Table 2. Timing of First, Second, and Third Vaccinations for Each Cohort (Dosage Group) over 12 wk

Cohort	Month 0	Month 1	Month 2	Month 3	Months 4–11
10 μg/rabies	Screening	1st	2nd	3rd	SAE follow-up
25 μg/rabies		1st	2nd	3rd	
50 μg/rabies			1st	2nd	3rd

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safety data reports as the trial progressed and the data analysis by Statistics Collaborative after study completion. DSMB membership included a statistician and four senior clinical research investigators with experience in conducting malaria vaccine trials, one of whom was a Kenyan. An experienced local clinician (Ambrose Misore, M.B.Ch.B.) served as the local medical monitor (LMM), reviewed all serious adverse events (SAEs) and safety data between dose escalations, and functioned as the patient advocate. In addition, the trial was monitored for regulatory compliance by representatives of the United States Army Medical Materiel Development Activity, GlaxoSmithKline Biologicals, and Pharmaceuticals Product Development (a contract research organization based in Wilmington, North Carolina, United States), all of whom made several visits to the study site. Initial approval of the study protocol and of subsequent protocol amendments was granted to the investigators by the Ethical Review Committee of the Kenya Medical Research Institute, Nairobi; by the Human Subjects Protection Committee of PATH, Seattle, Washington, United States; and by the United States Army HSRRB. The study was done under a Food and Drug Administration IND and was compliant with all relevant International Conference on Harmonization guidelines.

Vaccinations of the second and third cohorts were staggered from each other by 2 wk (Table 2). A safety report was produced prior to each dose escalation. The LMM and the DSMB reviewed all adverse events (AEs) occurring in the 7 d immediately following any vaccination that preceded a dose escalation. Written approval from the LMM and concurrence by the DSMB were required prior to any subsequent dose escalation.

Stopping rules were to be invoked when either of the following observations was made: (1) $> 20\%$ of subjects with "severe" (grade 3) general AEs (not local AEs) related to the vaccine; or (2) any SAE, including death, judged to be vaccine related.

Objectives

The primary objective was to assess the safety and reactogenicity of the FMP1/AS02A malaria vaccine in malaria-exposed 12- to 47-mo-old children living in western Kenya. The secondary objective was to assess the humoral immune response to the FMP1/AS02A malaria vaccine in malaria-exposed 12- to 47-mo-old children living in western Kenya.

Outcomes

The primary endpoints were (1) occurrence of solicited symptoms (based on a standardized questionnaire) during a 7-d follow-up period after each vaccination (postvaccination clinic visits occurred on study days 1, 2, 3, and 7 after each vaccination); (2) occurrence of unsolicited symptoms during a 30-d follow-up period after each vaccination; and (3) occurrence of SAEs during an 8-mo follow-up period following the first dose of study vaccine (i.e., 6 mo following the last vaccination). The secondary endpoints were anti-FMP1 (anti-MSP-1_{42} 3D7 strain antibody) titers as determined by an enzyme-linked immunosorbent assay (ELISA) on study days 0, 14, 30, 44, 60, 74, 90, 180, 270, and 364.

Assessment of primary endpoints (safety and reactogenicity). Following each vaccination, subjects were followed for occurrence of solicited symptoms for 7 d, unsolicited symptoms for 30 d, and SAEs for 8 mo (i.e., 6 mo after the last vaccination) or until resolution. Both local (injection-site pain and swelling) and general/systemic (fever, drowsiness, loss of appetite, and irritability/fussiness) symptoms were assessed (Table 3). After the final vaccination, subjects were followed monthly at home by field workers and were asked to return to the clinic every 3 mo until the end of the study for safety follow-up. An SAE was defined as any untoward medical occurrence that resulted in death, was life threatening, resulted in persistent or significant disability or incapacity, or required in-patient hospitalization (or prolongation of hospitalization). Important medical events that might jeopardize a subject or might require intervention to prevent one of the other outcomes listed above were considered SAEs. Serum creatinine, ALT, white blood cell count, lymphocyte count, platelet count, and hemoglobin were determined on study days 0, 14, 30, 44, 60, 74, and 90. Additional hemoglobin determinations were made on study days 180, 270, and 364. Normal ranges were calculated on the basis of previous data from the local pediatric population.

Assessment of malaria. A peripheral blood smear was obtained from any subject who presented to the Walter Reed Project's Kombewa Clinic with fever, a history of fever within 48 h, or an illness that the attending doctor suspected might be due to malaria infection. After Giemsa staining and examination by oil-immersion light microscopy, detection of asexual parasitemia of > 0 parasites/μL resulted in the diagnosis and treatment for malaria.

Assessment of secondary endpoints (humoral responses). Immunology samples were collected on study days 0, 14, 30, 44, 60, 74, 90, 180, 270, and 364; samples collected on study days 0, 30, and 60 were collected immediately prior to vaccination. Immune response to the FMP1/AS02A vaccine was determined by anti-FMP1 ELISA endpoint titers reported in optical density units (ODUs), the dilution yielding an ODU of 1.0 in our assay. This assay has been described in detail elsewhere [22].

Sample Size

This trial represents the first time to our knowledge that this vaccine candidate has been evaluated in a pediatric population. Sample size was chosen after weighing the need to detect any possible vaccine associated AEs against the need to limit the number of subjects exposed to an investigational product. Incorporation of the *Imovax*® Rabies vaccine comparison group enabled broad initial estimates of the incidence of local and general side effects in a population that suffers from significant comorbidity from exposure to endemic illnesses. The control cohort also served as a comparator for the longitudinal immune responses to the FMP1 antigen in a malaria-exposed population. Although comparative statistics for the safety variables were calculated, the study had low power to detect anything other than large differences in the incidence of local and general side effects between the vaccination groups.