Immunogenicity of rVSVΔG-ZEBOV-GP Ebola Vaccine (ERVEBO™) in African Clinical Trial Participants by Age, Sex, and Baseline GP-ELISA Titer: A *Post Hoc* Analysis of Three Phase 2/3 Trials

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and Research Council of Norway. The PREVAIL trial was supported by the National Institute of Allergy and Infectious Diseases and the Liberian Ministry of Health.

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Tables: 1

Highlights

1. In 3 phase 2/3 trials in Africa, vaccination with ERVEBO™ yielded robust immune responses.
2. Baseline seropositive participants had higher binding antibody concentrations postvaccination.
3. There was no association between immune response and age.
4. The immune response was robust & durable regardless of sex, age, or pre-existing antibody level.

Abstract

Background ERVEBO®, a live recombinant vesicular stomatitis virus (VSV) vaccine containing the *Zaire ebolavirus* glycoprotein (GP) in place of the VSV GP (rVSVΔG-ZEBOV-GP), was advanced through clinical development by Merck & Co., Inc., Rahway, NJ, USA in collaboration with multiple partners to prevent Ebola virus disease (EVD) and has been approved for human use in several countries.

Methods We evaluated data from three Phase 2/3 clinical trials conducted in Liberia (PREVAIL), Guinea (FLW), and Sierra Leone (STRIVE) during the 2013-2016 West African EVD outbreak to assess immune responses using validated assays. We performed a *post hoc* analysis of the association of vaccine response with sex, age (18-50 yrs & >50 yrs), and baseline (BL) GP-enzyme-linked immunosorbent assay (ELISA) titer (<200 & ≥200 EU/ml), including individual study (PREVAIL, FLW, or STRIVE) data and pooled data from all 3 studies. The endpoints were total IgG antibody response (EU/mL) measured by the GP-ELISA and neutralizing antibody response measured by the plaque reduction neutralization test (PRNT) to rVSVΔG-ZEBOV-GP at Days 28, 180, and 365 postvaccination.

Results In the overall pooled population, in all subgroups, and in each trial independently, GP-ELISA and PRNT geometric mean titers increased from BL, generally peaking at Day 28 and persisting through Day 365. Immune responses were greater in women and participants with BL GP-ELISA ≥200 EU/ml, but did not differ across age groups.

Conclusion These data demonstrate that rVSVΔG-ZEBOV-GP elicits a robust and durable immune response through 12 months post vaccination in participants regardless of age, sex, or

BL GP-ELISA titer. The higher immune responses observed in women and participants with preexisting immunity are consistent with those described previously and for other vaccines.

Trials were registered as follows: PREVAIL: ClinicalTrials.gov NCT02344407; FLW: Pan African Clinical Trials Registry PACTR201503001057193; STRIVE: ClinicalTrials.gov NCT02378753. Protocols V920-009, 011, and 018.

Key Words Ebola, vaccine, immunogenicity

Background

Ebola virus disease (EVD) is a rare, acute illness with a mortality rate ranging from 25% to 90%.[1] When used early, comprehensive medical care can improve the chances of survival.[1] In late 2020, the US Food and Drug Administration approved two biologicals for the treatment of EVD, Inmazeb (REGN-EB3), a cocktail of 3 monoclonal antibodies that target the glycoprotein on the surface of *Zaire ebolavirus*[2]; and Ebanga (Ansuvimab-zykl), a human monoclonal antibody that blocks binding of the virus to the cell receptor, thereby preventing entry into the cell.[3]

Although preventive measures such as avoiding direct contact with EVD-infected individuals and contaminated body fluids, are effective ways to prevent infection and stop the spread of EVD,[4] vaccination is an essential component of the public health response to outbreaks.[5] ERVEBO™, a live recombinant vesicular stomatitis virus (VSV) vaccine containing the *Zaire ebolavirus* glycoprotein (GP) in place of the VSV GP (rVSVΔG-ZEBOV-GP), was advanced through clinical development by Merck & Co., Inc., Rahway, NJ, USA in collaboration with multiple partners to prevent EVD. rVSVΔG-ZEBOV-GP has been approved for human use in several countries[6] based on high efficacy demonstrated in a ring-vaccination trial conducted in Guinea[7] and has been shown to be generally safe and well-tolerated with most adverse events reported as mild to moderate in the general population.[6]

An individual's immune response to a vaccination may be affected by characteristics such as their sex, age, and comorbid conditions, as well as factors such as preexisting immunity (e.g., due to prior infection or cross-reactive antibodies), concomitant medications, microbiota, or other behaviors (e.g., smoking, diet, and alcohol consumption) (reviewed in [8]). It has been observed that women may develop higher antibody responses to some vaccines compared with

men, including vaccines such as influenza, measles/mumps/rubella (MMR), Hepatitis A and B, Herpes virus, and yellow fever (reviewed in [9]). Additionally, women may report a higher number of adverse events associated with vaccinations, although the reasons for these differences have not been well-defined.[10] Certain populations may experience differences in efficacy and immunogenicity of vaccines based on immunosenescence,[11-13] the existence of pre-existing antibodies resulting from prior infection, vaccination with an antigenically similar virus, or due to the presence of maternal antibodies (reviewed in [14]). The objective of this post hoc analysis was to assess the immunogenicity of rVSVΔG-ZEBOV-GP in subgroups by sex, age, and pre-existing antibody level using Phase 2/3 clinical trial data (presenting individual study data side by side as well as pooled for the three studies)[15-17].

Methods

Study designs

Data were assessed from three Phase 2/3 clinical trials conducted during the 2013-2016 West African EVD outbreak. The Partnership for Research on Ebola Virus in Liberia trial (PREVAIL) was a randomized, placebo-controlled, Phase 2 trial in adults to evaluate safety and immunogenicity of two vaccines: a replication defective chimpanzee adenovirus 3 vector vaccine expressing *Zaire ebolavirus* glycoprotein (ChAd3-EBO-Z) and rVSVΔG-ZEBOV-GP.[16] PREVAIL randomized participants in Liberia from January 2015 to June 2016 with a 60-month follow-up period. The Front Line Worker Trial (FLW) trial was an open-label, non-randomized, single arm safety and immunogenicity trial conducted in Conakry, Guinea that enrolled front-line healthcare workers between March 2015 and July 2016, including personnel working in Ebola or non-Ebola health facilities and services.[15] The Sierra Leone Trial to Introduce a Vaccine Against Ebola (STRIVE) was a randomized, open-label, Phase 2/3 single-arm trial with phased

vaccine introduction, no placebo, and concurrent evaluation of vaccine safety and efficacy conducted in Sierra Leone. Healthcare and frontline response workers in 5 districts were randomized to immediate vaccination (within 7 days of enrollment (April 2015 through August 2015) or deferred (18–24 weeks later; September 2015 through December 2015) and followed for 6 months postvaccination to assess vaccine safety and efficacy.[18] The STRIVE immunogenicity substudy was conducted at one site (Connaught Hospital, Freetown) and enrolled participants from June 2015 through Sept 2015.

The trials included in this analysis were conducted in accordance with the International Conference on Harmonization (ICH), good clinical practice requirements, and applicable country and/or local statutes and regulations regarding ethical committee review, informed consent, and the protection of human participants in biomedical research. Participants in each trial provided written informed consent prior to any procedures being conducted. Trials were registered as follows: ClinicalTrials.gov NCT02344407; Pan African Clinical Trials Registry PACTR201503001057193; ClinicalTrials.gov NCT02378753.

Procedures

Immune responses in each of the three studies were assessed as previously described using validated GP-ELISA and PRNT assays. The GP-ELISA was developed by the US Army Medical Research Institute of Infectious Diseases and Filovirus Animal Nonclinical Group [15-17]. The GP-ELISA was qualified and validated and all testing described was conducted post-validation of the assay.[19] The PRNT assay was established and validated at Q2 Solutions as previously described.[17] All clinical samples were gamma irradiated with 50 kGy using a standardized process prior to clinical testing to minimize possible Ebola virus exposure risk while handling EVD samples under clinical laboratory biosafety level-2 containment. Compared with non-

irradiated samples, gamma irradiation was associated with an approximate 20% increase in antibody level in GP-ELISA responses for negative clinical sera (i.e., pre-vaccination) and an approximate 20% decrease in antibody level in GP-ELISA responses for positive clinical sera (i.e., postvaccination) and an approximate 20% decrease in antibody titer units in PRNT responses postvaccination.[20,21] Endpoints were total IgG antibody response to rVSVΔG-ZEBOV-GP measured by the GP-ELISA (ELISA units per milliliter [EU/mL]) and neutralizing antibody response to rVSVΔG-ZEBOV-GP measured by the plaque reduction neutralization test (PRNT) at Days 28, 180, and 365 postvaccination.

Statistics

Analyses were conducted under the assumption that data from studies using: 1) the same vaccine (rVSVΔG-ZEBOV-GP); 2) same nominal dose of 2×10^7 pfu; 3) same single intramuscular injection vaccination schedule; and 4) populations from West Africa of individuals ≥18 years of age with similar baseline and clinical characteristics can be pooled. In addition, data from each study[15-17] were evaluated individually as a subgroup in the analysis and shown for comparison. The primary immunogenicity populations from the PREVAIL, FLW, and STRIVE trials comprised the full analysis set (FAS) population, which included all rVSVΔG-ZEBOV-GP-vaccinated participants with serology data who had a serum sample collected within an inclusive day range of approximately 1-3 weeks. Participants with missing or out-of-day range assays were excluded by time point. Participants from PREVAIL receiving the ChAd3-EBO-Z vaccine or placebo were not included in the analysis.

For both the GP-ELISA and PRNT assays, analyses included calculation of geometric mean titer (GMT) at baseline, 28 days, 180 days, and 365 days (PREVAIL and STRIVE trials only) postvaccination. Seroresponse was defined two ways for the GP-ELISA: 1) ≥200 EU/mL and

≥2-fold increase from baseline, which was the definition that best differentiated vaccine from placebo recipients in the PREVAIL clinical trial[22] and 2) ≥4-fold increase from baseline, which is a frequently used historical definition of seroresponse. The 95% confidence intervals (CI) for geometric mean titers (GMTs) were based on analysis of variance, and the 95% CI for seroresponse was based on the exact binomial method. For GMTs, all sera with evaluable results were included; however, a baseline evaluable result was required for calculation of seroresponse. GP-ELISA uses a titer with a reference standard and is reported as a concentration (EU/mL). Separate analyses were conducted for the GP-ELISA and PRNT by study and time point up to Day 365 postvaccination with no data imputation. Statistical analyses were conducted in SAS v9.4 (Cary, NC). While not a formal statistical comparison, data with non-overlapping confidence intervals are characterized throughout the manuscript as being different, while data with overlapping confidence intervals are characterized as similar.

Results

There were 2,199 participants included in this post hoc analysis: 477 from the PREVAIL trial, 1,217 from the FLW trial, and 505 from the STRIVE trial. In the pooled population the majority were men (1487/2199; 67.6%), and the mean age was about 34 years with a small number older than 50 years (227/2199; 10.3%; Table 1). Most participants (1812/2199; 82.4%) entered their respective study with a baseline GP-ELISA below 200 EU/ml (Table 1).

GP-ELISA geometric mean titers increased significantly from baseline, peaking at Day 28 and persisting through Day 365 (last timepoint measured) in the total population and in all subgroups (Figure 1). PRNT geometric mean titers also increased significantly from baseline to Day 28, decreased slightly at Day 180 and showed an increase again at Day 365 (last timepoint measured) in the total population and in all subgroups (Figure 2). When comparing GP-ELISA

and PRNT GMTs by study, the FLW study had slightly higher post baseline geometric mean titers compared with PREVAIL and STRIVE at some but not all timepoints (Figure 1A and Figure 2A). There were higher immune responses in women compared with men (Figure 1B and 2B). GP-ELISA titers were higher in participants with baseline GP-ELISA ≥200 EU/ml compared with participants with baseline GP-ELISA <200 EU/mL at all time points postvaccination (Figure 1C). However, no impact of baseline GP-ELISA titer was observed for the PRNT results (Figure 2C). The GP-ELISA geometric mean titers (Figure 1D) and PRNT GMTs (Figure 2D) were similar across the age groups except for the PRNT at Day 180 where participants >50 years of age displayed higher GMTs.

Figure 3 shows GP-ELISA seroresponse ≥2-fold increase from baseline and ≥200 EU/ml in the total population and by subgroup. In the total population and in all subgroups except those with pre-existing antibodies, the proportion of participants who met the definition of seroresponse was ≥95% at any time postvaccination. In participants with pre-existing antibodies, the proportion of participants who met the definition of seroresponse was a high of 74% at Day 28 and decreased over time while the proportion of participants in the group without pre-existing antibodies who met the definition of seroresponse remained at ≥89% at all 3 timepoints (Figure 3). At day 180 and day 365 postvaccination, there was a slightly higher proportion of women compared with men who met the definition of seroresponse, but there were no apparent differences between age groups who met the definition of seroresponse at any of the time points measured (Figure 3).

Figure 4 shows GP-ELISA seroresponse ≥4-fold increase from baseline in the total population and by subgroup. In the total population, 87% of participants met the definition of seroresponse. As when measured using GP-ELISA seroresponse ≥2-fold increase from baseline and ≥200 EU/ml, there were differences observed between some subgroups. Specifically, a higher

proportion of participants with antibodies <200EU/mL compared with participants with pre-existing antibodies ≥200EU/mL, and more women than men met the definition of seroresponse defined as ≥4-fold increase from baseline when measured at all time points measured postvaccination (Figure 4).

When seroresponse was measured using PRNT and defined as ≥4-fold increase from baseline (Figure 5), the overall rates of seroresponse were lower than those observed by GP-ELISA with 57% of the total population demonstrating seroresponse at any timepoint postvaccination. As was seen with the GP-ELISA, more women than men met the definition of seroresponse at all time points measured postvaccination. However, the differences between participants with pre-existing antibodies ≥200 EU/mL and without pre-existing antibodies ≥200 EU/mL that were observed with the GP-ELISA were not observed with the PRNT (Figure 5). As with the GMTs, a difference was observed between age groups at Day 180 (with participants >50 years of age demonstrating higher seroresponse rates compared with younger individuals), but no differences were observed at other time points.

Discussion

The results of the current post hoc analysis, which assessed the impact of sex, age, and baseline titers indicating possible prior exposure to Ebola virus showed that overall, there was a robust immune response to the vaccine in the integrated analysis population and all subgroups, with most peaking at Day 28 and persisting through Day 365. Interestingly the PRNT titers showed a slight decline at Day 180 but then rose by Day 365, as observed in initial phase I testing of rVSVΔG-ZEBOV-GP[23]. The reason for this is not well understood but could reflect assay variability or maturing antibody responses over time. Potently neutralizing antibodies have been isolated from EBOV survivors after months or even years following natural

infection[24,25]. A longitudinal study of B cell responses in survivors found neutralizing antibody responses increased slowly over 1 year and were marked by significant somatic hypermutation, indicative of B cell maturation[26]. Unlike EBOV infection, which can persist after an initial acute phase, rVSVΔG-ZEBOV-GP vaccination leads to only transient vaccine viremia in adults that typically resolves within a few days [23,27-29]. Therefore, it is unlikely that increases in neutralizing responses observed after Day 180 are the result of ongoing vaccine replication, but instead may reflect the intricate process of antibody affinity maturation. Khurana et al, found high affinity antibodies induced through 56 days after rVSVΔG-ZEBOV-GP vaccination were associated with neutralizing activity in a phase I study[30]. Further studies with samples at later timepoints are needed to uncover the molecular mechanisms underlying this potential dynamic.

There were generally higher immune responses in women compared with men, although these studies were not designed to assess any sex related differences. In addition, higher GP-ELISA immune responses were observed in participants with baseline GP-ELISA ≥200 EU/ml, although those differences were not observed in the PRNT assay and the relevance of those higher titers vis a vis protection is not clear. A previous analysis by Grais et al. [32] showed that GP-ELISA provided a wider range and better differentiation for estimating correlates of protection for rVSVΔG-ZEBOV-GP than PRNT, suggesting that GP-ELISA is at least as relevant as PRNT for predicting protection. This analysis suggested that a dual criteria (serostatus cutoff titer and fold-rise over baseline) may be the most relevant way to assess responses, taking into account the presence of individuals with pre-existing GP-ELISA antibody titers. Despite some observed differences in immunogenicity between subgroups in the current analysis, no differences in efficacy have been reported for these subgroups although it is not

clear that sufficient data have been collected to enable such an analysis. There did not appear to be a difference in immune responses between age groups.

In the current analysis, 12.6% of participants had baseline GP-ELISA results ≥200 EU/ml. Since previous vaccination with an experimental Ebola virus vaccine or Marburg virus vaccine and self-reported history of EVD were exclusion criteria for the trials included in this analysis[16,18,31], the elevated baseline GMT level in some participants may indicate possible prior mildly symptomatic or asymptomatic infection with *Zaire ebolavirus* unknown to the participants, prior infection with a related filovirus, or cross-reactive antibodies unrelated to filoviruses.[32] Previous investigations of seroprevalence of Ebolaviruses and Marburg virus in different regions of Africa showed a wide range of Ebola virus exposure (from 5.3% to 32.4%).[33-38] A recent systematic review assessed population exposure rates based on known previous contact or exposure and also revealed a large range of exposures (0% to 46%) across regions and different populations in Africa (the general population with no known outbreak exposure or contact [0% to 24%], those with household or known case contact [0% to 46%], and those in outbreak areas but no known case contact [1% to 18%].[32,39] The proportion of participants included in the current analysis falls within the range of the population with known contact, which is reasonable since half of the population included were frontline healthcare workers who may have experienced exposure. As noted, we observed a slightly higher increase in post-baseline geometric mean titers in participants in the FLW study, which may reflect prior unrecognized exposure. Because seropositivity is fairly common in Africa, it is important from a public health and clinical point of view to know that post-vaccination titers are similar in groups that are seropositive and seronegative prior to vaccination.

We observed a higher magnitude of GP-ELISA immune response at Day 28 and Day 180 in participants with baseline serum levels ≥200 EU/mL compared with participants with baseline serum levels <200 EU/mL. This may be due to boosting and is important evidence that pre-existing antibodies do not inhibit the ability of this live-attenuated replicating vaccine to induce an immune response, consistent with data from a two-dose regimen administered one month apart in which a boost effect was noted.[29] Conversely, we observed a lower GP-ELISA seroresponse rate, defined as ≥200 EU/mL and ≥2-fold increase from baseline, at Day 28 postvaccination in participants with baseline serum levels ≥200 EU/mL. Moreover, the long-term (i.e., Day 365) GP-ELISA seroresponse rate in participants with baseline serum levels indicating ≥200 EU/mL in the current analysis was similar to that of participants with baseline serum levels <200 EU/mL at Day 365, signifying that the ability to differentiate between groups decreases over time.

A multitude of studies have assessed whether sex differences affect the immune response to vaccines including influenza, Hepatitis B, Herpes virus, Yellow Fever, Rabies, Smallpox, and others (reviewed in [10]). The results have been inconsistent, although adult women tend to have a greater immune response and an increase in adverse events associated with vaccination compared with men.[10] Our results are consistent with this trend of a higher immune response in women compared with men starting at Day 28 and persisting through Day 365. Also, as has been previously reported, women as well as participants with a history of arthritis were identified as being at increased potential risk for the development of arthritis postvaccination. It is unclear whether these differences in immune response between women and men translate into differences in protection.

Limitations

293 This was a post hoc analysis of multiple studies, so the analyses were not powered to assess
294 statistical significance. Therefore, the results should be interpreted with caution.

295 Efficacy was not assessed in any of the studies summarized in this paper and efficacy
296 based on subgroups was not assessed in the Ebola ça Suffit trial.[7] However, one may be able
297 to extrapolate efficacy based on population-based correlate of protection data[40] to this
298 subgroup analysis. In the Grais et al. paper, we proposed that GP-ELISA responses of ≥2-fold
299 increase from baseline and ≥200 EU/ml may be associated with protection at a population level.
300 Applying that thinking to these subgroup analyses, one would conclude that all subgroup
301 populations had a robust response to rVSVΔG-ZEBOV-GP and are likely to be protected from
302 EVD.

303 Conclusions

304 In conclusion, the results of this post hoc analysis of data from 3 trials in African participants
305 demonstrated that vaccination with rVSVΔG-ZEBOV-GP produces a robust immune response in
306 participants regardless of sex, age, or pre-existing antibody level.

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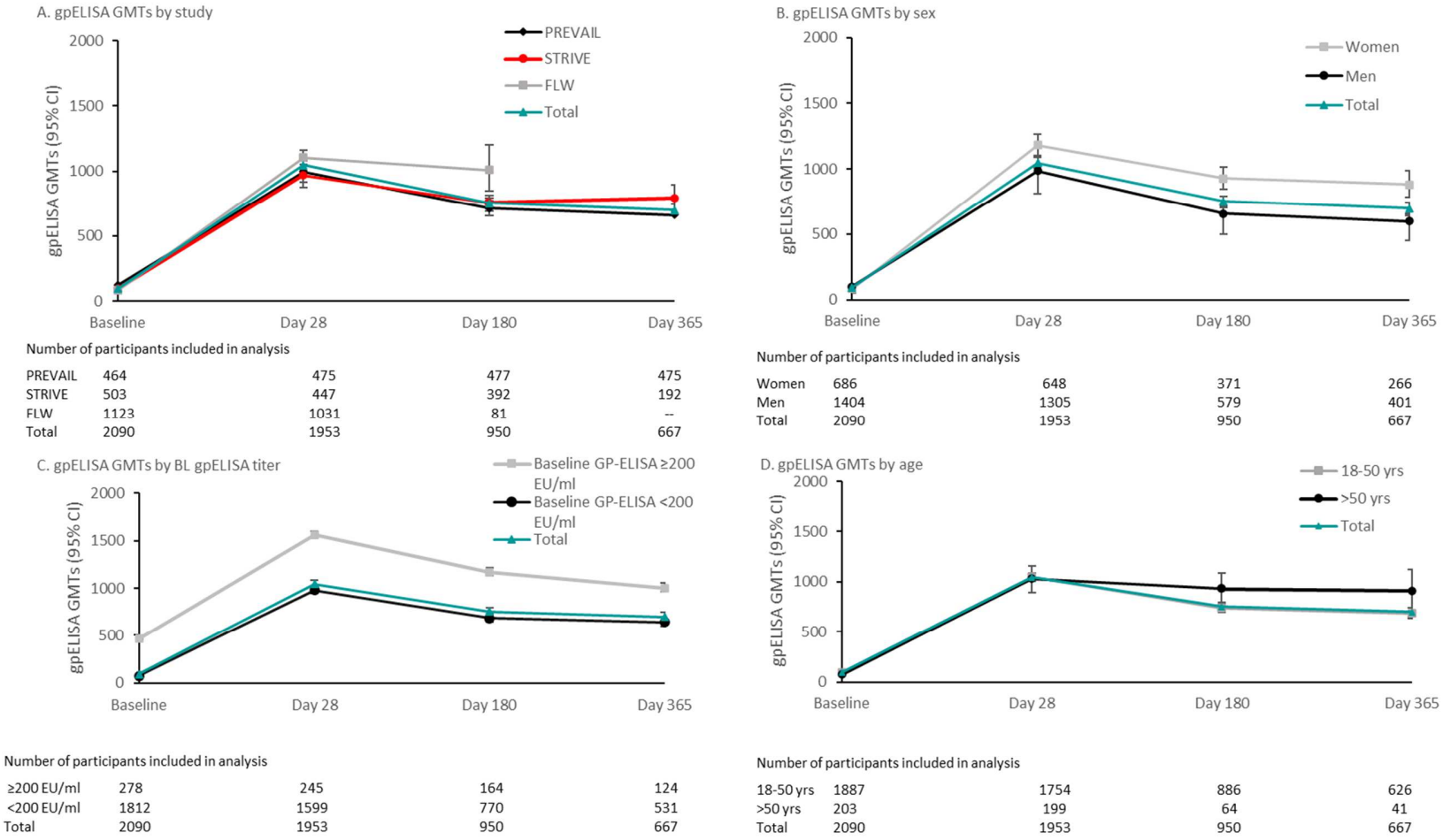
Tables and Figures

Table 1. Baseline characteristics of the pooled population and by individual study

	TOTAL	PREVAIL	FLW	STRIVE
Participants in population	**N=2,199**	n=477 (21.7%)	n=1,217 (55.3%)	n=505 (23.0%)
Sex, n (%)				
Men	1,487 (67.6)	299 (62.7)	896 (73.6)	292 (57.8)
Women	712 (32.4)	178 (37.3)	321 (26.4)	213 (42.2)
Age, years; n (%)				
18 to 50	1,972 (89.7)	453 (95.0)	1052 (86.4)	467 (92.5)
>50	227 (10.3)	24 (5.0)	165 (13.6)	38 (7.5)
Mean (SD)	33.9 (10.9)	31.3 (9.9)	34.9 (11.4)	33.9 (9.9)
Range, years	18 to 77	18 to 75	18 to 75	18 to 77
Baseline GP-ELISA, n (%)				
<200	1,812 (82.4)	367 (76.9)	1018 (83.6)	427 (84.6)
≥200	278 (12.6)	97 (20.3)	105 (8.7)	76 (15.0)
Missing data	109 (5.0)	13 (2.8)	94 (7.7)	2 (0.4)

FLW=front line worker; GP-ELISA= glycoprotein enzyme-linked immunosorbent assay; SD=standard deviation' STRIVE=Sierra Leone Trial to Introduce a Vaccine Against Ebola; PREVAIL= Partnership for Research on Ebola Virus in Liberia trial

444 Figure 1. GP-ELISA geometric mean titers over time following vaccination with rVSVΔG-ZEBOV-GP. (A) by study.
445 (B) by sex. (C) by baseline GP-ELISA titer. (D) by age.



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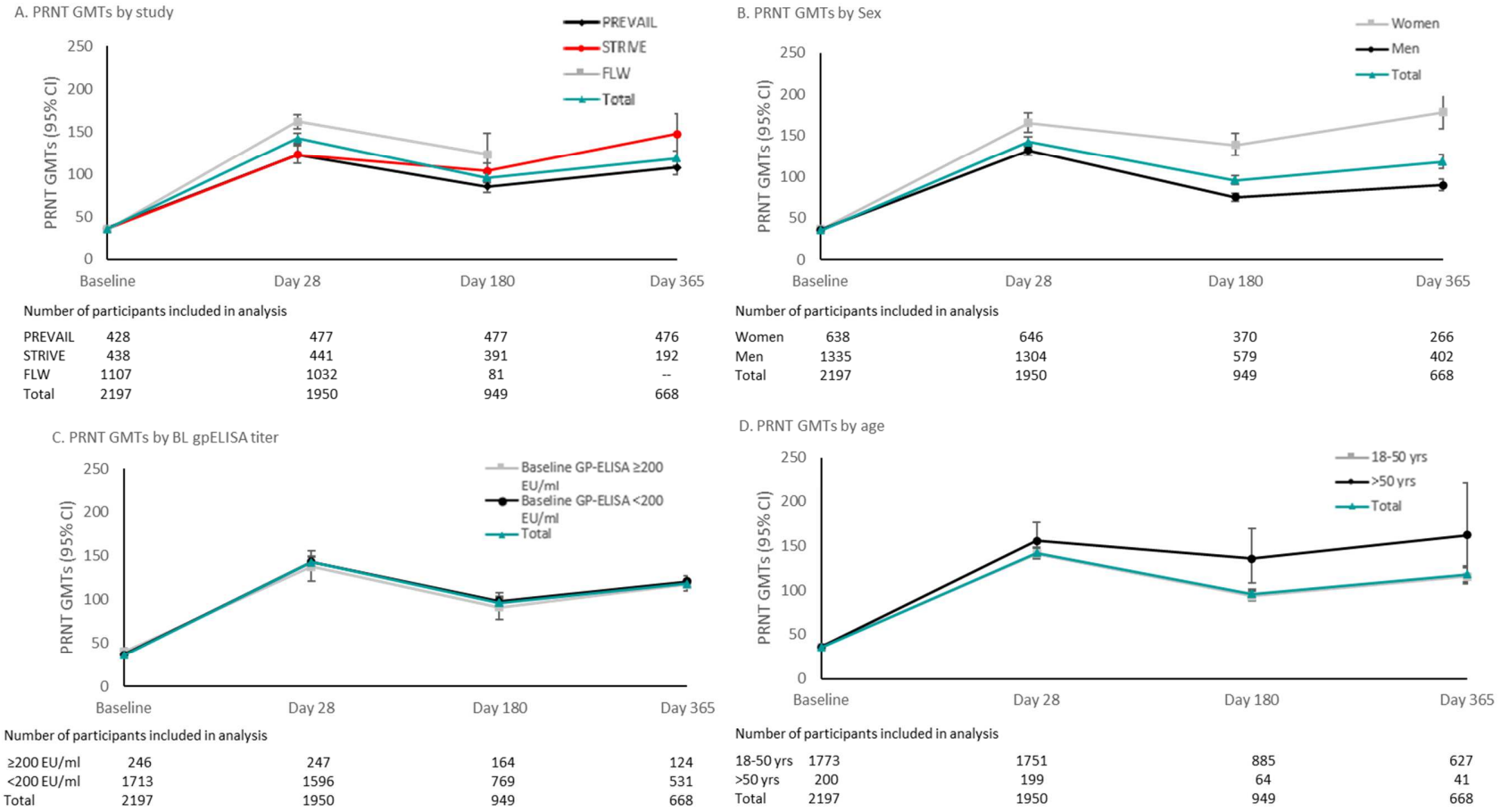
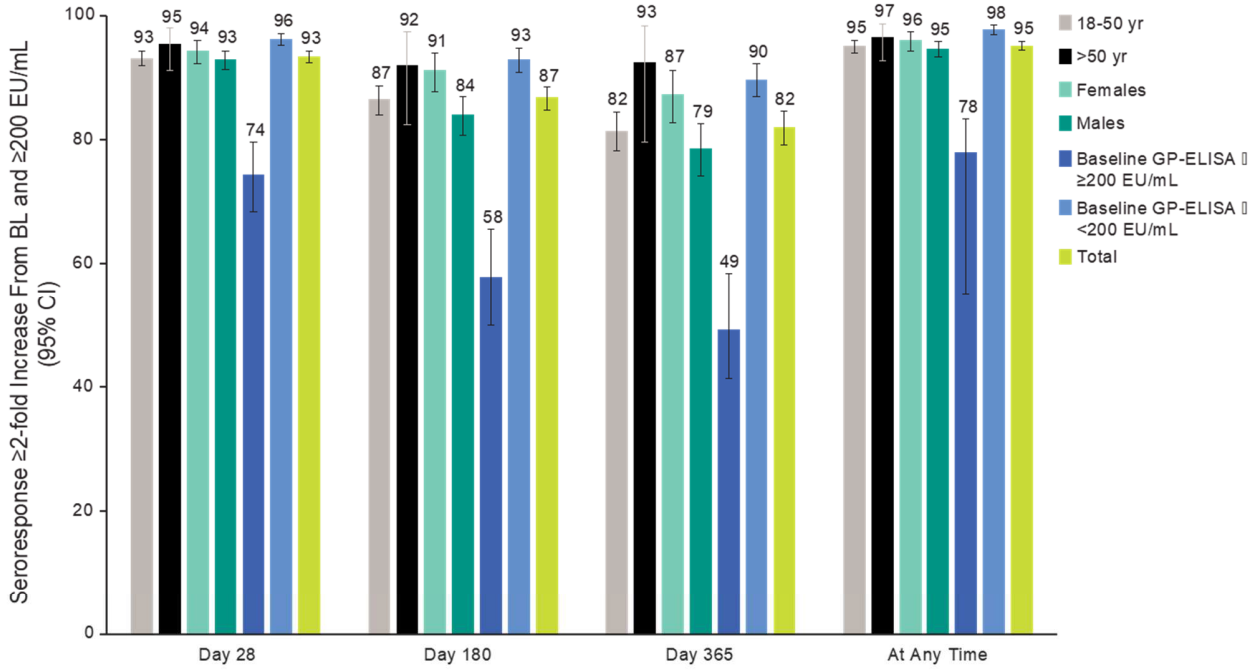
447 CI=confidence interval; EU=ELISA units; GMTs=geometric mean titers; GP-ELISA= glycoprotein enzyme-linked immunosorbent assay

Figure 2. PRNT geometric mean titers over time following vaccination with rVSVΔG-ZEBOV-GP. (A) by study. (B) by sex. (C) by baseline GP-ELISA titer. (D) by age.



CI=confidence interval; EU=ELISA units; GMTs=geometric mean titers; PRNT= plaque reduction neutralization test

Figure 3. GP-ELISA seroresponse, defined as ≥2-fold increase from baseline and ≥200 EU/ml, by subgroup



Number of participants included in analysis

18-50 yrs	1669	871	615	1669
>50 yrs	175	63	40	177
Women	622	365	262	637
Men	1222	569	393	1239
≥200 EU/ml	245	164	124	251
<200 EU/ml	1599	770	531	1625
Total	1844	934	655	1876

BL=baseline; CI=confidence interval; EU=ELISA units; GP-ELISA=glycoprotein enzyme linked immunosorbent assay

Figure 4. GP-ELISA seroresponse, defined as ≥4-fold increase from baseline, by subgroup

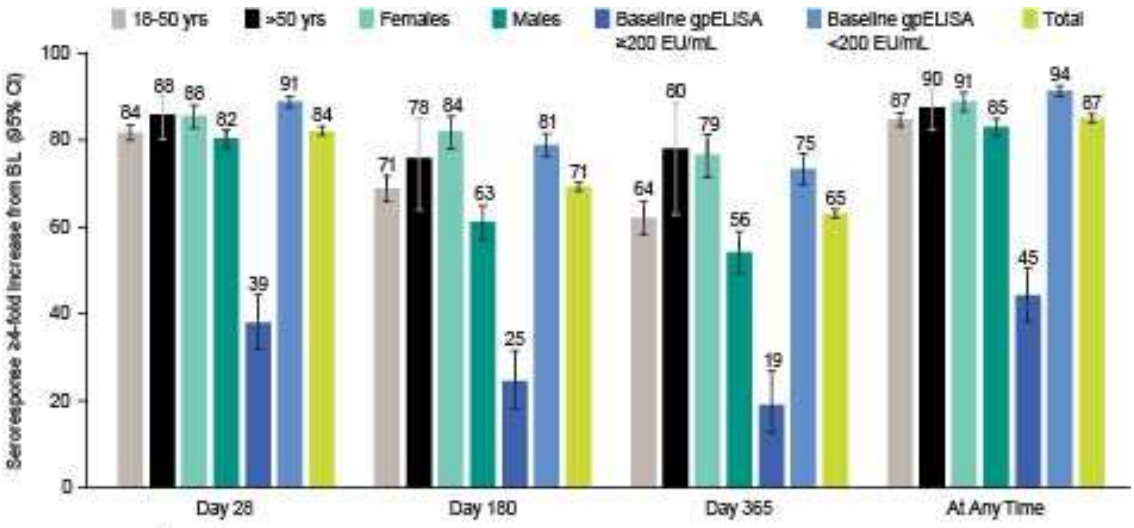


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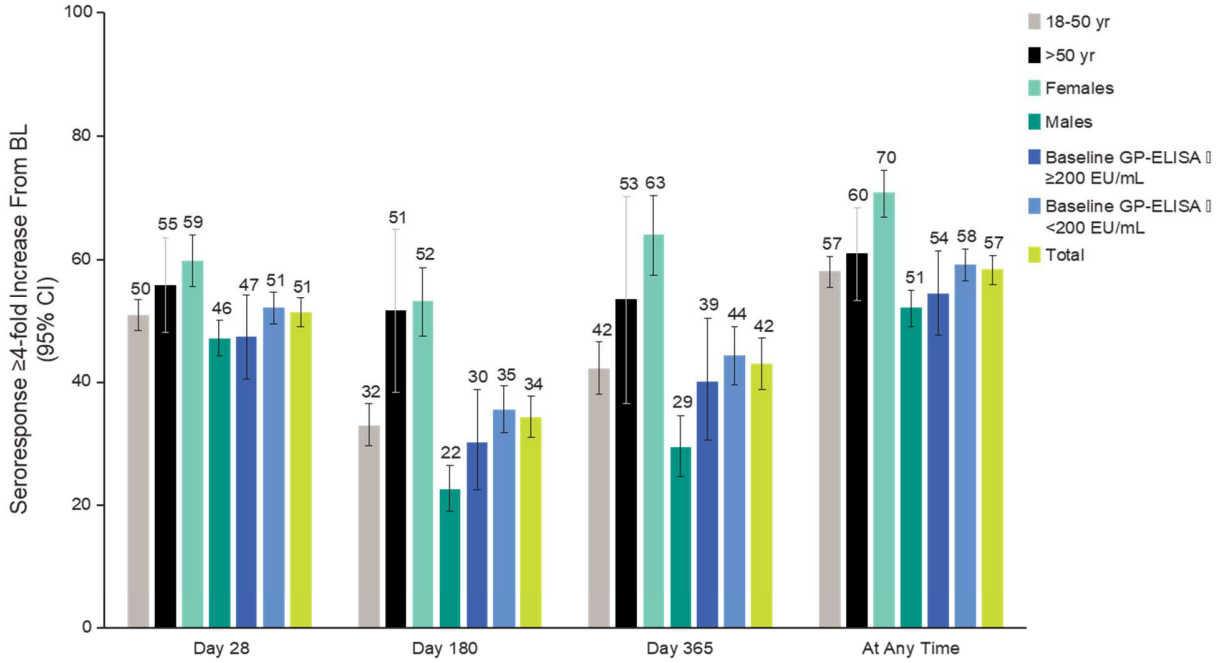
Figure 5. PRNT seroresponse, defined as ≥4-fold increase from baseline, by subgroup



Number of participants included in analysis

18-50 yrs	1557	782	552	1586
>50 yrs	173	61	38	175
Women	574	324	230	590
Men	1156	519	360	1117
≥200 EU/ml	217	138	104	220
<200 EU/ml	1500	691	474	1527
Total	1730	843	590	1761

BL=baseline; CI=confidence interval; EU=ELISA units; GP-ELISA=glycoprotein enzyme linked immunosorbent assay; PRNT=plaque reduction neutralization assay