immunization, whereas serious adverse events were monitored throughout the 12-mo study.

Blood was collected at screening, on immunization days, 14 d after each immunization, and on study days 90, 180, 272, and 364 to determine complete blood count, alanine aminotransferase (ALT), and serum creatinine.

Adverse events were graded by severity and judged for relatedness to study vaccines. Mild adverse events were easily tolerated, causing minimal discomfort and not interfering with daily activities. Moderate adverse events were sufficiently discomforting to interfere with normal activities. Severe adverse events prevented normal daily activities. Swelling, erythema, fever, and limitation of arm motion had specific definitions not based on interference with daily activities. Injection site swelling and erythema were graded based on their widest dimension: mild, >0–20 mm; moderate, >20–50 mm; and severe, >50 mm. Fever was classified as severe if the oral temperature was ≥39 °C , whereas severe limitation of arm motion was classified as abduction limited to 30°. For laboratory tests, toxicity grading was adapted to normal reference ranges determined for the local adult population.

Antibody responses to MSP-1_{42}. Antibody responses to MSP-1_{42} were measured by ELISA [6]. Briefly, cGMP-purified bulk MSP-1_{42} [3] was used as plate antigen, and serial dilutions of each sample, along with positive and negative controls, were made to yield a linear range of dilutions that could be analyzed with curve-fitting software (SoftMax Pro v4.1, Molecular Devices, Sunnyvale, California, United States) to calculate the theoretical dilution that would give an optical density of 1.0 in the endpoint assay; the reciprocals of these calculated dilutions were reported as the sample titers. To compare antibody responses to different alleles of MSP-1_{42}, recombinant MSP-1_{42} of the 3D7 and FVO alleles were prepared as described previously [6,13]. Preparation of recombinant MSP-1_{42} of the Camp/FUP allele will be described elsewhere.

Antibody responses to fragments of MSP-1_{42}. MSP-1 fragment-specific antibody responses were assessed by a standard ELISA [6]. Fragments of MSP-1_{42} corresponding to MSP-1_{19} (3D7 and FVO alleles) and the two epidermal growth factor (EGF)-like domains that comprise MSP-1_{19}, EGF1 (3D7-Camp/FUP [14] and FVO alleles) and EGF2 (3D7 and FVO-Camp/FUP alleles), were expressed as glutathione S-transferase fusion proteins and purified to homogeneity [13].

Sample Size

This phase I trial was not powered to detect differences between groups. A sample size of 20 malaria vaccine recipients was based on general acceptance of this size for initial assessment of safety, tolerability, and immunogenicity of investigational vaccines. Inclusion of a comparator vaccine group of 20 permitted broad estimates of the incidence of local and general side effects and immune responses to natural infection.

Randomization

Randomization was done in blocks of four without stratification. Opaque, sealed randomization envelopes containing sequential codes linked to vaccine assignment were prepared by Statistics Collaborative (Washington, D. C., United States). Codes were assigned in the order that participants arrived at the clinic on the day of first immunization.

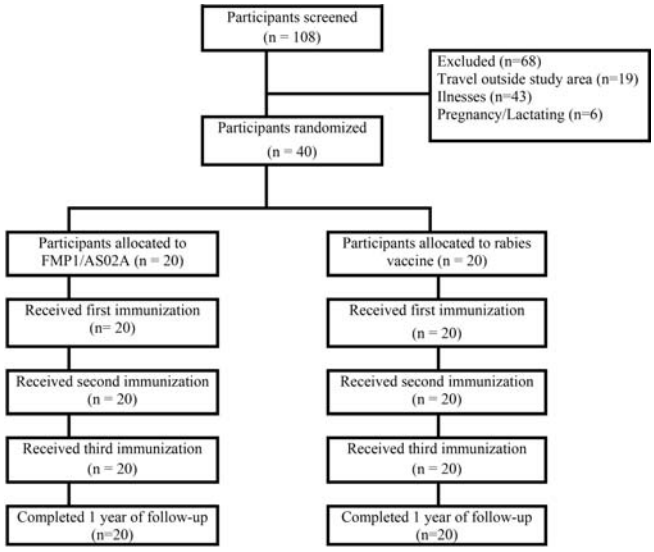

Figure 1. Trial Profile

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Blinding

The only people at the study site with access to the randomization codes during the study were two study pharmacists, who had no contact with study participants and did not reveal vaccine assignments to anyone else. Study participants and investigators who assessed outcomes were blinded to vaccine assignment. Vaccines were prepared in a secure room communicating with the vaccine administration room through a small window with a sliding door. Reconstituted FMP1/AS02A is off-white and Imovax is pink. To reduce potential bias, syringes containing the vaccines were wrapped with opaque tape to conceal their contents from participants and vaccinators. Vaccines were administered by physicians who did not participate in assessing outcomes.

Ethical Compliance

The protocol was approved by institutional review boards of the University of Bamako Faculty of Medicine, the University of Maryland, the United States Army Surgeon General, and the National Institute of Allergy and Infectious Disease. Separate written informed consent was obtained for screening and for enrollment. Consent of illiterate participants was documented by their thumbprints and by signatures of independent witnesses. Permission to conduct the study was granted by the Republic of Mali Ministry of Health, and the trial was monitored by the United States Army Medical Materiel Development Activity and the World Health Organization.

Statistical Methods

Adverse event rates were analyzed using SAS version 8.2 (SAS Institute, Cary, North Carolina, United States). Fisher's exact test was used to compare rates between vaccine groups. Confidence intervals (CIs) for geometric mean MSP-1_{42} antibody titers were estimated by using $\log_{10}$-transformed values, calculating the 95% CI based on the normal distribution, and then converting the limits to the original scale for presentation. All tests were two-sided, and no correction of p-values was made for additional analyses. MSP-