participants across the Phase I and
60 II clinical studies (all p-values > 0.80). Similarly, no differences are observed in Africa across trials and sites in
61 term of weight, however BMI appears significantly higher in East African participants (+6%, p-value=0.007) than in
62 West African ones. European participants were significantly older than African (41 vs 29 years, p-value < 0.001)
63 and consequently participants in EBL2002 tended to be older (34 vs 27 years, p-value < 0.001). BMI and weight
64 (p-values <0.001 in both cases) were significantly higher in European participants (+13% and +18%, respectively)
65 than in African participants.

66 Figure 1 shows the dynamics of antibody concentrations (median and interquartile ranges) 7 days after the

Table 1 – Demographic and baseline characteristics of participants

	Phase I trials			Phase II trials				Total
	Europe	East Africa		Europe	East Africa	West Africa	West Africa	
	UK EBL1001	Kenya EBL1003	Tanz./Ug. EBL1004	UK/France EBL2001	Ken./Tanz. EBL2002	BFA/IVC	Sierra Leone EBL3001	
Part., no.	14	15	15	71	79	58	235	487
Sex								
Men	4 (29%)	11 (73%)	10 (67%)	32 (45%)	45 (57%)	44 (75%)	203 (86%)	349 (72%)
Women	10 (71%)	4 (27%)	5 (33%)	39 (55%)	34 (43%)	14 (24%)	32 (14%)	138 (28%)
Age (yrs)	37.6 (9.3)	23.7 (2.8)	26.5 (6.8)	41.2 (14.7)	34.1 (13.5)	34.1 (10.8)	27.2 (10.0)	31.3 (12.4)
BMI (kg/m²)	26.1 (3.3)	22.5 (4.1)	22.9 (4.2)	25.4 (4.5)	23.8 (4.0)	23.0 (3.4)	21.9 (3.3)	23.0 (3.9)
Weight (kg)	73.7 (13.7)	63.3 (12.7)	63.5 (11.7)	74.7 (14.7)	63.9 (10.2)	67.2 (9.9)	62.4 (9.4)	65.4 (11.7)

Data are n (%) or mean (SD). Only healthy adults receiving Ad26.ZEBOV followed by MVA-BN-Filo 56 days later were selected within each of the 6 trials. Part.: Participants, no.: number, yrs: years, kg: kilograms, m: meter, UK: United Kingdom, Tanz.: Tanzania, Ug.: Uganda, BFA: Burkina Faso, IVC: Ivory Coast.

67 second vaccination for each study according to the assay used to quantify the binding antibodies. In addition, Ta-
68 ble 2 summarizes antibody concentrations observed at predefined sampling time points. Only participants who
69 had received both the first and second vaccinations were included in both the descriptive and the modeling an-
70 alyzes. Similar kinetics were observed in all studies, with the highest binding antibody concentrations observed
71 at 21 days post-dose 2 (hereafter referred to as "peak"), followed by a biphasic decline up to 1 year after the first
72 vaccination. Furthermore, the longer-term dynamics observed in EBL3001 suggest a durable immune response
73 after the biphasic decline.

74

75 **Mechanistic model of the humoral response** To better identify the factors associated with the dynamic
76 of the antibody response and to predict its duration, we used a model initially applied by (16) in Phase I trials
77 evaluating the two-dose heterologous Ad26.ZEBOV, MVA-BN-Filo vaccine regimen. In this mechanistic model, an-
78 tibodies are assumed to be produced by plasma cells (antibody-secreting cells, ASCs) divided into two distinct sub-
79 populations characterized by their lifespan: short-lived (SL) and long-lived (LL). For various infectious diseases, a
80 rapid expansion of antigen-specific ASCs in blood peaking on day 7 post-infection or vaccination, followed by a
81 fast depletion is observed (21, 22). Therefore, strictly decreasing dynamics were considered from 7 days after
82 the second vaccination for the two compartments of plasma cells assuming no additional exposure to the anti-
83 gen. A schematic diagram of the mathematical model used to describe the humoral response from 7 days after
84 the second vaccination is displayed in Figure 2. This simple model relied on three biological processes. LL and
85 SL ASCs decay with time at rate δ_L and δ_S , respectively, and produce antibodies at rates θ_L and θ_S , respectively.

Table 2 – Ebola glycoprotein-specific antibody concentrations (in log₁₀ ELISA units/mL) in each trial from 7 days after the second vaccination to study completion.

	Phase I trials			Phase II trials		
	EBL1001 (n=14) ¹	EBL1003 (n=15) ¹	EBL1004 (n=15) ¹	EBL2001 (n=71) ¹	EBL2002 (n=137) ²	EBL3001 (n=235) ³
Day 64 (7 days after the 2nd vaccination, MVA-BN-Filo)						
Number Part.	14	15	15			
Positive Part. ⁴	14 (100%)	15 (100%)	15 (100%)			
Missing data	0 (0%)	0 (0%)	0 (0%)			
Mean [IQR]	3.19 [2.99 ; 3.48]	3.33 [2.90 ; 3.73]	3.09 [2.55 ; 3.58]			
Day 78 (21 days after the 2nd vaccination, MVA-BN-Filo)						
Number Part.	14	15	15	70	137	231
Positive Part. ⁴	14 (100%)	15 (100%)	15 (100%)	70 (100 %)	137 (100%)	231 (100%)
Missing data	0 (0%)	0 (0%)	0 (0%)	1 (1%)	0 (0%)	4 (2%)
Mean [IQR]	3.88 [3.64 ; 4.10]	4.21 [3.96 ; 4.45]	4.03 [3.80 ; 4.31]	4.00 [3.79 ; 4.43]	3.88 [3.62 ; 4.16]	3.60 [3.34 ; 3.88]
Day 156 (155 days after the 1st vaccination, Ad26.ZEBOV)						
Number Part.						42 ⁵
Positive Part. ⁴						42 (100%)
Missing data						1 (2%)
Mean [IQR]						2.73 [2.54 ; 3.00]
Day 180 (179 days after the 1st vaccination, Ad26.ZEBOV)						
Number Part.	12	15	15		23 ⁶	
Positive Part. ⁴	12 (100%)	15 (100%)	15 (100%)		23 (100%)	
Missing data	2 (14%)	0 (0%)	0 (0%)			
Mean [IQR]	3.47 [3.29 ; 3.60]	3.00 [2.67 ; 3.20]	2.97 [2.71 ; 3.25]		2.70 [2.47 ; 2.85]	
Day 240 (239 days after the 1st vaccination, Ad26.ZEBOV)						
Number Part.	13	15	15			
Positive Part. ⁴	13 (100 %)	15 (100%)	15 (100%)			
Missing data	1 (7%)	0 (0%)	0 (0%)			
Mean [IQR]	3.35 [3.20 ; 3.41]	2.66 [2.22 ; 2.93]	2.83 [2.56 ; 3.09]			
Day 360/365 (1 year after the 1st vaccination, Ad26.ZEBOV)						
Number Part.	12	15	15	51	134	207
Positive Part. ⁴	12 (100%)	15 (100%)	15 (100%)	51 (100%)	134 (100%)	205 (99%)
Missing data	2 (14%)	0 (0%)	0 (0%)	20 (28%)	3 (2%)	28 (12 %)
Mean [IQR]	3.24 [3.09 ; 3.33]	2.61 [2.40 ; 2.96]	2.74 [2.46 ; 3.06]	3.07 [2.89 ; 3.28]	2.54 [2.26 ; 2.78]	2.44 [2.11 ; 2.68]
Day 540 (539 days after the 1st vaccination, Ad26.ZEBOV)						
Number Part.						33 ⁵
Positive Part. ⁴						33 (100%)
Missing data						10 (23%)
Mean [IQR]						2.43 [2.15 ; 2.68]
Day 720 (2 years after the 1st vaccination, Ad26.ZEBOV)						
Number Part.						190
Positive Part. ⁴						184 (97%)
Missing data						45 (19 %)
Mean [IQR]						2.45 [2.19 ; 2.69]

IQR: Interquartile range = 75% confidence intervals, Part.: Participants. ¹ Participants receiving the 2nd vaccination in the protocol-defined window of 57 ± 1 day. ² Participants receiving the 2nd vaccination in the protocol-defined window of 57 ± 3 days. ³ Participants receiving the 2nd vaccination in the protocol-defined window of 57 ± 1 week. ⁴ Refers to the number of participants with antibody concentration above the lower limit of quantification (LLOQ), expressed as n/N (%) where n is the number of participants with concentration above the LLOQ (i.e., 36.11 EU/mL for Battelle lab, 26.22 EU/mL for Focus lab and 36.11 EU/mL for Q² Solutions lab) at that timepoint and N the the total number of participants with data at the first and the second vaccination and at that time point. ⁵ Refers only to 43 participants enrolled in a substudy to receive a third dose (Ad26.ZEBOV) two years after the first vaccination. ⁶ Refers to participants enrolled in EBL 2002 having an additional timepoint, initially scheduled for participants who do not receive a second vaccination because of a study pause.

86 Finally, antibodies are assumed to decay over time at rate δ_{Ab} . Since the baseline level of ASCs is unknown, the
87 parameters $\phi_L = \theta_L L_0$ and $\phi_S = \theta_S S_0$ were defined, which represent the influx of LL and SL ASCs, respectively (see
88 [Mathematical model of antibody kinetics](#) for more details).

89

90 **Quality of model prediction** Using parameter estimations obtained by Pasin et al. (16) on humoral response
91 observed in Phase I trials, we evaluated the robustness of the model and its predictive abilities.

92 First, we looked at the capacity of the model to capture the dynamic of the antibodies during the early phase
93 following vaccination, based on the previously estimated parameters, in a new population of participants. Fixing
94 the antibody, short- and long-lived ASCs half-lives at 24 days, 3.0 days and 6.0 years, respectively, as well as the
95 SL ASC influx parameter at 2,755 ELISA units/mL/day and the LL ASC influx parameter at 16.6 and 70.7 ELISA
96 units/mL/day for African and European participants (see [Methods - Evaluation of the model quality of prediction](#)
97 for more details), only random effects (i.e. individual deviation from population mean) for the 487 participants
98 were evaluated using empirical Bayes estimates (EBEs).

99 When restricted from 7 days post-second vaccination to the peak of individual dynamics, the model predictions
100 fit well with observed antibody concentrations (see Table 3). The overall percent of observations falling within the
101 95% individual prediction intervals (also referred as the percent of coverage) was evaluated at 100%. The root
102 mean squared error (RMSE) was consistent with a relatively small average difference between observations and
103 predictions estimated at 0.012 \log_{10} ELISA units/ml (RMSE=0.028 \log_{10} ELISA units/ml in Phase I studies accounting
104 for two observations). The model provided similar goodness of predictions when observations extended to the
105 first year following vaccination were used to update EBEs (Supplementary Table 1). The percent of coverage was
106 evaluated at 100% and the RMSE at 0.045 \log_{10} ELISA units/ml (RMSE=0.066 and 0.038 \log_{10} ELISA units/ml in Phase
107 I and Phase II studies, respectively). These results confirmed the ability of the model, estimated using only data
108 from Phase I trials, to capture the antibody response in all additional participants included in Phase II trials.

109 Then, we looked at the ability of the model to predict antibody concentrations beyond the peak of the dynam-
110 ics. Individual parameters assessed for the early part of the dynamics of humoral responses were then used to
111 predict both short-term antibody responses between the peak and 1 year after the first vaccination, and long-term
112 antibody responses between 1 year and 2 years. As described in Table 2, while participants from all trials were
113 included in the analysis of short-term predictions, only participants from the EBL3001 clinical trial contributed to

Table 3 – Evaluation of the robustness and the quality of prediction of the model developed by Pasin et al. (16). The model was estimated on Phase I data and individual parameters were assessed, for each participant of Phase I and Phase II trials, using observation from 7 days post-second vaccination (day 64) to the peak of individual dynamics.

	All trials	Phase I trials			Phase II trials		
		EBL1001	EBL1003	EBL1004	EBL2001	EBL2002	EBL3001
Time of Peak¹							
Mean [95% CI]	15.0 [5.0 ; 21.0]	13.4 [11.6 ; 14.6]	14.4 [13.0 ; 18.2]	13.4 [12.0 ; 14.0]	14.3 [13.0 ; 16.3]	14.0 [12.4 ; 15.0]	16.0 [3.9 ; 22.2]
Number of timepoints²							
Mean [Min-Max]	1.09 [1.0 ; 2.0]	2.0 [2.0 ; 2.0]	2.0 [2.0 ; 2.0]	2.0 [2.0 ; 2.0]	1.0 [1.0 ; 1.0]	1.0 [1.0 ; 1.0]	1.0 [1.0 ; 1.0]
Predictions from 7 days post-2nd vaccination to the peak							
RMSE ³	0.012	0.034	0.023	0.026	0.005	0.006	0.006
Coverage (%)	100	100	100	100	100	100	100
Bias ³	0.002	0.018	-0.003	0.006	0.001	0.001	0.000
95% PI width	0.410	0.547	0.502	0.510	0.389	0.389	0.387
Short-term forecast from the peak to 1 year							
RMSE ³	0.471	0.218	0.539	0.361	0.442	0.460	0.515
Coverage (%)	98.1	100	93.3	100	100	98.7	97.6
Bias ³	0.251	0.014	0.239	0.077	0.293	0.224	0.328
95% PI width	2.297	2.097	2.124	2.184	2.502	2.358	2.298
Long-term forecast beyond 1 year							
RMSE ³	0.518						0.518
Coverage (%)	97.8						97.8
Bias ³	0.253						0.253
95% PI width	2.479						2.479

CI: Confidence interval, PI: Prediction interval, RMSE: Root mean squared error. ¹Time delay in days (Mean [95% CI]) from 7 day post-vaccination (day 64) to the peak (first local maximum). ²Number of observations from 7 days post-vaccination to the peak. ³Criteria calculated on the median of individual predictions.

114 the analysis of long-term predictions because they were the only ones with a follow-up beyond 12 months. As
 115 shown in Figure 3 and Table 3, the model demonstrated a high quality of short- and long-term predictions with a
 116 total of 98% of the observed antibody concentrations falling within the 95% individual prediction intervals. Never-
 117 theless, the high width of 95% prediction intervals (2.297 and 2.479 log₁₀ ELISA units/mL for short- and long-term
 118 forecast, respectively) highlights a large uncertainty in individual model parameters and explain the high percent
 119 of coverage. Plots of individual predictions for the more participants having at least two observations from 7 days
 120 post-second vaccination are given in Supplementary Figures 1-3. Similar work was done to evaluate the ability of
 121 the model to predict long-term antibody concentrations beyond 12 months when data from 7 days post-second
 122 vaccination to 1 year were used to estimate individual parameters (see Supplementary Figures 4-6). These results
 123 highlight the benefit of using an additional short-term observation to improve long-term predictions (beyond 1
 124 year after the second vaccination). The uncertainty of predictions was much lower (65% reduction in the size of
 125 prediction intervals) leading to a fair but smaller coverage (90% vs 98%) and a significant improvement of the
 126 quality of the predictions (RMSE: 0.298 instead of 0.518; bias: -0.017 instead of 0.253).

127

128 **Additional insight on longevity of the humoral immune response** The model performed well in forecast-
129 ing short- and long-term humoral response. However, the increase in RMSE and the width of 95% prediction
130 intervals, and the decrease in the percent coverage beyond 12 months (see Table 3 and Supplementary Table 1)
131 motivated an update of the parameters using all available data from both the Phase I and Phase II trials. First,
132 the model was modified to include laboratory effects (Battelle, Focus or Q² Solutions) in the observation model.
133 Among the four observation models tested (see [Methods - Update and re-estimation of the model](#) for more de-
134 tails), none was able to outperform the model without adjustment for laboratories. However, since laboratory
135 effects reflects an observable reality, the adjusted observation model was constrained and the model including a
136 scaling factor between the observations and the compartment Ab in natural scale, providing the lowest corrected
137 Bayesian Information Criteria (BICc), was chosen. We secondly focused on the half-life of LL ASCs, $\log(2)/\delta_L$. The
138 estimation of the lower bound of the loss rate of LL ASCs δ_L was performed with a profile likelihood. Thanks to
139 the longer follow-up available, the previous estimation of 5 years for the lower bound of the half-life of LL ASCs
140 was updated to 15 years (Figure 4). The method used to achieve this estimation is described in Supplementary
141 Methods. In other words, since long-lived antibody secreting cells are non-proliferating cells (23), half of these
142 antibody-secreting cells, which are produced at 7 days after the second vaccination, should persist for at least 15
143 years. Given this result, further estimations were performed with the parameter δ_L as fixed at the value corre-
144 sponding to a lifespan of 15 years.

145 The application of three algorithms of covariate selection (SCM, COSSAC and SAMBA; see [Methods - Update](#)
146 [and re-estimation of the model](#) for more details) enabled us to identify factors influencing the dynamics of the
147 humoral response. Although methods of covariate search differ from one algorithm to another, the adjustment
148 of the biological parameters of the model for baseline characteristics, including demographics, selected by the
149 different methods were quite consistent. All procedures led to the selection of an effect of continent, sex and age
150 on antibody responses. The same covariates were identified in the model without adjustment for laboratories.

151 The best model estimated an effect of continent on ϕ_L (see Table 4 presenting a summary of parameter
152 estimations). The mean value of ϕ_L was estimated at 36.6 ELISA units/mL/days in Europe compared to 10.2 ELISA
153 unit/mL/days in African participants. These results are in accordance with those previously obtained with Phase I
154 trial data (16).

155 By adding more information with Phase II trial data, we also identified sex as another significant covariate
 156 for explaining the inter-individual variability of the decay rate of antibodies. Indeed, we estimated that antibodies
 157 have a significantly higher half-life in women (p-value estimated by Wald test <0.001) with an increase of the decay
 158 rate of 41% (95% confidence interval (CI): [24%; 60%]) for men as compared to women.

159 We also found that older age was associated with a decrease of the influx of short-lived ASCs (parameter ϕ_S).
 160 For example, a 31 year old participant (ages are assumed to be centered, see Table 1) displayed a mean value
 161 of SL ASCs influx of 3057 ELISA units/mL/days (see Table 4). Each additional year from this mean age induces a
 162 division of the resulting influx of SL ASCs by 7% (95% CI: [5%; 9%]). Therefore, for a participant 10 years older, its
 163 influx of SL ASCs will then be divided by 49% (see Table 4 footnote), corresponding to a decrease of the peak of its
 164 dynamics of 0.23 [0.22; 0.25] \log_{10} EU/mL.

165
 166 Once the optimal structure was identified, we estimated the value of the parameters of the model as shown
 167 in Table 4, providing the model parameters estimated by Pasin et al. (16) on Phase I data as well as the model
 168 parameters obtained on combined Phase I and II data. Figure 5 displays the dynamics estimated by the model,
 169 highlighting the goodness-of-fit of the data (the reader can refer to Supplementary Figures 7 and 8 for additional
 170 results about model estimation and its goodness-of-fit).

171 Compared with the estimates we obtained from the Phase I data, the new estimates show a decrease in the
 172 magnitude of the ϕ_L parameter. This decrease is likely due to the significant increase in the half-life of the LL
 173 ASC from 6 to 15 years. Nevertheless, the mean ϕ_L remained four times higher in Europe than in Africa, similar
 174 to the approximation previously obtained using Phase I data (16), where the mean ϕ_L was 4.3 times higher in
 175 Europe than in Africa. For the dynamics of SL ASCs, the parameter estimates remained quite stable, between the
 176 newly estimated model and the earlier estimates. As noted above, the information gained from longer follow-up
 177 allowed an update of the lower bound of the LL ASC decay rate. Similarly, the use of 443 additional participants
 178 improved the precision of the model parameter estimates. Indeed, the confidence intervals in the new estimates
 179 have become narrower, for each parameter ϕ_L , ϕ_S , or δ_S , and are mostly included within the confidence intervals
 180 of the old estimates. Moreover, comparison of the model estimates showed a slight increase in inter-individual
 181 variability for parameters ϕ_L and δ_{Ab} in the new model compared with the old one. The latter may be due to the use
 182 of additional data collected in a more heterogeneous population than in Phase I studies. However, adjustment of

Table 4 – Model parameters estimated on Phase I participants by Pasin et al. (16) and the new estimates obtained on pooled Phase I and Phase II data and considering adjustment for laboratory effects.

Parameter	Meaning	Phase I data		Phase I & II data	
		Mean	95% CI	Mean	95% CI
Fixed Effects					
δ_{Ab}	antibody decay rate (day ⁻¹)	0.029	[0.027 ; 0.033]		
Women				0.0251	[0.0223 ; 0.0283]
Men				0.0353	[0.0296 ; 0.0421]
$\log(2)/\delta_{Ab}$	antibody half-life (days)	24	[22 ; 26]		
Women				27.6	[24.5 ; 31.1]
Men				19.6	[16.4 ; 23.4]
δ_S	SL ASCs decay rate (day ⁻¹)	0.231	[0.15 ; 0.36]	0.333	[0.326 ; 0.340]
$\log(2)/\delta_S$	SL ASCs half-life (days)	3.0	[1.9 ; 4.7]	2.08	[2.04 ; 2.13]
δ_L	LL ASCs decay rate (year ⁻¹)	3.16×10^{-4}	$[1.46 ; 7.03] \times 10^{-4}$	1.25×10^{-4}	
$\log(2)/\delta_L$	LL ASCs half-life (years)	6.0	[2.7 ; 13]	15.0	
ϕ_S	SL ASCs influx (EU/mL/day)	2755	[1852 ; 4100]		
Mean Age (31.3 years)				3057	[2418 ; 3865]
FC Δ Age = + 1year ¹				0.934	[0.915 ; 0.954]
ϕ_L	LL ASCs influx (EU/mL/day)				
African Part.		16.6	[13.7 ; 20.1]	10.2	[9.01 ; 11.4]
Eur. Part.		70.7	[54.0 ; 92.7]	36.6	[27.3 ; 49.2]
α	scaling factor - Lab effects				
α_{focus}				1.04	[0.93 ; 1.16]
α_{Q2sol}				1.00	[0.98 ; 1.02]
Random Effects					
ω_{ϕ_S}	Sd of RE on ϕ_S	0.92	[0.83 ; 1.01]	0.84	[0.56 ; 1.13]
ω_{ϕ_L}	Sd of RE on ϕ_L	0.85	[0.78 ; 0.92]	0.88	[0.81 ; 0.96]
$\omega_{\delta_{Ab}}$	Sd of RE on δ_{Ab}	0.30	[0.24 ; 0.36]	0.35	[0.29 ; 0.41]
Error Model					
σ_{Ab}	Sd of error model	0.10	[0.10 ; 0.10]	0.107	[0.101 ; 0.112]

CI: Confidence interval, EU: ELISA units, Eur.: European, FC: Fold change, LL ASCs: long-lived antibody secreting cells, Part.: Participants, RE: Random effects, SL ASCs: short-lived antibody secreting cells, Sd: Standard deviation. ¹ Represents the multiplicative factor to apply to the value of ϕ_S , obtained for the mean age, for an increase in participant age of 1 year: $\phi_S(\text{Mean Age} + 1 \text{ year}) = \phi_S(\text{Mean Age}) \times \text{FC}(\Delta\text{Age}=+1)$. Therefore, the percentage of decrease of ϕ_S for a participant X years older than the mean age is given by $100 \times (1 - \text{FC}(\Delta\text{Age}=+1))^X$.

183 the parameter ϕ_S for the age of the participants reduced the unexplained inter-individual variability for the same
 184 parameter by 24%. Finally, it can be noticed the absence of effects of laboratory adjustment, with the two scaling
 185 factors α_{focus} and α_{Q2sol} estimated as non-significantly different from 1.00, at 1.04 and 1.00, respectively.
 186 In order to evaluate the impact of fixing the parameter δ_L , we performed a model averaging analysis (24, 25),
 187 which integrates model uncertainty in the value of δ_L in the calculation of the parameter confidence intervals.
 188 Results shown in Supplementary Tables 2 and 3 indicate very stable estimation.

189 We examined the ability of our model to predict the response for new participants by performing Monte Carlo

190 cross-validation (MCCV) and using RMSE and percent coverage as quality criteria for prediction. (See [Methods -](#)
191 [Update and re-estimation of the model](#) for more details). The results of this analysis are summarised in Figure
192 [6](#), where these two criteria are displayed as functions of the percent of participants used in the training dataset.
193 Finally, despite the wide range of percentages tested for the split of train and test, the quality of the model pre-
194 diction was very stable. The mean RMSE gradually decreased from 0.0870 to 0.0828 \log_{10} ELISA units/mL until it
195 reached the values of 0.0843 \log_{10} ELISA units/mL when 100% of the data are used to estimate the models. The
196 mean percent coverage remained higher than 95% even when only 20% of participants were used to estimate the
197 model. Consequently, the model showed reasonably good quality in predicting the humoral immune response
198 from 7 days after the second vaccination to two years after the first vaccination.

199 DISCUSSION

200 With this modeling work we evaluated the quality of long-term predictions of the mechanistic model developed by
201 Pasin et al. ([16](#)) which considers two populations of ASCs. We demonstrated with new data and a longer follow-up
202 from phase 2 trials that the model, developed with a small number of participants from phase 1 trials, provides
203 long-term predictions of the antibody response with a high validity. Re-estimation of the model with a longer-term
204 follow-up allowed us to update the value of the lower limit of the LL ASC half-life and showed that the longevity
205 of LL plasma cells is much longer than previously estimated.

206 One advantage of this modelling work is the ability to distinguish the half-life of the antibodies from that of
207 ASCs. The estimated values obtained for the half-life of the antibodies (δ_{Ab}) were in the range of those reported in
208 the literature of 20 to 50 days ([26](#), [27](#), [28](#), [29](#), [30](#), [31](#), [32](#)). References on ASC half-lives are more difficult to figure
209 out because these cells are circulating in various compartments (lymphoid, bone marrow) and the survival of the
210 cells may vary greatly according to the infectious agents ([33](#)). Hence, estimates of short-lived ASC vary from days
211 ([34](#)) to several weeks ([3](#), [35](#)). The half-life of long-lived ASCs is even more variable and reflects the variability of
212 the antibody dynamics according to infectious agents and type of vaccines ([36](#)). The updated estimate for the
213 long-lived ASCs calculated in the present study is particularly encouraging with a half-life of at least 15 years. This
214 is comparable to the half-life estimated for long-lived ASCs induced by the Hepatitis A virus vaccine ([3](#)). The long-
215 term humoral immunity is maintained through the memory B cells and the long-lived plasma cells ([37](#)). These
216 latter cells, residing preferentially in the bone marrow, produce antibodies in absence of antigenic stimulation.

217 Interestingly, it has been recently demonstrated that these cells are intrinsically long-lived and can maintain the
218 secretion of antibodies without replenishment of the pool of memory B cells (38). Specific niches in the bone
219 marrow promote cell survival through various factors (36).

220 The model was able to capture inter-individual variation of the antibody dynamics. A part of this variability
221 was associated with the geographic region, age and sex. The cause of the influence of geographic region on
222 the humoral response to vaccine is still unknown. Concomitant malaria infection is suspected to play a role in
223 compromising the immune response (39, 40, 41). Nevertheless, these results should be interpreted cautiously,
224 as simple nonspecific cross-reactivity within the assays used could also be responsible for this association (40).
225 Chronic parasitic infections, such a schistosomiasis (42), have also been suspected to play a role in dampening
226 immune response to vaccination (43). Both hypotheses are currently explored in the EBOVAC consortia. The
227 less pronounced decline of antibodies observed in women is consistent with several reports from other vaccines,
228 including SARS-CoV-2 (44, 45), Ebola (46) and Flu (47). The biological mechanism behind the lower clearance of
229 binding antibodies in women remains an area of research and may differ from vaccine to vaccine (47). It could
230 also be a limitation of the modelling work in identifying precisely enough which compartment is influenced by sex
231 differences in absence of more immunological measurements. The influence of age on the response to vaccine is
232 known (48), but the characterisation of its effect through the production of short-lived antibody secreting cells is
233 novel and requires further confirmation. Pritz et al. (49) has noted an age-related decline in the number of plasma
234 cells in human bone marrow. Here also, identifiability of this effect could has been compromised because of a
235 restricted range of participant ages because none of them were older than 65 years.

236 In conclusion, the dynamical model constructed from early Phase I data has demonstrated its predictive ca-
237 pacity, with longer follow-up and updated estimates giving promising results for the duration of the immune re-
238 sponse. Nevertheless, the simplicity of this model significantly limits its use to fully describe the immune response
239 induced by a multi-dose vaccine strategy. Indeed, the model focuses exclusively on the decrease in antibody con-
240 centrations following vaccination, without modeling the establishment of the immune response triggered by each
241 injection. Thereafter, more complex versions of this model have been proposed to model the establishment, re-
242 activation, and persistence of the humoral immune responses induced by vaccination (50, 51). In particular, these
243 models integrate the stimulation of the immune system triggered by vaccine antigens and the role of immunolog-
244 ical memory resulting from interactions with memory B cells and plasma cells.

245 The main findings obtained in this modeling work are not necessarily specific to the Ad26.ZEBOV, MVA-BN-Filo
246 vaccine strategy. Some parameters, such as the long-lived ASCs half-life, could be found with other vaccine regi-
247 mens. Nevertheless, the value and the interpretation of the parameters are dependent on the model structure
248 and assumptions. For instance, in the modeling work conducted by Clairon et al. (51) to model antibody dynam-
249 ics induced by mRNA vaccine strategies against SARS-CoV-2, the absence of long-term persistence of antibody
250 responses required an adaptation of the model for monophasic trajectories. In our work, the biphasic nature of
251 the model fitted antibody trajectories perfectly, resulting in the estimation of the long-lived ASCs half-life.

252 The sporadic nature of Ebola outbreaks makes the identification of correlates of protection difficult (52). Cur-
253 rently, neither a universal Ebola immune correlate of protection, nor a binding antibody concentration threshold
254 which would ensure a high probability of protection against EBOV, has been identified. Nevertheless, studies per-
255 formed in non-human primates (14) identified vaccine-induced binding antibody concentrations as the immune
256 parameter most highly correlated with survival after EBOV challenge. Due to the shorter disease course and full
257 lethality of the Ebola disease model in non-human primates, the extrapolation of these results from animals to hu-
258 mans remains difficult. Consequently, no protective threshold of post-vaccination binding antibody concentration
259 was derived for the Ad26.ZEBOV, MVA-BN-Filo vaccine.

260 METHODS

261 **Ethics statement** The Phase I UK trial protocol and study documents were approved by the UK National Re-
262 search Ethics Service. The Phase I Kenya trial protocol and study documents were reviewed and approved by the
263 local Ethics Committee and the Kenyan regulatory authority. The Phase I Uganda/Tanzania trial protocol and study
264 documents were reviewed and approved by the Tanzanian Medical Research Coordinating Committee of the Na-
265 tional Institute for Medical Research, the Tanzania Food and Drugs Authority, the Uganda Virus Research Institute
266 Research and Ethics Committee, the Uganda National Council for Science and Technology, the Uganda National
267 Drug Regulatory Authority, and the Ethics Committee of the London School of Hygiene and Tropical Medicine. The
268 Phase II UK/France trial protocol and study documents were approved by the French national Ethics Committee
269 (CPP Ile de France III; 3287), the French Medicine Agency (150646A-61), the UK Medicines and Healthcare Products
270 Regulatory Agency (MHRA), and the UK National Research Ethics Service (South Central, Oxford; A 15/SC/0211).
271 The Phase II Kenya/Uganda/Burkina Faso/Ivory Coast trial protocol and study documents were approved by local

272 and national independent Ethics Committees and Institutional Review Boards. The Phase II Sierra Leone trial pro-
273 tocol and study documents were approved by The study was approved by the Sierra Leone Ethics and Scientific
274 Review Committee, the Pharmacy Board of Sierra Leone, and the London School of Hygiene & Tropical Medicine
275 ethics committee.

276 These trials were conducted in accordance with the principles of good clinical practice and the Declaration of
277 Helsinki, and all participants gave formal, written consent before undergoing any trial-related procedure.

278

279 **Immunogenicity measurements** We considered data from six studies aiming at evaluating the safety, toler-
280 ability and immunogenicity of two-dose vaccine regimens with Ad26.ZEBOV and MVA-BN-Filo. Ad26.ZEBOV is a
281 monovalent, recombinant, E1/E3-deleted, replication-defective, adenovirus type 26 vector vaccine encoding Ebola
282 virus Mayinga variant GP, produced in PER.C6 human cells and injected as a single dose of 5×10^{10} viral particles.
283 MVA-BN-Filo is a recombinant, replication-defective, modified vaccinia Ankara vector vaccine encoding Mayinga
284 variant GP, Sudan virus Gulu variant GP, Marburg virus Musoke variant GP, and Tai Forest nucleoprotein. This
285 multivalent vaccine was produced in chicken fibroblasts and injected at a dose of 1×10^8 Infectious Units (Inf. U).
286 Three of the six studies are randomized, observer-blinded, placebo-controlled Phase I trials on healthy volunteers
287 aged 18 to 50 years. These studies were performed in four countries: the United-Kingdom (UK), Kenya, Tanzania
288 and Uganda. Results of the trials were previously described by Milligan et al. (53) and Winslow et al. (54) for the
289 UK (study registered at ClinicalTrials.gov, NCT02313077, and labelled EBL1001 here), Mutua et al. (55) for Kenya
290 (study registered at ClinicalTrials.gov, NCT02376426, and labelled EBL1003 here), and Anywaine et al. (56) for
291 Tanzania/Uganda (study registered at ClinicalTrials.gov, NCT02376400, and labelled EBL1004 here). In addition,
292 we considered data from two randomized, observer-blinded placebo-controlled, parallel-group Phase II trials on
293 healthy volunteers aged 18 to 65 or 75 years. These studies were performed in 6 countries: the UK, France, Kenya,
294 Uganda, Burkina Faso and Ivory Coast. We refer to Pollard et al. (17) for detailed description of results in the Eu-
295 ropean trial and to Barry et al. (18) for the African trial (two studies registered at ClinicalTrials.gov, NCT02416453
296 and NCT02564523, and labelled EBL2001 and EBL2002 here respectively for the European and African studies).
297 The last study is a combined open-label, non-randomized stage 1, and a randomized, observer-blinded, placebo-
298 controlled stage 2 Phase II trial on healthy adults. This study conducted in Sierra Leone also aimed to evaluate
299 the long-term immunogenicity and the humoral immune memory induced by the vaccine regimen. Results of this

300 trial were described by Ishola et al. (19) (study registered at ClinicalTrials.gov, NCT02509494, and labelled EBL3001
301 here).

302 In Phase I trials, participants were equally randomized into four vaccination regimens: two with MVA-BN-Filo
303 as first vaccination at day 1, followed by Ad26.ZEBOV on day 29 or 57, and two with Ad26.ZEBOV as prime vaccine
304 on day 1, followed by MVA-BN-Filo on day 29 or 57. Within each regimen, participants received either active
305 vaccine or placebo in a 5:1 ratio. In the study EBL2001, participants in Cohorts I-III were equally randomized into
306 three parallel groups in which they received Ad26.ZEBOV as first vaccine on day 1, followed by MVA-BN-Filo on
307 day 29, 57 or 85. This first cohort was excluded from the analysis as participants were enrolled to provide data
308 only on safety and the timing of anti-Ebola virus GP ASCs responses. Within each group, participants received
309 active vaccines or placebo in a 14:1 or 10:3 ratios in cohorts II and III respectively. In the study EBL2002, healthy
310 adults (Cohort I) were equally randomized into the same three parallel groups with an active vaccine: placebo
311 ratio of 5:1. Adults HIV-infected patients (Cohort IIa) and healthy children (Cohorts IIb and III) were not included in
312 the analysis. Finally, in the study EBL3001, participants received either Ad26.ZEBOV as first vaccination on day 1
313 followed by MVA-BN-Filo on day 57, or MenACWY vaccine on day 1 and placebo on day 57, with a ratio of 1:0 and
314 3:1 in stage 1 and 2 respectively. In this work, only participants receiving Ad26.ZEBOV as first vaccination on day 1
315 and MVA-BN-Filo as second vaccination in the protocol-defined window of $57 \pm X$ days (Ad26/MVA D57; with $X=1$
316 for Phase I trials and EBL2001, 3 for EBL2002 and 7 for EBL3001) were included. Based on these criteria, a total of
317 487 participants over all studies were included (among the 725 participants enrolled to receive Ad26/MVA D57, a
318 total of 238 participants were excluded for not receiving their second dose ($n=108$) or outside the protocol-defined
319 window ($n=130$)), 44 of whom were in Phase I studies, 71 in EBL2001, 137 in EBL2002 and 235 in EBL3001. In
320 addition, the 168 participants receiving placebo as vaccine strategy were excluded.

321 Participants were followed up to 1 year after the first vaccination in all the studies, with longitudinal immuno-
322 genicity measurements performed on blood samples. As shown in Figure 7, for the vaccine regimen of interest,
323 immunogenicity samples were collected in all participants immediately before the administration of the first vac-
324 cination (Ad26.ZEBOV) on day 1, before the second vaccination (MVA-BN-Filo) on day 57, then 21 days after the
325 second dose at day 78 and 1 year after the first dose (at day 360 or 365 according to the trial). In Phase I trials,
326 additional samples were taken at days 7, 29, 64, 180 and 240, while immunological assays were done on blood
327 samples taken at day 180 and day 156 in EBL2002 and EBL3001 respectively. Participants enrolled in EBL3001

328 were additionally followed up to 2 years after the first vaccination, with blood samples collected every 6 months
329 after the first year. We analyzed total IgG Ebola virus GP-specific binding antibody concentrations measured by
330 an Ebola virus GP (Kikwit strain) Filovirus Animal Non-Clinical Group (FANG) ELISA assay. The FANG ELISA assays
331 were performed at three different accredited laboratories: (a) at Battelle Biomedical Research Center (Columbus,
332 OH, USA; hereafter referred to as Battelle) for the studies EBL1001 and EBL1004, (b) at Focus Diagnostics (San
333 Juan Capistrano, CA, USA; hereafter referred to as Focus) for the study EBL1003, and (c) at Q² Solutions laboratory
334 (San Juan Capistrano, CA, USA; formerly Focus Diagnostics; hereafter referred to as Q² Solutions) for the studies
335 EBL2001, EBL2002 and EBL3001. Particular attention has been paid in this work to account for a possible system-
336 atic difference in measurements induced by the distinct ELISA assays and thus between studies. Being interested
337 in the longevity of the long-term immunity induced by the two-dose heterologous vaccine, similarly to Pasin et
338 al. (16), we mainly focused our analysis on immunogenicity measurements assessed after the second vaccination.

339

340 **Statistical analysis** A preliminary descriptive analysis was performed on the baseline and demographic char-
341 acteristics of the 487 participants to describe and summarize the basic features of the data. Statistical differences
342 among groups of participants were evaluated using classic t-tests (Welch's t-test in case of unequal variance, iden-
343 tified by a F-test, and Student t-test otherwise) implemented in R, and p-values were adjusted for test multiplicity
344 with Benjamini and Hochberg correction (20) using the built-in R function *p.adjust*. Because of the difference of
345 antibody concentrations measured by the distinct laboratories, comparisons of immunogenicity data between
346 trials were not possible.

347 Finally, Spearman correlations between antibody concentrations measured 21 days after the second vaccina-
348 tion and longer-term humoral responses were evaluated integrating adjustment for test multiplicity on p-values.

349

350 **Mathematical model of antibody kinetics** To analyze the humoral immune response induced by the two-
351 dose heterologous vaccine regimen Ad26.ZEBOV, MVA-BN-Filo against Ebola virus and evaluate the long-term
352 immunogenicity, we used a mechanistic model divided into three parts. First of all, a mathematical model based
353 on ordinary differential equations is defined to describe the dynamics of plasma cells and antibodies (3). As shown
354 in Figure 2, antibodies are assumed to be produced by two plasma cell populations differentiated by their lifes-
355 pan: short- and long-lived antibody secreting cells (ASCs). Consequently, the ordinary differential equation (ODE)

356 system contains three compartments: the short-lived ASCs (labelled S), the long-lived ASCs (labelled L) and the
 357 antibodies (Ab). Based on the hypothesis that antibody secreting cells peaked at day 7 post-infection/vaccination
 358 (21, 22), time was rescaled to consider only the antibody dynamics from 7 days after the second vaccination (day
 359 64) during which plasma cells only decrease over time ($t = \text{time observation} - 64$). As demonstrated by Pasin et al.
 360 (16), the model can be written as a single equation (1).

$$361 \quad \begin{cases} \frac{dAb}{dt} = \phi_S e^{-\delta_S t} + \phi_L e^{-\delta_L t} - \delta_{Ab} Ab \\ Ab(t=0) = Ab_0 = Ab_{D64} \end{cases} \quad (1)$$

362 with δ_S , δ_L and δ_{Ab} representing the average decay rates of SL ASCs, LL ASCs and antibodies, respectively. The
 363 parameters ϕ_S and ϕ_L are, respectively, the influx of SL and LL ASCs defined as $\phi_S = \theta_S S_0$ and $\phi_L = \theta_L L_0$, where
 364 $S_0 = S(t=0) = S_{D64}$ and $L_0 = L(t=0) = L_{D64}$ are the initial conditions at 7 days after the second vaccination
 365 and θ_S and θ_L are their respective antibody production rates. The initial antibody concentration Ab_0 is defined by
 366 the individual measure of antibody concentration at 7 days after the second vaccination. Keeping in mind that
 367 the antibody concentration can be unobserved at day 64 for some participants (see Figure 7) while the decrease
 368 of the dynamics of ASCs is still assumed to start 7 days post-vaccination, an individual lag-time T_i was introduced
 369 in equation (1). This lag-time represents the individual time interval between day 64 and the first observation
 370 following this specific time. The equation can then be written as follows.

$$371 \quad \begin{cases} \frac{dAb}{dt} = \phi_S e^{-\delta_S(t+T_i)} + \phi_L e^{-\delta_L(t+T_i)} - \delta_{Ab} Ab \\ Ab(t=0) = \widetilde{Ab}_0 = Ab_{D64+} \end{cases} \quad (2)$$

372 Based on this equation, time was rescaled for each individual such that the initial condition ($Ab(t=0)$) coincided
 373 with the first observation following day 64 (Ab_{D64+}), given: $\text{time} = \text{time observation} - 64 - T_i$. Therefore, for a
 374 participant with a first measurement at day 64 and observations at days {64, 78, 180, 240, 360}, the lag-time is null
 375 ($T_i=0$), rescaled time points of observations are given by {0, 14, 116, 179, 296} and Ab_{D64+} is equal to measurement
 376 at day 64, Ab_{D64} . For a participant with a first measurement at day 78 and observations at days {78, 180, 240, 360},
 377 the lag-time $T_i = 78 - 64 = 14$, rescaled time points of observations are then given by {0, 102, 162, 282} and
 378 Ab_{D64+} is equal to measurement at day 78, Ab_{D78} . We estimated the five following biological parameters $\Psi =$
 379 ($\phi_S, \delta_S, \phi_L, \delta_L, \delta_{Ab}$). To account for inter-individual variability, we used a statistical model on which the five model
 380 parameters are assumed to be log-transformed, to ensure their positivity. Each parameter is then described by

381 a mixed-effects model which depends on covariates. Each individual parameter Ψ_k^i for the participant i can be
 382 defined as follows, for $k = \{1, \dots, 5\}$.

$$383 \quad \log(\Psi_k^i) = \log(\Psi_{k,0}) + \beta_k Z_k^i + u_k^i \quad (3)$$

384 where Ψ_0 is the fixed effect, Z_k and β_k are, respectively, the vectors of explanatory variables and regression co-
 385 efficients related to the biological parameter Ψ_k , and u_k^i is the individual random effect assumed to be normally
 386 distributed with the variance ω_k^2 . Random effects were assumed to be independent from each other. Based on
 387 results obtained in the previous work (16), we assumed random effects on the influx parameters, ϕ_L and ϕ_S , and
 388 on the decay rate of antibodies δ_{Ab} .

389 For the observation model, we modeled the observed IgG binding antibody concentrations against the Kikwit gly-
 390 coprotein from the six studies by the antibody ODE-compartment. We assumed an additive error model normally
 391 distributed on the \log_{10} value of the antibody concentrations, with a variance σ_{Ab}^2 . The antibody concentration for
 392 patient i at the j th time is given by

$$393 \quad Y(t_{ij}) = \log_{10} [Ab(\Psi^i, t_{ij})] + \varepsilon_{ij} \quad \varepsilon_{ij} \sim \mathcal{N}(0, \sigma_{Ab}^2) \quad (4)$$

394

395

396 **Model estimation** Mathematical and practical identifiability has been assessed in previous work (16). Thus
 397 the parameter δ_L was estimated by profile likelihood (57) which consists of defining a grid of values for the parame-
 398 ter, sequentially setting the parameter δ_L at one of those different values, and estimating the model by maximizing
 399 the log-likelihood, given that value of δ_L . The resulting profile shows the maximum possible log-likelihood for each
 400 value of δ_L and has its maximum at the maximum likelihood estimate $\hat{\delta}_L$. Other parameters were estimated by a
 401 population approach in which the model estimation relies on the estimation of the vector of population parame-
 402 ters including the fixed effects (Ψ_0), the regression coefficients (β), the standard deviation of random effects (ω)
 403 and the standard deviation of the error model (σ_{Ab}). Model estimation was performed by the Monolix ®software
 404 versions 2019R1 and 2019R2. This software uses the Stochastic Approximation Expectation-Maximization (SAEM)
 405 algorithm (58, 59) to estimate the population parameters with likelihood computed by importance sampling (60)

406 and the Fisher information matrix calculated by stochastic approximation. Once population parameters are re-
 407 estimated, individual parameters are computed as Empirical Bayes estimates (EBEs) representing the most likely
 408 values of the individual parameters, given individual data and population parameters. EBEs are calculated as
 409 the mode of the conditional parameter distribution by Markov-Chain Monte-Carlo (MCMC) procedure (61) using
 410 the Metropolis-Hasting algorithm (62) to compute the conditional distribution and the Nelder-Mead Simplex algo-
 411 rithm (63) to maximize it.

412

413 **Evaluation of the model quality of prediction** The mechanistic model described by equations 2, 3 and 4, ini-
 414 tially estimated on Phase I data by Pasin et al. (16), was validated on data from the six trials according to its quality
 415 of prediction. To this end, a two-step approach was applied: first, the robustness of the model was assessed by
 416 evaluating its ability to predict antibody dynamics from 7 days post-second vaccination to the peak of the dynam-
 417 ics (i.e., the first local maximum) for all Phase I and Phase II participants. Then, the ability of the model to forecast
 418 short-term (i.e, from the peak to 1 year after the first vaccination) and long-term antibody concentration (i.e, be-
 419 yond 1 year following the first vaccination) was evaluated. Because validation of the mechanistic model estimated
 420 on Phase I data is sought here, no modification of the observation model defined in equation 4 was considered
 421 here to account for possible laboratory-induced effects in the measurement of antibody concentrations.

422 To investigate the robustness of the model initially estimated on Phase I data, only data restricted to the first year
 423 following the first vaccination were used to stay in the scope of applicability of the model (see Table 2 for a detailed
 424 description of the number of observations available at each time point). Consequently, for each participant the
 425 peak of its dynamics was sought during the first year (see Table 3 for a detailed description of individual times of
 426 peak in each trial). Assuming fixed effects and regression coefficients of the population parameters ($\Psi_{k,0}$ and β_k ,
 427 $\forall k \in \{1, \dots, 5\}$), distribution of random effects ($\sigma_k, \forall k \in \{1, \dots, 5\}$), as well as the standard deviation of the error
 428 model (σ_{Ab}) as fixed to previously obtained values, we evaluated individual parameters for the 487 participants,
 429 via the variables u_k^j , using the empirical Bayes estimates (EBEs) approach implemented in Monolix. As shown in
 430 Table 4, we fixed the decay rate of antibodies (δ_{Ab}), SL ASCs (δ_S) and LL ASCs (δ_L) at values corresponding to half-
 431 lives of 24 days, 3 days and 6 years, respectively. The parameter ϕ_S was fixed at 2,755 ELISA units/mL/day while ϕ_L
 432 was fixed at 16.6 ELISA units/mL/day for African participants and 70.7 ELISA units/mL/day for Europeans. East and
 433 West African participants were assumed to share the same value of LL ASCs influx. Finally, standard deviations of

434 the inter-individual variability on the three parameters ϕ_S , ϕ_S and δ_{Ab} were chosen as $\omega_{\phi_S} = 0.92$, $\omega_{\phi_L} = 0.85$ and
 435 $\omega_{\delta_{Ab}} = 0.30$. The parameter σ_{Ab} was fixed at 0.10 (see (16) or Table 4). To stay consistent with the model built on
 436 Phase I data, we included an adjustment for geographic region in the statistical model (binary variable equal to 0
 437 in Africa and 1 in Europe) on ϕ_L , as shown on the following equation:

$$438 \quad \log(\phi_L^i) = \log(\phi_{L,0}) + \beta_{\phi_{L,Eur}} \times \mathbb{1}_{i \in Eur} + u_{\phi_L}^i \quad (5)$$

439 For each individual, the 95% prediction interval (64) of the antibody dynamics was calculated and the percent cov-
 440 erage, defined as the percent of observations falling within the prediction interval, was assessed. Through these
 441 results, we highlighted the ability of the model to predict the very first antibody concentration measurements
 442 from 7 days post-second vaccination. Once these predictions were validated, individual parameters estimated on
 443 the early phase of the follow-up were used in the second step to quantify both the short- and long-term forecast
 444 skills of the model. To this end, we used the model to make individual predictions of antibody concentration be-
 445 tween the peak and 2 years after the first vaccination. Predictions were then compared to observations and the
 446 percent of observations falling within the 95% individual prediction intervals was quantified.

447 Thereafter, the two-step approach was also applied for evaluating: first, the ability of the model to predict antibody
 448 dynamics from 7 days post-second vaccination to 1 year after the first vaccination (instead of the peak), and
 449 second, its ability to forecast antibody concentration beyond 1 year. This additional analysis was performed to
 450 identify whether the estimation of individual parameters on a longer follow-up can improve long-term predictions.
 451 K-means clustering for longitudinal data (65) was performed to identify distinct trajectories of the dynamics of
 452 the humoral response. Using the kml R package (66), trajectories of antibody concentration from 7 days after the
 453 second vaccination to 2 years after the first vaccination were sequentially clustered into two and more clusters.
 454 Thereafter, we evaluated the percent coverage and the RMSE to investigate potential differences in prediction
 455 abilities according to underlying trajectories for each resulting partition.

456

457 **Update and re-estimation of the model** Once the quality of prediction of the mechanistic model was evalu-
 458 ated, an update of the model was performed in order to improve biological knowledge about the longevity of the
 459 long-term immune response induced by the two-dose heterologous vaccine regimen, Ad26.ZEBOV, MVA-BN-Filo.
 460 The low number of participants included in the three Phase I trials (177 participants, of whom only 44 received

461 the Ad26/MVA D57 vaccine regimen) as well as the short-term follow-up of their immune response up to 1 year
 462 after the first vaccination tended to limit the precision of the estimation of the model parameters in the work
 463 conducted only on Phase I trials. Despite the validation of the model according to its quality of prediction on ad-
 464 ditional data coming from the three Phase II trials (EBL 2001, 2002 and 3001), a re-estimation of the model using
 465 antibody dynamics from the 487 participants was performed to enhance and reinforce our understanding of the
 466 underlying biological processes leading to the long-term immunity following vaccination against Ebola. To account
 467 for the difference in measurements induced by the three distinct ELISA assays performed at Battelle, Focus and
 468 Q² Solutions laboratories, we assumed in the observation model an adjustment for laboratory effects, as shown
 469 on the following equation:

$$Y(t_{ij}) = \log_{10} [\alpha \times Ab(\Psi^i, t_{ij})] + \varepsilon_{ij} \quad \varepsilon_{ij} \sim \mathcal{N}(0, \sigma_{Ab}^2)$$

$$\alpha = \begin{cases} 1 & \text{if } i \in \text{Battelle} \\ \alpha_{focus} & \text{if } i \in \text{Focus} \\ \alpha_{Q2sol} & \text{if } i \in \text{Q}^2 \text{ Solutions} \end{cases} \quad (6)$$

471 with α representing the proportional scaling factor (in natural scale) between the three laboratories, consider-
 472 ing Battelle as the reference, and where the two parameters α_{focus} and α_{Q2sol} are estimated with the five other
 473 biological parameters (Ψ). To ensure their positivity, both of them are assumed to be log-transformed. Further
 474 investigations with other link-functions between Y and Ab were conducted to model laboratory effects, such as a
 475 proportional relationship in log10 scale, or with more complex functions like nonlinear sigmoid functions applied
 476 either in natural or log10 scale. The function leading to the best model (i.e., lowest BICc value) before any covariate
 477 adjustment was kept. We also tested whether the accuracy of the three assays differed using different measure-
 478 ment error models. However, this modeling did not improve the fit and was therefore not retained (result not
 479 shown).

480 Participants from Phase I clinical studies, being monitored only during the first year following the first vaccination,
 481 provided information only on the early phase of the humoral response. In particular, the lack of information on
 482 the long-term immunity made the estimation of the decay rate of the long-lived ASCs difficult. Long-lived ASCs
 483 are persistent plasma cells with a lifespan ranging from several months to the end of an individual's lifetime
 484 (67, 68, 69, 23), therefore only an approximation of the lower bound of the confidence interval of their half-life
 485 ($\log(2)/\delta_L$) was possible. Using additional data from Phase II studies and, in particular, the humoral response

486 measurements beyond 1 year, we performed a profile likelihood to identify whether enough information was
487 available to precisely estimate the parameter δ_L . Considering the statistical model found by Pasin et al. (16), the
488 model was estimated for multiple values of LL ASCs half-life ranging from 1 to 40 years. The profile likelihood was
489 then drawn by maximizing the log-likelihood, computed by importance sampling (60), for each of those related
490 models.

491 As a first estimation, a sequential Bayesian estimation was envisaged, that is using information provided by
492 Phase I studies only through informative prior distribution for parameters. Maximum a posteriori (MAP) esti-
493 mates, corresponding to a penalized maximum likelihood estimation (70), should then be obtained using humoral
494 responses from only the 443 Phase II participants. However, the difference in sampling between Phase I and II
495 studies, in particular the absence of data from 7 to 21 days after the second vaccination for Phase II participants
496 (see Table 2), made estimation of the model difficult. The lack of information at the early stage of the dynamics
497 induced practical identifiability issues for the parameters δ_S and ϕ_S . To tackle this difficulty, all data were used
498 to update the model. Random effects found on Phase I trials were kept, considering inter-individual variability on
499 the parameter δ_{Ab} as well as on the ASCs influx, ϕ_L and ϕ_S .

500 The statistical model was updated by performing a covariate selection. We applied the classic stepwise co-
501 variate modeling (SCM) algorithm (71, 72) which is a stepwise procedure with a forward selection followed by a
502 backward elimination. In the forward selection, each parameter-covariate relationship is tested in turn and the
503 relationship improving the model criteria (a corrected version of the Bayesian information criterion, BICc) the
504 most is kept (the lower the better). Then the addition of a second covariate is tested. In the backward elimination,
505 the removal of each parameter-covariate relationship selected in the first step is tested in an univariate manner.
506 To verify the robustness of the results, two other algorithms of covariate selection in non-linear mixed effects
507 models were performed, using BICc as model selection criteria: 1) the conditional sampling use for stepwise ap-
508 proach based on correlation tests (COSSAC) (72), and 2) the stochastic approximation for model building algorithm
509 (SAMBA) (73). The three algorithms were independently applied on an initial model without any covariates and
510 tested the addition of the seven following potential covariates: Sex (=0 for women and =1 for men), Age, Weight,
511 BMI, Continent (=0 for Africa and =1 for Europe), Region (=0 for East Africa, =1 for West Africa and =2 for Europe)
512 and EBL3001 (=1 for participants from EBL3001 and 0 otherwise). Covariates such as Age, BMI and Weight were
513 centered around the mean value of the studied population (see Table 1). The parameter δ_L , facing some iden-

514 tifiability issues due to the lack of measurements beyond two years, was removed from the covariate selection
 515 procedure. Based on their definition, the parameters α_{focus} and α_{Q2sol} were also excluded from this selection. The
 516 statistical significance of selected covariates was then evaluated using a Wald test. EBL3001 was the only study
 517 which had a follow-up beyond 1 year after the first vaccination and which was conducted in a single country (Sierra
 518 Leone). Therefore, the robustness of the results was analyzed to verify the short term relevance of the selected
 519 covariates. To this end, the same procedure was performed on the model already adjusted for the selected co-
 520 variates but considering only data up to 1 year after the first vaccination. At the end of the covariate selection
 521 procedure, an optimal model was obtained with the following statistical model (see section [Results - Additional](#)
 522 [insight on longevity of the humoral immune response](#) for more details).

$$523 \quad \begin{cases} \log(\phi_L^i) = \log(\phi_{L,0}) + \beta_{\phi_{L,Eur}} \times \mathbb{1}_{i \in Eur} + u_{\phi_L}^i \\ \log(\phi_S^i) = \log(\phi_{S,0}) + \beta_{\phi_{S,Age}} \times (Age_i - \overline{Age}) + u_{\phi_S}^i \\ \log(\delta_{Ab}^i) = \log(\delta_{Ab,0}) + \beta_{\delta_{Ab,Men}} \times \mathbb{1}_{i \in Men} + u_{\delta_{Ab}}^i \end{cases} \quad (7)$$

524 where Age_i and \overline{Age} are respectively the age of the participant i and the average age of the participants and
 525 with $u_{\phi_L}^i \sim \mathcal{N}(0, \omega_{\phi_L}^2)$, $u_{\phi_S}^i \sim \mathcal{N}(0, \omega_{\phi_S}^2)$ and $u_{\delta_{Ab}}^i \sim \mathcal{N}(0, \omega_{\delta_{Ab}}^2)$. Once the optimal model selected, its goodness of fit
 526 was checked and the robustness of the convergence of the estimation was assessed by using the convergence
 527 assessment tool implemented in Monolix which evaluated the robustness of the SAEM algorithm for numerous
 528 initial conditions.

529 The predictive quality of the newly estimated model was assessed by performing a Monte-Carlo cross-validation
 530 (74). Participants from the overall dataset were randomly split into a training and a testing dataset, given a partic-
 531 ular train-test split percentage. We ensure that the same ratio of participants in each trial was maintained within
 532 each of the two sub-datasets. Once the model was fitted on training data, EBEs resulting from this model were
 533 evaluated on test data, followed by the prediction of the individual antibody dynamics. Two criteria were then
 534 calculated on the testing dataset to estimate how accurately the predictive model performs: the percent cover-
 535 age (the higher the better) and the RMSE (the lower the better). For each of the seven train-test split percentages
 536 {20%, 30%, 40%, 50%, 60%, 70%, 80%}, the procedure was replicated 1,000 times.

537 **AVAILABILITY STATEMENT**

538 **Data availability statement** Janssen has an agreement with the Yale Open Data Access (YODA) Project to
539 serve as the independent review panel for the evaluation of requests for clinical study reports and participant-
540 level data from investigators and physicians for scientific research that will advance medical knowledge and public
541 health. Data will be made available following publication and approval by YODA of any formal requests with a
542 defined analysis plan. For more information on this process or to make a request, please visit the Yoda Project
543 site at <http://yoda.yale.edu>. The data sharing policy of Janssen Pharmaceutical Companies of Johnson & Johnson
544 is available at <https://www.janssen.com/clinical-trials/transparency>.

545 **Code availability statement** The original code (mlxtran models and R codes) developed in this work is avail-
546 able and free-of-cost on github (Inria SISTM Team) at the following link: <https://github.com/sistm/ModelingEbola>.
547 [git](#)

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564 **COMPETING INTERESTS**

565 Chelsea McLean and Macaya Douoguih declare no Competing non-Financial Interests but are full-time employees
566 of Janssen Vaccines and Prevention, B.V., and may hold shares of Johnson & Johnson. Viki Bockstal declares no
567 Competing non-Financial Interests but was a full-time employee of Janssen Vaccines and Prevention at the time
568 of the study and may hold shares of Johnson & Johnson. The other authors declare no Competing Financial or
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570 **AUTHOR CONTRIBUTION**

571 R.T. and M.D. coordinated the project and acquired funding, R.T. and M.P. supervised the work and formulated
572 research goals and aims, R.T., M.P. and M.A. designed the methodological framework, M.A. analyzed the data, car-
573 ried out the implementation of the computer code and performed formal analysis, V.B. performed data curation,
574 M.A., M.P. and R.T. wrote the original manuscript. All authors contributed to the writing of the manuscript, read
575 and approved the final manuscript.

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577 **FIGURE LEGENDS**

578 - **Figure 1. Dynamics of Ebola GP-specific binding antibody concentrations, in \log_{10} scale (ELISA units/mL,**
 579 **EU/mL) for each clinical study from 7 days after the second vaccination.**

580 Each subplot represents the antibody dynamics measured in one of the three accredited laboratories: Bat-
 581 telle (left side), Focus (middle) and Q² Solutions (right side). Each color corresponds to a clinical study
 582 (red: EBL1001, dark blue: EBL1003, light blue: EBL1004, orange: EBL2001, turquoise: EBL2002, light green:
 583 EBL3001). Solid and dashed lines represent medians in European and African participants, respectively. Cir-
 584 cles correspond to Phase I studies and triangles to Phase II studies. Error bars correspond to 25th-75th
 585 confidence intervals. The vertical dotted line represents the first year after the first vaccination (309 days
 586 after the 2nd vaccination). The horizontal dot-dashed lines represent the LLOQ values considered for each
 587 laboratory (36.60 EU/mL, 26.22 EU/mL and 36.11 EU/mL, at Battelle, Focus and Q² Solutions, respectively).

588 - **Figure 2. Schematic diagram of the model describing the humoral immune response from 7 days after**
 589 **the 2nd vaccination.**

590 S and L stand for short- and long-lived ASCs, respectively and Ab for antibodies. The parameters δ_S , δ_L
 591 and δ_{Ab} are respectively the decay rates of SL ASCs, LL ASCs and antibodies while θ_S and θ_L represent the
 592 production rates of antibodies by SL and LL ASCs.

593 - **Figure 3. Individual antibody concentrations predicted by the model, estimated on Phase I data, for**
 594 **a random sample of participants from the 6 clinical studies.**

595 Each subplot represents the individual antibody dynamics (in \log_{10} ELISA units/mL) from 7 days after the 2nd
 596 vaccination. For each participant, the vertical dashed line represents the time limit (individual peak of dy-
 597 namics) between the predictions (on the left) and the forecasts (short-term in blue and long-term in orange).
 598 Plain dots correspond to observations used to evaluate individual parameters while circles are observations
 599 not used in parameter estimation. Shaded areas correspond to 95% individual prediction intervals (account-
 600 ing for the uncertainty on the individual parameter estimation and the measurement error) and the solid
 601 lines correspond to the prediction of the model.

602 - **Figure 4. Profile likelihood on parameter δ_L .**

603 The y-axis corresponds to the non-penalized log-likelihood computed by importance sampling for several
604 values of LL ASCs half-life which needs to be maximized. The blue dotted vertical line represents the lower
605 bound of the LL ASCs half-life estimated by profile likelihood by (16) on Phase I data. The red dashed vertical
606 line represents the newly estimated lower bound using both Phase I and II data.

607 - **Figure 5. Individual antibody concentrations estimated by the model for random sample of partici-**
608 **pants from the 6 clinical studies.**

609 Each subplot represents the individual antibody dynamics (in \log_{10} ELISA units/mL) from 7 days after the 2nd
610 vaccination to 2 years. Colored circles correspond to observations used to estimate the model. The thick
611 solid lines correspond to the individual dynamics and the 95% individual confidence intervals (accounting
612 for the uncertainty of the estimation of the individual parameters only) are delimited by the shaded areas.

613 - **Figure 6. Evaluation of the ability of the model to predict unseen data using Monte-Carlo cross-validation.**

614 The predictive quality was assessed by the evaluation of two criteria: the RMSE (left side) and the percent
615 coverage (right side). The x-axis corresponds to the percent of participants randomly selected for the training
616 dataset and the y-axis to the value of criteria calculated on the testing dataset. One hundred replicates were
617 performed for each train-test split percentage. Solid lines display the values of criteria and dashed lines, the
618 95% confidence intervals. The horizontal red dotted line on the right side displays the threshold of 95%.

619 - **Figure 7. Design of EBOVAC 1 (EBL 1001, 1003, 1004 and 3001) and EBOVAC 2 (EBL 2001 and 2002) trials**
620 **for participants receiving Ad26, MVA D57 as vaccine regimen.**

621 Immunogenicity measurements provide the concentration of IgG binding antibodies against Ebola, as mea-
622 sured by ELISA (ELISA units/mL).