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# A Phase II, Randomized, Safety and Immunogenicity Study of a Re-Derived, Live-Attenuated Dengue Virus Vaccine in Healthy Adults

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*Abstract.* Two formulations of a new live tetravalent dengue virus (DENV) vaccine produced using re-derived master seeds from a precursor vaccine and that same precursor vaccine as a control were compared in a placebo-controlled, randomized, observer-blind, phase II trial of 86 healthy adults. Two vaccine doses were administered 6 months apart; a third dose was offered to a subset. Symptoms and signs of dengue-like illness reported after vaccination were mild to moderate, transient, and occurred with similar frequency among recipients of the new DENV vaccine and placebo, except for rash. Neither dengue nor vaccine-related serious adverse events were reported. The first DENV vaccine dose was moderately immunogenic; the second dose increased the potency and breadth of the neutralizing antibody response. Tetravalent response rates to the new formulations were 60% and 66.7% in unprimed subjects. A third dose did not increase tetravalent antibody rates. The new DENV vaccine candidates merit additional evaluation.

## INTRODUCTION

Dengue, one of the world's most prevalent and important arboviral diseases, occurs after infection by any of four antigenically distinct but serologically related dengue virus (DENV) types (DENV-1, DENV-2, DENV-3, and DENV-4). An estimated 3.6 billion people live at risk of infection in more than 120 dengue-endemic countries. Approximately 70–500 million infections occur annually, resulting in over 2 million severe illnesses.[1] Vaccination against DENV in conjunction with strategic vector control is considered to be the most viable long-term option for reducing the global dengue burden.[2–5]

The Walter Reed Army Institute of Research (WRAIR) in collaboration with GlaxoSmithKline Vaccines (GSK) developed a live-attenuated tetravalent dengue virus vaccine candidate comprised of four live virus strains representing each of the four DENV types attenuated by serial passage in primary dog kidney (PDK) cells.[6,7] A safe, well-tolerated, and immunogenic preparation of the vaccine candidate was identified in a phase II trial conducted in the United States in adult subjects.[8] The vaccine candidate was then evaluated in two phase I/II clinical trials of flavivirus-naïve children in Thailand who were administered two doses 6 months apart. The first trial was an open-label study of seven seronegative children, and the second trial was a randomized study of 51 seronegative infants from 12 to 15 months of age.[9,10] The vaccine safety profile was clinically acceptable in both studies. Immune responses to all four DENV types were reported in more than one-half of the infants and all of the children 1 month after the second dose. All of the above trials used lyophilized monovalent vaccines that were combined into a tetravalent preparation at the time of administration.

Herein, we report the first clinical evaluation of a new WRAIR-GSK live-attenuated DENV candidate vaccine. The new candidate was prepared from re-derived vaccine strains using the same manufacturing process, except that each strain has three additional passages in fetal rhesus lung (FRhL) cells, monovalent bulks were formulated with a carbohydrate stabilizer rather than human serum albumin, and the final vaccine was lyophilized as a tetravalent product.

## MATERIALS AND METHODS

**Study design.** This study was a phase II, randomized, single-center, observer-blind, controlled, parallel-group trial conducted in the United States. The study was designed to evaluate the safety and immunogenicity of two formulations of a new live-attenuated tetravalent DENV vaccine compared with a precursor live-attenuated tetravalent DENV vaccine and a cell culture medium placebo.

The study was conducted in two stages. The first stage was an observer-blind evaluation of the above four treatment groups followed for 6 months after administration of a first vaccine dose and 3 months after administration of a second vaccine dose. Subjects were randomly allocated to treatment groups using a 1:1:1:1 ratio. The randomization was performed at GlaxoSmithKline Vaccines, Rixensart, Belgium, using a standard Statistical Analysis System (SAS) program (SAS Institute Inc., Cary, NC).

During this first stage, although the vaccine preparer/administrator was aware of some treatment assignments because of a unique method for preparation of the precursor vaccine (monovalent vials mixed into a tetravalent mixture), no volunteer or investigator was aware of treatment assignments until data collection was completed and the first-stage database was frozen for analysis.

The second stage was an open-label evaluation of a subset of subjects in the two new vaccine treatment groups who consented to receive a third dose of the same formulation used for their primary immunization. The third dose was given 5–12 months after the second dose.

The institutional review board, US Army Human Subjects Research Review Board, Office of the Surgeon General approved the study protocol and supporting documents. The study was conducted between April of 2006 and March of 2008 in accordance with the provisions of the Declaration

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of Helsinki, Good Clinical Practices, and US regulations. The US Army Medical Materiel Development Activity (USAMMDA) and GSK monitored the conduct of the trial and verified the data. Internal audits by separate teams from the US Army and GSK were also conducted. Written informed consent was obtained from each volunteer before the performance of any study procedures.

**Role of the sponsor and development partners.** The study was designed by the US Army and GSK. The USAMMDA, as the sponsor's representative, monitored and reported on subject safety. Investigators collected and encoded the data into a GSK database, and a GSK statistician analyzed the data according to a pre-specified and mutually approved plan. All authors had complete and unfettered access to the data, reviewed the manuscript, and can vouch for the document's accuracy and completeness. The study was jointly funded by the US Army Medical Research and Materiel Command and GSK.

**Vaccines.** In this trial, two different formulations of a new live-attenuated tetravalent DENV vaccine designated TDEN (formulations F17 and F19) were compared with the legacy live-attenuated tetravalent DENV vaccine candidate designated F17/Pre[10,11] and a cell culture medium placebo. The TDEN vaccine was made using virus seeds re-derived from the F17/Pre vaccine: DENV-1 (West Pac 74, 45AZ5, PDK-27), DENV-2 (S16803, PDK-50), DENV-3 (CH53489, PDK-20), and DENV-4 (341750, PDK-6). Briefly, purified viral RNA from each F17/Pre vaccine virus strain was extracted and used to transfect FRhL cells, the production cell substrate for the F17/Pre vaccine. Progeny viruses from the transfection were then used to produce master and working seeds and finally, new monovalent vaccine lots; these lots were formulated with a carbohydrate stabilizer into tetravalent blends at a target viral concentration per strain and then lyophilized. The purpose of this re-derivation was to enhance the purity of the seed viruses and the traceability of the resulting TDEN vaccine components. Laboratory evaluation and testing in non-human primates of TDEN virus seeds and tetravalent vaccine lots compared with F17/Pre virus seeds and monovalent lots found no major phenotypic or genotypic variations (Eckels K and others, unpublished data).

The vaccines in this study were formulated to have comparable *in vitro* potency, except that TDEN F19 was planned to contain approximately 10-fold less DENV-4 than TDEN F17 and F17/Pre. Table 1 shows the results of *in vitro* potency tests for the vaccine treatments performed as previously described.[9] The lyophilized placebo contained cell culture medium (EMEM) mixed with an equal volume of stabilizer. After hydration with water for injection (0.5 mL), vaccine or placebo doses having identical appearances were administered subcutaneously into the upper outer triceps/deltoid area.

**Study subjects.** Male and female subjects between 18 and 45 years of age were provided the study details, and after informed consent, they were enrolled by staff from the Clinical Trials Center, WRAIR. All were screened for hepatitis B virus surface antigen (HBsAg) and antibodies to hepatitis C virus (HCV) and the human immunodeficiency virus (HIV); they were excluded from participation if positive. Clinically significant laboratory abnormalities at screening, receipt of immune-modifying drugs within 90 days of enrollment, or a history of chronic disease were exclusion criteria. Additional screening tests included a complete blood count (CBC; including white blood cell [WBC] differential and platelet count), blood urea nitrogen (BUN), creatinine, alanine aminotransferase (ALT), and aspartate aminotransferase (AST). Female subjects were of non-childbearing potential or had been abstinent or used adequate contraceptive precautions for 30 days before vaccination, had a negative pregnancy test within 48 hours before vaccination, and agreed to continue such precautions for 60 days after vaccination.

Subjects were not screened for antibodies to DENV. Instead, subjects were retrospectively classified as being DENV-primed or unprimed based on the presence of detectable neutralizing antibodies to any DENV type in a pre-vaccination blood sample (see data analysis below). Proceeding in this manner was considered appropriate, because the precursor vaccine candidate had been safely administered to a small number of DENV primed individuals; such individuals can be at risk for dengue, and they comprise a large proportion of the target population for vaccine prophylaxis.

**Safety assessments.** In both study stages, safety was assessed in the following manner. We solicited adverse events (AEs) by asking subjects to record injection site symptoms (injection site pain, redness, and swelling) and general symptoms (fever, fatigue, headache, pain behind the eyes, abdominal pain, nausea, vomiting, muscle ache, joint ache, diffuse rash on the trunk, photophobia, and pruritis) on diary cards for 21 days after each vaccination. Intensities of each AE were scored as grades 1–3, with grade 3 fever defined as an oral body temperature > 39°C (> 102.2°F), grade 3 redness and swelling defined as > 20-mm diameter around the injection

TABLE 1

Passage history and *in vitro* potency ($log_{10}$ focus forming units per milliliter) of the DENV strains in vaccine candidates that were evaluated previously and in this trial

| Vaccine formulation | Pre-transfection DENV-1 45AZ5 PDK27 FRhL-3 | Pre-transfection DEN-2 S16803 PDK50 FRhL-3 | Pre-transfection DEN-3 CH53489 PDK20 FRhL-3 | Pre-transfection DEN-4 341750 PDK6 FRhL-3 |
|---|---|---|---|---|
| F17/Pre, dose 1 (after in-clinic mixing)* | 6.1 | 6.3 | 4.8 | 6.0 |
| F17/Pre, dose 2 (after in-clinic mixing)* | 6.2 | 6.3 | 5.0 | 6.1 |
| F17/Pre, dose 1 (after in-clinic mixing)† | 5.1 | 5.3 | 4.9 | 5.1 |
| F17/Pre, dose 2 (after in-clinic mixing)† | 4.8 | 5.3 | 4.7 | 4.9 |
| | Post-transfection DEN-1 45AZ5 PDK27 FRhL-6 | Post-transfection DEN-2 S16803 PDK50 FRhL-6 | Post-transfection DEN-3 CH53489 PDK20 FRhL-6 | Post-transfection DEN-4 341750 PDK6 FRhL-6 |
| F17 (at release) | 4.9 | 5.3 | 4.7 | 5.0 |
| F19 (at release) | 4.9 | 5.2 | 4.6 | 4.4 |

*Historical benchmark (average of three independent tetravalent blends).[10]

†This trial is the average of seven doses, 1 blend retained or four doses, two blends retained; the differences from the historical benchmark reflect dilutions implemented to match release potency of F17 lyophilized vaccine at release.

site, and all other grade 3 AEs defined as those events preventing normal daily activity.

Investigators conducted active surveillance for AEs of potential cardiac origin occurring after vaccination (chest pain, irregular heart beat or palpitations, shortness of breath, dizziness or loss of consciousness, irregular heart rhythm, pericardial friction rub, and abnormal electrocardiogram [ECG] or elevated troponin I level). If an AE of potential cardiac origin was suspected, the investigator was requested to exclude other (non-vaccine) potential etiologies, and the subject was referred to a cardiologist for definitive assessment. This evaluation was designed to address the theoretical potential for a live DENV vaccine to cause AEs similar to the atypical cardiac manifestations reported after natural DENV infections.[12]

Subjects were requested to consult an investigator if they developed fever. Subjects were suspected of having dengue if they had (1) fever > 39°C or fever ≥ 38°C measured at least one time on two successive days and (2) at least two of the following signs or symptoms concurrently (nausea, vomiting, headache, eye pain, muscle ache, joint ache, abdominal pain, sore throat, or dengue-like rash). Any subject with suspected dengue underwent additional evaluation (history, physical examination, and clinical laboratory: CBC with WBC differential and platelet count, ALT, and AST) and testing for DENV viremia. Confirmed dengue was a suspected case that was (1) positive for any of the following clinical laboratory abnormalities: absolute neutrophil count (ANC) ≤ 1,000 cells/μL, AST or ALT > 2.5 times the upper limit of normal, hemoconcentration during fever or 1 day after defervescence (peak hematocrit ≥ 1.2 times baseline), or thrombocytopenia < 100,000 cells/μL, and (2) positive for DENV viremia.

On the day of vaccination and again 30 days after each vaccination, subjects were questioned by the investigator about AEs, and a physical examination was performed to evaluate for any dengue-like signs, including rash, hemorrhage (skin, conjunctival, or mucosal), conjunctival injection, hepatomegaly, splenomegaly, or lymphadenopathy. Subjects were also randomly assigned to return for an evaluation on study days 2, 5, 8, or 12 and then again on study days 5, 8, 12, or 14 after each vaccination. Therefore, subjects were assessed on a total of three occasions after each vaccination.

Investigators collected AEs spontaneously reported by subjects that occurred over a 31-day follow-up period after each vaccine or placebo dose. These unsolicited AEs were coded with the use of the *Medical Dictionary for Regulatory Activities*.[13] Investigators also recorded any serious adverse events (SAEs), defined as medically significant events, including those events resulting in hospitalization, disability, or death, throughout the study period.

There were safety laboratory assessments (CBC including WBC, differential and platelet counts, neutrophil count, hematocrit, and ALT and AST) on each vaccination day, two times between days 2 and 14 after vaccination as assigned randomly, and again, 30 days after vaccination. We defined alert laboratory values requiring follow-up as follows: platelet count < 100,000 cells/μL, ANC < 1,000 cells/μL, and ALT or AST > 2.5 times the upper limit of normal.

We systematically evaluated subjects for the presence of dengue viremia during the 31-day period after each vaccine dose in both study stages. Viremia was also measured any time that dengue was suspected. Detection of DENV RNA as a measure of viremia was performed by reverse-transcriptase quantitative polymerase chain reaction (RT-qPCR) using an assay protocol modified from the work by Sadon and others.[14]

The limit of detection for the RT-qPCR assay was expressed in genome equivalents per milliliter: 6,500 Geq/mL for DENV-1, 160,000 Geq/mL for DENV-2, 1,250 Geq/mL for DENV-3, and 33,000 Geq/mL for DENV-4.

**Assays for immune response.** To characterize DENV vaccine immunogenicity, anti-DENV neutralizing antibodies were measured on the day of each vaccination and 1 month after each dose.

A microneutralization assay referred to as MN90 was initially used to measure the dengue antibody titer required to neutralize 90% of the viral input. By reducing the virus input into the neutralization reaction, the assay was refined to estimate a 50% microneutralization endpoint; consequently, all available serum specimens were retested by the qualified MN50 assay as follows. Antibodies to each DENV type were measured at the Pilot Bioproduction Facility, WRAIR, using a 96-well quantitative microneutralization assay (MN50) performed in Vero cells (World Health Organization [WHO], National Institute for Biological Standards and Control [NIBSC]-011038011038) with an initial input of 50 plaque-forming units (PFU) each DENV-2, DENV-3, and DENV-4 and 100 PFU DENV-1. The parental virus strains to be neutralized were DENV-1 WP74, DENV-2 S16803, DENV-3 CH53489, and DENV-4 341750. Seven threefold serial dilutions from 1:3.3 to 1:2,430 were tested per sample. The MN50 titers were automatically processed by an EXCEL spreadsheet, which used a log midpoint linear regression program model to derive the MN50 titer. The MN50 titer is the reciprocal of the serum dilution that neutralizes ≥ 50% of dengue virus, leading to a reduction of 50% of the optical density measured by enzyme-linked immunosorbent assay on fixed cells after 4 days of incubation. Seropositivity was defined as a titer ≥ 1:10.

**Data analyses.** This study was exploratory; thus, analyses were descriptive, with no statistical comparisons performed. The primary safety analysis was performed on all enrolled subjects who received at least one vaccine dose (total vaccinated cohort), and the primary immunogenicity analysis was based on the according-to-protocol cohort (ATP; i.e., subjects who met all eligibility criteria, complied with protocol procedures, had no elimination criteria during the study, and had data available for at least one immunogenicity endpoint). Analyses were stratified based on pre-vaccination DENV antibody status (i.e., primed or unprimed). An unprimed subject was defined as having a DENV neutralizing antibody titer < 1:10 to all four DENV serotypes, whereas a primed subject was defined as having a DENV neutralizing antibody titer ≥ 1:10 to any DENV type.

The overall percentages of subjects reporting an AE after vaccine administration (21 days after each vaccination for solicited AEs and 31 days after each vaccination for spontaneously reported symptoms) were tabulated with exact 95% confidence intervals (CIs) by type of AE and intensity (any grade and grade 3). All SAEs occurring during the study were listed for each treatment group. The proportion of subjects with dengue-like physical examination signs detected up to 31 days after each vaccination was tabulated with exact 95% CI.

The proportion of subjects in each group with dengue viremia at each time point within 31 days after vaccination was tabulated after each vaccination. A test for viremia was considered positive if the assay value was greater than or equal

to the limit of detection, negative if the assay value was zero, and undetermined if the assay value was > 0 and less than the limit of detection.

We calculated the following immunogenicity parameters by group with seropositivity rate and geometric mean titer (GMT) for each DENV type as well as the tetravalent antibody seroconversion rate. The seropositivity rate was defined as the percentage of subjects with neutralizing antibody titer ≥ 1:10. GMT calculations were performed by taking the antilog of the mean of the log titer transformations, with a value of five given to titers < 1:10. GMTs were calculated with 95% CIs, and seropositivity rates and tetravalent seroconversion rates were calculated with exact 95% CIs. Antibody titers were also summarized by reverse cumulative curves.

## RESULTS

**Study population.** Eighty-six subjects (twenty-one to twenty-two subjects per group) were enrolled in the study for the first stage starting in April of 2006. All received dose 1 of the candidate vaccine or placebo (Figure 1 shows subject disposition), and 74 subjects (86%) received dose 2. Eleven subjects were withdrawn: one withdrawal was because of an SAE (axonal demyelinating polyneuropathy/bilateral upper and lower extremities 171 days after dose 1 of F19) assessed by the investigator as unrelated to the study treatment. The other 10 subjects moved or were otherwise lost to follow-up. The first stage was completed by June of 2007. In this first stage, 82 subjects were included in the ATP cohort for immunogenicity analysis with the MN90 assay (Figure 1); however, MN50 immunogenicity results were available for only 77 subjects, because the specimens of 5 subjects were consumed in preliminary testing (F17/Pre $N = 1$, F17 $N = 1$, F19 $N = 3$).

Twenty-one subjects (nine subjects from the F17 group and twelve subjects from the F19 group) entered the study's second stage in September of 2007 (Figure 1). These 21 subjects received a third dose of vaccine, and follow-up visits were completed by March of 2008. Of these 21 subjects, MN50

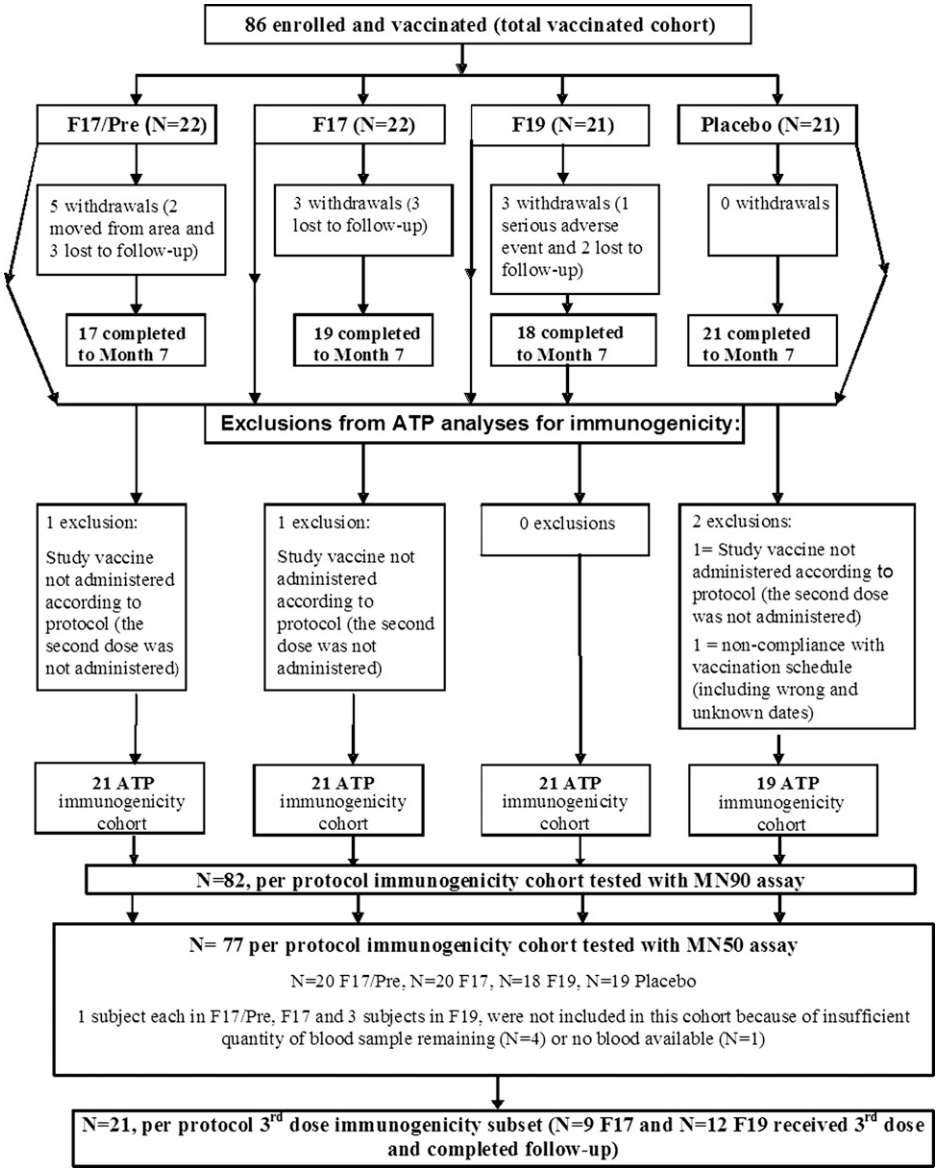


FIGURE 1. Subject disposition. ATP = according to protocol cohort; $N$ = number of subjects.

immunogenicity results for all time points (for all three doses) were available for 17 subjects (F17 $N = 8$, F19 $N = 9$).

The mean age in the total vaccinated cohort was 34.3 years (median = 35.5 years, range = 20–45 years): 59% were males. Most subjects were either African American (57%) or Caucasian (36%). The above attributes were similar in each group. At baseline, in the ATP cohort with MN50 data available, 14 of 77 subjects (18%) were categorized as primed to DENV, with primed subjects evenly distributed among treatment groups (3–4 primed subjects per group in the ATP cohort). Among the 14 primed subjects in the per-protocol cohort, 11 subjects were primed to all four DENV types, and the other 3 subjects were primed to only one DENV type. Of the 17 subjects in the subset of the ATP cohort who received the third dose and had MN50 data available, there were 4 subjects primed to DENV at baseline: 2 subjects per F17 and F19 groups.

**Vaccine safety.** Most subjects (89–100% per group) returned a symptom diary card after vaccination. The incidence of solicited injection site symptoms, all of which were assumed to be causally related to vaccination, is presented in Table 2. Most injection site symptoms were transient, lasted 2 days or less, and mild to moderate in severity; there were few reports of grade 3 symptoms. Injection site symptoms did not seem to increase with dose 2 or in the subset receiving a third dose of TDEN F17 or F19.

The incidence of each solicited general AE is reported per subject considering both doses 1 and 2 (Table 3). The incidence of fever is shown after each dose and both doses. Most reported general symptoms were graded mild or moderate and were transient. Headache was the most frequently reported general symptom (ranging from 33.3% to 54.5% after dose 1 among the four groups and from 16.7% to 37.5% after dose 2) followed by fatigue (ranging from 22.7% to 36.4% after dose 1 among the four groups and from 15% to 25% after dose 2).

As with injection site symptoms, there were no notable differences between groups, including the placebo group, for incidence of any general symptom. This finding was also true for reports of grade 3 general symptoms; these reports were rare, most being reported by one or no subjects per group. In the subset receiving a third dose of TDEN F17 or F19, there were very few reports of general symptoms (Table 4).

During the first study stage, 10–15 subjects in each group (15 [68.2%] subjects in the F17/Pre group, 10 [45.5%] subjects in the F17 group, 15 [71.4%] subjects in the F19 group, and 12 [57.1%] subjects in the placebo group) reported at least one unsolicited symptom during the 31-day follow-up period

TABLE 2
Incidence of solicited injection site symptoms during the 21-day follow-up after each vaccination (total vaccinated cohort)

| Symptom and type | F17/Pre | | | | F17 | | | | F19 | | | | Placebo | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *n* | % | 95% CI lower | 95% CI upper | *n* | % | 95% CI lower | 95% CI upper | *n* | % | 95% CI lower | 95% CI upper | *n* | % | 95% CI lower | 95% CI upper |
| | ($N = 22$) | | | | ($N = 22$) | | | | ($N = 21$) | | | | ($N = 21$) | | | |
| Dose 1 | | | | | | | | | | | | | | | | |
| Pain | | | | | | | | | | | | | | | | |
| Any | 7 | 31.8 | 13.9 | 54.9 | 2 | 9.1 | 1.1 | 29.2 | 4 | 19.0 | 5.4 | 41.9 | 5 | 23.8 | 8.2 | 47.2 |
| Grade 3 | 0 | 0.0 | 0.0 | 15.4 | 0 | 0.0 | 0.0 | 15.4 | 0 | 0.0 | 0.0 | 16.1 | 1 | 4.8 | 0.1 | 23.8 |
| Redness | | | | | | | | | | | | | | | | |
| Any | 8 | 36.4 | 17.2 | 59.3 | 3 | 13.6 | 2.9 | 34.9 | 4 | 19.0 | 5.4 | 41.9 | 4 | 19.0 | 5.4 | 41.9 |
| Grade 3 | 1 | 4.5 | 0.1 | 22.8 | 1 | 4.5 | 0.1 | 22.8 | 0 | 0.0 | 0.0 | 16.1 | 0 | 0.0 | 0.0 | 16.1 |
| Swelling | | | | | | | | | | | | | | | | |
| Any | 6 | 27.3 | 10.7 | 50.2 | 4 | 18.2 | 5.2 | 40.3 | 5 | 23.8 | 8.2 | 47.2 | 2 | 9.5 | 1.2 | 30.4 |
| Grade 3 | 0 | 0.0 | 0.0 | 15.4 | 1 | 4.5 | 0.1 | 22.8 | 1 | 4.8 | 0.1 | 23.8 | 1 | 4.8 | 0.1 | 23.8 |
| | ($N = 16$) | | | | ($N = 19$) | | | | ($N = 18$) | | | | ($N = 20$) | | | |
| Dose 2 | | | | | | | | | | | | | | | | |
| Pain | | | | | | | | | | | | | | | | |
| Any | 7 | 43.8 | 19.8 | 70.1 | 1 | 5.3 | 0.1 | 26.0 | 1 | 5.6 | 0.1 | 27.3 | 2 | 10.0 | 1.2 | 31.7 |
| Grade 3 | 0 | 0.0 | 0.0 | 20.6 | 0 | 0.0 | 0.0 | 17.6 | 0 | 0.0 | 0.0 | 18.5 | 0 | 0.0 | 0.0 | 16.8 |
| Redness | | | | | | | | | | | | | | | | |
| Any | 1 | 6.3 | 0.2 | 30.2 | 0 | 0.0 | 0.0 | 17.6 | 2 | 11.1 | 1.4 | 34.7 | 2 | 10.0 | 1.2 | 31.7 |
| Grade 3 | 0 | 0.0 | 0.0 | 20.6 | 0 | 0.0 | 0.0 | 17.6 | 0 | 0.0 | 0.0 | 18.5 | 0 | 0.0 | 0.0 | 16.8 |
| Swelling | | | | | | | | | | | | | | | | |
| Any | 1 | 6.3 | 0.2 | 30.2 | 0 | 0.0 | 0.0 | 17.6 | 0 | 0.0 | 0.0 | 18.5 | 1 | 5.0 | 0.1 | 24.9 |
| Grade 3 | 0 | 0.0 | 0.0 | 20.6 | 0 | 0.0 | 0.0 | 17.6 | 0 | 0.0 | 0.0 | 18.5 | 0 | 0.0 | 0.0 | 16.8 |
| | | | | | ($N = 8$) | | | | ($N = 12$) | | | | | | | |
| Dose 3 | | | | | | | | | | | | | | | | |
| Pain | | | | | | | | | | | | | | | | |
| Any | | | | | 0 | 0.0 | 0.0 | 36.9 | 0 | 0.0 | 0.0 | 26.5 | | | | |
| Grade 3 | | | | | 0 | 0.0 | 0.0 | 36.9 | 0 | 0.0 | 0.0 | 26.5 | | | | |
| Redness | | | | | | | | | | | | | | | | |
| Any | | | | | 1 | 12.5 | 0.3 | 52.7 | 2 | 16.7 | 2.1 | 48.4 | | | | |
| Grade 3 | | | | | 0 | 0.0 | 0.0 | 36.9 | 0 | 0.0 | 0.0 | 26.5 | | | | |
| Swelling | | | | | | | | | | | | | | | | |
| Any | | | | | 0 | 0.0 | 0.0 | 36.9 | 1 | 8.3 | 0.2 | 38.5 | | | | |
| Grade 3 | | | | | 0 | 0.0 | 0.0 | 36.9 | 0 | 0.0 | 0.0 | 26.5 | | | | |

$N$ = number of subjects per group with data available; $n$ = number of subjects with the specified symptom.

TABLE 3
Incidence of solicited general symptoms during the 21-day follow-up period after vaccination (total vaccinated cohort)

| Symptom | Type | F17/Pre | | | | F17 | | | | F19 | | | | Placebo | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | *n* | % | 95% CI lower | 95% CI upper | *n* | % | 95% CI lower | 95% CI upper | *n* | % | 95% CI lower | 95% CI upper | *n* | % | 95% CI lower | 95% CI upper |
| | | (*N* = 22) | | | | (*N* = 22) | | | | (*N* = 21) | | | | (*N* = 21) | | | |
| Fever post-dose 1 | Any | 4 | 18.2 | 5.2 | 40.3 | 2 | 9.1 | 1.1 | 29.2 | 2 | 9.5 | 1.2 | 30.4 | 1 | 4.8 | 0.1 | 23.8 |
| | Grade 3 | 0 | 0.0 | 0.0 | 15.4 | 0 | 0.0 | 0.0 | 15.4 | 0 | 0.0 | 0.0 | 16.1 | 1 | 4.8 | 0.1 | 23.8 |
| | | (*N* = 16) | | | | (*N* = 19) | | | | (*N* = 18) | | | | (*N* = 20) | | | |
| Fever post-dose 2 | Any | 1 | 6.3 | 0.2 | 30.2 | 2 | 10.5 | 1.3 | 33.1 | 1 | 5.6 | 0.1 | 27.3 | 4 | 20.0 | 5.7 | 43.7 |
| | Grade 3 | 0 | 0.0 | 0.0 | 20.6 | 0 | 0.0 | 0.0 | 17.6 | 0 | 0.0 | 0.0 | 18.5 | 0 | 0.0 | 0.0 | 16.8 |
| Combined (after both doses) | | (*N* = 22) | | | | (*N* = 22) | | | | (*N* = 21) | | | | (*N* = 21) | | | |
| Fever | Any | 5 | 22.7 | 7.8 | 45.4 | 4 | 18.2 | 5.2 | 40.3 | 3 | 14.3 | 3.0 | 36.3 | 5 | 23.8 | 8.2 | 47.2 |
| | Grade 3 | 0 | 0.0 | 0.0 | 15.4 | 0 | 0.0 | 0.0 | 15.4 | 0 | 0.0 | 0.0 | 16.1 | 1 | 4.8 | 0.1 | 23.8 |
| Abdominal pain | Any | 0 | 0.0 | 0.0 | 15.4 | 5 | 22.7 | 7.8 | 45.4 | 4 | 19.0 | 5.4 | 41.9 | 4 | 19.0 | 5.4 | 41.9 |
| | Grade 3 | 0 | 0.0 | 0.0 | 15.4 | 0 | 0.0 | 0.0 | 15.4 | 1 | 4.8 | 0.1 | 23.8 | 0 | 0.0 | 0.0 | 16.1 |
| Arthralgia | Any | 6 | 27.3 | 10.7 | 50.2 | 4 | 18.2 | 5.2 | 40.3 | 3 | 14.3 | 3.0 | 36.3 | 3 | 14.3 | 3.0 | 36.3 |
| | Grade 3 | 0 | 0.0 | 0.0 | 15.4 | 1 | 4.5 | 0.1 | 22.8 | 0 | 0.0 | 0.0 | 16.1 | 1 | 4.8 | 0.1 | 23.8 |
| Fatigue | Any | 10 | 45.5 | 24.4 | 67.8 | 8 | 36.4 | 17.2 | 59.3 | 6 | 28.6 | 11.3 | 52.2 | 7 | 33.3 | 14.6 | 57.0 |
| | Grade 3 | 0 | 0.0 | 0.0 | 15.4 | 1 | 4.5 | 0.1 | 22.8 | 1 | 4.8 | 0.1 | 23.8 | 1 | 4.8 | 0.1 | 23.8 |
| Headache | Any | 14 | 63.6 | 40.7 | 82.8 | 9 | 40.9 | 20.7 | 63.6 | 10 | 47.6 | 25.7 | 70.2 | 9 | 42.9 | 21.8 | 66.0 |
| | Grade 3 | 0 | 0.0 | 0.0 | 15.4 | 1 | 4.5 | 0.1 | 22.8 | 1 | 4.8 | 0.1 | 23.8 | 0 | 0.0 | 0.0 | 16.1 |
| Muscle ache | Any | 6 | 27.3 | 10.7 | 50.2 | 5 | 22.7 | 7.8 | 45.4 | 4 | 19.0 | 5.4 | 41.9 | 4 | 19.0 | 5.4 | 41.9 |
| | Grade 3 | 0 | 0.0 | 0.0 | 15.4 | 1 | 4.5 | 0.1 | 22.8 | 0 | 0.0 | 0.0 | 16.1 | 2 | 9.5 | 1.2 | 30.4 |
| Nausea | Any | 6 | 27.3 | 10.7 | 50.2 | 4 | 18.2 | 5.2 | 40.3 | 3 | 14.3 | 3.0 | 36.3 | 2 | 9.5 | 1.2 | 30.4 |
| | Grade 3 | 0 | 0.0 | 0.0 | 15.4 | 0 | 0.0 | 0.0 | 15.4 | 0 | 0.0 | 0.0 | 16.1 | 0 | 0.0 | 0.0 | 16.1 |
| Pain behind eyes | Any | 4 | 18.2 | 5.2 | 40.3 | 7 | 31.8 | 13.9 | 54.9 | 5 | 23.8 | 8.2 | 47.2 | 3 | 14.3 | 3.0 | 36.3 |
| | Grade 3 | 0 | 0.0 | 0.0 | 15.4 | 0 | 0.0 | 0.0 | 15.4 | 0 | 0.0 | 0.0 | 16.1 | 0 | 0.0 | 0.0 | 16.1 |
| Photophobia | Any | 4 | 18.2 | 5.2 | 40.3 | 3 | 13.6 | 2.9 | 34.9 | 4 | 19.0 | 5.4 | 41.9 | 4 | 19.0 | 5.4 | 41.9 |
| | Grade 3 | 0 | 0.0 | 0.0 | 15.4 | 0 | 0.0 | 0.0 | 15.4 | 0 | 0.0 | 0.0 | 16.1 | 1 | 4.8 | 0.1 | 23.8 |
| Pruritus | Any | 9 | 40.9 | 20.7 | 63.6 | 3 | 13.6 | 2.9 | 34.9 | 3 | 14.3 | 3.0 | 36.3 | 4 | 19.0 | 5.4 | 41.9 |
| | Grade 3 | 0 | 0.0 | 0.0 | 15.4 | 0 | 0.0 | 0.0 | 15.4 | 0 | 0.0 | 0.0 | 16.1 | 0 | 0.0 | 0.0 | 16.1 |
| Rash | Any | 7 | 31.8 | 13.9 | 54.9 | 3 | 13.6 | 2.9 | 34.9 | 3 | 14.3 | 3.0 | 36.3 | 0 | 0.0 | 0.0 | 16.1 |
| | Grade 3 | 0 | 0.0 | 0.0 | 15.4 | 0 | 0.0 | 0.0 | 15.4 | 0 | 0.0 | 0.0 | 16.1 | 0 | 0.0 | 0.0 | 16.1 |
| Vomiting | Any | 2 | 9.1 | 1.1 | 29.2 | 2 | 9.1 | 1.1 | 29.2 | 0 | 0.0 | 0.0 | 16.1 | 0 | 0.0 | 0.0 | 16.1 |
| | Grade 3 | 0 | 0.0 | 0.0 | 15.4 | 0 | 0.0 | 0.0 | 15.4 | 0 | 0.0 | 0.0 | 16.1 | 0 | 0.0 | 0.0 | 16.1 |

*N* = number of subjects per group with data available; *n* = number of subjects with the specified symptom.

after each vaccination. Most of the unsolicited AEs reported by DENV vaccine recipients were related to common infections, including bronchitis, a fungal rash, nasopharyngitis, pharyngitis, rhinitis, tonsillitis, or upper respiratory tract infection, gastrointestinal disorders, or respiratory symptoms (cough, nasal congestion, pharyngolaryngeal pain, sinus congestion, or wheezing). Among placebo recipients, most reports were respiratory or musculoskeletal in nature, although disorders of the nervous system were also reported. There was only one report of a grade 3 unsolicited AE that was assessed by the investigator as treatment-related (back pain in a placebo recipient). During the second study stage, 6 of 9 (66.7%) F17 recipients and 3 of 12 (25.0%) F19 recipients reported at least one unsolicited symptom during the 31-day follow-up period for dose 3; only mild pruritis 5 days after vaccination in one subject, based on the history elicited by the investigator (although not recorded in the subject's diary card), was assessed as potentially related to treatment.

For all groups, most abnormal hematological and biochemical levels were also abnormal at baseline. None of the alert values reported after vaccination were associated with suspected dengue. During the study's first stage, there were no alert values reported after doses of F17/Pre or TDEN F17. One F19 vaccine recipient had a serum neutrophil level of 998 cells/μL (alert value defined as < 1,000 cells/mm$^3$) 14 days after dose 1, which returned to normal the next day. One placebo recipient had an AST of 247 U/L (alert value defined as 2.5 times > 50 U/L) 8 days after dose 2, which returned to normal within 4 days. No one in the study's second stage had an alert safety laboratory value.

During study stage 1, investigators examined all subjects during the follow-up visits to find treatment emergent dengue-like signs. They were uncommon and occurred in all treatment groups, including placebo, except for rash/generalized rash, which occurred only in recipients of DENV vaccines (Table 5).

During the study, there were five subjects who had an AE of potential cardiac origin. A cardiologist evaluated all five subjects by history and physical examination, serial ECGs, serial troponin measurements, and in some cases, additional evaluations, including modified stress tests and echocardiograms. None of these events were confirmed as cardiac in origin; none were considered to be caused by the study treatment.

There were four SAEs reported during the entire study: axonal demyelinating polyneuropathy, cerebral bleed secondary to a physical assault, Hodgkin's lymphoma, and postoperative hypertension after surgery required to repair a fracture sustained in a car accident. None of these events were assessed by the investigator as related to vaccination.

**DENV viremia.** During study stage 1, DENV-1, DENV-2, and DENV-3 viremia were not detected during the 31-day post-vaccination period; DENV-4 viremia was detected in five subjects (29.4%) in the F17/Pre group and one subject (5.3%)

TABLE 4

Incidence of solicited general symptoms during the 21-day follow-up period after the third dose (total vaccinated cohort)

| Symptom and type | F17 ($N = 8$) | | | | F19 ($N = 12$) | | | |
|---|---|---|---|---|---|---|---|---|
| | *n* | % | 95% CI lower | 95% CI upper | *n* | % | 95% CI lower | 95% CI upper |
| Abdominal pain | | | | | | | | |
| Any | 0 | 0.0 | 0.0 | 36.9 | 1 | 8.3 | 0.2 | 38.5 |
| Grade 3 | 0 | 0.0 | 0.0 | 36.9 | 0 | 0.0 | 0.0 | 26.5 |
| Arthralgia | | | | | | | | |
| Any | 0 | 0.0 | 0.0 | 36.9 | 0 | 0.0 | 0.0 | 26.5 |
| Grade 3 | 0 | 0.0 | 0.0 | 36.9 | 0 | 0.0 | 0.0 | 26.5 |
| Fatigue | | | | | | | | |
| Any | 1 | 12.5 | 0.3 | 52.7 | 0 | 0.0 | 0.0 | 26.5 |
| Grade 3 | 0 | 0.0 | 0.0 | 36.9 | 0 | 0.0 | 0.0 | 26.5 |
| Fever | | | | | | | | |
| Any | 3 | 37.5 | 8.5 | 75.5 | 0 | 0.0 | 0.0 | 26.5 |
| Grade 3 | 0 | 0.0 | 0.0 | 36.9 | 0 | 0.0 | 0.0 | 26.5 |
| Headache | | | | | | | | |
| Any | 2 | 25.0 | 3.2 | 65.1 | 0 | 0.0 | 0.0 | 26.5 |
| Grade 3 | 0 | 0.0 | 0.0 | 36.9 | 0 | 0.0 | 0.0 | 26.5 |
| Muscle aches | | | | | | | | |
| Any | 0 | 0.0 | 0.0 | 36.9 | 0 | 0.0 | 0.0 | 26.5 |
| Grade 3 | 0 | 0.0 | 0.0 | 36.9 | 0 | 0.0 | 0.0 | 26.5 |
| Nausea | | | | | | | | |
| Any | 1 | 12.5 | 0.3 | 52.7 | 1 | 8.3 | 0.2 | 38.5 |
| Grade 3 | 0 | 0.0 | 0.0 | 36.9 | 0 | 0.0 | 0.0 | 26.5 |
| Pain behind eyes | | | | | | | | |
| Any | 0 | 0.0 | 0.0 | 36.9 | 0 | 0.0 | 0.0 | 26.5 |
| Grade 3 | 0 | 0.0 | 0.0 | 36.9 | 0 | 0.0 | 0.0 | 26.5 |
| Photophobia | | | | | | | | |
| Any | 0 | 0.0 | 0.0 | 36.9 | 1 | 8.3 | 0.2 | 38.5 |
| Grade 3 | 0 | 0.0 | 0.0 | 36.9 | 0 | 0.0 | 0.0 | 26.5 |
| Pruritus | | | | | | | | |
| Any | 0 | 0.0 | 0.0 | 36.9 | 0 | 0.0 | 0.0 | 26.5 |
| Grade 3 | 0 | 0.0 | 0.0 | 36.9 | 0 | 0.0 | 0.0 | 26.5 |
| Rash | | | | | | | | |
| Any | 0 | 0.0 | 0.0 | 36.9 | 0 | 0.0 | 0.0 | 26.5 |
| Grade 3 | 0 | 0.0 | 0.0 | 36.9 | 0 | 0.0 | 0.0 | 26.5 |
| Vomiting | | | | | | | | |
| Any | 1 | 12.5 | 0.3 | 52.7 | 0 | 0.0 | 0.0 | 26.5 |
| Grade 3 | 0 | 0.0 | 0.0 | 36.9 | 0 | 0.0 | 0.0 | 26.5 |

$N$ = number of subjects per group with data available; $n$ = number of subjects with the specified symptom.

TABLE 5

Incidence of dengue-like physical examination signs per subject overall (considering doses 1 and 2) during the 31-day follow-up period after vaccination (total vaccinated cohort)

| Physical sign | F17/Pre ($N = 22$) | | F17 ($N = 22$) | | F19 ($N = 21$) | | Placebo ($N = 21$) | |
|---|---|---|---|---|---|---|---|---|
| | *n* | % | *n* | % | *n* | % | *n* | % |
| Rash | 3 | 13.6 | 1 | 4.5 | 3 | 14.3 | 0 | 0 |
| Gen. rash | 1 | 4.5 | 0 | 0 | 2 | 9.5 | 0 | 0 |
| Skin hemorrhage | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 4.8 |
| Conjunctival hemorrhage | 0 | 0 | 0 | 0 | 0 | 0 | 1 | 4.8 |
| Conjunctival injection | 2 | 9.1 | 1 | 4.5 | 2 | 9.5 | 1 | 4.8 |
| Mucosal hemorrhage | 2 | 9.1 | 1 | 4.5 | 1 | 4.8 | 1 | 4.8 |
| Lymphadenopathy | 4 | 18.2 | 6 | 27.3 | 7 | 33.3 | 4 | 19.0 |
| Gen. lymphadenopathy | 0 | 0 | 1 | 4.5 | 0 | 0 | 1 | 4.8 |
| Hepatomegaly | 2 | 9.1 | 3 | 13.6 | 6 | 28.6 | 4 | 19.0 |
| Splenomegaly | 0 | 0 | 1 | 4.5 | 1 | 4.8 | 2 | 9.5 |

Gen. rash = generalized rash involving at least 50% of the body surface; Gen. lymphadenopathy = palpable lymph nodes in four or more of the following locations: cervical, axillary, inguinal, or other areas (right and left sides are considered as separate locations); N = number of subjects per group with data available; n = number of subjects with the specified symptom.

in the F17 group between days 5 and 14 post-vaccination. The magnitude of the DENV-4 viremia ranged from 4.6 to 5.2 log Geq/mL of serum. There were no cases of viremia reported in the F19 or placebo groups. Five of these six subjects with DENV-4 viremia were unprimed before the first vaccination (four subjects in the F17/Pre group and one subject in the F17 group).

There were no alert laboratory findings associated with these six instances of viremia, and there were no symptoms associated with the viremia in the TDEN recipient. However, four of five F17/Pre vaccine recipients with DENV-4 viremia reported symptoms in temporal association with the day that viremia was detected as follows: day 8 post-dose 1 (4.6 log Geq/mL) with grade 1 arthralgia, headache, fatigue, muscle aches, and pain behind the eyes, all lasting 4 days, with rash occurring 1 day later; day 13 post-dose 1 (4.9 log Geq/mL) with fever and symptoms as presented in the case history below; day 14 post-dose 2 viremia (4.6 log Geq/mL) with generalized rash; day 8 post-dose 2 (5.2 log Geq/mL) with grade 2 arthralgia and grade 1 muscle aches for 1 day, grade 2 fatigue for 2 days, and rash and conjunctival injection noted by the investigator on day 13; day 15 post-dose 2 (5.2 log Geq/mL) with no symptoms.

**Case history of a febrile illness after DENV vaccine.** Based on the occurrence of fever and other symptoms, dengue was suspected in two subjects, both after dose 1. One subject received F17/Pre vaccine, and the other received placebo. These subjects were evaluated by an investigator with history, physical, and laboratory testing (CBC, ALT/AST, and viremia). Neither case met the clinical and laboratory criteria for confirmed dengue. Nevertheless, the case of the subject who received F17/Pre vaccine is briefly described below, because he was confirmed to have 4.9 log Geq/mL DENV-4 viremia on day 13 after vaccine dose 1.

The affected subject was primed at baseline for all four DENV types. He developed fever and complained of sore throat on day 12, which resolved after 2 days. From day 9 to 16 after dose 1, the subject also reported grades 1–2 headache, nausea, pain behind the eyes, photophobia, rash, pharyngitis, and musculoskeletal pain lasting anywhere from 3 to 12 days.

**Immunogenicity.** Immunogenicity was characterized separately for unprimed and primed subjects, because live DENV vaccines can elicit anamnestic multivalent antibody responses after a single dose in individuals having baseline antibody to DENV or other flaviviruses. DENV neutralizing antibody seropositivity rates and GMTs for unprimed subjects with MN50 data available are presented in Tables 6 and 7, and reverse cumulative curves are shown in Figures 3–6.

In unprimed subjects, both TDEN formulations were moderately immunogenic after a single dose; seropositivity rates at 1 month after dose 1 were highest for DENV2 (68.8% F17, 86.7% F19) and somewhat less, although similar, for the other three DENV types (Table 6). Seropositivity rates increased 1 month after dose 2 for all DENV types; responses to DENV2 were again the highest (80.0% F17, 100% F19). Response rates to the other types seemed to be somewhat less, in the range from 66.7% to 83.3%. The rates of seropositivity after doses 1 and 2 of F17/Pre were similar to those rates observed after the TDEN vaccines. No unprimed subject in the placebo group was seropositive after dose 2. In general, there was a slight increase in GMTs for unprimed subjects in all vaccine groups from doses 1 to 2 (Table 7).

Seroconversion rates to each DENV type (i.e., mono-, bi-, tri-, and tetravalent responses) per group and dose are shown

TABLE 6
Seropositivity rates to each DENV type in unprimed subjects administered DENV vaccines (ATP cohort with MN50 data available)

| Virus type and vaccine group | Time points | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Post-dose 1 | | | Post-dose 2 | | | Pre-dose 3 | | | Post-dose 3 | | |
| | *N* | *n* ≥ 1:10 | % ≥ 1:10 (95% CI) | *N* | *n* ≥ 1:10 | % ≥ 1:10 (95% CI) | *N* | *n* ≥ 1:10 | % ≥ 1:10 (95% CI) | *N* | *n* ≥ 1:10 | % ≥ 1:10 (95% CI) |
| DENV-1 | | | | | | | | | | | | |
| F17/Pre | 16 | 7 | 43.8 (19.8–70.1) | 14 | 13 | 92.9 (66.1–99.8) | | | ND | | | ND |
| F17 | 16 | 6 | 37.5 (15.2–64.6) | 15 | 11 | 73.3 (44.9–92.2) | 6 | 4 | 66.7 (22.3–95.7) | 6 | 5 | 83.3 (35.9–99.6) |
| F19 | 15 | 10 | 66.7 (38.4–88.2) | 12 | 10 | 83.3 (51.6–97.9) | 7 | 5 | 71.4 (29.0–96.3) | 7 | 7 | 100 (59.0–100) |
| DENV-2 | | | | | | | | | | | | |
| F17/Pre | 16 | 14 | 87.5 (61.7–98.4) | 14 | 14 | 100 (76.8–100) | | | ND | | | ND |
| F17 | 16 | 11 | 68.8 (41.3–89.0) | 15 | 12 | 80.0 (51.9–95.7) | 6 | 4 | 66.7 (22.3–95.7) | 6 | 5 | 83.3 (35.9–99.6) |
| F19 | 15 | 13 | 86.7 (59.5–98.3) | 12 | 12 | 100 (73.5–100) | 7 | 4 | 57.1 (18.4–90.1) | 7 | 7 | 100 (59.0–100) |
| DENV-3 | | | | | | | | | | | | |
| F17/Pre | 16 | 8 | 50.0 (24.7–75.3) | 14 | 10 | 71.4 (41.9–91.6) | | | ND | | | ND |
| F17 | 16 | 8 | 50.0 (24.7–75.3) | 15 | 10 | 66.7 (38.4–88.2) | 6 | 1 | 16.7 (0.4–64.1) | 6 | 5 | 83.3 (35.9–99.6) |
| F19 | 15 | 9 | 60.0 (32.3–83.7) | 12 | 10 | 83.3 (51.6–97.9) | 7 | 3 | 42.9 (9.9–81.6) | 7 | 4 | 57.1 (18.4–90.1) |
| DENV-4 | | | | | | | | | | | | |
| F17/Pre | 16 | 6 | 37.5 (15.2–64.6) | 14 | 11 | 78.6 (49.2–95.3) | | | ND | | | ND |
| F17 | 16 | 9 | 56.3 (29.9–80.2) | 15 | 11 | 73.3 (44.9–92.2) | 6 | 2 | 33.3 (4.3–77.7) | 6 | 5 | 83.3 (35.9–99.6) |
| F19 | 15 | 8 | 53.3 (26.6–78.7) | 12 | 8 | 66.7 (34.9–90.1) | 7 | 3 | 42.9 (9.9–81.6) | 7 | 4 | 57.1 (18.4–90.1) |

Seropositivity is DENV antibody titer ≥ 1:10 dilution based on MN50 assay. *N* = number of subjects per group with immunogenicity data available at a specified time point; *n* = number of subjects seropositive for the specified DENV type; ND = not done; post-dose 1 = blood sample taken 1 month after dose 1 administration; post-dose 2 = blood sample taken 1 month after dose 2 administration; post-dose 3 = blood sample taken 1 month after dose 3 administration; pre-dose 3 = blood sample taken before dose 3 administration (at the dose 3 visit).

in Figure 2. Tetravalent seroconversion rates among the unprimed subjects were modest 1 month after dose 1 for any DENV vaccination (37.5% F17/Pre, 37.5% F17, and 40.0% F19). One month after dose 2, tetravalent rates increased (71.4% F17/Pre, 60.0% F17, and 66.7% F19).

In unprimed subjects, acknowledging the small groups sizes, there were no striking differences among the reverse cumulative curves of neutralizing antibody titers by DENV treatment after dose 2 for any DENV type (Figures 3–6).

Among the 14 primed subjects in the per-protocol immunogenicity cohort with MN50 data available (3–4 subjects per group), the tetravalent rate was 100% 1 month after dose 2 (data not shown), with high GMTs to each DENV type regardless of DENV treatment.

Individual antibody titers of subjects in study stage 2 who had MN50 data available at 1 month post-dose 2 and pre- and post-dose 3 visits are provided in Table 8. The tetravalent response rates before and 1 month after dose 3 (stage 2) in unprimed subjects were 16.7% and 66.7% in six F17 recipients and 28.6% and 57.1% in seven F19 recipients. Among the primed subjects who were in study stage 2 (two subjects in F17 and two subjects in F19), the tetravalent rate was 100% on the day of dose 3 and 1 month later. There was no important change in antibody level to any DENV type for any subject.

## DISCUSSION

In this first clinical evaluation of the new WRAIR-GSK TDEN live vaccine candidate that is the successor to the live tetravalent DENV vaccine previously in development, we found that the new candidate had a clinically acceptable

TABLE 7
GMTs to each DENV type in unprimed subjects administered DENV vaccines (ATP cohort with MN50 data available)

| Virus type and vaccine group | Time points | | | | | | |
|---|---|---|---|---|---|---|---|
| | Post-dose 1 | | Post-dose 2 | | Pre-dose 3 | | Post-dose 3 | |
| | *N* | GMT (95% CI) | *N* | GMT (95% CI) | *N* | GMT (95% CI) | *N* | GMT (95% CI) |
| DENV-1 | | | | | | | | |
| F17/Pre | 16 | 22 (7–74) | 14 | 198 (59–664) | | ND | | ND |
| F17 | 16 | 11 (6–19) | 15 | 50 (14–177) | 6 | 15 (4–57) | 6 | 39 (7–226) |
| F19 | 15 | 79 (20–317) | 12 | 118 (38–368) | 7 | 35 (6–187) | 7 | 73 (19–281) |
| DENV-2 | | | | | | | | |
| F17/Pre | 16 | 147 (47–457) | 14 | 666 (376–1,179) | | ND | | ND |
| F17 | 16 | 58 (19–175) | 15 | 156 (46–527) | 6 | 33 (5–203) | 6 | 104 (15–720) |
| F19 | 15 | 195 (56–677) | 12 | 226 (70–729) | 7 | 30 (6–158) | 7 | 194 (56–675) |
| DENV-3 | | | | | | | | |
| F17/Pre | 16 | 17 (7–44) | 14 | 75 (20–283) | | ND | | ND |
| F17 | 16 | 12 (7–21) | 15 | 31 (9–103) | 6 | 7 (3–20) | 6 | 34 (5–226) |
| F19 | 15 | 21 (8–56) | 12 | 52 (15–177) | 7 | 12 (4–38) | 7 | 19 (5–69) |
| DENV-4 | | | | | | | | |
| F17/Pre | 16 | 25 (7–93) | 14 | 279 (57–1,381) | | ND | | ND |
| F17 | 16 | 43 (10–177) | 15 | 70 (17–293) | 6 | 29 (2–521) | 6 | 46 (4–535) |
| F19 | 15 | 35 (9–140) | 12 | 46 (12–179) | 7 | 23 (3–168) | 7 | 31 (4–231) |

GMT = geometric mean neutralizing DENV antibody titer calculated on all subjects; *N* = number of subjects with available results; ND = not done; post-dose 1 = blood sample taken 1 month after dose 1 administration; post-dose 2 = blood sample taken 1 month after dose 2 administration; post-dose 3 = blood sample taken 1 month after dose 3 administration; pre-dose 3 = blood sample taken before dose 3 administration (at the dose 3 visit).

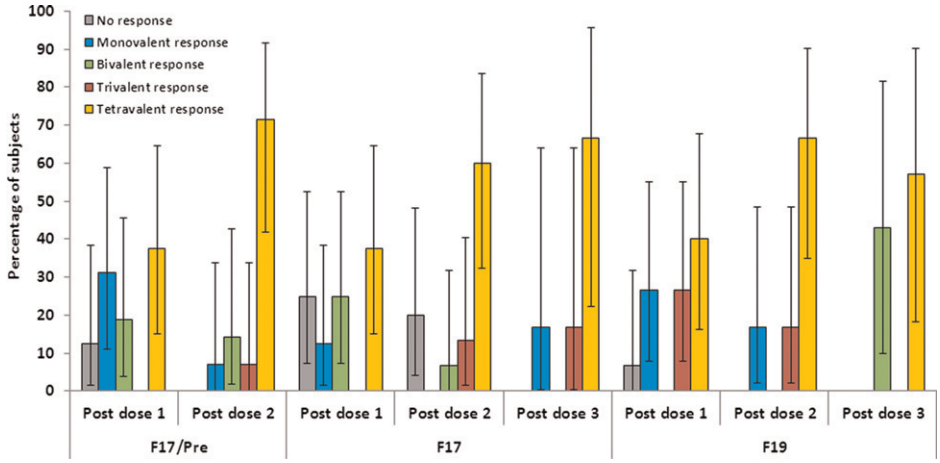


FIGURE 2. Mono-, bi-, tri-, and tetravalent responses to DENV types in unprimed subjects per group and dose (ATP cohort). Percentages of initially unprimed subjects in each group (F17/Pre, F17, and F19) having seroconverted for none (grey), one (blue), two (green), three (red), or four (orange) DENV types after each dose of the candidate vaccine are shown. Blood samples were taken from subjects 1 month after each dose.

safety profile in a small number of healthy adults. In addition, we found that it was moderately immunogenic after two doses were administered 6 months apart in persons with no baseline neutralizing antibodies to DENV.

The new TDEN vaccine candidate was re-derived by transfection and amplification of the WRAIR's attenuated DENV strains used in the precursor vaccine to improve their quality characteristics. Although non-clinical testing conducted before this clinical trial identified no phenotypic or genotypic changes relative to the precursor vaccine, the trial was designed to explore whether there were important clinical differences between the new candidate and its precursor. The intent to assess potential clinical differences between the new TDEN candidate and the F17/Pre candidate vaccine, while controlling for responses that might be affected by the trial's environment, mandated our use of two control groups: F17/Pre with a virus content adjusted to the level of the new TDEN F17 vaccine and a saline placebo.

This clinical trial was also intended to explore whether the response to the TDEN vaccine was conditioned by its DENV-4 content. The DENV-4 strain had the fewest cell culture passages compared with the other three strains; moreover, in clinical trials of the precursor vaccine F17/Pre, the DENV-4 strain was the most common cause of documented viremia. Consequently, we blended and finished two lots of the TDEN vaccine, F17 and F19, which differed in their DENV-4 content. Although F19 was intended to have 10-fold less DENV-4, the potency testing at release showed that the measured reduction in F19 DENV-4 content was fourfold. Nevertheless, we believed this difference was sufficient to potentially affect interactions between the DENV strains and the clinical responses in terms of fever and other symptoms related to virus replication, viremia, and the humoral immune response to each DENV type.[15]

Lastly, with respect to design of this clinical trial, we retained the two-dose schedule for primary vaccination used in previous trials of the precursor F17/Pre vaccine. These previous trials had clearly shown the need for two vaccine doses to generate tetravalent antibody responses in unprimed subjects. We also retained the 6-month interval between doses, because pilot experiments conducted with the precursor vaccine at the WRAIR had suggested that intervals of 1 or 3 months between doses was ineffective (Gibbons R and others, unpublished data), whereas a 6-month interval had allowed the second vaccine dose to be consistently immunogenic.

Because this study was the first clinical evaluation of a new vaccine candidate, it was conducted in healthy adults. In contrast to our preceding early-phase live DENV vaccine clinical trials, we elected to enroll subjects without first screening to exclude those individuals with baseline antibodies to DENV or other flaviviruses. This enrolment better represents the profile of potential vaccine recipients who may be at highest risk for dengue, while acknowledging that the responses of primed and unprimed subjects may be different in terms of both reactogenicity and immunogenicity. We did not know whether priming increased or reduced reactogenicity, but we did know that priming increased immunogenicity. In light of our study location in metropolitan Washington, DC, we expected the proportion of enrollees who were primed to DENV to comprise less than 20% of the total enrolled cohort.

The total vaccinated cohort had a median age of 35.5 years, with a maximum age of 45 years. African Americans comprised 57% of the cohort. Although the effects of age and race on response to live DENV vaccines are not fully understood, it is important to note that increasing age in general diminishes responses to many vaccines, and others have hypothesized that persons of African ancestry may have less clinically overt responses to wild-type DENV infection.[16] The prevalence of priming to DENV in the ATP immunogenicity cohort with MN50 data was 18%; this prevalence allowed us to have 15–16 unprimed subjects and 3–4 primed subjects per treatment group, a minimally adequate sample size to preliminarily assess the potential of the vaccine candidate to offer effective immunization based on its immunogenicity in unprimed adults.

The most important endpoints for safety were injection site and general symptoms that we solicited from subjects by asking them to maintain a diary for 21 days after each vaccination. Of these symptoms, we considered the absence of fever to be the most objective and reliable measure of overall attenuation.[17] Although the group sizes in the total vaccinated cohort for

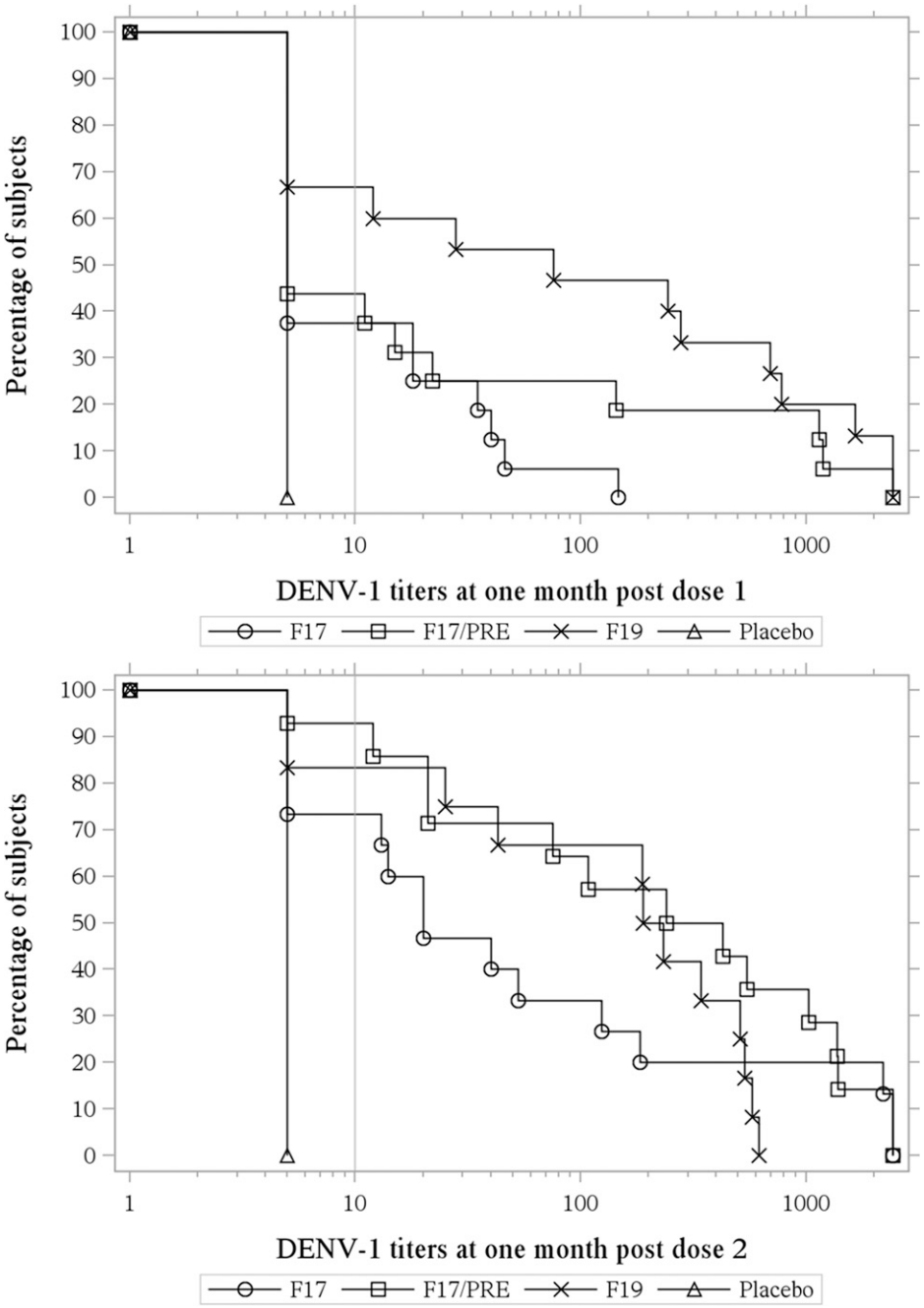


FIGURE 3. Reverse cumulative curves of DENV-1 neutralizing antibody titers 1 month after dose 1 and dose 2 for each vaccine group for unprimed subjects (ATP cohort for immunogenicity).

each treatment were small (21 or 22 subjects in study stage 1), they were appropriate for an initial clinical trial.

In study stage 1, most solicited injection site symptoms reported in the first stage of the study were mild to moderate and lasted 2 days or less. Their frequency within the DENV vaccine groups did not seem to differ from placebo or increase when a second dose was administered. The F17/Pre group had the highest proportion of subjects reporting pain, particularly after dose 2 (43.8%; acceptable for a parenterally administered vaccine); although this result may have been a chance finding, the presence of human serum albumin in the F17/Pre formulation and its absence in the other formulations may have been contributory. In study stage 2, when a small number of subjects received a third TDEN dose, the occurrence of solicited injection site symptoms was very low.

We found no major differences among treatment groups in the incidence of solicited general symptoms in study stage 1. Grade 3 solicited general symptoms were reported by < 5% (zero to one subjects per group; the one exception was in the placebo group). The incidence of fever by group after dose 1, dose 2, and overall did not suggest any difference among treatments. Rash was the only symptom reported exclusively in DENV groups (31.8% F17/Pre, 13.6% F17, and 14.3% F19); its occurrence in recipients of live DENV vaccines has been noted previously.[18,19] In addition to soliciting subjects for the occurrence of symptoms that could be expected in response to

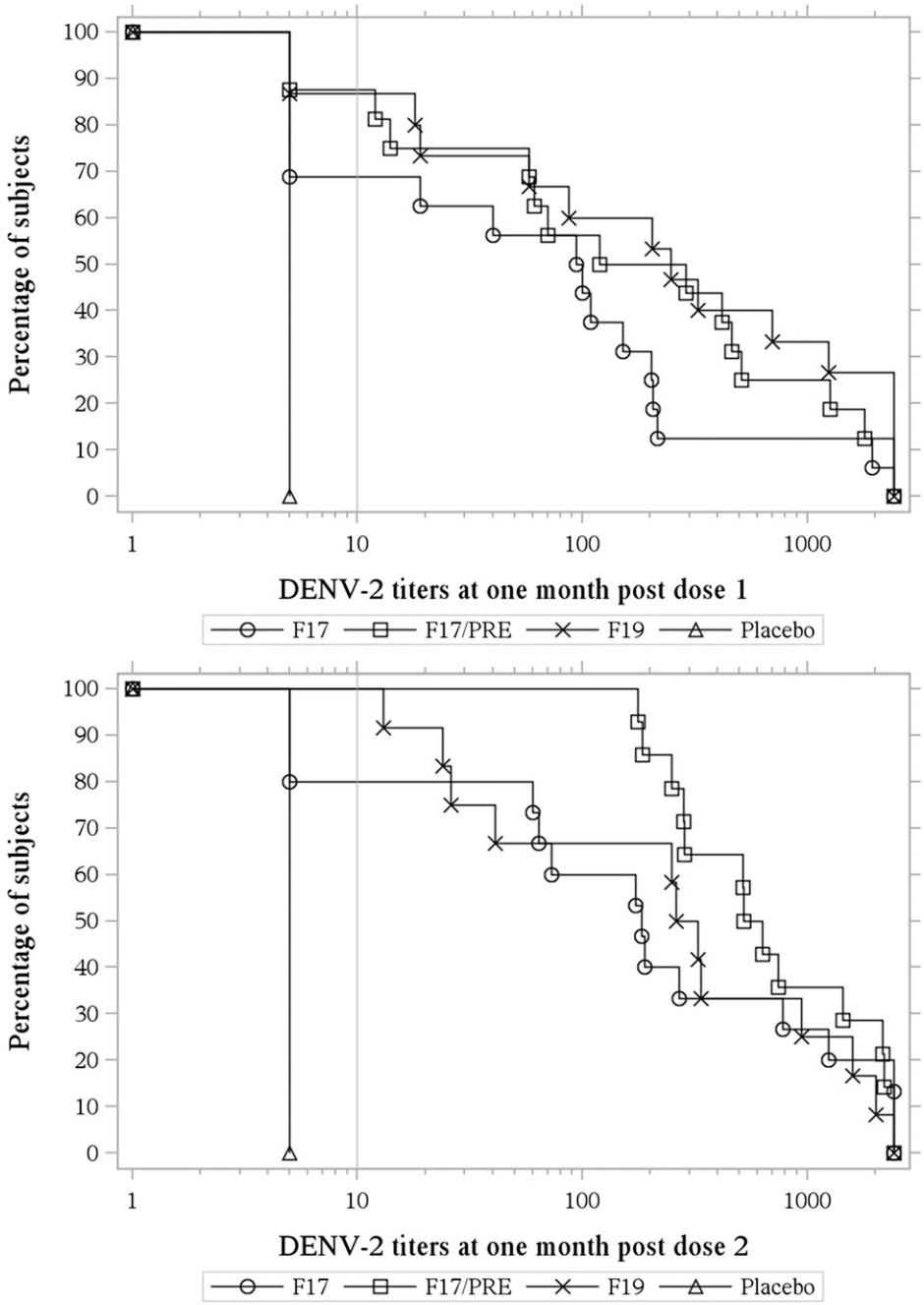


FIGURE 4. Reverse cumulative curves of DENV-2 neutralizing antibody titers 1 month after dose 1 and dose 2 for each vaccine group for unprimed subjects (ATP cohort for immunogenicity).

a live DENV vaccine, we also collected all spontaneous reports of adverse events (unsolicited symptoms) for 31 days after each vaccination and SAEs throughout the study. There were no clinically important differences in the incidence of unsolicited AEs among treatment groups in either study stage. Only one grade 3 causally related unsolicited symptom was reported during stage 1 (back pain in placebo group). In study stage 2, the occurrence of solicited general symptoms was low. Throughout the entire study, there were only four SAEs; none were assessed by an investigator as related to the study treatment. Overall, the findings with respect to solicited and unsolicited symptoms after vaccination support that the new TDEN vaccine candidate as either formulation is attenuated and has a reactogenicity profile that compares favorably with the precursor vaccine and saline placebo.

To extend the evaluation of clinical responses to vaccination during the 2 weeks after administration, the period when clinical responses to replication of the vaccine viruses were expected to be maximal, we evaluated all members of the study cohort on 2 randomly selected days (from the set of days 2, 5, 8, 10, 12, and 14) and 1 month after vaccination during study stage 1 for dengue-like physical examination finding, hematologic and serum chemistry tests, DENV viremia, and AEs of potential cardiac origin. We performed these

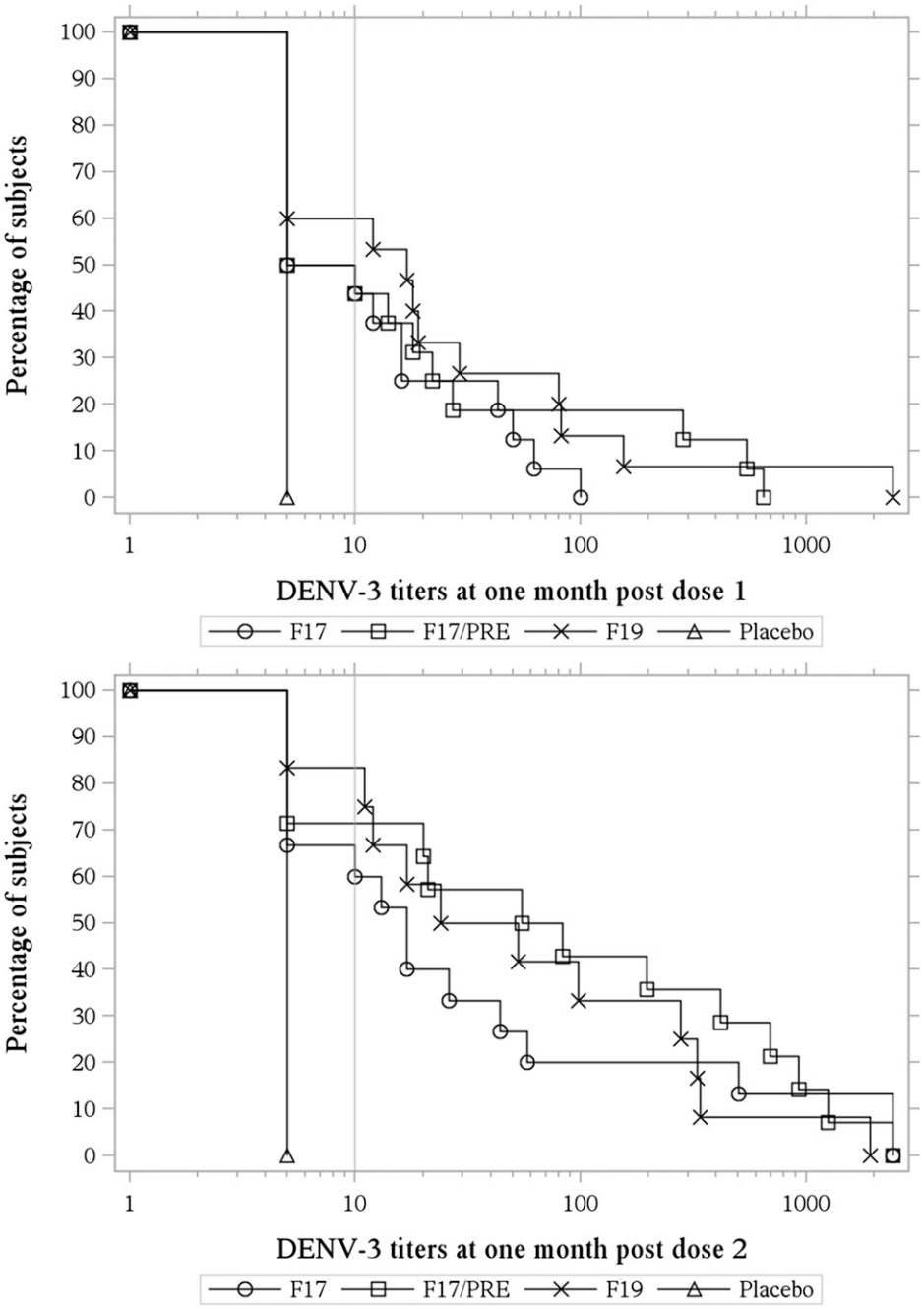


FIGURE 5. Reverse cumulative curves of DENV-3 neutralizing antibody titers 1 month after dose 1 and dose 2 for each vaccine group for unprimed subjects (ATP cohort for immunogenicity).

same observations on the subset of subjects who received a third dose of TDEN vaccine during study stage 2.

During the entire study, there were no important differences among groups in terms of dengue-like physical examination signs; the incidence of rash and generalized rash was low in the DENV groups, but no rash was observed in the placebo group. Of all dengue-like signs, rash seems to be the one with the most specific potential association with receipt of a live DENV vaccine relative to placebo.[18,19] Our clinical impression is that these rashes are not bothersome to vaccine recipients unless they are accompanied by pruritis. With respect to hematologic or serum chemistry laboratory results that differed from baseline, there were few abnormal results during the entire study; consequently, there were no notable differences among groups in the proportion of subjects who reached this endpoint.

In a previous clinical trial of the F17/Pre vaccine candidate, one adult subject reported transient palpitations approximately 3 weeks after vaccination (Gibbons R and others, unpublished data). This report, in conjunction with reports of myocarditis after dengue and fatal myopericarditis after smallpox vaccination, prompted us to include clinical evaluations designed to detect AEs of potential cardiac origin in this study. During the entire study, there were no AEs of cardiac origin confirmed by

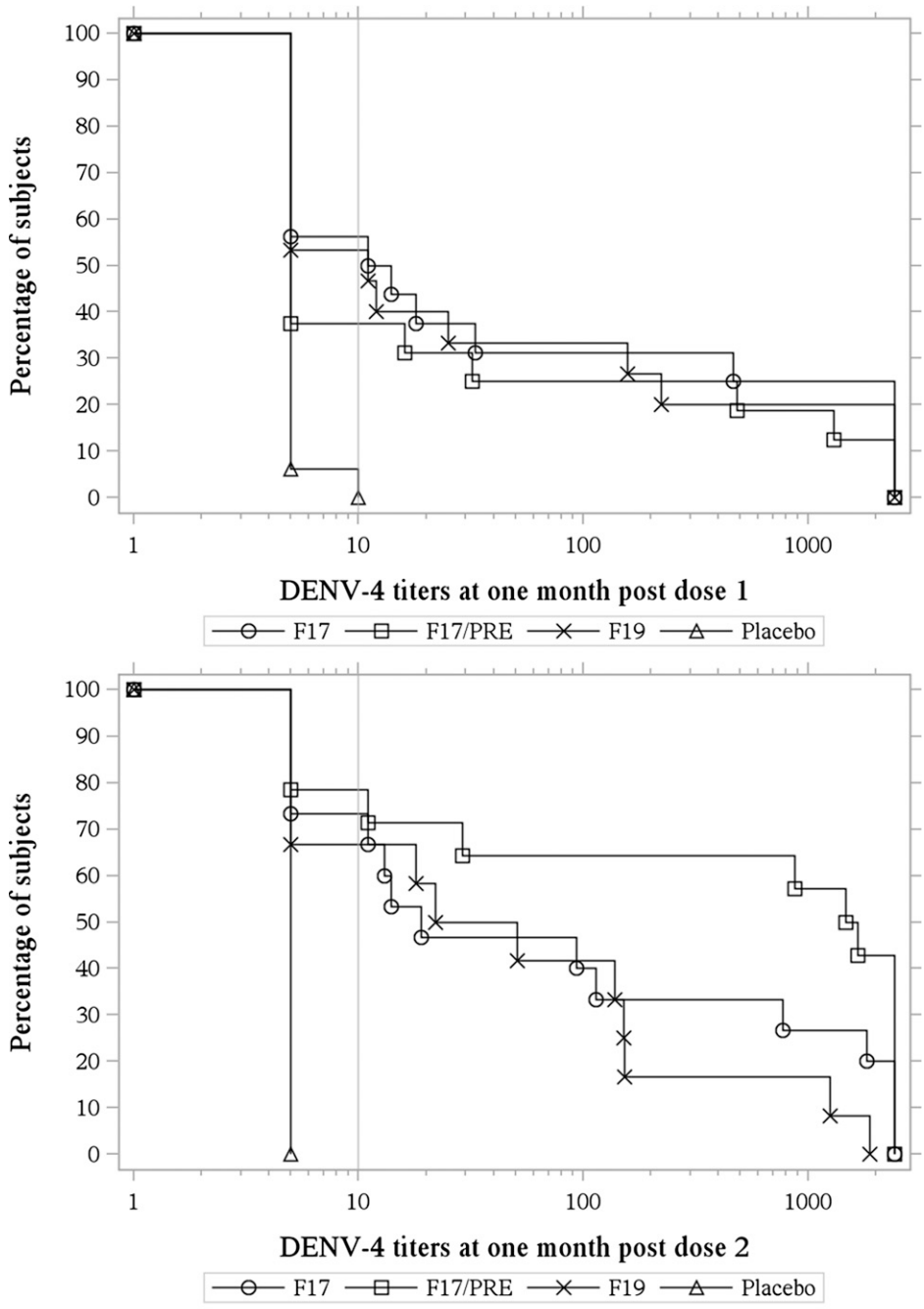


FIGURE 6. Reverse cumulative curves of DENV-4 neutralizing antibody titers 1 month after dose 1 and dose 2 for each vaccine group for unprimed subjects (ATP cohort for immunogenicity).

a Walter Reed Army Medical Center cardiologist. This type of evaluation has been included in subsequent expanded phase II trials of the TDEN vaccine candidate.

The screening for DENV viremia in this trial was more intensive than we have performed in earlier trials of the F17/Pre vaccine candidate; all subjects in this trial were tested two times on randomly assigned days during the 2 weeks after each vaccine dose, whereas formerly, we tested subjects only on day 10 or when they developed illness suspected to be dengue. During study stage 1, we detected DENV-4 viremia in five recipients of F17/Pre vaccine and one recipient of F17 vaccine (from 7 to 15 days after vaccination). The single instance of viremia occurring in the TDEN recipient was subclinical, because it was not associated with symptoms, dengue-like physical signs, or clinical laboratory abnormalities. However, four of five subjects in the F17/Pre group with DENV-4 viremia did have symptoms, although only one had fever (on a single day). None of these four subjects had a clinically notable illness; none met the pre-specified case definition for dengue. The levels of DENV4 viremia in these

TABLE 8
Individual DENV antibody titers from subjects with data available for all three doses (ATP cohort)

| Groups and subject ID | Primed | Post-dose 2 | | | | Pre-dose 3 | | | | Post-dose 3 | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | DENV-1 | DENV-2 | DENV-3 | DENV-4 | DENV-1 | DENV-2 | DENV-3 | DENV-4 | DENV-1 | DENV-2 | DENV-3 | DENV-4 |
| F17 | | | | | | | | | | | | | |
| 14 | | 2,202 | > 2,430 | 504 | > 2,430 | 147 | 236 | 53 | 1,522 | 166 | 210 | 77 | 1,550 |
| 29 | | < 10 | < 10 | < 10 | < 10 | < 10 | < 10 | < 10 | < 10 | 460 | < 10 | 828 | 14 |
| 35 | Yes | 549 | >2,430 | 1,551 | 228 | 426 | 930 | 1,327 | 310 | 552 | 1,416 | 1,893 | 228 |
| 49 | Yes | 1,208 | 672 | 494 | 213 | 638 | 129 | 137 | 49 | 819 | 583 | 418 | 202 |
| 65 | | 13 | 184 | 13 | 14 | 10 | 31 | < 10 | < 10 | 33 | 73 | 25 | 14 |
| 67 | | 20 | 776 | 17 | 13 | 11 | 234 | < 10 | < 10 | 15 | 219 | 12 | 13 |
| 88 | | 53 | 189 | 26 | 1,825 | 26 | 32 | < 10 | 651 | 20 | 58 | 16 | 485 |
| 95 | | < 10 | 64 | < 10 | 93 | < 10 | < 10 | < 10 | < 10 | < 10 | 1,281 | < 10 | < 10 |
| F19 | | | | | | | | | | | | | |
| 20 | | 233 | 1,588 | 277 | 1,255 | 20 | 449 | 63 | 204 | 105 | 466 | 89 | 350 |
| 34 | | 512 | 26 | 17 | < 10 | 202 | < 10 | < 10 | < 10 | 253 | 19 | < 10 | < 10 |
| 46 | | < 10 | 326 | < 10 | < 10 | < 10 | 66 | < 10 | < 10 | 11 | 250 | < 10 | < 10 |
| 54 | | 618 | 337 | 98 | 22 | 399 | 67 | 60 | < 10 | 571 | 157 | 110 | 24 |
| 61 | Yes | > 2,430 | 1,372 | 1,417 | 1,134 | > 2,430 | 939 | 1,435 | 1,343 | > 2,430 | 800 | 1,405 | 1,172 |
| 64 | | 25 | 24 | 11 | 1,887 | 11 | < 10 | < 10 | 944 | 38 | 171 | 21 | 1,080 |
| 71 | | < 10 | 41 | < 10 | < 10 | < 10 | < 10 | < 10 | < 10 | 15 | 1,540 | < 10 | < 10 |
| 75 | Yes | > 2,430 | 1,780 | 1,536 | 628 | > 2,430 | 482 | 1,392 | 437 | > 2,430 | 790 | 1,605 | 326 |
| 82 | | 188 | 261 | 53 | 51 | 133 | 87 | 19 | 27 | 119 | 115 | 40 | 22 |

Post-dose 2 = blood sample taken 1 month after dose 2 administration; post-dose 3 = blood sample taken 1 month after dose 3 administration; pre-dose 3 = blood sample taken before dose 3 administration (at the dose 3 visit).

F17/Pre vaccine recipients were in line with those levels reported in children who received F17/Pre vaccine[9,10]; however, these levels of viremia were substantially less than those levels determined by bioassay in log median mosquito infectious doses per milliliter in patients with clinically overt dengue caused by wild-type infection.[20] Overall, the findings with respect to dengue physical examination signs, clinical laboratory determinations, and viremia after vaccination further support the acceptable safety profile of the new TDEN vaccine candidate. The detection of DENV-4 viremia in one TDEN vaccine recipient in contrast to five F17/Pre vaccine recipients also suggests that the re-derivation and serial passage of the DENV-4 seed virus for the TDEN vaccine candidate may have created a more attenuated DENV-4 strain. More clinical experience with the TDEN vaccine candidate will be required to confirm this observation.

We assessed vaccine immunogenicity as a surrogate for clinical benefit. In subjects who were seronegative for a DENV type at baseline, we considered that the detection of neutralizing antibodies at or above the assay positive threshold indicated activation of an adaptive immune response. In subjects who were already seropositive for a DENV type, we assessed whether there was an increase in antibody titer and whether primed subjects with less than tetravalent antibody responses were promoted to acquire tetravalent neutralizing antibodies. A dengue vaccine will ideally elicit protection against disease caused by any of the four DENV types; nevertheless, as with other multivalent live vaccines, there is a potential for interference between the DENV types in any tetravalent live DENV vaccine, resulting in an incomplete or diminished tetravalent immune response profile.[2,15] Although we do not know if generation of sustained tetravalent neutralizing antibodies after vaccination is associated with protection, we assume that such responses acutely after vaccination are favorable, whereas their absence is not.

In this trial, we used a microneutralization assay to quantitate the 50% effective dose of neutralizing antibodies to each DENV type in contrast to the 50% plaque reduction neutralization test (PRNT) that we have used in all previous work. Access to a qualified, high-throughput, serotype-specific neutralization assay is essential for development of a DENV vaccine.[2,21] The microneutralization test that we used was developed at the WRAIR Pilot Bioproduction Facility to overcome many of the PRNT's limitations, such as its low throughput, unacceptable labor-intensive nature, and high degree of interassay variability.[22] This new assay was shown to be specific and sensitive for the detection of anti-DENV neutralizing antibodies (e.g., limit of the blank < 1:3.3; limit of detection < 1:7; limit of quantification ≤ 1:10 for all four serotypes). The precision of the assay was estimated to range from 39% to 59% depending on serotype. Performance characteristics of this assay are described in another publication under preparation (De La Barrera R and others, unpublished data).

We found that the TDEN vaccine candidates were modestly immunogenic across all DENV types after a single dose and moderately immunogenic across all types after two doses. Overall, dose 2 expanded and broadened the immunogenicity responses (seropositivity rates, GMTs, and tetravalent seroconversion) elicited after dose 1. There were no clear differences in the immunogenicity of any of the three DENV vaccine candidates. As has been the case in all prior studies of the F17/Pre vaccine candidate and as seen with other live dengue vaccines in development,[23] we found that most vaccine recipients failed to develop tetravalent responses after the first vaccine dose. Among unprimed subjects in this trial who received a TDEN vaccine formulation, the tetravalent vaccine response increased from 37.5–40.0% after dose 1 to approximately 60.0–66.7% after dose 2. In a previous adult study of F17/Pre vaccine candidates, Sun and others[8] observed a 63% tetravalent antibody rate 1 month after dose 2 measured by the PRNT. In two subsequent studies of the F17/Pre vaccine candidate in children[9] and infants,[10] 100% of the DENV unprimed children and 54% of the DENV unprimed infants had tetravalent antibody 1 month after the second dose.

We found that a third dose administered 5–12 months after the second dose was ineffective at boosting the immune responses to any of the four DENV types. Tetravalent response rates and GMTs did not increase from 1 month after dose 2 to 1 month after dose 3; however, they did increase from pre-dose 3 to 1 month after dose 3 among the small number of subjects tested. In a study of the F17/Pre vaccine candidate administered on the same two-dose schedule given 6 months apart to infants, it was found that, by 1 year after the second dose, seropositivity rates decreased to all but one of the DENV types. This study suggested that DENV neutralizing antibody titers determined 1 year after administration of vaccine dose 2 may underestimate immunity based on the observed loss of detectable anti-DENV-4 antibody in subjects who had antecedent DENV-4 vaccine viremia.[10] The impact of declining levels of DENV neutralizing antibodies on protective immunity is unknown and warrants additional study.

## CONCLUSION

This study found the WRAIR-GSK re-derived, live-attenuated, tetravalent DENV vaccine candidate to have a clinically acceptable safety and immunogenicity profile in a small number of healthy adult subjects, including several subjects who were immunologically primed at the time of vaccination. Although administration of a third dose after at least a 5-month interval was well-tolerated, it offered limited additional immunogenicity. There were no important differences observed between the two TDEN formulations evaluated. Both formulations should be evaluated in a larger number of healthy adult subjects and then, children.

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