Partnership for Research on Ebola VACcinnation (PREVAC)

Statistical Data Analysis Plan

10 April 2020

NCT02876328

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1 Introduction

This data analysis plan (DAP) is intended to provide a description of the general analytic strategy and the statistical methods that will be used to summarize the 12 month follow-up results of PREVAC. This DAP updates the one dated 22 September 2017 which described interim analyses to be performed for the independent Data and Safety Monitoring Board (DSMB) as well final analyses.

This DAP was prepared by members of the trial steering committee prior to unblinding and after 12 months of follow-up for all randomized participants was completed.

The DAP describes primary and sensitivity analyses which will be carried out for participants enrolled in Version 4.0 of PREVAC.

2 Study Design Overview and Protocol History

Version 1.0 of the PREVAC Protocol

Version 1.0 of PREVAC described the plan to compare three vaccine strategies with placebo in adults and children for immunogenicity and safety. The three vaccine strategies were:

1) Ad26.ZEBOV (prime) (0.5 mL) followed by an MVA boost (0.5 mL) at 56 days;

2) rVSV (prime) (1 mL) with a placebo (1 mL) boost at 56 days; and

3) rVSV (prime) (1 mL) with a rVSV boost (1 mL) at 56 days.

There were two placebo groups, one with a syringe fill volume of 0.5 mL to match the fill volumes of the Ad26.ZEBOV and MVA vaccines and the other with a fill volume of 1 mL to match the fill volume for the rVSV vaccine. We referred to this plan as the 5-arm randomization.

Version 1.0 of PREVAC was not implemented.

Version 2.0 of the PREVAC Protocol

In January 2017, prior to beginning Version 1.0 of PREVAC, the NIH informed the co-sponsor and collaborators in PREVAC of a suspension of enrolment in a study of the rVSV vaccine in at-risk health care workers at the NIH Clinical Center. The investigator of the NIH study found that among 9 participants enrolled and vaccinated with the rVSV vaccine, 3 developed signs and symptoms of arthritis in the knee 10-14 days following vaccination. The lot of vaccine in the NIH study was the same lot of rVSV vaccine provided for PREVAC.

The investigation by Merck into the vaccine lot and discussions of next steps, which potentially could include the preparation of a new vaccine lot, was likely going to take

some time. Because the sites in Guinea and Liberia were prepared to begin enrolment, the PREVAC team decided to modify the PREVAC protocol and begin the trial with the Ad26.ZEBOV/MVA-BN-Filo vaccine strategy and matching placebo (Figure 1). Version 2.0 of the protocol was issued on February 27, 2017. The plan was to move to the 5 arm randomization as soon as possible after Merck completed their review of the batch of rVSV vaccine to be used in PREVAC. Enrollment in Version 2.0 of PREVAC began on March 27, 2017 and ended on July 18, 2017 with 537 participants enrolled, 496 adults and 41 children. These participants were enrolled by 2 sites in Guinea, Landreah and Maferinyah, and by one site in Liberia, Redemption.

Version 3.0 of the PREVAC Protocol

Merck's investigation of the lot of the rVSV vaccine used in the NIH study determined that the lot met all manufacturing parameters and all criteria associated with the product. Merck also reviewed reports of arthritis from all completed and ongoing studies of the rVSV vaccine and concluded that the reports of arthritis were generally consistent with other reports of arthritis from their program.

At the request of the PREVAC team, Merck provided another lot of the vaccine for PREVAC that is different from the lot used in the NIH study. The certificate of analysis of the new rVSV vaccine lot indicated a test result of 1.3×10^8 pfu/mL, a dose which was higher than that used in the PREVAIL I trial in Liberia (2×10^7).

Variation in titer/potency in live virus vaccines is common. Vaccine manufacture and release for potency is based upon defined specifications and always encompasses a range, routinely with a lower and upper limit. The lower limit, referred to as the nominal dose, is determined during development and is defined by the lowest dose for which there is demonstrated efficacy. A test result higher than the nominal dose is routine amongst live attenuated vaccines because the lower limit for potency must still be valid at the end of shelf-life in order to ensure that the vaccine is still efficacious up until its defined expiry.

Since there were limited numbers of children in previous studies of the rVSV vaccine and the dose used was similar to PREVAIL I (the diluted dose), it was decided to use a measured approach and give the rVSV vaccine at a 2-fold dilution (approximately 5×10^7 pfu/mL). Version 3.0 of the protocol used this dose of the Merck rVSV vaccine. We refer to this as the diluted dose. The 5-arm Version 3.0 design is depicted in Figure 2.

Both adults and children were enrolled under Version 3.0; however, the focus was on the enrollment of children. The enrolment of children began with those 12-17 years, and proceeded to those 5-11 and 1-4 years in a sequential fashion after safety reviews by the DSMB. When the DSMB determined that the two prime vaccines (both the diluted rVSV vaccine and the Ad26.ZEBOV vaccine) were safe in all age groups, the design was modified to enroll adults and children with the undiluted rVSV vaccine (Version 4.0).

Two sites in Guinea, Landreah and Marferinyah, and one site in Liberia, Redemption, enrolled 1,450 participants (663 adults and 787 children) in Version 3.0 of PREVAC.

Version 4.0 of the PREVAC Protocol

The design of Version 4.0 of the PREVAC protocol was similar to Version 3.0 except the undiluted rVSV vaccine was used. This design is depicted in Figure 3.

In Version 4.0, like Version 3.0, the enrolment of children began with those 12-17 years, and proceeded to those 5-11 and 1-4 years in a sequential fashion after safety reviews by the DSMB.

Two sites in Guinea, Landreah and Marferinyah, one site in Liberia, Redemption, 2 sites in Mali, UCRC and CVD, and one site in Sierra Leone, Mambolo, enrolled 2,802 participants in Version 4.0. Of these 2,802 participants, 1,401 were adults and 1,401 were children. In each of 3 age groups, 1-4, 5-11 and 12-17 there were 467 children enrolled.

Follow-up and Data Collection

The follow-up visit schedule was the same for Versions 2.0, 3.0 and 4.0. Following randomization, all participants were seen at 7, 14, 28, 56 and 63 days, and at 3, 6, and 12 months. Participants continue to be seen at 24, 36, 48 and 60 months.

The data collection plan is also the same with three exceptions for children: 1) under Version 4.0, temperature was measured each day in the week after prime and booster vaccination; 2) clinical significant laboratory abnormalities were identified by the site and reported on the case report form; and 3) there was greater standardization across sites for the daily collection of symptoms and injection site reactions each day of the week after prime and booster vaccination.

Summary

Table 1 summarizes the different versions of the PREVAC protocol that have been planned and implemented. Version 1.0 was not implemented. In versions 2.0, 3.0 and 4.0, data on safety and immunogenicity will be available for the rHAd26 prime/MVA boost vaccine and matching placebo. Versions 3.0 and 4.0 will also provide safety and immunogenicity data for the two rVSV vaccine strategies and matching placebo.

Table 1. Summary of PREVAC Protocol Versions

PREVAC Protocol Version	Date of Protocol	No. of Vaccine Groups	Vaccine Groups
1.0	October 8, 2016	5	rHAd26/MVA boost, rVSV with and without boost, and matching placebo groups
2.0	February 27, 2017	2	rHAd26/MVA boost and matching placebo
3.0	May 5, 2017	5	rHAd26/MVA boost, diluted rVSV with and without boost, and matching placebo groups
4.0	March 10, 2018	5	rHAd26/MVA boost, undiluted rVSV with and without boost, and matching placebo groups

Figure 1: PREVAC Version 2.0 Design

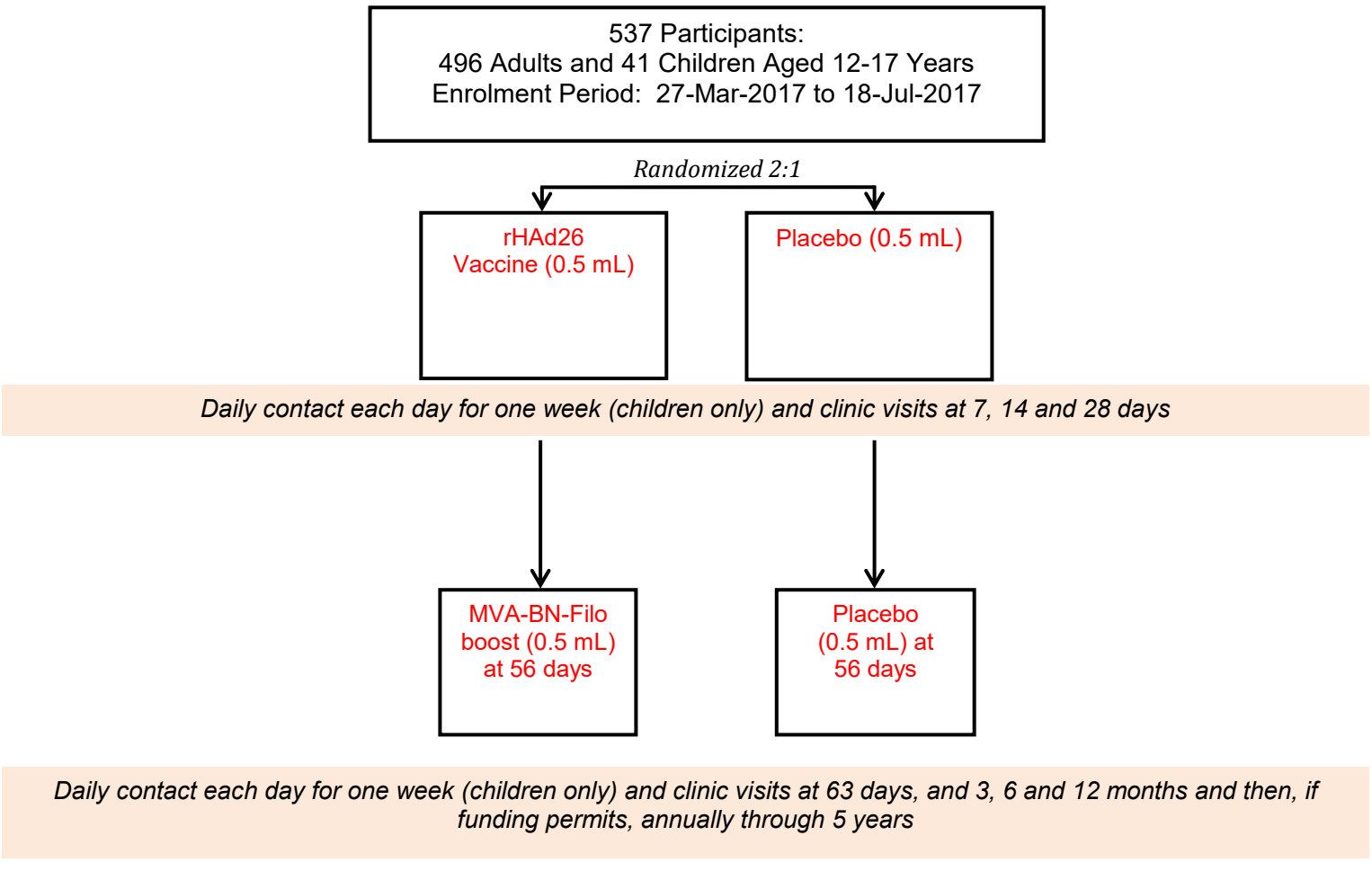


Figure 2: PREVAC Version 3.0 Design

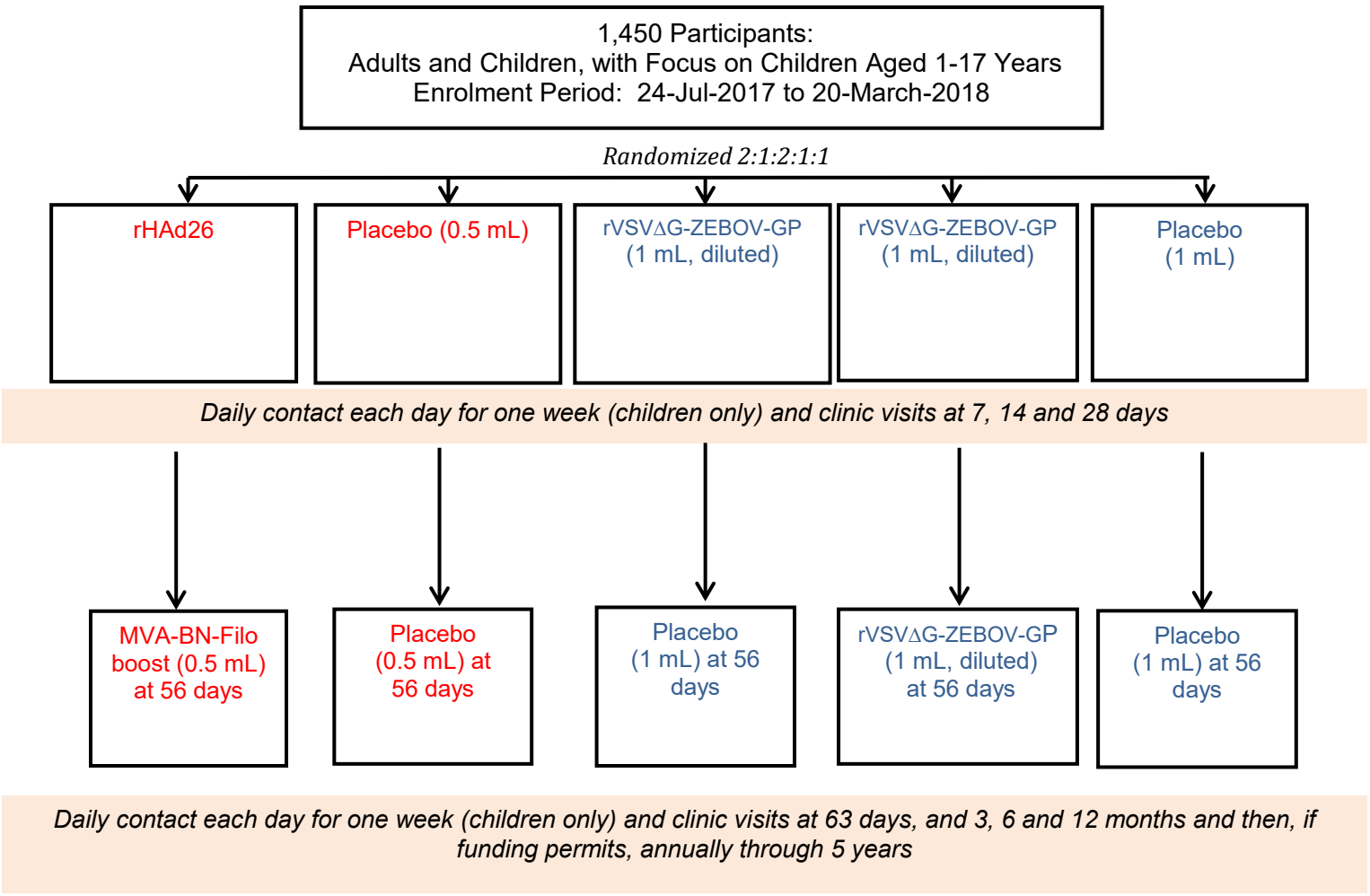
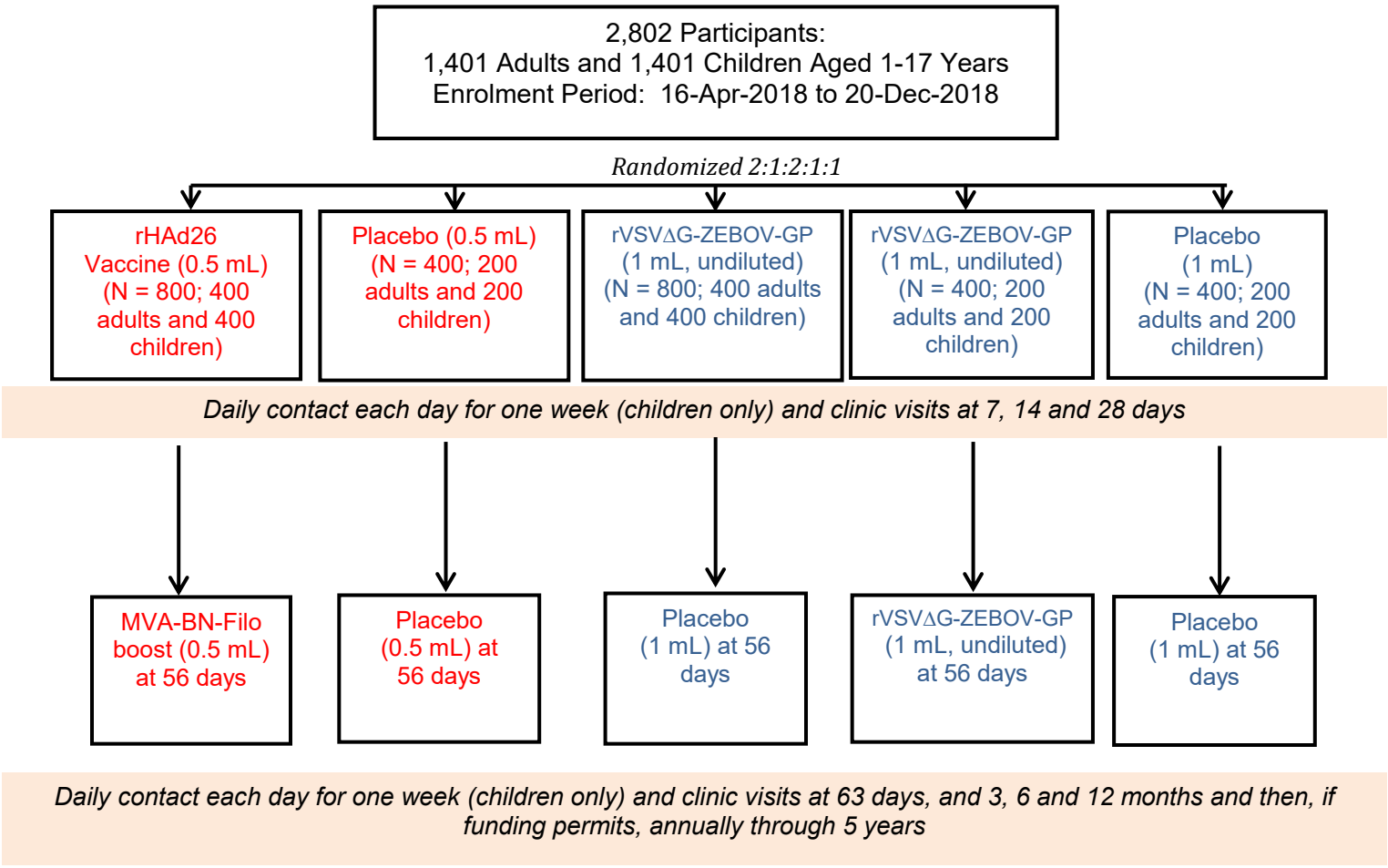


Figure 3: PREVAC Version 4.0 Design



2.1 Analysis Set

The analysis set for both adults and children, which will be analyzed separately and combined, includes all participants who were randomized (and, who by definition, received the prime vaccination). This analysis set will be used for all analyses.

All participants enrolled in Version 4.0 met the eligibility criteria.

2.1.1 Baseline Characteristics

Baseline characteristics will be summarized by vaccine/placebo group (4 groups) and overall. Baseline characteristics will be shown separately for adults and children.

The following baseline characteristics will be summarized:

- Age
- Gender
- Body mass index (adults)
- Arm circumference (children, 1-4 years)
- HIV (%) (adults)
- Syphilis (%)
- Ebola IgG titer; median, IQR, percent “elevated”
- Site and country of enrolment

Summaries for continuous-valued outcomes by treatment group will include N and mean, SD or median, 25th and 75th percentiles. Summaries for categorical outcomes will include N and %.

P-values for comparisons of baseline characteristics between treatment groups will not be provided.

2.1.2 Data Completeness (to be used in Flow Diagram)

The following reports will be provided for adults and children by treatment group:

- Follow-up visit attendance (N and % of those alive).
- Number and percent of those alive with a baseline, 28 day, 6 month and 12 month antibody result.
- Number and percent who withdrew consent.

Note: Among 1,401 adults, 1,335 (95.3%) attended the 12 month visit. Among 1,401 children, 1,352 (96.5%) attended the 12 month visit.

2.1.3 Booster Vaccination

The number and percent of participants who received the booster vaccination and the reason the booster was not received will be summarized, overall and by age group. In addition, the distribution of the number of days after randomization that the booster

vaccination was administered will be summarized.

Note: Among 1,401 adults randomized, 1,325 (95%) received a boost. Among 1,335 adults who attended the 12 months visit, 1,286 (96%) received a boost. Among 1,401 children randomized, 1,372 (98%) received a boost. Among 1,352 children who attended the 12 month visit, 1,334 (99%) received a boost.

2.1.4 Immunogenicity outcomes

The primary endpoint specified in the protocol by the PREVAC group is the percentage of antibody responders at 12 months. An antibody responder at 12 months is defined as a participant who experiences a 4-fold increase in antibody level from baseline (i.e., a .6 increase on the $\log_{10}$ scale) and for whom the antibody level at 12 months is ≥ 200 EU/mL. With this definition, participants with a missing baseline or 12 month result will be excluded. Also, with this definition, there will be no exclusion of participants with elevated antibody levels at entry.

Each of the 3 vaccine strategies will be compared with the pooled placebo group at the 0.0167 (2-sided) level of significance. Analyses will be carried out for adults and children separately and combined.

This same definition will be used for defining responders at 7, 14, 28, 56, and 63 days and at 3 and 6 months. For the comparisons with the pooled placebo group at 7, 14, 28, and 56 days, the two rVSV strategies will be combined. The two pair-wise comparisons at these time-points, each vaccine strategy versus pooled placebo, will be carried out at the 0.025 (2-sided) level of significance.

As a sensitivity analysis, for the above analyses based on responders, participants with an antibody level ≥ 200 EU/mL at baseline will be excluded.

The following additional summaries of antibody levels will be carried out, separately for children and adults:

- Geometric mean titers at 7, 14, 28, 56, and 63 days, and 3, 6 and 12 months separately for adults and children. Comparison groups will be defined as above for version 4.0 participants at day 63 and months 3 and 6. For 7, 14, 28, and 56 days, the rVSV vaccine groups with and without the boost will be combined. Analysis of covariance with stratification by site and with baseline $\log_{10}$ antibody level as a covariate will be used to compare each vaccine group versus the pooled placebo for $\log_{10}$ antibody levels at each follow-up visit (geometric mean ratios will be cited).

- Fold increase from baseline to 14 and 28 days, and 6 and 12 months (<2, 2 to < 4, 4 to < 8, 8 to <16, ≥ 16).

- Box plots of antibody levels at 14 and 28 days, and 6 and12 months. For days 14 and 28 the rVSV vaccine groups with and without the boost will be combined.

During the course of the study changes were made in the reagents used for the FANG assay. The manufacturer did not note that these would lead to different results. However, studies comparing samples run on assays using the different reagents found differences between the FANG results run obtained with the old versus new reagents. found that not to be the case. Accordingly, prior to carrying out the primary analysis, levels made after the change in reagents were recalibrated based on the analysis of paired samples. This recalibration will be documented in a supplement appendix to the manuscript prepared based on this analysis plan.

In addition, since 3 laboratories were used for serology analyses rather than one as originally planned, subgroup analyses that are described in section 2.1.6 include a subgroup analysis by laboratory.

2.1.5 Safety outcomes

2.1.5.1 Primary safety endpoint

The primary safety endpoint is the occurrence of a serious adverse events (SAEs) at any time over the 12 month follow-up period. In addition, the number and percentage with an SAE through 28 days of follow-up and through 3 months of follow-up will be summarized (i.e., through one month after prime and booster vaccination).

For the SAEs through 12 months, event counts, overall and by system organ class according to MedDRA®, and percents (with 95% CIs) will be provided by vaccine group. P-values calculated via the Mantel-Haenzel chi-square test with stratification by site will be provided for comparing each vaccine strategy to the pooled placebo. In addition, time to event analyses, e.g., Kaplan-Meier curves and proportional hazards regression, will be carried out for death and for the composite outcome of death or SAE. These analyses will be performed for the full analysis set.

Note: One participant had an SAE considered related to the vaccine. **The great majority of participants received the booster vaccination (see above).**

2.1.5.2 Safety listings

Line listings by vaccine group will be provided for SAEs. Listings will include the vaccine group label, diagnosis, relatedness to vaccine as judged by the medical officer, and time from vaccination (prime and booster) to onset, age, and sex.

2.1.5.3 Malaria events

Malaria events are reported in 3 different ways:

- Malaria is reported as an SAE if the participant was hospitalized due to malaria.
- As part of the hospitalization for an SAE due to a reason other than malaria, a malaria test may be performed and be reported as positive.
- At each follow-up visit, participants are asked about a malaria diagnosis since

the last visit and it is noted whether it was confirmed by a malaria test.

Analyses will be performed for each definition of a malaria event, and of malaria reported as any of these events. Confirmed malaria events will be summarized by vaccine group and compared between vaccine and placebo groups using the Mantel-Haenszel chi-square test with stratification by site.

2.1.5.4 Appendicitis events

Appendicitis SAE events will be reported separately by site, gender and treatment group.

2.1.5.5 Injection site reactions, targeted symptoms and grade 3 or 4 adverse events at scheduled follow-up visits following prime vaccination

The number and percentage of participants who report an injection site reaction (any redness or swelling induration) within 30 minutes after prime vaccination (baseline), one week after prime vaccination (day 7), 2 weeks after prime vaccination (day 14), and one month after prime vaccination (day 28) will be summarized. Similarly, the number and percentage of participants who report an injection site reaction 30 minutes after booster vaccination (day 56), one week after booster vaccination (day 63), and one month after booster vaccination (month 3) will be summarized by vaccine group. For these analyses, proportions will be compared between each vaccine strategy and the pooled placebo using the Mantel Haenszel chi-square test stratified by site.

For safety outcomes, comparisons may also be made between each active vaccine and its matching placebo.

Pain/tenderness with activity, itching at injection site, and targeted symptoms are graded as not present or grade 1-4. For each of these, the number and percent of participants with each reported symptom or any of the symptoms will be summarized. For each symptom, the number and percent with each grade will also be summarized. Participants with unsolicited symptoms will be listed. The percent with fever (body temperature > 38.0 °C) will also be summarized. These summaries will be carried at the time points mentioned above. For these analyses, proportions will be compared between each vaccine strategy and the pooled placebo using the Mantel Haenszel chi-square test stratified by site. Comparisons may also be made between each active vaccine and its matching placebo.

Note: Analyses of safety data after day 56 will be carried out on all participants, including those who did not receive the booster vaccination. As previously noted, a very high percentage of participants received the booster vaccination. Among participant who did not receive the booster, most did not complete questions concerning symptoms at Day 63 and 3 months.

2.1.5.6 Daily diary for children

For children, injection site reactions, targeted symptoms of any grade severity and

unsolicited grade 3 or 4 adverse events are assessed each day during the week following prime and booster vaccination. The daily diary results will be summarized as described in the previous section. In addition, the number and percent of children reporting the different events at least once will be summarized.

2.1.5.7 Pregnancies

The number of pregnancies discovered in the first 30 days after the prime or booster vaccination, and the outcomes of these pregnancies, will be summarized by vaccine group.

2.1.5.8 Complete blood count and serum chemistries

Complete blood counts and serum chemistries were measured for children using blood collected at baseline, day 7 and day 63. Laboratory tests were carried out at the vaccination site.

Results will be summarized by vaccine group. At day 7 and day 63 (7 days after the booster vaccination), change from baseline will be summarized by vaccine group, and compared between each active vaccine group and placebo using a linear regression model with vaccine indicators, adjusted for baseline levels. For each vaccine versus placebo comparison, the estimated mean difference, the standard error (SE) of the difference and P-value will be provided. For the analyses using day 7 results, the two rVSV groups will be combined.

All of these analyses will be stratified by site (local site laboratory).

2.1.5.9 Weight and mid-upper arm circumference for children

Weight and arm circumference changes will be summarized for children using a linear regression models with vaccine indicators, adjusted for baseline level, age and site.

2.1.6 Subgroup analyses

The effects of each vaccine strategy compared to placebo on SAEs, injection site reactions, targeted symptoms and the percent of participants with a positive antibody response at 12 months will be assessed for subgroups defined by age (1-4, 5-11, 12-17, 18-29, ≥30) gender, country, site (vaccination center), laboratory performing serology, and HIV status.

These analyses will be conducted using logistic or Cox proportional hazards regression models that include interaction terms for the vaccine group and the subgroup variable. The interaction p-value for age will be assessed both with categorical age (4df) and continuous age (1df). Hazard ratios or odds ratios (each vaccine group versus placebo) and 95% confidence intervals (CIs) will be cited as well as the p-value for the interaction.