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# Phase 1 randomized double-blind safety and immunogenicity trial of *Plasmodium falciparum* malaria merozoite surface protein FMP1 vaccine, adjuvanted with AS02A, in adults in western Kenya

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# Phase 1 randomized double-blind safety and immunogenicity trial of *Plasmodium falciparum* malaria merozoite surface protein FMP1 vaccine, adjuvanted with AS02A, in adults in western Kenya<sup>☆,☆☆</sup>

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## Abstract

We report the first trial of candidate malaria vaccine antigen FMP1, a 42 kDa fragment from the C-terminus of merozoite surface protein-1 (MSP-1) from the 3D7 strain of *Plasmodium falciparum*, in an endemic area. Forty adult male and female residents of western Kenya were enrolled to receive 3 doses of either FMP1/AS02A or Imovax<sup>®</sup> rabies vaccine by intra-deltoid injection on a 0, 1, 2 month schedule. Thirty-seven volunteers received all three immunizations and 38 completed the 12-month evaluation period. Slightly more recipients of the FMP1/AS02A vaccine experienced any instance of pain at 24 h post-immunization than in the Imovax<sup>®</sup> group

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(95% versus 65%), but otherwise the two vaccines were equally safe and well-tolerated. Baseline antibody levels were high in both groups and were boosted in the FMP1/AS02A group. Longitudinal models revealed a highly significant difference between groups for both the average post-baseline antibody responses to MSP-1<sub>42</sub> ( $F_{1,335} = 13.16$ ;  $P < 0.001$ ) and the Day 90 responses to MSP-1<sub>42</sub> ( $F_{1,335} = 16.69$ ;  $P < 0.001$ ). The FMP1/AS02A vaccine is safe and immunogenic in adults and should progress to safety testing in children at greatest risk of malaria. Published by Elsevier Ltd.

**Keywords:** Malaria; Vaccine; Merozoite surface protein; FMP1; AS02A; Randomized controlled trials

## 1. Introduction

*Plasmodium falciparum*, mankind's ancient nemesis, kills three children a minute [1]. Despite widespread efforts to implement antimalarial drugs, bed nets and vector control, the worldwide toll of malaria is undiminished [2]. Natural immunity to malaria, acquired after multiple episodes of malaria, does not prevent parasitemia, but does limit disease severity and death [3,4]. A blood stage vaccine that accelerates the acquisition of such immunity to *P. falciparum* malaria would offer enormous benefit to the public health, particularly for infants and children living in endemic areas who suffer the most morbidity and mortality due to malaria [5].

The 42 kDa fragment of merozoite surface protein 1 (MSP-1) is a leading erythrocytic stage candidate antigen for a malaria vaccine [6]. *P. falciparum* MSP-1 is a 195 kDa protein that is proteolytically cleaved to yield four fragments, one of which is the carboxy-terminal 42 kDa fragment known as MSP-1<sub>42</sub> [7,8]. Further cleavage of the 42 kDa fragment into 19 and 33 kDa fragments is important for merozoite invasion [9]. Three lines of evidence support the candidacy of MSP-1<sub>42</sub> kDa as a malaria vaccine. First, epidemiologic studies have demonstrated that antibody to the 19 kDa protein contained within MSP-1<sub>42</sub> is associated with diminished *P. falciparum* disease severity [10,11]. Second, antibodies raised in animals against MSP-1<sub>42</sub> or the 19 kDa fragment inhibit the growth of *P. falciparum* in vitro [12]. Third, immunization of New World monkeys with recombinant MSP-1 formulated with a potent adjuvant confers protection against blood stage challenge with *P. falciparum* [13–15].

MSP-1<sub>42</sub> of the 3D7 clone of *P. falciparum* was cloned and expressed in *E. coli*, purified and formulated according to Good Manufacturing Practices (GMP). The final product was named *falciparum* malaria protein 1 (FMP1) [16]. Preclinical evaluation of FMP1 in the rhesus monkey safety and immunogenicity model identified AS02A as a safe, well-tolerated, and highly immunogenic adjuvant [17]. FMP1 formulated with GlaxoSmithKline's proprietary adjuvant AS02A proved safe and immunogenic in two previous trials conducted in 60 malaria naïve volunteers in the USA ([18] and Cumming's unpublished data). The study presented here, the first Phase 1 trial of FMP1/AS02A in a malaria-experienced population, provides further safety and immunogenicity data as part of a clinical development plan that aims to develop vaccines to prevent disease in children.

## 2. Materials and methods

### 2.1. Study design and population

This Phase 1 double-blind, randomized, controlled trial evaluated the safety and immunogenicity of the FMP1/AS02A candidate malaria vaccine versus Imovax® Rabies in Kenyan adults. The primary objective was to assess the safety and reactogenicity of the FMP1/AS02A vaccine in malaria-experienced adults. The primary endpoints were the occurrence of solicited signs and symptoms during an 8-day period after each vaccination (day of vaccination and post-vaccination days 1, 2, 3, and 7), occurrence of unsolicited symptoms during a 30-day follow-up period after each vaccination, and the occurrence of serious adverse events (SAEs) during the 1-year study period. The secondary objective was to quantify the humoral immune response. The secondary endpoint, the anti-FMP1 antibody titer, was determined by enzyme-linked immunosorbent assay (ELISA) just prior to and 14 days after each immunization and on days 90, 180, 272 and 364 after the first immunization.

The trial was carried out in Kombewa Division, western Kenya. The area is part of the Lake Victoria basin where most of the population belongs to the Luo ethnic group and is engaged in subsistence fishing and farming. *P. falciparum* in this region is holoendemic and transmission is intense, principally via *Anopheles gambiae* mosquitoes [19]. Before enrollment, volunteers gave a detailed medical history and underwent a physical examination and laboratory evaluation to determine if they had any underlying disease that might confound their evaluation. Volunteers were eligible for enrollment if they were 18–55 years of age, male or female, in good health and agreed to participate for 12 months. Exclusion criteria included any evidence of chronic illness or of hematological, renal or hepatic pathology. Specific exclusion criteria were as follows: prior receipt of an investigational malaria vaccine or rabies vaccine, recent or planned use of any investigational drug, vaccine, immunoglobulin or any blood product, use of immunosuppressant drugs, confirmed or suspected immunodeficiency, history of surgical splenectomy, alcohol or drug abuse, pregnancy or concurrent participation in another clinical trial. Laboratory exclusion criteria were serum alanine aminotransferase (ALT)  $\geq 84$  IU/L, serum creatinine  $\geq 2.0$  mg/dL, absolute lymphocyte count  $\leq 1.0 \times 10^3/\mu\text{L}$ , a positive urine or plasma beta-human chorionic gonadotropin ( $\beta$ -HCG), or a hemoglobin (Hgb)  $< 10$  g/dL for men or  $< 8$  g/dL for women.

## 2.2. Vaccines

The expression, purification, biochemical and immunological characterization of *E. coli* produced, GMP-manufactured FMP1 antigen has been described [16]. The adjuvant system AS02A consists of an oil-in-water emulsion containing two immunostimulants, 3-deacylated monophosphoryl lipid A (MPL) (Corixa Inc., Seattle, WA) and QS21 (Antigenics Inc., Lexington, MA). The AS02A adjuvant was manufactured by GlaxoSmithKline Biologicals (Rixensart, Belgium) under GMP conditions and was provided in prefilled syringes. Immediately prior to immunization, the lyophilized FMP1 antigen was reconstituted in AS02A to yield a milky white fluid containing a final dose of 50 µg of FMP1 in a volume of 0.5 mL of AS02A. The comparator vaccine, Imovax® Rabies, is an inactivated rabies vaccine produced by Aventis Pasteur, S.A., as a sterile, stable, freeze-dried suspension of inactivated rabies virus prepared from strain PM-1503-3M obtained from the Wistar Institute, Philadelphia, PA. Immediately prior to immunization, the rabies vaccine was reconstituted with sterile water for injection to yield a clear pink liquid. Each 1 mL dose contained <100 mg of human albumin, <150 µg of neomycin sulfate, 20 µg of phenol red indicator and ≥2.5 IU of rabies antigen.

## 2.3. Immunizations and assessment of primary endpoints

Forty volunteers were randomized in a 1:1 ratio to receive either FMP1/AS02A or Imovax® Rabies vaccine. Randomization was carried out in blocks of four with no stratification. The only personnel at the study site with access to the randomization list during the study were the study Drug Manager and Pharmacist. The Medical Monitor also had one set of the randomization codes in a sealed envelope in the event that emergency unblinding was required. Volunteers were immunized at 0, 1, and 2 months in the left deltoid muscle.

Because the color and volume of the reconstituted FMP1/AS02A and Imovax® differed, blinding the individuals responsible for preparing the vaccine was not possible. However, individuals administering the vaccine and performing follow-up evaluations were blinded. The vaccine preparation area and the immunization area were physically and visually separated. All vaccines were prepared by a designated Drug Manager and Pharmacist who were not involved in vaccine administration or follow-up of volunteers. Following reconstitution of the vaccine the dose was drawn into a 1 mL tuberculin syringe and the barrel of the syringe was covered with opaque tape to mask its contents and labeled with the volunteer ID and randomization code. The vaccine was then handed to the immunization clinician who proceeded with administration. Following immunization, the volunteer was assessed by a separate group of clinicians who performed all the follow-up assessments.

After each vaccination, volunteers were followed for 8 days (day of vaccination and post-vaccination days 1, 2, 3, and 7) for solicited symptoms, 30 days for unsolicited AEs,

and until resolution of any SAE or pregnancy. The injection site was assessed for pain, swelling, erythema, and limitation of arm motion at the shoulder. Solicited general symptoms were: fever, nausea, headache, malaise, myalgia and joint pain. Pain was graded as follows: 0, absent; 1, painful to touch; 2, painful with limb movement; and 3, spontaneously painful. Swelling or erythema at the injection site was measured at the longest axis of involvement and graded as 0 if absent, 1 if ≥1 but ≤20 mm, 2 if >20 but ≤50 mm, and 3 if >50 mm. Limitation of arm motion was based upon the volunteer's active range of abduction; 0 if normal, 1 if >90 but ≤120°, 2 if >30 but ≤90°, and 3 if ≤30°. All other symptoms were graded according to the extent of interference with daily activity; 0, none; 1, easily tolerated; 2, interferes with daily activity; and 3, prevents daily activity. After the third administration of vaccine, volunteers were followed monthly by field workers at home and were asked to return to the clinic every 3 months until the end of the study for safety follow-up. SAEs were collected and reported throughout the 12-month period of the study. An SAE was defined as an event that was life threatening, or resulted in or prolonged hospitalization, or resulted in persistent or significant disability, or a congenital anomaly in the offspring of a vaccinated female. Serum creatinine, ALT, white blood cell count, platelet count, Hgb, and hematocrit were determined on days 0, 14, 30, 44, 60, 74, and 90. Additional Hgb determinations were made on days 180, 272 and 364. Normal ranges were calculated based upon the local population. β-HCG was determined within 48 h before each immunization.

## 2.4. Measurement of humoral responses

Immune response to the FMP1/AS02A vaccine was determined by anti-MSP-1<sub>42</sub> ELISA endpoint titers. For the ELISA, the MSP-1<sub>42</sub> capture antigen was diluted in antigen diluent (pH 7.4 phosphate buffered saline (PBS), 0.5% boiled casein) to a concentration of 0.5 µg/mL. Wells of 96-well plates were coated with 100 µL of diluted antigen overnight at 4 °C, and blocked with a solution of 0.5% boiled casein in PBS. All subsequent steps were carried out at room temperature. Sera were serially diluted in triplicate on the plates from 1:500 to 1:64,000 and incubated for 2 h, followed by horseradish peroxidase-conjugated goat anti-human IgG (KPL, Gaithersburg, MD) diluted 1:1000 in PBS diluent, for 1 h. After addition of 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonate) (ABTS) peroxidase substrate (Kirkegaard & Perry Laboratories Inc. Gaithersburg, MD), plates were incubated for 1 h, and the reaction stopped by addition of 10 µL of 20% SDS (Sigma, St. Louis, MO) stop solution. Plates were washed between all steps with an automated plate washer (Molecular Devices, Sunnyvale, CA) using four rinses of PBS with 0.05% Tween 20 (Sigma). Plates were then read by an automated plate reader (Vmax Molecular Devices™) at 414 nm, and the serial dilutions used to fit a 4-parameter curve using SoftMax Pro v4.1 (Molecular Devices). Results were expressed in titer values, the titer endpoint being defined

as the calculated serum dilution yielding an optical density of 1.0 in our assay. Two positive controls, one negative control, and blank wells were included on every plate for quality assurance. Plates not meeting pre-defined limits for control values were excluded and the samples re-tested.

Fine specificity of antibody responses was assessed using recombinant fragments of MSP-1<sub>42</sub> including MSP-1<sub>19</sub>, endodermal growth factor (EGF) domains 1 and 2 from the 3D7 allele of *P. falciparum*. The individual fragments from MSP-1<sub>42</sub> were expressed as GST-fusion proteins in *E. coli* and purified to >95% purity [15]. Microtiter plate (Immulon-2 HB; Thermo Labsystems, Franklin, MA) wells were coated with 0.8 pmol of recombinant antigen in 100  $\mu$ L of PBS and incubated overnight at 4 °C followed by blocking for 1 hr at room temperature with 300  $\mu$ L of blocking buffer (1% Fraction V BSA in PBS). Sera were diluted serially with blocking buffer and incubated for 2 h at room temperature. Plates were washed 4 times with wash buffer (0.05% Tween 20, 0.0025% Chlorohexidine in PBS) using a microplate washer (Skan-Washer 300 version B, Skatron Instruments, Sterling, VA). Goat anti-human IgG (H+L)-alkaline phosphatase conjugate diluted to 1:1000 (Promega, Madison, WI) was added to each well and incubated at room temperature for 1 h. *p*-Nitrophenyl phosphate substrate (Sigma, St. Louis, MO) was added to each well and the OD<sub>405nm</sub> was measured after 60 min with a microplate reader (Molecular Devices, Spectra-Max Plus 384, SoftMax Pro software). The antibody titer was calculated in the same way as the endpoint titer above. For the analyses comparing MSP-1<sub>42</sub> fragment-specific Ab titers, the plate antigens were normalized to one another. Equal molar coatings (0.8 pmol/well) for each ELISA plate antigen were confirmed by detecting with antibody against the GST moiety of the fusion protein (in the case of p19, EGF1, and EGF2), or mAb 5.2 (in the case of MSP-1<sub>42</sub> and p19).

### 2.5. Data safety monitoring board and regulatory affairs

An independent Data Safety Monitoring Board (DSMB) was appointed prior to the study start to review safety data during and after the course of this trial. DSMB membership included a statistician and four senior clinical investigators with experience in conducting malaria vaccine trials. The trial

was monitored for regulatory compliance by representatives from the United States Army Medical Materiel Development Activity, GlaxoSmithKline Biologicals, and Pharmaceuticals Product Development, a contract research organization based in Wilmington, NC.

### 2.6. Data handling and statistical analyses

Analyses and descriptive tables of the final database were made using SAS version 8.2 (SAS Institute, Cary, NC). Comparisons of the proportion of volunteers by group experiencing any symptom or event were made by Fisher's exact test without correction for multiple comparisons. Comparisons of serologic responses by group were made using geometric mean endpoint dilution titers. Confidence intervals for serologic responses were calculated by transforming individual data to base 10 logarithms, calculating the 95% confidence interval based upon the normal distribution, and then exponentiating the confidence limits. For volunteers who received all scheduled immunizations, group responses were compared using a longitudinal model, with a spatial power covariance structure (SAS, version 8.2), to assess the effect of vaccination on the mean level of antibody over time. A one-sided *t*-test (alternative hypothesis: FMP1 slope > Imovax® slope) was performed to determine if the two groups differed in the rate of antibody development between days 0 and 90.

## 3. Results

### 3.1. Study conduct and participant flow

The study was conducted from April 2002 to April 2003. A total of 199 people were briefed; of these 139 consented to screening. Of the 107 who returned for screening, 40 (14 females and 26 males) were enrolled and randomized to one of two treatment groups. The groups were comparable in baseline demography, clinical laboratory values and distribution of antibody to FMP1 (Table 1). All 40 participants received the first two immunizations. Three did not receive the third immunization but were followed as per protocol. Subject FMP1-1-032, who received Imovax®, was excluded from the third dose because of transient transami-

Table 1  
Clinical and demographic baseline characteristics of enrolled volunteers

Characteristics	Imovax® N = 20	FMP1/AS02A N = 20
Mean age in years (S.D.)	32.9 (11.4)	33.7 (9.6)
No. male	13	13
Mean WBC $\times 10^3/\mu$ L (S.D.)	6.0 (2.2)	5.1 (1.4)
Mean hemoglobin g/dL (S.D.)	12.9 (1.7)	13.5 (2.1)
Mean platelets $\times 10^3/\mu$ L (S.D.)	224 (67)	214 (68)
Mean lymphocyte count $\times 10^3/\mu$ L (S.D.)	2.3 (0.5)	2.2 (0.8)
Mean creatinine mg/dL (S.D.)	0.76 (0.15)	0.78 (0.15)
Mean ALT U/L (S.D.)	20.5 (14.0)	15.0 (4.7)
GMT anti-MSP-1 <sub>42</sub> antibody titer (95% CI) $\times 10^3$	24 (12–47)	18 (9–38)

GMT: geometric mean titer; CI: confidence interval; ALT: alanine amino-transferase.



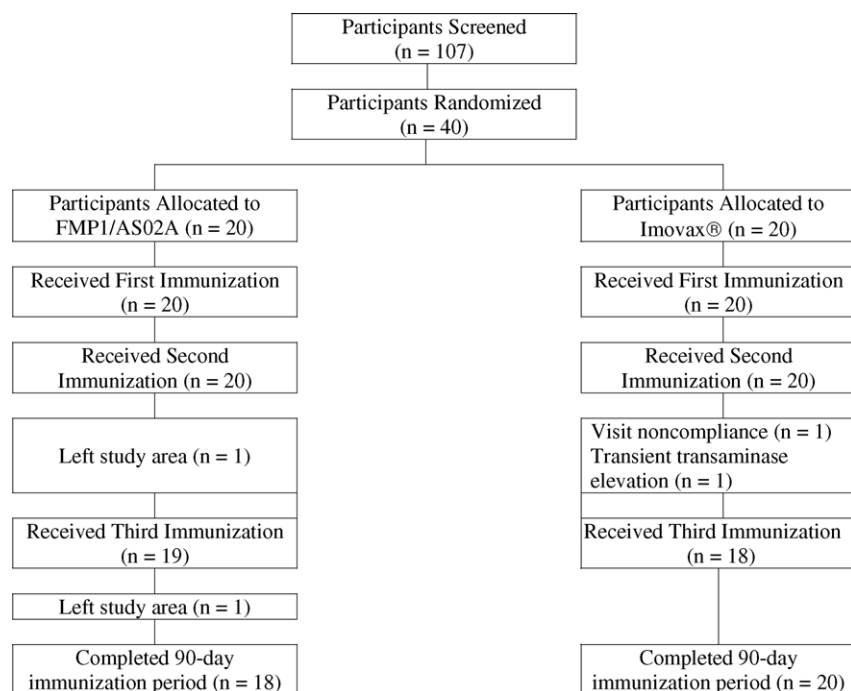


Fig. 1. CONSORT diagram summarizing the number of volunteers at each stage of the trial.

nase elevation. Subject FMP1-1-004 (Imovax®) missed the third immunization because of visit noncompliance, and subject FMP1-1-083 (FMP1/AS02A) was excluded because he left the study area (Fig. 1).

### 3.2. Safety and reactogenicity

The vaccines were well tolerated. No withdrawal due to vaccine side effects occurred. Table 2 summarizes the solicited signs and symptoms. The most common side effect related to immunization was pain at the site of injection. In general, more FMP1/AS02A vaccinees reported pain at the injection site within 24 h than did Imovax® vaccinees. The pain was more prominent with the first injection than with

subsequent doses. In most cases the pain resolved within 48 h. There were no other differences in solicited symptoms during the 8-day follow-up periods in vaccine-related signs and symptoms. There was no difference in the occurrence of Grade 3 local events between the FMP1/AS02A and Imovax® recipients by dose or by group. After three immunizations, 8 of 20 individuals in the Imovax® group and 9 of 20 in the FMP1/AS02A group experienced at least one Grade 3 local reaction. No Grade 3 solicited systemic reaction occurred in either group during the 8-day follow-up period. Three Grade 3 unsolicited AEs occurred during the three 30-day post-immunization follow-up periods, but none was causally related to immunization. In the Imovax® group, one volunteer experienced gastroenteritis and another experienced enteritis;

Table 2  
Number of volunteers who experienced solicited signs and symptoms during the 8-day follow-up after each immunization

	Imovax®			FMP1/AS02A		
	Dose 1 N=20	Dose 2 N=20	Dose 3 N=18	Dose 1 N=20	Dose 2 N=20	Dose 3 N=19
<b>Local</b>						
Pain	10	11	10	17*	11	13
Arm motion limitation	2	0	2	4	0	3
Swelling	0	0	2	0	2	5
Erythema	0	0	0	0	0	0
<b>Systemic</b>						
Fever	1	1	0	1	0	0
Headache	4	4	1	6	5	4
Arthralgia	1	2	0	0	3	1
Myalgia	3	2	0	1	1	1
Malaise	4	2	0	1	2	3
Nausea	0	0	0	0	3	0

\* P value = 0.04 compared to Imovax® Dose 1.

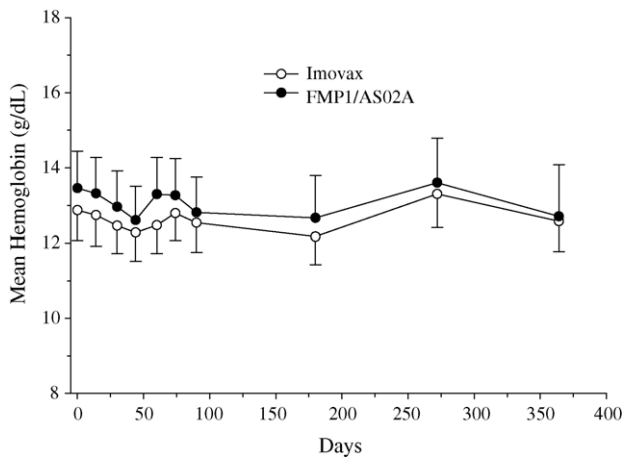


Fig. 2. Group mean hemoglobin levels over time. Error bars represent point wise 95% confidence interval of the mean.

in the FMP1/AS02A group, one volunteer injured his left knee.

Ten documented SAEs occurred in nine volunteers during the study; 7 in seven volunteers in the Imovax<sup>®</sup> group and 3 in two volunteers who received FMP1/AS02A. In the Imovax<sup>®</sup> group, three volunteers contracted malaria, two developed sepsis, one experienced a ruptured ectopic pregnancy, and one experienced gastroenteritis. In the FMP1/AS02A group, one volunteer contracted malaria, and another experienced two separate SAEs: gastroenteritis (possibly typhoid fever) and pneumonia. No SAE was deemed causally related to immunization.

In addition, volunteer FMP1-1-097, who received the FMP1/AS02A vaccine, presented on day 364, the last scheduled study visit, with symptoms of fatigue and history of heavy menstruation. She was pale and had a hemoglobin level of 5.3 g/dL. A Giemsa-stained thick and thin smear revealed no evidence of malaria. A stool sample showed evidence of hookworm infection. She was treated with mebendazole, folate, and iron supplementation and recovered uneventfully.

Group mean and group median safety laboratory values (WBC, Hgb, Hct, Plt, Cr and ALT) were unchanged in the 30-day post immunization follow-up period. Likewise, longitudinal models of the extended Day 90–Day 364 determinations of Hgb showed no change for group mean or median values (data not shown). Group mean Hgb values and point wise 95% CI are depicted for the 12-month period of follow up (Fig. 2).

### 3.3. Immunogenicity

The volunteers had high pre-existing antibody titers to FMP1 prior to immunization. The baseline GMT (in thousands) for FMP1/AS02A vaccinees who received all three immunizations was 17 (point wise 95% CI: 8–36) and for Imovax<sup>®</sup> vaccines 26 (point wise 95% CI: 13–51). Among FMP1/AS02A vaccinees, anti-FMP1 titers increased after each dose, peaking on day 90 at 46 (point wise

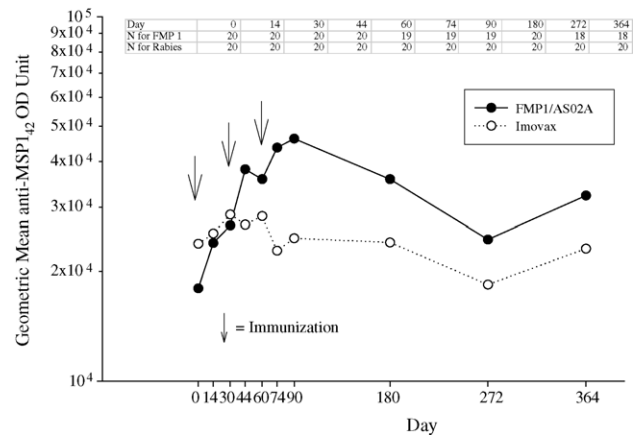


Fig. 3. Group geometric mean anti-MSP-142 titers.

95% CI: 28–77) versus Imovax<sup>®</sup> 25 (point wise 95% CI: 12–51), and remained higher than Imovax<sup>®</sup> vaccinees on day 364. Among Imovax<sup>®</sup> vaccinees, anti-FMP1 titers were relatively constant throughout (Fig. 3). Log<sub>10</sub>-transformed titer data were analyzed using longitudinal mixed models. These models account for correlations within volunteers, thus producing more precise estimates at each time point than point wise comparisons. Therefore, even though point wise comparisons do not show statistically significant differences, longitudinal models revealed a highly significant difference between groups for both the average post-baseline responses ( $F_{1,335} = 13.16$ ;  $P < 0.001$ ) and the Day 90 responses ( $F_{1,335} = 16.69$ ;  $P < 0.001$ ). Comparison of the anti-MSP-142 antibody titers at 0 and 90 days illustrates that among FMP1/AS02A vaccinees both low and high pre-existing titers were boosted in this semi-immune adult population (Fig. 4).

Proteolytic cleavage of MSP-142 into fragments MSP-133 and MSP-119, the latter containing two EGF domains, is felt to be important for invasion [9]. Therefore, in order to determine if immunization with FMP1/AS02A was able to induce more anti-MSP-142, anti-MSP-119, anti-EGF-1 or anti-EGF-2 antibodies, we measured immune responses against recombinant antigens of these peptides. Consistent with the antibody titers against MSP-142 (Fig. 3), at the specified time points we observed no significant differences in mean endpoint titers against these peptides between the two groups. We felt the most likely explanation for this was the high malaria transmission intensity in the study site, which in semi-immune adult volunteers may have overshadowed the inductive (recall) capacity of the vaccine. In order to minimize the effect of fluctuations in antibody titers due to intermittent infections, we reanalyzed the antibody titers as a function of time. Table 3 presents the rate of antibody acquisition between day 0 and 2 weeks and 4 weeks post third immunization (Day 74 and 90) that were analyzed by ELISA against fragments of MSP-142 3D7 (MSP-142, MSP-119, EGF-1 and EGF-2). The results show that for the MSP-142, MSP-119, and EGF-1 fragments, there are significant differences in the



Table 3

Mean slope of the ELISA Ab response ( $\Delta$ ELISA Ab titer/day) of the regression line for days 0, 74, and 90

Plate Ag	Imovax® <i>N</i> = 18 slope (S.E.)	FMP1 <i>N</i> = 19 slope (S.E.)	One sided <i>P</i> -value
MSP-1 <sub>42</sub>	39(46)	178(61)	0.04
MSP-1 <sub>19</sub>	−43(43)	121(30)	<0.01
EGF-1	18(16)	87(20)	<0.01
EGF-2	42(13)	61(22)	0.23

S.E.: standard error of the mean.

rates of anti-MSP-1 fragment antibody development between Imovax® and FMP1/AS02A groups ( $P=0.04$ ,  $<0.01$ , and  $<0.01$ , respectively). No significant difference was observed when sera were analyzed against the C-terminal most EGF-2 domain fragment of MSP-1<sub>42</sub>. This interesting observation, in light of the apparent prevalence of the FUP/Camp parasite strain in this study area in Kenya, suggests that vaccination with FMP1/AS02A failed to recall an immune response against EGF-2. One possible explanation is the lack of homology between the vaccine strain and the local FUP/Camp strain, even though their EGF-2 domains differ at only three amino acid positions [12,20].

#### 4. Discussion

This Phase 1 trial provided clear evidence of the safety and tolerability of FMP1/AS02A when given to adults subject to intense malaria transmission in western Kenya. With the exception of localized pain at the injection site at 24 h, detailed post-immunization follow-up revealed that volunteers receiving FMP1/AS02A reported a similar incidence and intensity of local side effects and general symptoms compared to those receiving the licensed comparator rabies vaccine. No local or systemic allergic event occurred. Of the 10 SAEs occurring during this 12-month study, three occurred in two volunteers who received FMP1/AS02A, but

neither the Principal Investigator, the Medical Monitor, nor the Data Safety Monitoring Board found any causal relationship with receipt of the vaccine.

With the ultimate goal of administering a malaria vaccine as part of the Expanded Program on Immunization (EPI) to infants at greatest risk of malaria, we have tested the FMP1/AS02A vaccine on an EPI-compatible 0-, 1- and 2-month schedule. This brisk schedule proved to be well tolerated and was not associated with any new or higher incidence of AEs than had been observed in initial trials of FMP1/AS02A given on a 0-, 1-, and 3-month schedule in healthy malaria-naïve adults ([18] and Cumming's unpublished data).

There was a statistically significant antibody response to a 3-dose regimen of FMP1/AS02A in a population with substantial baseline antibody to this immunogen and relative clinical immunity to malaria. This immunogenicity of the vaccine may have been lessened by the high levels of pre-existent antibody titers in this semi-immune population as suggested by the fact that the greatest rise in antibody responses was seen in individuals with low pre-existing antibody levels (Fig. 4). By the same token, we did not observe differences in endpoint titers against MSP-1<sub>42</sub> sub-fragments but we demonstrated a higher rate of antibody development against these peptides in FMP1/AS02A recipients. The FMP1/AS02A vaccine was highly immunogenic in the initial trials conducted in malaria-naïve adults in the USA ([18] and Cumming's unpublished data). These data suggest that this vaccine will induce an even greater fold increase in anti-FMP1 titers when evaluated in children with lower baseline antibody titers. An efficacy trial will be required to establish the relationship of antibody to FMP1 or to its subdomains and clinical immunity to malaria.

Malaria is a well-described cause of anemia in areas of intense transmission, particularly in infants and young children with minimal immunity to clinical disease. Malarial anemia is multifactorial in origin; it results from the combined

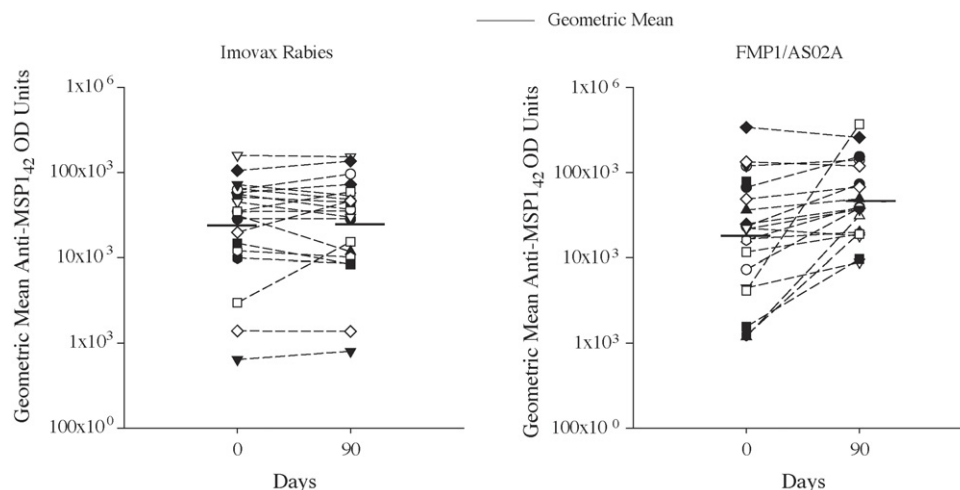


Fig. 4. Changes in anti-MSP-1<sub>42</sub> geometric mean OD units from Day 0 to Day 90.

effects of suppression of erythropoiesis, lysis of infected erythrocytes, and the accelerated destruction of uninfected erythrocytes [21,22]. Although there are no validated predictive models of human *falciparum* malaria [23], the observation of malaria-associated anemia in New World monkeys with prolonged *Plasmodium falciparum* parasitemia has led to speculation that malaria vaccines eliciting immunity that controls but does not eliminate parasitemia might themselves increase the risk of anemia in endemic populations [24]. Hemoglobin levels were stable in both groups for 365 days, suggesting that FMP1/AS02A had no adverse group effect on hemoglobin levels following immunization and exposure to malaria. A single instance of significant anemia not associated with concurrent malaria was noted on Day 364 in FMP1/AS02A-immunized volunteer FMP1-1-097, who had a history of menorrhagia and severe hookworm infection, and responded to iron, folate, and antihelminthic treatment.

The clinical development plan for FMP1/AS02A has two immediate goals: an expansion of the safety and immunogenicity profile in endemic populations, followed by determination of the preliminary efficacy of this vaccine for reduction of clinical malaria in children at risk of disease. Later in 2003, the DSMB, on the basis of the results presented here, endorsed a Phase I trial of FMP1/AS02A in children. Accordingly, we then conducted a Phase Ib dose-escalation trial of FMP1/AS02A in 135 Kenyan children 1–4 years of age that enabled selection of a safe, well-tolerated, immunogenic dose of FMP1/AS02A for an efficacy trial that began in April 2005. The results of these trials will be reported in future communications.

### Ethical review

Participants were recruited under a human use protocol approved by and executed in accordance with the guidelines of the Office of the Surgeon General, US Army, the Kenya Medical Research Institute, Nairobi, Kenya, and the Human Subjects Protection Committee of the Program for Appropriate Technology in Health. Informed consent was obtained from all participants in accordance with all applicable guidelines.

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**Potential conflicts of interest:** Drs. Jeffrey Lyon and Evelina Angov hold patents for production of the FMP1 antigen and together with Joe Cohen for the formulation of FMP1 with the proprietary AS02A adjuvant. Drs. Amanda Leach and Joe Cohen are employees of GlaxoSmithKline Biologicals, the manufacturer of AS02A. Jessica Milman was formerly an employee of the PATH's Malaria Vaccine Initiative, which provided funding for this trial.

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