Original article

Determinants of antibody persistence across doses and continents after single-dose rVSV-ZEBOV vaccination

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Word count: XXX/3500

Funding: Wellcome Trust through World Health Organization, IMI…

Registration: Trials and observational studies were registered at www.clinicaltrials.gov (Geneva: no. NCT02287480 and NCT02933931; Kilifi: NCT02296983) and Pan-African Clinical Trials Registry (Lambaréné PACTR201411000919191 and XXX).

Abstract

Background The recombinant vesicular stomatitis virus (rVSV) vaccine expressing the glycoprotein of Zaire Ebola virus (ZEBOV) is efficacious in the weeks following single-dose injection, but duration of immunity is unknown.

Methods Participants from the African and European phase I rVSV-ZEBOV trials vaccinated once in 2014-2015 with 300,000 (low dose, LD) or 10 to 50 million (high dose, HD) pfu of rVSV-ZEBOV are followed prospectively for ZEBOV-glycoprotein (GP) IgG antibody persistence; the primary outcome is ZEBOV-GP-specific IgG geometric mean concentrations (GMCs) measured yearly by ELISA, intrinsically and compared to one month after immunisation. We report GMCs at two years (Geneva, Switzerland) and one year (Lambaréné, Gabon; Kilifi, Kenya) after vaccination as well as factors associated with higher titres and persistence of seropositivity.

Findings Among 217 vaccinees (n=102, 75 and 40 in Geneva, Lambaréné and Kilifi, respectively), 197 returned at one year (n=95, 63 and 39) and 89 at two years (Geneva). Most (208/217, 97%) had seroconverted by day 28, with the remainder seroconverting at month 2-3. Day 28 seroconverters had a high probability (>92%) of remaining seropositive. Follow-up ZEBOV-GP IgG GMCs decreased slightly after their early peak but did not differ significantly from day 28 GMCs at any site, reaching similar values in LD and HD vaccinees. Predictors of higher GMCs included vaccine-related arthritis among LD vaccinees (p=0·0198), but not gender or age.

Interpretation Antibody responses to single-dose rVSV-ZEBOV vaccination are sustained across dose ranges and settings.

Funding The Wellcome Trust (through World Health Organisation) and Innovative Medicines Initiative.

Introduction

The live-attenuated recombinant vesicular stomatitis virus (rVSV) vaccine expressing the glycoprotein of Zaire Ebola virus (ZEBOV, rVSV-ZEBOV) has been granted Breakthrough Therapy Designation status by the US Food and Drug Administration and PRIME status by the European Medicines Agency after proving highly immunogenic[1,2] and efficacious[3] in the weeks following single-dose, post-exposure injection. The durability of the immune response to this single injection over longer periods and in Ebola-endemic areas, however, is yet undefined. Its importance increases as additional outbreaks occur[4] and more information is amassed on the persistence of Ebolavirus itself within human hosts: replication-competent Ebolavirus has been isolated from clinical specimens as many as nine months after transmission,[5] and viral RNA has been detected up to two years thereafter.[6] Indeed, the recent Wellcome Trust-CIDRAP Ebola Vaccine Team B initiative recommends that any vaccine used for immunisation of disease contacts should induce protection lasting at least 2 years.[7] This is challenging and has not yet been reported for rVSV-ZEBOV; antibody titres dropped in all participants already 6 months after a single dose of the recombinant chimpanzee adenovirus 3 (rChAd3)-ZEBOV vaccine.[8]

Despite the demonstration of field effectiveness of post-exposure rVSV-ZEBOV injection,[3] correlates of vaccine-induced protection against Ebolavirus disease (EVD) remain undefined: the WHO-sponsored Guinea ring vaccination trial documenting that field efficacy[3] did not harvest blood for immunogenicity analyses, and the mechanisms conferring protection in non-human primate (NHP) models have not yet been demonstrated to be the same in humans.[9] Although the identification of those immune mediators is only in its early phase, passive antibody transfer protects naïve NHP against lethal EBOV challenge,[10] and such antibodies are required for protection.[11] In humans, rVSV-ZEBOV-induced antibodies likely to contribute to protection include *in vitro* neutralising antibodies to the G protein.[12] Thus the presumed durability of rVSV-ZEBOV-induced protection is currently best estimated by antibody persistence in vaccinees.

Beginning in 2014, the VSV-Ebola Consortium (VEBCON)[1] conducted a large (n=115) phase I/II randomised, placebo-controlled trial in Geneva, Switzerland[1,2,13] with parallel dose-escalation trials in Lambaréné, Gabon (n=75), and Kilifi, Kenya (n=40);[1] early immunogenicity results of these investigator-initiated trials have been reported.[1,2] Thereafter vaccinees were invited to participate in prospective observational studies to determine antibody persistence. Here we present persistence data at one year for all three sites and at two years for Geneva vaccinees; we further explore factors associated with sustained or waning antibody concentrations.

Methods

Study designs, settings and populations

The phase I trials conducted in Geneva, Lambaréné and Kilifi healthy adults have been described extensively elsewhere (clinicaltrials.gov [NCT02287480 and NCT02296983] and Pan African Trials Registry [PACTR201411000919191]).[1,2] These studies were extended to 12 months through extra funding (Innovative Medicines Initiative [IMI]), followed in Geneva and Lambaréné by the implementation of four-year prospective observational follow-up studies (registered at clinicaltrials.gov [NCT02933931, Geneva; XXXXXXXXXXX, Lambaréné]). We report here antibody concentrations at one and two years, as compared to early (day 28 post-rVSV-ZEBOV vaccination) results to define persistence or waning.

In Geneva and Lambaréné, all adults who (1) participated in the phase I trials and (2) received a single dose of either 300,000 or 3, 10, 20 or 50 million plaque-forming units (pfu) of rVSV-ZEBOV according to protocol were eligible for follow-up in the prospective observational studies. As the randomised Geneva phase I trial had revealed no differences in reactogenicity, viraemia, nor early immunogenicity after vaccination with 10 to 50 million pfu,[1,2] Geneva volunteers receiving these doses were again grouped as “high-dose” (HD) participants.

The per-protocol (PP) follow-up population consists of volunteers from the three sites who adhere to the studies' protocols (*e.g.*, do not undergo further rVSV-ZEBOV vaccination) and who have no suspected or documented clinical exposure to *Ebolavirus* throughout the study period.

Laboratory analyses

Samples from all studies were frozen at -20˚C before transfer to the immunology laboratory at the US Army Medical Research Institute for Infectious Diseases (USAMRIID) in Frederick, Maryland, USA. ZEBOV-GP-specific antibodies were quantified by ELISA using the homologous Zaire-Kikwit strain glycoprotein following USAMRIID's standard operating procedure (SOP AP-03-35-00; USAMRIID ELISA).[1] To improve inter-assay comparisons, the relative amounts of ZEBOV-GP-specific antibodies previously reported as endpoint titres[1,2] were expressed as arbitrary ElU/mL compared to a common standard. These values were log (base 10) transformed and reported as the geometric mean concentrations (GMC) of arbitrary ElU/mL with 95% CIs, as indicated.

Outcomes and definitions

The primary follow-up outcome is ZEBOV-glycoprotein (GP)-specific GMC measured by ELISA at yearly time-points, as compared to one month after immunisation ("baseline"). Seropositivity after vaccination was experimentally defined by a ZEBOV-GP-specific IgG antibody titre ≥58·84 arbitrary ELISA units per mL (ElU/mL) (SOP AP-03-35-00; USAMRIID ELISA). In the absence of established correlates of protection, antibody persistence was arbitrarily defined by (1) the maintenance of seropositivity and/or (2) the ratio of anti-ZEBOV IgG GMCs at a given follow-up time point compared to Day 28.

Statistical analysis

Sample size: The sample size of the three follow-up cohorts was not calculated but pre-determined by the number of vaccinees (n= 102, 75 and 40 adults in Geneva, Lambaréné and Kilifi, respectively), and the defined eligibility criteria.

Analyses: GMCs of ZEBOV-GP-specific IgG antibodies and 95% confidence intervals were calculated for all volunteers with available data. Given the USAMRIID ELISA assay's limit of quantification of 58·84 ElU/mL, titres below this value were arbitrarily assigned a 50% value of 29·42 ElU/mL for statistical analyses. Comparisons between dose groups were performed with the Kruskal-Wallis test; pairwise comparisons were conducted with the Mann-Whitney test. Mean fold increases and decreases at years 1 and 2 were assessed (in comparison with values at days 28, 84, and 168); equality of fold increases was analysed via the Friedman test. Seropositivity persistence was assessed at each specified time point beyond day 28 and is reported with 95% confidence intervals. Comparisons between intervention groups use the chi-squared or Fisher's exact test for categorical data. Univariate logistic and linear regression models were conducted to assess associations between antibody persistence and vaccine dose, age, gender, ethnicity/setting, post-vaccination arthritis, and baseline seropositivity.

Role of the funding source

The funders of the early phase I trials (Wellcome Trust Foundation) and of the follow-up trials (Innovative Medicines Initiative) had no role in the study design, data collection, data analysis or data interpretation, or writing of the report. The corresponding author had full access to all study data and had final responsibility for the decision to submit for publication.

Results

Baseline demographic, clinical and immunologic characteristics

Volunteers' baseline demographic, clinical and immunological characteristics are described in Table 1, and flow from the phase 1 to the observational studies is depicted in Figure 1.

Among the 102 Geneva vaccinees, two had been lost to follow-up and one had received an extra-protocol, additional rVSV-ZEBOV injection before the six-month visit. Thus 99 volunteers were invited to participate in the observational study. Attendance at the one- and two-year visits was 95/99 (96%) and 89/99 (90%), respectively, with the remaining participants unavailable or unreachable on these occasions. At the two-year visit, 45/89 (51%) were LD vaccinees and 44/89 (49%) HD vaccinees. Most (95/99, 96%) Geneva vaccinees were seronegative pre-vaccination (Table 1 & Figure 1).

Among 75 Lambaréné adult phase I participants, 20, 39, and 16 had received 300,000 pfu, 3 or 20 million pfu, respectively. A total of 68 had completed their six-month visit and were invited for further follow-up: 63/68 (93%) completed their one-year visit, with the remainder lost to follow-up. Baseline seropositivity rates were much higher (17/68, 25%) in this Ebola-endemic area. In the Kilifi phase I trial, 40 had completed the six-month visit and 39/40 (98%) vaccinees (3 million pfu: 20; 20 million pfu: 19) completed their one-year visit (one lost to follow-up); only 1/39 (3%) was seropositive at baseline.

Early and follow-up (≥1 year) seropositivity following vaccination

Early seroresponses were dose-dependent at all sites. In Geneva, 48/48 (100%) HD vaccinees but only 41/51 (80%) LD vaccinees were seropositive on day 28 (p=0·001). Similarly, in Lambaréné, all HD vaccinees but only 17/20 (85%) LD vaccinees were seropositive on day 28 (p=0·025). In Kilifi, all vaccinees (3 or 20 million pfu) were seropositive by day 28. Thus, the vaccine dose significantly influenced early (D28) antibody responses, as described by us[1,2] and others.[14]

In the absence of established correlates of protection, seropositivity was taken as a first low-stringency marker for the persistence of vaccine-induced responses. Seropositivity rates at one and two years post-vaccination did not differ significantly among dose groups (Table S1). In volunteers vaccinated with 3, 10 or 50 million pfu, seropositivity persisted at high levels (Geneva: 43/44 [98%] at 2 years *vs* 48/48 [100%] at day 28, p=0·616; Lambaréné: 52/52 (100%); Kilifi: 20/20 (100%) at 1 year). Delayed responses were occasionally observed in volunteers vaccinated with 300,000 pfu (Geneva: from 41/51 (80%) at day 28 to 42/45 (93%) at two years (p=0·0644); Lambaréné: from 17/20 (85%) at day 28 to 15/16 (94%) at one year (p=0·4065), Table S1). Thus, seropositivity rates remained very high and their dose-dependency was lost as of one year post-vaccination.

Predicting long-term persistence of seropositivity

In Geneva, HD and LD vaccinees with early (D28) seropositivity had a 98% and 92% probability, respectively, of remaining seropositive in the following two years (p=0·3310). Table S2 further details the similarly high probabilities of seropositivity persistence for Geneva, Lambaréné and Kilifi.

Induction and persistence of ZEBOV-IgG GMCs

Unless protection only requires very low antibody concentrations, persistence of seropositivity is an unlikely correlate of protection. At the other extreme, maintenance of the GMCs seen at day 28, at which point a high-dose of rVSV-ZEBOV (20 million pfu) was effective in a post-exposure setting,[3] should be sufficient. Figure 2 illustrates and Table 2 lists GMCs for each dose group at each site over time, and Tables S3-5 provide GMC ratios across various time points. GMCs peaked between 1 and 3 months after vaccination in all dose groups. Baseline seropositivity, frequent in Lambaréné, did not influence follow-up GMCs compared to seronegative volunteers receiving the same dose, there or elsewhere (Figure 2 and Table 2). In all sites and across all doses, GMC peaks were followed by only mild, gradual decreases over time (Figures 2). Geometric mean concentrations plateaued between six and 12 months, and two years post-vaccination, GMCs among LD vaccinees in Geneva were not significantly lower than those of HD vaccinees (442·9 units/ml [95% CI 299·1—655·8] *vs* 661·3 units/ml [459·7—951·4], p=0·11; Table 2 and Figure 2).

Because comparing GMCs may mask antibody disappearance in a subset of subjects, individual values are illustrated at each time point in Figure S1, which indicates that the lower D28 GMCs in LD (300,000 pfu) vaccinees are due to a slower response in some volunteers to this low vaccine dose, while higher doses (≥ 3 million pfu) induce prompter responses. Further, antibody loss is rare after 12 months: it was observed in 3/45 (7%) LD and 1/44 (2%) HD Geneva vaccinees.

Determinants of antibody persistence

Univariate analyses detected a trend toward higher GMCs at one year in females at each site, though statistical significance was not reached (Table S6). No significant associations could be detected between age and GMCs (Table S7).

In the six months following vaccination, two Geneva volunteers with early arthritis experienced suspected, self-limited recurrences of arthritis.[1] Since then, no episodes were reported or detected. Yet the occurrence of post-vaccination arthritis was strongly associated with higher ZEBOV-GP-specific IgG GMCs throughout the two-year follow-up period (Figure 3). The association was most marked in the LD group: GMCs of those with arthritis were two- to threefold higher than those without arthritis at every time point, and by two years, these vaccinees had higher GMCs than those of HD vaccinees who never experienced arthritis (910·2 ElU/mL [95%CI 472.6-1753·1] *vs* 662·8 ElU/mL [95%CI 468·3-938·0], p=0·3434; Figure 3 and Table S8). High-dose volunteers with arthritis also transiently (day 28) achieved two-fold higher GMCs *vs* HD volunteers without, but this effect was not significant (1550·6 ElU/mL [95%CI 719·3-3342·9] *vs* 828·0 ElU/mL [566·0-1211·4], p=0·0780) and by two years, GMCs in the two groups had become identical (Figure 3, Table S8). A multivariate linear regression model constructed to investigate interactions between vaccine dose and arthritis did not reveal a significant difference in the GMC ratios of those with *vs* those without arthritis (p=0·1118) between LD and HD groups (Table S9). In other words, the presence of arthritis essentially "erased" the influence of the vaccine dose on antibody responses.

Discussion

The humoral response induced by a single injection of the replication-competent rVSV-ZEBOV vaccine persists for at least 1 to 2 years across continents and doses, with kinetics enabling the prediction of long-term antibody persistence in most vaccinees. The Geneva data indicate a 98% probability for early responders (*i.e.,* all participants receiving doses ≥10 million pfu) to remain seropositive at two years, with similar patterns in two African countries. Even at the lowest dose of 300,000 pfu, seroconverters had a 92% chance of remaining seropositive two years later. Thus, a single injection of rVZV-ZEBOV induces sustained antibody responses in almost all vaccinees.

The kinetics of ZEBOV-GP-specific IgG antibodies are classic for live-attenuated vaccines: after an early peak at two to three months after single-dose vaccination, during which ZEBOV-GP-specific antibodies are likely produced by short-lived plasmablasts,[15] antibody concentrations follow a weak slope, decreasing only mildly and gradually, with no significant differences in GMCs between 28 days and up to one or two years after vaccination. This reflects an effective switch from short-lived to long-lived plasmablasts following rVSV-ZEBOV immunization[15] and provides an immunological basis for potential long-lasting protection.

Protective humoral responses to natural, systemic viral infections can be extremely sustained, even lifelong: circulating, antigen-specific antibodies have been detected in patients as many as 65 and 75 years after measles and yellow fever infections with no interim exposure, respectively.[16,17] Some replication-competent vaccines appear to induce similar lifelong humoral immunity; antigen-specific IgG has been measured at consistent concentrations several decades after single-dose injection with the smallpox[18] and yellow fever[19] vaccines in patients without pathogen exposure. The present results allow some degree of cautious optimism regarding the long-term persistence of antibody responses. This does not reflect a strong immunogenicity of the G protein of Ebolavirus, a rather weak immunogen, as evidenced by the rapid disappearance of G-specific antibodies following a single dose of the same protein delivered by the rChAd3 vector.[8] We therefore presume that the sustainability of humoral responses to rVSV-ZEBOV essentially results from the strong influence of

rVSV on early immune responses[20]. This is of interest as it may similarly confer rVSV the capacity to induce sustained responses to glycoproteins from other emerging viruses such as Nippah or Lassa.[21]

The influence of rVSV is dose-dependent: vaccine doses below 3 million pfu, which induce lower cytokine responses,[20] lead to weaker early (D28) antibody responses.[1,2,14] But this may be a delay rather than a limitation: by one year, GMCs in Lambaréné and Geneva LD vaccinees resemble those of participants immunised with doses ten- and a hundred-fold higher, and by two years (Geneva) GMCs in LD vaccinees were 1.5-fold higher than on day 28. Thus, while high vaccine doses may contribute to early post-exposure protection, lower (less reactogenic[1,2,20]) doses could be attractive should preventive campaigns be considered necessary. We postulate that the induction of delayed but higher humoral responses by lower doses of rVSV-ZEBOV results from a facilitated escape of the vaccine load from lower early antiviral responses,[20] enabling longer antigen persistence/immune responses.

The same mechanism may be at play in vaccinees with arthritis. We have previously reported the onset of arthritis in the second week after immunisation in a significant proportion (>20%) of LD and HD Geneva vaccinees, an observation that remained much rarer at other sites. We subsequently showed that HD (but not LD) vaccinees with arthritis had significantly weaker cytokine/chemokine responses than their counterpart.[20] We now show that vaccine-induced arthritis is associated with significantly higher GMCs, especially in LD vaccinees, in whom the occurrence of arthritis was dose-independent. Low-dose *vs* HD vaccinees and HD vaccinees with *vs* without arthritis have in common weaker cytokine/chemokine responses than their respective counterparts. Thus, vaccine escape and persistence may contribute to the onset of rVSV-ZEBOV-induced arthritis and result in longer antibody responses, with the “benefit” of prolonged antigen presentation contributing less in HD than in LD vaccinees.

This report has limitations. Not all vaccinees were available for later-phase sampling, but missing samples are few. The immune correlate of protection against EVD has not been defined. IgM vaccine

antibodies were shown to contribute to *in vitro* virus neutralization in vitro, but over time they are replaced by IgG responses.[12] Should ZEBOV-GP-specific IgG concentration correlate with protection, the protective concentration remains unknown. We addressed this limitation by using low-stringency (seropositivity) and high-stringency (GMC ratio compared to day 28, at which protection has been observed) markers. Although a protective threshold would likely rank somewhere in between, the use of both markers generate the same conclusion: the overall durability of the humoral response in the two years following "one-shot" rVSV-ZEBOV vaccination. Given the logistic challenges inherent to vaccine campaigns in Ebola-endemic regions, the importance of single-injection vaccination goes beyond mere convenience.

Contributors

AH, STA, JFF,BL, SG, … and CAS collected the data. AH, STA, CC, JFF, FN, JB, BL, FA, SG, PB, PS, PK and CAS analysed and interpreted the data. AH, STA, the VEBCON and VSV-EBOLA consortia, PK and CAS made substantial contributions to the conception and design of the study. AH, CC and CAS wrote the report. All authors contributed to the revision of the report.

Declaration of interests

TM … All other authors declare no conflicts of interest.

Acknowledgments

We thank all volunteers for their generous participation in the phase I trials and follow-up studies; Wellcome Trust and IMI/SEFRI for funding; the team of the Clinical Trials Unit of the HUG–Hôpitaux Universitaires de Genève, including Prof Jules Desmeules and Dr Alain Matthey; Dr Julie-Anne Dayer; Prof Laurent Kaiser for continuous support; Beth-Ann Coller and Rituparna DAS (Merck); …

USAMRIID's research was funded by MCS-JVAP workplan B.30 and B.31 under USAMRIID project numbers 18836631, 17336629, and 17336630. Opinions, interpretations, and conclusions expressed in this manuscript are those of the authors and are not necessarily endorsed by the US Army.

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Table 1. Demographic, clinical and immunologic characteristics at follow-up enrolment (month 6 after immunisation).

	All	Geneva, Switzerland			Lambaréné, Gabon			Kilifi, Kenya	
Dose received		**300,000 pfu**	**10 million pfu**	**50 million pfu**	**300,000 pfu**	**3 million pfu**	**20 million pfu**	**3 million pfu**	**20 million pfu**
Group size at vaccination, n	217	51	35	16	20	39	16	20	20
Group size at one year, n	197	49	32	14	16	35	12	20	19
Age, years	XX	40±12	42±11	43±14	29±8	27±7	25±6	34±7	34±8
Sex									
Female (%)	67 (31)	27 (53)	14 (40)	5 (31)	6 (30)	4 (10)	1 (6)	6 (30)	4 (20)
Male (%)	150 (69)	24 (47)	21 (60)	11 (69)	14 (70)	35 (90)	15 (94)	14 (70)	16 (80)
Ethnic origin									
Black	114 (53)	0 (0)	2 (6)	0 (0)	20 (100)	39 (100)	16 (100)	17 (85)	20 (100)
White	98 (45)	51 (100)	29 (83)	15 (94)	0 (0)	0 (0)	0 (0)	3 (15)	0 (0)
Other	5 (2)	0 (0)	4 (11)	1 (6)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Clinical characteristics									
Vaccine-related arthritis (%)	25 (12)	13 (25)	8 (23)	3 (19)	0 (0)	0 (0)	0 (0)	1 (5%)	0 (0)
Immunologic characteristics									
Pre-vaccination seropositivity (%)	22 (10)	1 (2)	2 (6)	1 (6)	2 (10*)	8 (21)	7 (44)	0 (0)	1 (5)
Day 28 seropositivity (%)	208 (97)	48 (80)	34 (100*)	14 (100*)	17 (85)	39 (100)	16 (100)	20 (100)	20 (100)

*A sample from one volunteer in each of these dose groups was not available on this visit.

Table 2. Geometric mean concentrations of ZEBOV-GP-specific antibodies across doses and studies.

	Doses	N **Day 0 GMC** 95% CI	N **Day 28 GMC** 95% CI	N **Day 56 GMC** 95% CI N	N **Day 84 GMC** 95% CI N	N **Month 6 GMC** 95% CI N	N **Year 1 GMC** 95% CI N	N **Year 2 GMC** 95% CI N
Geneva	**300,000 pfu**	51 **30·0** 28·9-31·1 *0.1882**	51 **267·4** 175·3-407·9 ***<0.0001****	ND	50 **824·9** 576·7-1179·9 ***0.0062****	51 **583·9** 407·5-836·5 *0.1898**	49 **589·8** 400·3-869·0 ***0.0191****	45 **442·9** 299·1-655·8 *0.1080**
	10 million pfu	35 **32·6** 28·2-37·7	34 **821·2** 579·7-1163·5	ND	33 **1332·1** 955·9-1856·5	34 **747·6** 539·1-1036·9	32 **1029·3** 719·3-1472·9	31 **622·6** 409·2-947·2
	50 million pfu	16 **31·9** 28·4-36·0	14 **1383·0** 996·0-1920·4	ND	16 **1993·7** 1547·9-2567·8	15 **865·7** 630·6-1188·6	14 **1037·9** 804·2-1339·6	13 **763·7** 614·3-949·4
	10 or 50 million pfu	51 **32·4** 28·2-37	48 **956·1** 680·1-1344	ND	49 **1591·6** 1118·6-2064·3	49 **782·6** 521·7-1069·6	46 **1031·9** 749·1-1421·6	44 **661·3** 469·7-951·4
Lambaréné	**300,000 pfu**	19 **33·7** 27·6-41·1	20 **540·2** 254·4-1146·7	17 **809·9** 355·3-1846·1	17 **654·0** 333·1-1283·9	16 **375·2** 189·9-741·2	16 **505·9** 269·3-950·5	ND
	3 million pfu	39 **40·6** 29·2-56·5	39 **1245·0** 778·4-1991·2	37 **1330·7** 829·7-2134·1	35 **994·0** 629·0-1571·0	37 **684·6** 479·6-977·2	35 **638·2** 462·6-880·6	ND
	20 million pfu	16 **69·1** 39·7-120·2	16 **1503·0** 943·6-2394·0	13 **2589·5** 1625·2-4126·0	14 **1825·7** 1133·6-2940·2	15 **1514·4** 972·1-2359·3	12 **1496·3** 620·9-3605·7	ND
Kilifi	**3 million pfu**	20 **34·0** 25·1-46·2	20 **1005·2** 655·2-1542·1	20 **1054·9** 721·6-1542·3	20 **1018·9** 711-1460·2	20 **756·9** 520·1-1101·5	20 **667·9** 484·7-920·2	ND
	20 million pfu	18 **29·4** 29·4-29·4	20 **785·3** 571·8-1078·7	20 **944·6** 625·8-1425·7	20 **946·8** 695·5-1288·9	20 **877·9** 625·7-1231·9	19 **1083·1** 766·8-1529·8	ND

n: number of samples available at this time point; ND: not done

* Comparison of GMCs between low-dose (300,000 pfu) and high-dose (10 or 50 million pfu) vaccinees from the Geneva randomised controlled trial

Figure 1. Study flowchart.

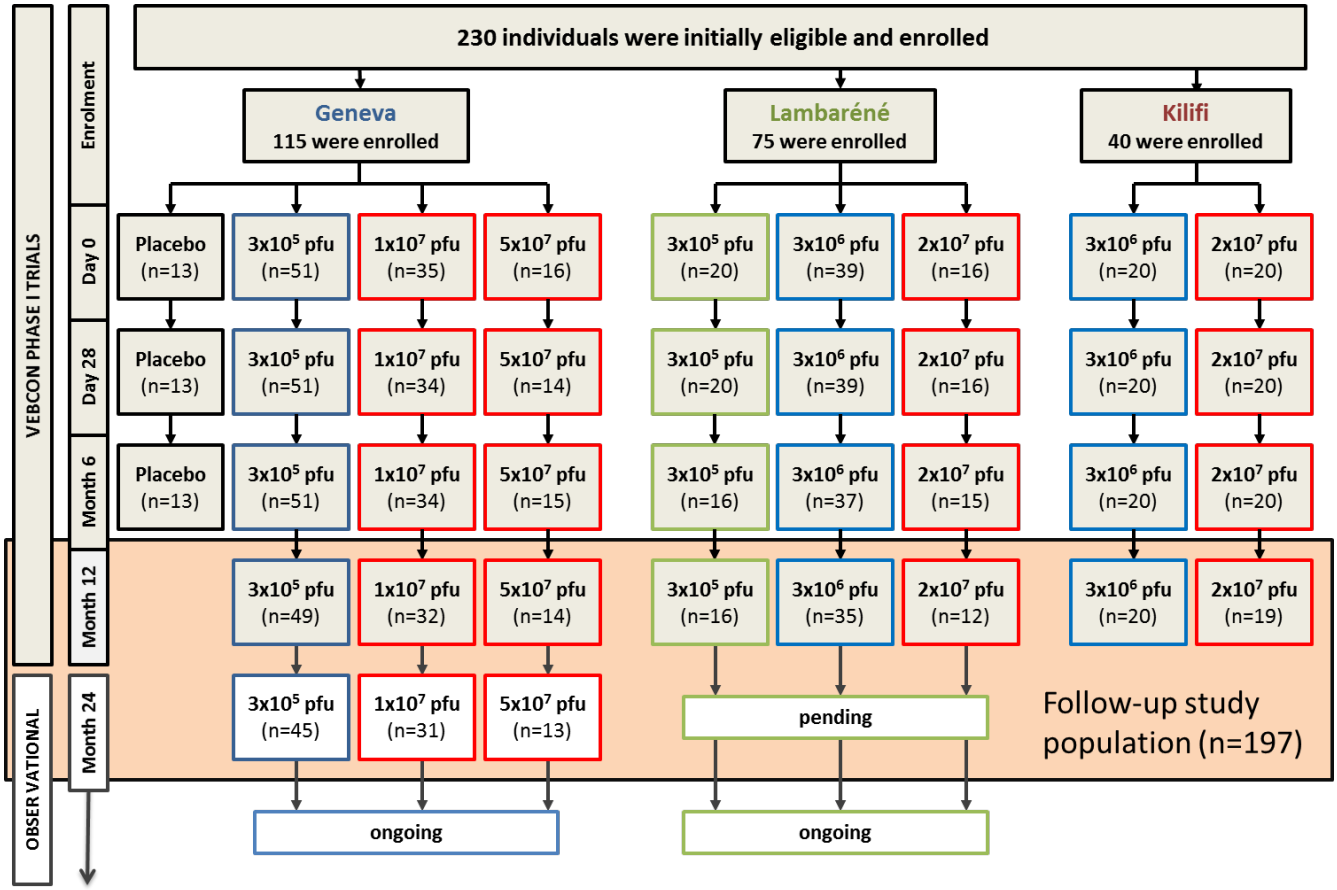

Figure 2. Geometric mean concentrations (with 95% confidence intervals) of ZEBOV-GP-specific antibodies in (a) Geneva, (b) Lambaréné, and (c) Kilifi.

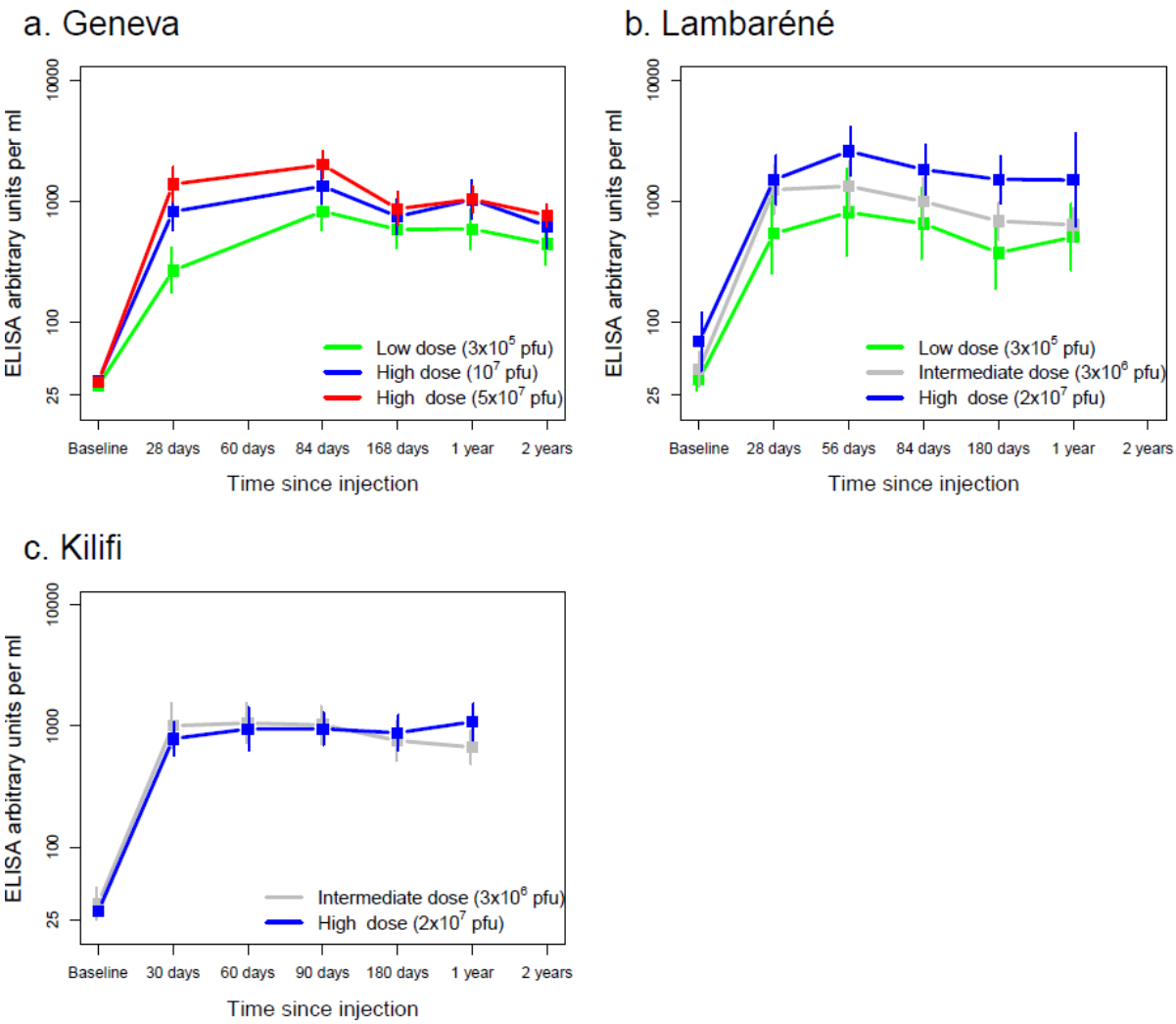

Figure 3. Geometric mean concentrations of ZEBOV-GP-specific antibodies in Geneva volunteers with and without post-vaccination arthritis by low (300,000 pfu) and high (10 or 50 million pfu) dose groups.