Supplementary Appendix

This appendix has been provided by the authors to give readers additional information about their work.

Supplement to: Thera MA, Doumbo OK, Coulibaly D, et al. A field trial to assess a blood-stage malaria vaccine. N Engl J Med 2011;365:1004-13.

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Supplementary Methods

Investigator and sponsor roles

The study was prospectively registered at ClinicalTrials.gov (NCT00460525). The Office of the Surgeon General of the United States Army was the study sponsor, and the U.S. Army was the vaccine developer and manufacturer. Permission to import and administer the investigational products was granted by the Republic of Mali Ministry of Health, and permission to undertake the trial was given by community leaders in Bandiagara, Mali. Site development and the conduct of the trial were supported by contract N01AI85346 and cooperative agreement U19AI065683 from the National Institute of Allergy and Infectious Diseases (NIAID), grant D43TW001589 from the Fogarty International Center, National Institutes of Health, and contract W81XWH-06-1-0427 from the United States Department of Defense and the United States Agency for International Development (USAID). Vaccine production and laboratory assays were supported by USAID, Washington, D.C. and by the Military Infectious Diseases Research Program, Fort Detrick, Maryland. GlaxoSmithKline Biologicals (GSK) provided the adjuvant for the trial. Data collection and quality were managed by the data coordinating center at the EMMES Corporation on behalf of NIAID. At the 6-month post-vaccination surveillance period, data quality checks were completed and the clinical data monitoring plan were executed. This activity was completed in October 2009, when all study databases were locked and released to the study investigators for unblinded analysis. Analysis was performed jointly by authors who are EMMES Corporation employees and by academic authors. The first draft of the manuscript was written by Mahamadou Thera and Christopher Plowe. All authors contributed to revision of the

manuscript, and these revisions were coordinated by Mahamadou Thera and Christopher Plowe. A scientific writer employed by GSK Biologicals (Julia Donnelly) suggested minor revisions of the manuscript that did not alter the content. A clinical trials agreement was in place among the sponsors (NIAID, GSK and the U.S. Army), and the data were subject to a cooperative agreement between NIAID and the investigators, which established full access to and ownership of the study data by the investigators.

Location and site details

The study was carried out in Bandiagara, Mali, 700 km northeast of Bamako on the Dogon plateau. Here the malaria transmission season is from July through December, with peak transmission in September and peak disease incidence in October. Previous reports of malaria incidence at the site report that 85% of Bandiagara children aged 0-10 years have at least one clinical episode of uncomplicated malaria during the malaria season, and the average number of clinical episodes of malaria per child and per transmission season is two, with a few children experiencing a maximum of four clinical episodes.[1] The incidence of severe malaria among children aged six years or less in Bandiagara was 2.5% (n=2284) in 2000.[2]

Study Participants: Screening and enrolment

Four hundred healthy children were included in the study. All were residents of the town of Bandiagara. Written informed consent was obtained from each child's parent or guardian separately for screening and for participation in the vaccine trial before study procedures were initiated. Nonliterate parents indicated consent using a thumbprint, and a signature was obtained from a literate witness. The participating children were drawn from the population of healthy children aged 1-6 years old (inclusive) at the time of first vaccination, healthy, and resident of Bandiagara. Children were screened by history, clinical examination, urine testing (dipstick testing for blood, glucose and protein) and blood tests (full blood count, alanine aminotransferase and serum creatinine). Children with clinically significant illness or out of range blood tests were excluded. Clinically evident immunosuppression was an exclusion criterion, but HIV testing was not performed. Children were referred to an appropriate service for management of illnesses identified at screening. Children were recruited following an IRB-approved public announcement on the local radio that invited potential participants to attend the research clinic for a screening visit.

Children were enrolled by study research physicians and the principal investigator at the time of the first immunization from May 28 to July 4, 2007. Children were provided with study photo identification cards.

Vaccines, randomization and treatment masking

Lyophilized FMP2.1 was manufactured at the Walter Reed Army Institute of Research and shipped to the site by an NIAID contractor in Maryland. $AS02_A$ was manufactured and shipped to the site by GlaxoSmithKline Biologicals in Rixensart, Belgium, and rabies vaccine was shipped to the site by the manufacturer.

Children were randomized to receive three doses of FMP2.1/$AS02_A$, i.e. FMP2.1 with the proprietary Adjuvant System $AS02_A$ comprising MPL (3-D-deacylated Monophosphoryl Lipid A) and QS21 (a triterpene glycoside purified from the bark of Quillaja Saponaria) or three doses of Chiron's RabAvert/Rabipur® rabies purified chicken embryo cell vaccine. In the description "FMP2.1/$AS02_A$", "FMP2.1" describes the ectodomain of the 3D7 clone of *P. falciparum*

AMA1, produced and purified in *E. coli* bacteria, "AS02" indicates the Adjuvant System, and the letter suffix "$_A$" indicates a pediatric dose formulation of the AS02 Adjuvant System. The acceptable interval between vaccinations was from 16 to 44 days.

Children were randomized to receive FMP2.1/AS02A (n=200) or rabies vaccine (n=200) in the order they were enrolled, with stratification for age by two-year increments (1-2 years, 3-4 years, 5-6 years), but without stratification for gender. Treatments were assigned to participant ID numbers using randomly varying blocks of size 6, 8 or 10. Randomization to either of the two vaccines was done using a computer-generated randomization list. The randomization list contained sequential codes linking a participant ID number to a vaccine assignment. Participant ID numbers were assigned to participants of each age stratum in the order in which they were enrolled in the trial.

Vaccine syringes were labeled with study numbers from a list of sequential codes linked to vaccine assignment that was generated by the study statistician. Measures were taken to keep children, their parents, and clinical investigators including the principal investigator and all other staff involved in measuring study outcomes blinded to treatment allocation. Access to the randomization list during the study was restricted to two study pharmacists who prepared the vaccines, had no contact with study participants and were instructed not to reveal vaccine assignments. Because the FMP2.1/AS02$_A$ vaccine appears opaque milky white compared to the slightly opaque, colorless suspension characterizing the rabies vaccine, and the different volumes of the two vaccines, blinding of the study pharmacists preparing the vaccine was not possible. However, the vaccine preparation room staffed by two study pharmacists was physically separated from the immunizing area. Immunizations were administered in dedicated vaccination rooms adjacent to the vaccine preparation room and connected by small pass-through sliding doors. Syringe barrels were covered with opaque tape. Vaccinators were physicians who did not evaluate participants after vaccination for reactogenicity or other adverse events. Each participant was vaccinated in a closed room out of view of anyone other than the vaccinators, so that each parent saw only the syringe that their child was injected with and never saw other participants being injected. Parents were not told that the vaccines vary with regard to volume.

Assessment of safety

After each vaccination, children were observed for at least 60 minutes after which solicited adverse events were recorded by study physicians. Study participants then returned to the research clinic 1, 2, 3, and 7 days after each vaccination so that study physicians could record solicited and unsolicited adverse events. Data relating to all clinical malaria episodes were reviewed and verified by both study principal investigators and at least one co-investigator. Non-serious episodes of clinical malaria detected in surveillance to determine efficacy were not considered to be adverse events. Severe adverse events (SAEs) were categorized according to the preferred term from the MedDRA® database, allocated before unblinding. Non-malaria SAEs were defined as those which excluded the MedDRA terms "*Plasmodium falciparum* infection", "Malaria" and "Cerebral Malaria". A grade was assigned all adverse events as follows; grade 1 (easily tolerated by the subject, causing minimal discomfort and not interfering with everyday activities), grade 2 (sufficiently discomforting to interfere with normal everyday activities), grade 3 (prevents normal, everyday activities). Blood tests for routine biochemistry (plasma alanine aminotransferase and creatinine) and hematology (full blood counts) were conducted at screening, on the days of vaccination, 7 days after each vaccination, and 30 days after the final

vaccination. Normal reference ranges were based on previous surveys done in healthy Malian children.[3] The definition of anemia as 8.4 g/dL (the lower limit of normal) was based on the rank of the 2.5th centile from the ordered distribution, following protocols established by the Clinical and Laboratory Standards Institute (formerly the National Committee for Clinical Laboratory Standards).

Monitoring for clinical malaria episodes

Both active and passive case detection methods were used to monitor for clinical malaria, although asymptomatic infections were not detected in real time and infections detected through active surveillance were not considered to be clinical episodes unless accompanied by fever and/or treatment-seeking for symptoms consistent with malaria. Active detection consisted of scheduled visits during the immunization phase followed by six additional scheduled monthly visits during the first post-vaccination malaria transmission season. These scheduled visits included physical examination, measurement of vital signs, collection of a malaria thick smear and hemoglobin determination at the very least. Children with any sign of malaria at the time of the visit (including but not limited to headache, body aches, fever, chills, and weakness) had their malaria smears read in real-time and were treated with antimalarial medication if the smear was positive, regardless of the parasite count. Other malaria smears collected from asymptomatic children were read retrospectively.

Summary of study procedures

Study Days	**-45 to –1 Screening**	**0**	**1-3**	**7**	**30**	**31-33**	**37**	**60**	**61-63**	**67**	**90**	**120**	**150**	**180-210**	**240**	**364-730**
Clinic Visit	**1**	**2**	**3-5**	**6**	**7**	**8-10**	**11**	**12**	**13-15**	**16**	**17**	**18**	**19**	**20-21**	**22**	**23-25**
Village & family information & discussion	●															
Written individual Screening Consent	●															
Check of inclusion/exclusion criteria	●	●														
Check of contraindications to immunization		●			●			●								
Written individual Study Consent		●														
Medical history	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
Physical examination	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
Vaccination		●			●			●								
Post-vaccination recording of solicited AE		●	●	●	●	●	●	●	●	●						
Recording of unsolicited AE occurring up to one month post-vaccination		●	●	●	●	●	●	●	●	●	●					
Recording of medication		●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
Recording of SAEs during the study period		●	●	●	●	●	●	●	●	●	●	●	●	●	●	●
Urine analysis for blood, glucose and protein	●															
CBC	●	●		●	●		●	●		●	●					
Serum chemistry (Creatinine, ALT)	●	●		●	●		●	●		●	●					
Hepatitis B surface antigen	●															
Serum and cells for anti-AMA-1 response		●			●			●			●		●		●	●
Venous blood for parasite genotyping	●	●		●	●		●	●		●	●		●		●	●
Fingerstick blood for parasite genotyping, malaria smear and hemoglobin		●			●			●			●	●	●	●	●	●

Passive case detection consisted of continuous availability of free, expeditious, high quality medical care at the Bandiagara research clinic, including microscopic diagnosis of malaria. This

outpatient research clinic was staffed by study physicians and laboratory personnel during the day and physicians were on-call at night. Parents were encouraged to bring their children to the research clinic whenever the child was ill. When a child presented after the research clinic had closed, a guard telephoned the on-call study physician who would return to the research clinic to evaluate the child and call in the on-call laboratory staff. Near-exclusive use of the research clinic for illnesses was achieved by availability of study staff, proximity of the research clinic to children's place of residence (all children reside within 2 km of the study clinic), and the fact that the only other modern health facility in the town is a maternity clinic that does not treat children.

Children requiring hospitalization were admitted to the pediatric ward of the Bandiagara district hospital adjacent to the study clinic and followed by study staff daily. This ward was staffed by 24-hour nursing and on-call physicians.

Criteria for severe malaria were based on WHO diagnostic criteria modified to include two additional criteria based on a study of severe malaria at the site,[2] including: coma (Blantyre Coma Score <2), seizure (one or more witnessed by the investigators), obtundation (depressed consciousness with Blantyre Coma Score <2), parasitemia >500,000/mm^3, lethargy or prostration, (clinical judgment or child $\geq$ 7 months unable to sit unassisted), severe anemia (hemoglobin $\leq$ 5 g/dl), respiratory distress (intercostal muscle retraction, deep breathing, grunting), hypoglycemia (glucose $\leq$ 40 mg/dl), jaundice, renal insufficiency as indicated by lack of urination for $\geq$ 1 day, gross hematuria, state of shock (systolic blood pressure $\leq$ 50 mm Hg, rapid pulse, cold extremities), and inability to eat or drink or protracted vomiting (added based on our experience at the site).

Laboratory methods

IgG antibodies to the *P. falciparum* 3D7 AMA1 vaccine antigen were measured by ELISA in the clinical immunology laboratory at the Division of Malaria Vaccine Development of the Walter Reed Army Institute of Research in Silver Spring, Maryland. Results were reported in optical density units that were converted to units of micrograms per milliliter based on a standard curve. Plates were coated overnight at 4º C with the FMP2.1 recombinant AMA1 antigen (100 mL/well, 0.5 mg/mL), after which they were blocked with a 0.5% boiled casein buffer for 1 hour at 22º C. Test samples were added to the plate, serially diluted in eight sequential 2-fold serial dilutions (done in triplicate) and incubated for 2 hours at 22º C. Secondary antibody (Affinity Purified Antibody Peroxidase Labeled Goat Anti-Human IgG (c), KPL, Gaithersburg, Maryland, United States: Cat#074-1002) at a 1:4,000 dilution, was added and incubated for 1 hour at 22º C, after which substrate (ABTS Peroxidase Substrate System (2-Component), KPL: Cat#50-62-01) was added and incubated for an additional hour at 22º C. A stop solution (20% SDS) was added and the plates were read using a Spectromax 340PC Plate Reader (Molecular Devices, Sunnyvale, California, United States). Between each incubation step the wells were washed in PBS using a SkanWasher Plate Washer (Molecular Devices) with four washing cycles of 400 mL each.

Thick and thin blood smears for parasite identification and density readings were made at the study site and stained with Giemsa. Smears were read in real time at the study site for children with symptoms of malaria for treatment purposes, but all smears were ultimately read in Bamako at the Malaria Research and Training Center. Smears were read in duplicate with a third reader serving as a tiebreaker if the first two readings were >25% discordant. The final result was the mean density of the two readings. If a third reader was involved, the mean of the closest two

readings was recorded as the final result. All smears were read prior to unblinding study results. Slide readers underwent a rigorous training and evaluation process at the Malaria Research and Training Center in Bamako with annual retraining. Malaria smears were made for all children at scheduled visits and when any child presented with a malaria symptom.

Data analysis

A study analysis plan was developed by the study sponsor, investigators and statistical consultant prior to unblinding. The primary analysis was the hazard ratio of first or only episode of malaria meeting the primary case definition (defined as fever $\geq$37.5ºC with parasitemia above 2500/microliter), according to vaccination group. The intention-to-treat cohort included all children who received the first vaccination and were thus enrolled in the study. Data from the intention-to-treat cohort were analyzed from the time of first vaccination (randomization) to six months after the assigned date of the third immunization. The per-protocol cohort included all children who received all 3 vaccinations within acceptable time limits (defined in the protocol prior to study start). Data from the per-protocol cohort were analyzed from two weeks after the assigned date of the third vaccination to six months after the assigned date of the third immunization. No adjustment was made for antimalarial medication in either analysis. Secondary analyses included efficacy against multiple clinical malaria episodes, efficacy against other case definitions of malaria (varying parasitemia thresholds with and without fever $\geq$37.5ºC), and efficacy against anemia (hemoglobin <8.4g/dL, defined in the protocol based on local norms prior to study start) for the intention-to-treat cohort.

Statistical Analysis

The primary analysis estimated the hazard ratio for first or only episode of clinical malaria in the intention-to-treat cohort (all randomized children, and data collected starting on the day of the first vaccination). Secondary efficacy analyses included the hazard ratio for the first or only clinical episode of malaria (using the primary endpoint definition) with an AMA1 genotype matching that of the vaccine strain;[4] the hazard ratio for multiple clinical malaria episodes; and the same endpoints in the per-protocol cohort (children who received all three doses of the vaccine to which they were randomized and completed at least 14 days of follow-up after the third vaccination, and data collected starting 14 days after the third vaccination). Hazard ratios were estimated using a standard Cox regression model unadjusted for other covariates (unless indicated). Efficacy against multiple clinical episodes was assessed using Poisson regression which was not adjusted for other covariates. Cumulative incidence of clinical malaria for six months after the third vaccine dose was estimated by the Kaplan-Meier method to account for loss to follow-up. Fisher's exact test was used to compare proportions. All P-values presented are two-sided.

The relationship between anti-AMA1 antibodies and risk of clinical malaria was examined by estimating the hazard ratio for the first or only episode of clinical malaria. To estimate vaccine-induced (as opposed to naturally-acquired) anti-AMA1 antibodies, for each individual log-transformed antibody level at baseline was subtracted from the level at the time-point at least 2 weeks after the third vaccination that was closest in time to but not after the first malaria episode. The change in antibody level, unadjusted for other covariates, was included as a time-dependent, continuous variable in Cox regression analysis in FMP2.1/AS02$_A$ recipients.

Cumulative parasite density was estimated for each individual by measuring AUC for the entire study period and also for the period from two weeks after the third vaccination through the end of follow-up. All recorded episodes of parasitemia were included irrespective of presence or absence of symptoms of malaria; parasite density was assumed to decline linearly to zero three days after a treated malaria episode. AUC was compared between vaccine groups using a continuity-adjusted Wilcoxon-Mann-Whitney test.

Area-under-the curve of parasitemia

The endpoint of asexual *P. falciparum* parasitemia measured as area under the curve (AUC) was specified in the study protocol and statistical analysis plan as an exploratory endpoint. AUC was estimated for each individual, using the trapezoidal rule, from the time of enrollment up to day 240 (intention-to-treat analysis) and also for the period from two weeks after the scheduled data of the third vaccination through the end of the follow-up period (per-protocol analysis). Parasite density was assumed to decline linearly to zero 3 days after a treated malaria episode, consistent with the rapid rate of parasite clearance seen when highly efficacious artemisinin combination therapies are used. AUC was compared between the vaccine groups using a continuity-adjusted Wilcoxon-Mann-Whitney test. Comparison of AUCs is similar to comparing average parasite densities over the 240-day period, but takes into account presumed continuous parasitemia between consecutive measurements of positive parasitemia when no treatment has occurred, and accounts for the rapid decline of parasitemia following treatment with highly efficacious, rapid-acting artemisinin-based combination therapy. Supplementary Figure 2 showing cumulative AUC is essentially a summation of the individual AUCs shown in Supplementary Figure 1 below.

Supplementary Results

Safety and Reactogenicity

Throughout the entire study period of approximately 8 months, 1347 unsolicited adverse events were recorded for children who received the malaria vaccine and 1316 for children who received the control vaccine. Five subjects experienced adverse events with an intensity of severe or greater (not including serious adverse events), three in the vaccine group and two in the control group, none of which were judged to be associated with the vaccine.

All but two (99.0%) of the children who received the FMP2.1/AS02$_A$ malaria vaccine experienced local reactions (95% CI, 96.4 to 99.0), compared to 82.1% of children who received rabies vaccine (95% CI, 76.1 to 87.1). These local reactions were more likely to be graded as moderate or severe in the malaria vaccine group (98.0%) than in the control group (70.6%). As with previous trials of this vaccine[3, 5] most of these reactions were local swelling, which was graded as severe when it was at least 20 millimeters at its widest dimension, and which was typically noted only on physical examination and not of concern to parents of study participants.

Malaria vaccine recipients were also more likely to experience solicited systemic symptoms, which were reported by 68.3% of malaria vaccine recipients (95% CI 61.4 to 74.7), compared with 29.9% of rabies vaccine recipients (95% CI, 23.6 to 36.7). Fever was the commonest solicited symptom in both treatment groups, and was recorded in 61.3% of children in the

malaria vaccine group and 25.9% of those the rabies vaccine group. Irritability/fussiness and loss of appetite were also both reported more frequently in the malaria vaccine group.

Per-protocol results
Results of the per-protocol data analysis for vaccine efficacy against first and multiple episodes of different definitions of clinical malaria are reported below in Supplementary Table 2, and are similar to results of the intention-to-treat analysis.

Area-under-the-curve of parasitemia
The Supplementary Figure below shows a spaghetti plot of individual parasitemia curves for the control and vaccine groups. Figure 3 in the manuscript essentially depicts a cumulative summation of the areas under these individual curves with normalization for the number of observations. Clustering of parasitemias at approximately monthly intervals in the spaghetti plots represents the monthly surveys done to measure asymptomatic parasitemia. Parasitemia was assumed to remain positive between consecutive measured parasitemias unless treatment was given. Following treatment parasitemia was assumed to decline to zero in three days, as occurs with the highly effective artemisinin-based combination treatment used to treat malaria. Both asymptomatic parasitemias and clinical infections were included in AUC calculations.

Supplementary Discussion

Evidence of cross-protection
The ability of a monovalent AMA1 vaccine to provide even a marginal signal of overall efficacy in a setting where only 3% of parasite strains have AMA1 fully identical to the vaccine strain and more than 200 unique AMA1 variants circulate at low frequencies in the population[4, 6] provides evidence of at least partial cross-protection against parasites with AMA1 that differs from that of the vaccine strain. Detailed molecular analyses of AMA1 from both clinical episodes and asymptomatic infections will measure cross-protection and vaccine-induced selection, and may inform the design of more-efficacious next-generation AMA1 vaccines.

Heterogenous risk of malaria
Previous entomological surveys at the study site have suggested some variation in transmission risk, with higher risk near the river. Although the locations of children's homes were not recorded in this trial, randomization would be expected to balance any such heterogeneity of risk between the vaccine and control groups. The close similarity of baseline characteristics shown in Table 1 of the manuscript (including near-identical hemoglobin levels, which might be different if malaria risk were unbalanced), suggests that the randomization was effective in this regard.

AMA1 antibody responses and efficacy
Previous AMA1 vaccine trials at this site in adults and children demonstrate that AMA1 antibody titers increase over the course of a lifetime of exposure, and that adults have baseline titers that are many-fold higher than those seen in children.[3, 5] The idea underlying the analysis of AMA1 antibodies and efficacy against clinical malaria is therefore that the baseline titer reflects only these naturally acquired AMA1 antibodies, while the peak titer (Day 90), as well as the most recent titer prior to a clinical episode ("Day K") reflect a combination of both naturally-acquired and vaccine-induced AMA1 antibodies. To estimate the vaccine-attributable AMA1

antibodies we subtracted the baseline titer from the Day K titer. This measure is approximate because the day K titer should reflect both the increase in antibodies caused by vaccination and any increase caused by natural exposure occurring between the baseline and Day K measurements. However, the observation that the (Day K – Baseline) titer correlated significantly with efficacy against clinical malaria in the vaccinated group, while neither the baseline nor the peak or Day K titer correlated with efficacy, supports the conclusion that the increase in antibodies between baseline and Day K is related to efficacy.

Area-under-the-curve measurement of parasite density

The question of how to measure the impact of malaria vaccines on malaria infection (as opposed to clinical malaria disease) has long vexed the malaria vaccine community. Various methods have been used, each of which has significant drawbacks. For example, Genton et al. reported that a multi-antigen blood-stage vaccine that had no efficacy against clinical malaria did reduce the geometric mean parasite density of microscopy-positive blood samples collected at two-weekly intervals starting four weeks after immunization.[7] This approach fails to account for a vaccine's ability to reduce the prevalence of any parasitemia—a small number of individuals with high breakthrough parasitemias in a vaccine group, the rest of whom were completely protected, could have the same mean parasitemia as a control group in which many individuals had modest parasitemias. In an effort to better capture the parasite burden over time, Sagara et al. compared the rate of parasitemia >3000 sexual parasites per microliter per day at risk in a trial of an AMA1 vaccine that showed no efficacy.[8] This method was essentially a hybrid attempt to examine both risk of clinical malaria (hence the parasitemia threshold) and cumulative parasite burden.

We have previously used area-under-the-curve (AUC) to measure cumulative gametocytemia following drug treatment.[9] Although comparison of AUCs for asexual parasite density has not been validated as a measure of vaccine efficacy, we felt that this novel method was a promising approach to capture the overall effect of a vaccine on parasite density over time, and included it as an exploratory endpoint in the study protocol and statistical analysis plan prior to the study. We suggest that a significantly lower median AUC in individuals who received the FMP2.1/AS02$_A$ vaccine provides an additional suggestion of a beneficial effect of the vaccine.

The observation that AUC was significantly reduced by the vaccine while parasitemia prevalence was not is consistent with the notion that a blood-stage vaccine would exert its effect through controlling the level of parasite density through inhibiting parasite multiplication in the blood while not completely preventing infection.[10]

Study power

Efficacy against the primary clinical endpoint was not statistically significant ($P = 0.18$). However, the study was designed to have 90% power to find significant efficacy (i.e., with $P<0.05$) if the vaccine reduced malaria incidence by 20% from an incidence of 75% in control vaccine recipients. The observed incidence was only 54.4%; the power of the study to find significant efficacy for a true incidence reduction of 20% from 54.4%, assuming about 4% loss to follow-up as was observed, was only about 59%. The study may therefore have been underpowered to detect overall efficacy against clinical malaria in light of a lower-than-expected incidence of clinical malaria.

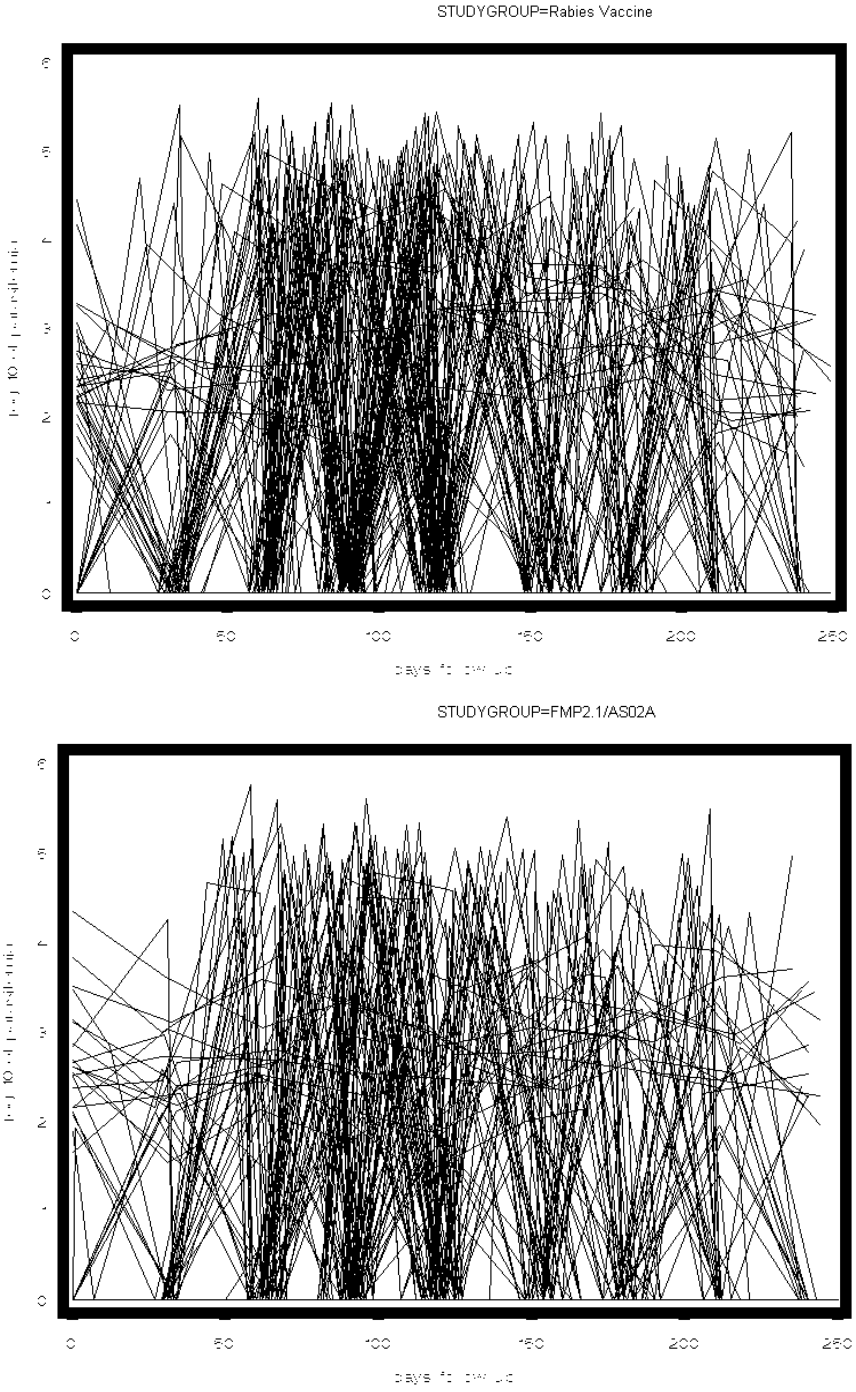
Supplementary Table 1. Proportion With Any Solicited Adverse Events in the Intention-to-Treat Cohort, According to Vaccine Group

	FMP2.1/AS02$_A$ Vaccine (n=199)				Rabies Vaccine (n=201)					
	Any Severity		Moderate or Severe		Any Severity		Moderate or Severe			
	No. of Subjects	Percent (95% CI)	No. of Subjects	Percent (95% CI)	No. of Subjects	Percent (95% CI)	No. of Subjects	Percent (95% CI)	P value*	P value**
Systemic										
Drowsiness	5	2.5 (0.8-5.8)	1	0.5 (0.0-2.8)	1	0.5 (0.0-2.7)	0	0.0 (0.0-1.8)	0.121	0.50
Irritability/fussiness	20	10.1 (6.2-15.1)	4	2.0 (0.6-5.1)	3	1.5 (0.3-4.3)	1	0.5 (0.0-2.7)	<0.001	0.21
Loss of appetite	33	16.6 (11.7-22.5)	1	0.5 (0.0-2.8)	4	2.0 (0.5-5.0)	0	0.0 (0.0-1.8)	<0.001	0.50
Vomiting	9	4.5 (2.1-8.4)	0	0.0 (0.0-1.8)	6	3.0 (1.1-6.4)	2	1.0 (0.1-3.5)	0.444	0.50
Fever	122	61.3 (54.2-68.1)	44	22.1 (16.5-28.5)	52	25.9 (20.0-32.5)	17	8.5 (5.0-13.2)	<0.001	<0.001
Local										
Site pain	190	95.5 (91.6-97.9)	63	31.7 (25.3-38.6)	108	53.7 (46.6-60.8)	2	1.0 (0.1-3.5)	<0.001	<0.001
Swelling	193	97.0 (93.6-98.9)	193	97.0 (93.6-98.9)	142	70.6 (63.8-76.8)	142	70.6 (63.8-76.8)	<0.001	<0.001
Erythema	16	8.0 (4.7-12.7)	16	8.0 (4.7-12.7)	0	0.0 (0.0-1.8)	0	0.0 (0.0-1.8)	<0.001	<0.001
Limitation of arm motion	92	46.2 (39.2-53.4)	36	18.1 (13.0-24.2)	6	3.0 (1.1-6.4)	0	0.0 (0.0-1.8)	<0.001	<0.001
Reported limitation of arm motion	92	46.2 (39.2-53.4)	24	12.1 (7.9-17.4)	8	4.0 (1.7-7.7)	1	0.5 (0.0-2.7)	<0.001	<0.001
Aggregate										
Any local	197	99.0 (96.4-99.9)	195	98.0 (94.9-99.4)	165	82.1 (76.1-87.1)	142	70.6 (63.8-76.8)	<0.001	<0.001
Any systemic	136	68.3 (61.4-74.7)	46	23.1 (17.4-29.6)	60	29.9 (23.6-36.7)	19	9.5 (5.8-14.4)	<0.001	<0.001
Any symptom	198	99.5 (97.2-100.0)	196	98.5 (95.7-99.7)	174	86.6 (81.1-91.0)	147	73.1 (66.4-79.1)	<0.001	<0.001

*P for difference in frequency of Any Severity among treatment groups
**P for difference in frequency of Moderate or Severe among treatment groups

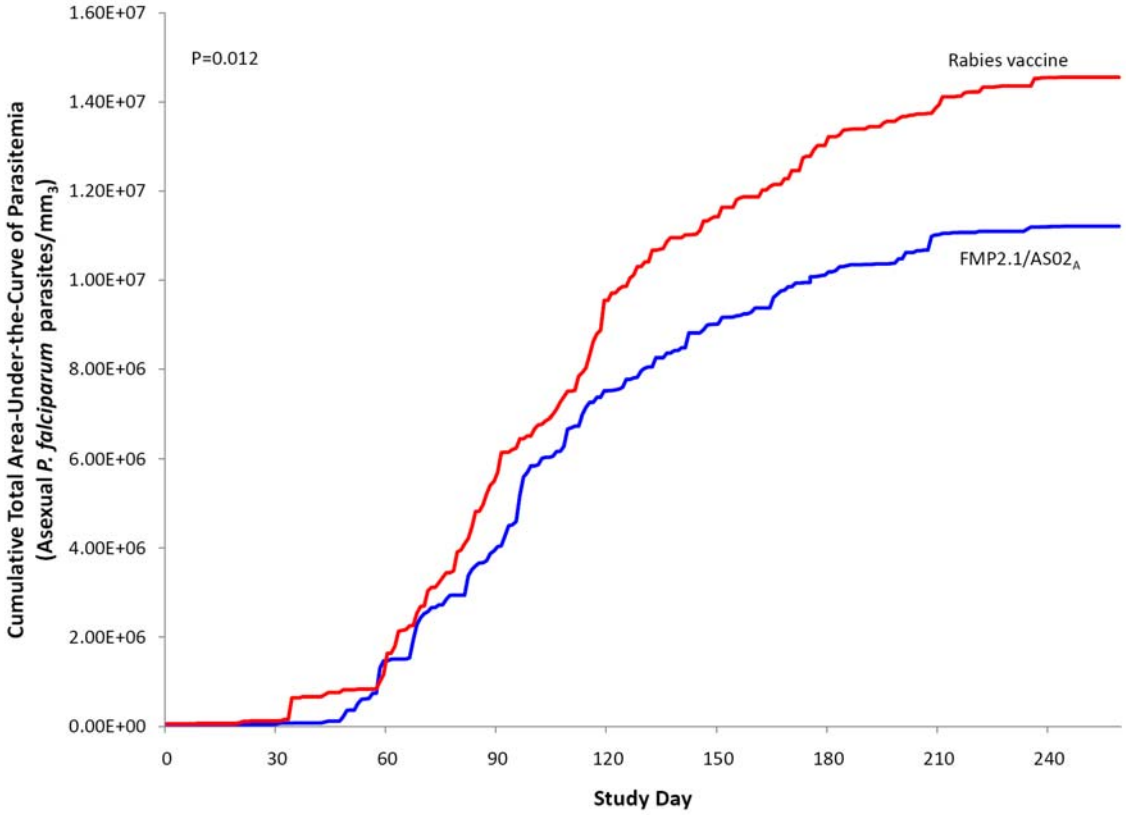
Supplementary Table 2. Vaccine Efficacy in the Per-protocol Cohort Against First and Multiple Episodes Using Different Definitions of Clinical Malaria.

Definition of clinical malaria		No. of Episodes		First Episode		No. of Episodes		Multiple Episodes	
Temperature	$FMP2.1/AS02_A$	$FMP2.1/AS02_A$	Rabies	Percent (95%CI)	P value	$FMP2.1/AS02_A$	Rabies	Percent (95%CI)	P value
Any	$> 0/mm^3$	100	117	20.2 (-4.2 – 38.9)	0.10	138	172	18.5 (-1.96 - 34.9)	0.07
	$\geq 100/mm^3$	99	116	19.7 (-5.0 - 38.6)	0.11	136	170	18.8 (-1.8 - 35.2)	0.07
	$\geq 1000/mm^3$	95	110	17.7 (-8.4 – 37.4)	0.17	127	159	18.9 (-2.5 - 35.7)	0.08
	$\geq 2500/mm^3$	92	107	17.3 (-9.3 – 37.4)	0.18	122	151	17.9 (-4.2 - 35.3)	0.11
	$\geq 5000/mm^3$	91	106	17.6 (-9.1 – 37.7)	0.18	120	147	17.0 (-5.6 - 34.8)	0.13
	$\geq 10{,}000/mm^3$	84	101	21.7 (-4.6 – 41.4)	0.10	105	139	23.3 (1.2 - 40.5)	0.04
	$\geq 20{,}000/mm^3$	73	91	23.2 (-4.5 – 43.6)	0.09	92	118	20.7 (-4.1 - 39.6)	0.10
	$\geq 50{,}000/mm^3$	50	58	13.3 (-26.6 – 40.6)	0.46	57	71	18.3 (-15.8 - 42.4)	0.26
	$\geq 100{,}000/mm^3$	21	28	23.9 (-34.0 – 56.8)	0.34	23	31	24.5 (-29.5 – 56.0)	0.31
$> 37.5^oC$	$> 0/mm^3$	90	97	9.0 (-21.3 – 31.7)	0.52	111	128	11.8 (-13.8 - 31.6)	0.33
	$\geq 100/mm^3$	89	96	8.7 (-21.9 – 31.6)	0.54	109	127	12.7 (-12.8 - 32.4)	0.30
	$\geq 1000/mm^3$	87	92	6.3 (-25.6 – 30.1)	0.66	103	121	13.4 (-12.6 - 33.4)	0.28
	$\geq 2500/mm^3$	84	90	7.2 (-24.9 – 31.1)	0.622	99	117	13.9 (-12.5 - 34.2)	0.272
	$\geq 5000/mm^3$	83	89	7.5 (-24.8 – 31.4)	0.612	98	116	14.1 (-12.4 - 34.3)	0.269
	$\geq 10{,}000/mm^3$	77	86	13.2 (-18.1 – 36.2)	0.367	89	110	17.8 (-8.8 - 37.8)	0.171
	$\geq 20{,}000/mm^3$	68	76	12.2 (-21.9 – 36.7)	0.438	78	92	13.7 (-16.7 - 36.2)	0.339
	$\geq 50{,}000/mm^3$	45	47	3.0 (-46.1 – 35.5)	0.886	49	55	9.3 (-33.3 - 38.3)	0.620
	$\geq 100{,}000/mm^3$	19	21	7.7 (-71.8 – 50.4)	0.802	20	24	15.2 (-53.6 - 53.1)	0.588



Supplementary Figure 1. Individual Area-Under-the-Curve (AUC) of Parasitemia.

Top, rabies vaccine group; bottom, FMP2.1/AS02A malaria vaccine group.



Supplementary Figure 2. Cumulative Mean Area-Under-the-Curve of Parasitemia, Intention-to-Treat Cohort.

Cumulative total area-under-the-curve (AUC) of *Plasmodium falciparum* parasitemia was calculated for each study group. All recorded episodes of parasitemia were included irrespective of presence or absence of symptoms of malaria to provide an estimate of the overall impact of the FMP2.1/AS02$_A$ malaria vaccine on the total parasite burden over time. Parasite density was assumed to decline linearly to zero three days after a treated malaria episode. AUC was compared between the vaccine groups using a continuity-adjusted Wilcoxon-Mann-Whitney test. Immunizations were on Study Days 0, 30 and 60.

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