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A Randomized Controlled Phase 2 Trial of the Blood Stage AMA1-C1/Alhydrogel Malaria Vaccine in Children in Mali

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Abstract

A double blind, randomized, controlled Phase 2 clinical trial was conducted to assess the safety, immunogenicity, and biologic impact of the vaccine candidate Apical Membrane Antigen 1-Combination 1 (AMA1-C1), adjuvanted with Alhydrogel[®]. Participants were healthy children 2-3 years old living in or near the village of Bancoumana, Mali. A total of 300 children received either the study vaccine or the comparator. No impact of vaccination was seen on the primary endpoint, the frequency of parasitemia measured as episodes >3000 per μ L per day at risk. There was a negative impact of vaccination on the hemoglobin level during clinical malaria, and mean incidence of hemoglobin <8.5 g/dL, in the direction of lower hemoglobin in the children who received AMA1-C1, although these differences were not significant after correction for multiple tests. These differences were not seen in the second year of transmission.

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Keywords

Malaria; Vaccine; AMA1

1. Introduction

Malaria remains a primary cause of morbidity and mortality in children in sub-Saharan Africa, with an estimated 881 000 malaria deaths in 2006, of which 91% were in Africa and 85% were in children under 5 years of age. [1]. An effective malaria vaccine is needed to combat this disease. During the last 10 years, several malaria vaccine candidates have reached the stage of clinical testing in malaria exposed populations, and one vaccine has shown 35% efficacy against uncomplicated malaria [2]. Apical membrane antigen-1 (AMA1) is a surface protein expressed during the asexual blood stage of *P. falciparum*, and is a leading vaccine candidate, with several formulations being tested in malaria endemic areas in Africa [3-5]. Preclinical studies have shown that vaccination with AMA1 induces antibodies and protection against homologous parasite challenge in both rodent and monkey models of malaria infection [6-9].

The AMA1-Combination 1 (C1) vaccine was developed by the Malaria Vaccine Development Branch of the National Institute of Allergy and Infectious Diseases (NIAID), National Institutes of Health, USA. The vaccine is a combination of an equal mixture of the correctly folded ectodomain portion of recombinant AMA1 from two divergent clones of *P. falciparum*, FVO and 3D7, adjuvanted with Alhydrogel®. The combination vaccine was chosen because of sequence polymorphism of the AMA1 gene and the strain specific antibody response to recombinant AMA1 [10,11]. It is hoped that the inclusion of more than one polymorphic protein will induce broader protection against infection with diverse strains of parasites. Phase 1 studies in malaria naïve adults in the US and in semi-immune adults in Mali have shown that the vaccine was well tolerated and immunogenic at a dose of 80 µg [12,13]. A Phase 1 clinical trial of this vaccine in children 2-3 years old in a malaria-endemic setting also showed that the vaccine was well tolerated and immunogenic at the doses of 20 and 80 µg [14]. Based on these Phase 1 studies, a Phase 2 study was implemented in Bancoumana, Mali to assess the impact of this vaccine on malaria parasitemia and to further evaluate its safety and immunogenicity.

2. Methods

This was a double blind; randomized, controlled clinical trial (www.clinicaltrials.gov NCT00341250) designed to assess the safety, immunogenicity, and biologic impact of the blood stage malaria vaccine candidate AMA1-C1, adjuvanted with Alhydrogel®. The primary biologic impact endpoint was the rate of episodes of *P. falciparum* parasitemia >3000/µL per time at risk occurring during the transmission season after vaccination. Secondary biological impact endpoints included the rate of clinical episodes of malaria per time at risk using axillary temperature ≥ 37.5°C and varying *P. falciparum* density cutoffs, time to first event for the primary endpoint and clinical malaria using the same cutoffs, four measures of hemoglobin/anemia, and three measures of *P. falciparum* density. Adverse events and antibody responses were also summarized and analyzed by vaccination group.

2.1. Participants

Participants were healthy children 2-3 years old living in or near the village of Bancoumana, Mali, an area with intense seasonal malaria transmission from July - November [15]. Enrolled children were available for the initial duration of the trial (52 weeks) and had normal screening labs and physical examination. Specific exclusion criteria were identical to the preceding Phase 1 study in children in Donéguébougou, Mali, which was conducted under the same clinical trial protocol and is described in Dicko et al [14]. Children were enrolled in two cohorts of 60

(May–June 2006) and 240 (July–August 2006) and were followed initially for 52 weeks. Children who were available and whose parent or guardian gave consent were re-enrolled for extended follow up from November/December 2007 to January 2008, to assess possible impact on potential safety issues identified after the end of initial follow up.

2.2. Ethics

Community and individual consent were obtained prior to screening and enrollment. The study protocol was reviewed and approved by the NIAID Institutional Review Board, and by the Ethics Committee of the Faculty of Medicine, Pharmacy and Dentistry (FMPOS), University of Bamako. The study was conducted under Investigational New Drug application BB-IND#10944, and was monitored for regulatory compliance by the Regulatory Compliance and Human Subject Protection Branch of NIAID and the Initiative for Vaccine Research of WHO. Safety data for the first cohort were reviewed prior to enrollment of the second cohort by the Data Safety Monitoring Board (DSMB) of NIAID and by the local medical monitor.

2.3. Interventions

AMA1-C1 is a combination of two recombinant allelic proteins (AMA1-FVO and AMA1-3D7), consisting of amino acids 25 through 545 of the published sequences of each line's AMA1 gene (GenBank accession number AJ277646 for FVO and accession number U65407 for 3D7). Protein production and vaccine formulation are described in detail elsewhere [14,16]. Each 0.5 mL dose of vaccine contains about 424 µg of aluminum (Alhydrogel®, HCl Biosector, Denmark) onto which 80 µg of recombinant AMA1-C1 has been bound. The comparator vaccine, Hiberix® (GlaxoSmithKline, Uxbridge, UK), is a noninfectious vaccine containing purified capsular polysaccharide of *Haemophilus influenzae* type b (Hib) covalently bound to tetanus toxoid. Each 0.5 mL dose contains 10 µg of purified polysaccharide covalently bound to approximately 30 µg tetanus toxoid. Both vaccines were administered IM in the thigh muscles on Days 0 and 28, in alternating legs.

2.4. Outcomes

2.4.1. Safety and Tolerability—Local and adverse events were recorded as described in Dicko et al [14]. Briefly, children were seen for follow up 1, 2, 3, 7, and 14 days after each vaccination, at study weeks 12, 22, 30, 42, and 52, and at two time points during the extended follow up, in addition to unscheduled visits at any time. Solicited and unsolicited injection site reactions and systemic adverse events and laboratory adverse events were recorded and graded as described. In addition to the above, clinic records for the period during which the children were off protocol were reviewed and data from these visits were abstracted. Illnesses recorded in the clinic records were counted as adverse events and graded by severity, with those which required parenteral treatment or which were described in the clinic record as severe graded as severe (Grade 3).

2.4.2. Biologic Impact—Parasitologic follow up began two weeks after the second vaccination on Study Day 42, and ended on Study Day 154. These time periods overlapped but were not concurrent for the two cohorts, since they were vaccinated at different times. During parasitologic follow up children were seen weekly. At the initial visit and every fourth weekly (monthly) visit, blood was obtained for malaria smears, hemoglobin, and anti-AMA1 antibody, regardless of symptoms. If the child was febrile (axillary temperature $\geq 37.5^{\circ}\text{C}$) or had a history of recent fever, or if hemoglobin was <8.5 g/dL, slides were read immediately and the child was treated for malaria if positive. At weekly visits blood was obtained for malaria smears and hemoglobin only if the child was febrile or had a history of recent fever. At unscheduled visits blood was obtained if febrile as at the weekly visits, unless the child was already being followed up for a known cause of fever. Hemoglobin was measured using a

hemoglobin analyzer Hemocue®, except at the time points when a complete blood count (CBC) was performed. Malaria smears were read separately by two blinded certified microscopists, with discrepancies resolved by a third certified microscopist. *P. falciparum* density was calculated using individual white blood cell (WBC) counts when available within 2 weeks, otherwise average WBC counts of 7,500/μL were assumed.

2.4.3. Immunogenicity—Antibody responses to the AMA1 antigens were measured in plasma by enzyme linked immunosorbent assay (ELISA) at Days 0, 14, 42, 98, 154, 210, 294, and 364, and at two time points during the extended follow up period (November/December 2007 and January 2008). The ELISA technique was described previously [12,17]. A human anti-AMA1 standard was made using a pool of plasma from individuals enrolled in a previous US vaccine trial [18]. The standard using this pool was assigned 460.9 units on AMA1-FVO and 578.0 units on AMA1-3D7. The minimal detection level of this assay was 38 ELISA units and all data below that limit of detection were assigned a value of one half the limit of detection (i.e., 19 units) for analysis.

2.5. Randomization and Blinding

Participants were block randomized in a 1:1 ratio to receive either the study vaccine or the control (Hiberix®), with block sizes varying from 4 to 6. Randomization codes were created by the study statistician, and randomization occurred at the time of first vaccination. A copy of the randomization code was provided to the pharmacist who used coded labels for the vaccines, and to the medical monitor and DSMB. Both participants and investigators were unaware of treatment assignment until completion of the initial phase of the study. Since the appearance of the vaccines was slightly different, opaque tape was placed over the syringe so that vaccinators were unable to see the contents. In addition, physicians who administered the vaccine were not involved in clinical evaluation of volunteers. Unblinding of the investigators occurred after databases to the end of the parasitologic follow up period (Day 154) were cleaned and finalized, and after the data analysis plan was final.

2.6. Statistical Methods

2.6.1. Safety

Initial analyses: Adverse events (AEs) were summarized by grade and relationship to vaccination. For AEs observed in the initial part of the study (up to Day 364), exact Wilcoxon-Mann-Whitney (WMW) tests were performed to examine relationships between vaccine group and AEs, with a separate test done for each category of AE and an overall test done for any specific AE. For each AE tested (including the overall category), each subject was defined as having a response of no AE, grade 1 AE, grade 2 AE, grade 3 AE or serious AE (SAE) based on the subject's highest grade of AE in that category. In order to maintain a low threshold for detecting safety concerns no correction was made for multiple comparisons. Additionally, Fisher's exact test was used for testing types of SAE. Wilcoxon signed rank tests were also performed to test whether severity of AEs increased after second vaccination for the AMA1-C1/Alhydrogel group, comparing AEs observed from Day 0 to 28 and those observed from Day 29 to 56. All children for whom data were available were included in the safety analysis.

Extended Analyses: As described above, an extended follow-up period was added to the study in order to examine potential safety issues observed after the end of initial follow-up. This analysis was limited to the impact of vaccination on hemoglobin and anemia, on the frequency and severity of malaria disease, and on parasite density. The statistical methods used were the same as for initial Biologic impact (below), except that all children for whom data were available were included in the analysis, since the primary purpose was for safety.

2.6.2. Biologic impact—As specified in the protocol, subjects were excluded from the biologic impact and immunogenicity analyses who either did not receive the second vaccination or who had treatments or events which may have interfered with the immune response. The primary biologic impact endpoint was rate of *P. falciparum* >3000/μL per day at risk. Time at risk was the period of parasitologic follow up (Study Day 42-154 for each subject), minus 28 days after each treatment for malaria, and minus time after missed visits or time away from the village for more than one week. In addition, in order to prevent double counting episodes where a child may have had asymptomatic parasitemia and a second parasitemic episode within a week, parasitemic episodes >3000/μL occurring within 7 days of a previous episode were also excluded, regardless of whether or not treatment was given. Subjects were stratified by subcohort, defined as a set of subjects who received their first vaccination on the same day, and a stratified WMW test was performed, and the associated Hodges-Lehmann estimate and 95% confidence intervals on the rate ratio were determined [19]. Secondary biological endpoints were analyzed as follows. Stratified WMW tests were used to compare frequency of clinical episodes per time at risk. Kaplan-Meier curves were drawn to show time to first event, with stratified logrank tests performed on each of the malaria event definitions. Stratified WMW tests were used to compare the groups for measures of hemoglobin and anemia and for measures of parasite density.

2.6.3. Immunogenicity—Subjects who did not receive both vaccinations or who had events which may have interfered with the immune response were excluded from the analysis, as described above. In addition, for the graphical representation of responses over time (Fig. 3a and 3b), subjects who were missing immune responses for any day were excluded for all days. For each subject included in the analysis, the arithmetic average of the FVO and 3D7 ELISA responses at each day was used as that subject's AMA1-C1 antibody response for that day, because the ELISA responses for the two allelic AMA1 were highly correlated, as in the previous study [14]. Since vaccinations for the two cohorts occurred at different times in the transmission season (just before and just after the start of the season), antibody responses were represented graphically by Cohort. WMW tests were done to compare Day 42 antibody levels for the AMA1-C1/Alhydrogel and Hiberix groups. Spearman rank correlation test was used to test if antibody level on Day 42 (two weeks after second vaccination) was correlated with the primary biological endpoint (rate of parasitemia >3000/μL per day at risk).

2.6.4. Sample size—Sample size calculations were based on the primary biologic impact endpoint (frequency of parasitemia >3000 per μL per day). Data from a previous epidemiologic survey [20] were used to estimate the frequency of parasitemia >3000/μL in this age group. The study was powered to detect a 56% reduction in this rate in 50% of those receiving the study vaccine [21]. To account for variability in the surveys, a bootstrap replication was done and a sample size of 105 subjects in each group gave 80% power in over 95% of bootstrap replications to detect a difference at a 0.05 level. This was increased to 150 per group to allow for loss to follow up and for vaccinations occurring slightly after the start of the transmission season.

Data were double entered and reconciled using Microsoft Access and analyzed using SAS (SAS Institute Inc. North Carolina, USA) or R software (R Core Development Team, Vienna Austria).

3. Results

3.1. Participant Flow and Baseline Data

Five hundred eighty four children were screened for inclusion in the study, of whom 300 (167 males and 133 females) were enrolled (Fig. 1). Reasons for exclusion were concurrent illness

(n=102), positive serology for hepatitis B or C (n=12), hemoglobin <8.5 g/dL and/or malnutrition (n=73), other abnormal screening laboratory tests (n=23), or other conditions (n=74). Cohort 1 (60 volunteers) received their first vaccinations in May and June, 2006, and Cohort 2 (240 volunteers) received their first vaccinations in July and August; second vaccinations were 28 days later for all volunteers. A total of 21 subjects were excluded from the immunogenicity and biologic impact analyses, either because they did not receive both vaccinations (n=17) or because of treatments or events which may have interfered with the immune response (n=4), as detailed in Table 1. All volunteers who received the first vaccination were included in the adverse event analysis. Baseline characteristics at enrollment are shown in Table 2.

A total of 46 children missed the 2 visits of the extended follow up, 27 in the AMA1-C1 group and 19 in the Hiberix group; however, any subject for whom we had data was included in the extended follow up analyses, including two children who died during the period off protocol.

3.2. Safety

Adverse events to Day 364 are summarized in Table 3. Both vaccines were well tolerated. All local adverse events were mild or moderate except for one child who experienced Grade 3 swelling at the injection site after the first dose of Hiberix (Table 4). There were no significant differences between groups for total AE. There were 65 types of AEs observed, but only 22 of these occurred with sufficient frequency (>5) to detect significant differences between groups and only one out of 22 showed significance: mumps was more frequent in the AMA1-C1/Alhydrogel group than the Hiberix group (p=0.0145). There was no significant increase in AE severity with the second dose of vaccine.

No serious adverse events related to vaccination occurred, although one case of epilepsy judged by the investigators to be unrelated due to the timing of the event was reported by the sponsor to FDA as possibly related. That subject received the first dose of AMA1-C1 vaccine on 23 July 2006 and the second dose on 20 August 2006; the serious adverse event occurred on 12 September 2006. The 14 SAEs occurring during the study and extended follow up are shown in Table 5. There were no significant differences between vaccine groups in SAEs related to hepatitis A (p=0.371) or malaria (including severe, complicated or suspected (not confirmed by microscopy) cases) (p=0.121).

Subsequent to completion of initial follow up to one year after enrollment (Day 364), 4 events occurred, all in children who had received the AMA1-C1 vaccine. Two of these events were hospitalizations due to severe malaria, and two were deaths presumed due to malaria (Table 5). For this reason, and to further investigate the possible adverse impact on hemoglobin of vaccination (see Biologic Impact below), the protocol was amended and follow up was extended to the end of a second transmission season. No significant increase in severity of malaria for the AMA1-C1/Alhydrogel vaccine group was seen in the data to Day 364 or in the extended follow up period.

3.3. Biologic Impact

No impact of vaccination was seen on the primary biologic endpoint, rate of *P. falciparum* >3000/μL per day at risk, or on the secondary endpoints of clinical malaria and *P. falciparum* density (Table 6). For all these outcomes the frequency of events was similar in the two groups. The median rate of *P. falciparum* >3000/μL was 0.0156 in the AMA1-C1 vaccine group as compared to 0.0136 in the Hiberix group; the rate ratio estimate by Hodges-Lehmann method also showed similar rates in both groups (RR= 0.98; 95% CI [0.83, 1.14], p=0.67). The median rate of malaria episodes (fever and parasitemia>0/μL) was 0.014 in the AMA1-C1/Alhydrogel vaccine group as compared to 0.012 in the Hiberix group (p=0.34). There was no

correlation between Day 42 anti-AMA1 antibody levels and the rate of *P. falciparum* >3000/ μ L (Spearman correlation, for average FVO and 3D7 responses $r=0.0909$, $p = 0.1149$). As shown in Figure 2 both groups had similar median time to first *P. falciparum* >3000/ μ L (85 days for the AMA1-C1 group and 79 days for the Hiberix group), and the stratified logrank test showed no significant difference between the groups ($p = 0.33$). Similarly there was no significant difference in time to the four clinical malaria endpoints between the 2 groups (data not shown).

The impact of vaccination on four measures of hemoglobin and anemia during the parasitologic follow up period is shown in Table 7. We considered four summary measures for hemoglobin/anemia for each subject: (i) minimum hemoglobin for any type of visit, (ii) mean hemoglobin at monthly visits, (iii) mean hemoglobin during clinical malaria, and (iv) number of episodes of hemoglobin <8.5 g/dL at any time point. Only mean hemoglobin during clinical malaria and number of episodes of hemoglobin <8.5 g/dL showed significant differences between the groups (p values 0.004 and 0.029 respectively), both in the direction of lower hemoglobin in the children who received AMA1-C1. These two effects were not significant if adjusted for the 16 secondary biologic impact tests done (Holm's adjusted p -value: 0.059 and 0.430 respectively) [22]. Unadjusted significance was also seen for the minimum Hb when data from Day 1 to Day 364 and data from all subjects on whom data were available were included: minimum Hb ($p=0.0121$), mean Hb during clinical malaria ($p=0.0044$), and number of episodes of Hb <8.5 g/dL ($p=0.0096$). No significant differences between groups were seen for all hemoglobin measurements during the extended follow-up (data not shown).

3.4. Immunogenicity

Immune responses over time for both cohorts are shown in Figure 3. Peak antibody levels for both cohorts were seen at Day 42, two weeks after second vaccination. Cohort 1 Day 42 geometric mean anti-AMA1 antibody levels were 866.8 antibody units for the AMA1-C1 group versus 35.4 for the Hiberix group (WMW, $p<0.00001$), and for Cohort 2 were 901.0 and 61.1 ($p<0.00001$). Antibody levels were consistently higher for the AMA1-C1 group for every time point after Day 0, decreasing in both groups in May/June 2007, prior to the start of the second transmission season, and then increasing again in the second transmission season.

4. Discussion

Malaria vaccines which target the blood stage of infection are expected to work through reducing parasite density, thereby reducing the severity of disease. These vaccines are not intended to prevent infection, but instead are intended to reduce morbidity and mortality due to severe malaria, particularly in children. In early trials intended to demonstrate proof of concept, surrogate endpoints must be chosen due to the prohibitive sample sizes required to demonstrate a reduction in severe malaria [23]. In an area such as Bancoumana, children generally develop sufficient natural immunity to cease being at significant risk of clinical malaria after 5 years of age [20]. Furthermore, the age-specific incidence of clinical malaria closely parallels the incidence of parasite density >3,000/ μ L in such areas [20]. Thus, parasite density >3,000/ μ L was chosen as the primary biologic impact endpoint for this trial. This endpoint also has the advantage of being precise, and of avoiding potential error due to the inclusion of fever in a clinical case definition. While error in microscopy can lead to a reduction in specificity and thus power to detect a protective effect, this error is reduced at higher parasite densities [24].

The entomological inoculation rate (EIR) in Bancoumana was not measured during this study. However, the incidence rate of malaria in the control group was consistent to that seen in an earlier study [15]. Importantly it was also similar to that measured using the same methodology in the same age group in Donéguébougou, the village used for Phase 1 studies of this vaccine.

In Donéguébougou, the EIR was 137.3 measured by landing catches and 19.2 by spray catches [20]. As the observed rate in Bancoumana in the control group matched the expected rate derived from the Donéguébougou data, this provides additional confidence that the transmission season in Bancoumana during this trial was not exceptional and that the trial was powered to meet the objectives. Since there is no generally agreed upon surrogate endpoint for blood stage vaccine efficacy, additional secondary endpoints were also analyzed, including clinical malaria using varying parasite density cutoffs. Inclusion of these endpoints allows greater comparability among studies. The similarity of the results when these secondary endpoints were examined increases confidence that the study was adequately powered and that a true effect was not missed.

The two cohorts were enrolled sequentially for safety reasons. Because the study was a blocked randomized study, exactly half of the subjects were randomized to each treatment arm within each cohort. So even though the two cohorts had different exposure experiences (i.e. different periods during the transmission season), combining the cohorts in the analysis will not bias the tests on the main outcome measure, rate of *falciparum* parasitemia > 3000/uL. In addition, although the two cohorts were vaccinated over a month apart there were not large differences in the rates of parasitemia > 3000/uL between the cohorts.

The only secondary endpoints for which a statistically significant difference was demonstrated during the protocol specified period of biologic impact were two measures of anemia, with both in the direction of a negative impact of vaccination. However, a total of 16 secondary endpoints were analyzed, and when a correction for multiple tests was made, these p values were non-significant. The differences in measures of anemia were not seen in the second transmission season. It is plausible that vaccination with AMA1-C1 induced antibody which caused destruction of red blood cells, particularly during malaria infection; however, no correlation of anemia with peak antibody titers was seen (data not shown). Further analysis of antibody levels with hemoglobin is planned, and hemoglobin will be monitored as critical safety readout in future trials, particularly in children.

There was one statistically significant AE effect (mumps was more likely in the AMA1-C1 group, $p=0.0145$). This effect should not be over interpreted since we would expect to see at least one significant effect 67.6% of the time purely by chance when testing 22 independent null effects. To ensure a high sensitivity for detecting safety issues, we chose not to correct for multiple comparisons when performing the primary statistical tests.

The occurrence of four serious illnesses thought to be due to malaria in children receiving AMA1-C1 in the second transmission season is of concern. However, when safety data overall were analyzed there was no significant increase in SAEs related to malaria, or in clinical malaria graded as moderate or severe in either the first or second transmission season. The fact that these cases (including two deaths) occurred after the close of initial follow up highlights the value of rapid case detection and treatment in preventing such outcomes.

The vaccine was moderately immunogenic; immune responses decreased rapidly after the peak at Day 42 but antibody titers in the vaccine group remained higher with a small “boost” during the second transmission season. Antibody levels also increased during both transmission seasons in the control group, reflecting exposure due to malaria infection. Previous Phase 1 studies showed a much higher increase in antibody levels after first and second vaccination with AMA1-C1/Alhydrogel in Malian adults compared to Malian children [13,14]. Antibodies levels in this study were higher than those seen in the previous Phase 1 study in Malian children of the same age [14]. The children in this study were vaccinated during the malaria transmission season while the children in the Phase 1 study were vaccinated well before the start of the season, which may account for the difference in responses. Based on studies with purified

antibody from other human trials, the geometric mean level of antibody observed in the vaccinated groups following the second vaccination would not give substantial levels (<20%) of in vitro growth inhibition [18]. To date no direct correlation has been shown between in vitro and in vivo growth inhibition, but given these data, it is not surprising that the antibody induced by vaccination did not have a direct impact on parasite levels. A more immunogenic formulation of the vaccine may be more likely to be protective, and is expected to enter clinical trials in Malian children in the near future. There was a possibility that vaccination with AMA1 could prime for immune responses (either antibody or cellular) induced by subsequent natural infection that could modulate the course of parasitemia and decrease the likelihood of a clinical episode. The results in this paper suggest that at least in this case this was not achieved.

AMA1 is a highly polymorphic protein, with at least 64 known polymorphisms in the amino acid sequence [25-29]. Although the AMA1-C1 vaccine contained two divergent proteins, it is possible that vaccination with additional allelic proteins is required for protection in the field, or that an AMA1 protein designed to target conserved epitopes is needed [30,31]. Studies of AMA1 parasite genotypes detected during the study are ongoing [32-34].

Epidemiologic studies conducted in malaria endemic populations show conflicting results as to which antigens or combinations of antigens are likely to be protective [3-5]. It is possible or even likely that a blood stage vaccine will require a combination of proteins, to counter both antigen polymorphism and individual variability in responses. In the absence of a human blood stage challenge model or a predictive animal model, blood stage malaria vaccine development is empiric and demonstration of protection requires Phase 2b studies in the target population.

Results of a Phase 2 study of FMP1 (the FVO allelic protein of MSP1-42) adjuvanted with ASO2A also showed no impact on the primary outcome of that study, clinical malaria [4]. Results for a second Phase 2 study of a single allelic protein of AMA1, FMP2, also adjuvanted with ASO2A, are expected soon. Results of these studies, as well as the study presented here, need to be further analyzed to determine which strategies are likely to be most effective in the further development of a blood stage vaccine for *P. falciparum* malaria.

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Figure 1.
Study Flow Chart

Figure 2. Kaplan-Meier curve of time to first parasite density > 3000/ μ L

Kaplan-Meier curves for time from Day 42 until first observed parasitemia > 3000/uL for AMA1-C1 (solid line, n=139) or Hiberix (dashed line, n=140) groups. Subjects that were included in the biologic impact analysis are shown.

Figure 3.

Figure 3a: Cohort 1: Geometric mean of (arithmetic) average of anti-FVO and anti-3D7 AMA1 antibody.

Red is the 80 ug AMA1-C1/Alhydrogel group (n=26) and blue is the Hiberix group (n=28). The horizontal axis is Study Day up to Day 154. Day 0 occurred between May 28 and June 2 2006. After day 154, responses shown are as follows: the values at Day 324 are the responses in May/June 2007, the values at Day 503 are from November/December 2007, and responses at Day 545 are from January 2008. Dashed lines are pointwise 95 percent confidence intervals for each group using t-statistics on the log transformed ELISA values.

Figure 3b: Cohort 2: Geometric mean of (arithmetic) average of anti-FVO and anti-3D7 AMA1 antibody

Red is the 80 ug AMA1-C1/Alhydrogel group (n= 92) and blue is the Hiberix group (n=95). The horizontal axis is Study Day up to Day 154. Day 0 occurred between July 23 and August

14 2006. After Day 154, responses shown are as follows: the values at Day 324 are the responses in May/June 2007, the values at Day 503 are from November/December 2007, and responses at Day 545 are from January 2008. Dashed lines are pointwise 95 percent confidence intervals for each group using t-statistics on the log transformed ELISA values.

Table 1
Exclusion from biologic impact and immunologic analyses

Study ID	Reason for exclusion	Cohort	Vaccine Group
MV-04-42V	tetanus toxoid on Study Day 11	1	AMA1-C1/Alhydrogel
MV-04-067X	missed second vaccination (hepatitis A)	1	Hiberix®
MV-04-086T	missed second vaccination (hepatitis A)	1	AMA1-C1/Alhydrogel
MV-04-112W	refused second vaccination	2	Hiberix®
MV-04-116A	withdrew consent on Study Day 21	2	AMA1-C1/Alhydrogel
MV-04-123H	refused second vaccination	2	AMA1-C1/Alhydrogel
MV-04-125K	refused second vaccination	2	Hiberix®
MV-04-155T	Withdrawn prior to second vaccination (mistaken inclusion, born before Sept 1, 2001)	2	AMA1-C1/Alhydrogel
MV-04-163B	received extra dose of vaccine due to mistaken identity	2	AMA1-C1/Alhydrogel
MV-04-164C	missed second vaccination (fracture)	2	AMA1-C1/Alhydrogel
MV-04-183Y	missed second vaccination (hepatitis A)	2	AMA1-C1/Alhydrogel
MV-04-194K	tetanus toxoid on Study Day 12	2	Hiberix®
MV-04-214G	refused second vaccination	2	Hiberix®
MV-04-260G	missed second vaccination (transfusion on Study Day 12)	2	AMA1-C1/Alhydrogel
MV-04-263K	refused second vaccination	2	Hiberix®
MV-04-266P	missed second vaccination (asthma)	2	Hiberix®
MV-04-285J	refused second vaccination	2	Hiberix®
MV-04-290Q	refused second vaccination	2	Hiberix®
MV-04-297X	refused second vaccination	2	AMA1-C1/Alhydrogel
MV-04-300A	refused second vaccination	2	AMA1-C1/Alhydrogel
MV-04-302C	refused second vaccination	2	Hiberix®

Table 2**Baseline characteristics at enrolment**

	AMA1-C1/Alhydrogel	Hiberix®
Age in years¹	2.93±0.55	2.93±0.56
Gender-Male (%)	56.7	52.7
Ethnic groups		
Malinke (%)	92.0	89.3
Peuhl (%)	4.7	6.7
Others (%)	3.3	4.0
Parasitemic²	18.0	23.3
Hemoglobin¹	10.40±1.0	10.26±1.0

¹ mean±SD

² % with *P. falciparum* parasites detected by microscopy

Table 3
Summary of All Adverse Events occurred from Day 0 to Day 364

	All adverse events			Definitely, probably, or possibly related AEs			Unlikely or not related AEs		
	All	AMA1 ¹	Hib ²	All	AMA1 ¹	Hib ²	All	AMA1 ¹	Hib
All Adverse Events									
Number of participants with any AE	293	148	145	68	31	37	293	148	145
Number of AEs	3033	1515	1518	79	36	43	2954	1479	1475
Grade 1 or 2 (and not SAE)									
Number of participants with any AE	293	148	145	68	31	37	293	148	145
Number of AEs	2720	1351	1369	78	36	42	2642	1315	1327
Grade 3 (and not SAE)									
Number of participants with any AE	170	86	84	1	0	1	170	86	84
Number of AEs	299	158	141	1	0	1	298	158	140
Serious Adverse Events									
Number of participants with any AE	10	5	5	0	0	0	10	5	5
Number of AEs	14	6	8	0	0	0	14	6	8

¹ AMA1-C1/Alhydrogel;
² Hiberix (comparator)

Table 4
Number of Injection Site Local Adverse Events^I [Grade 1, Grade 2, Grade 3]

	Vaccination #1		Vaccination #2	
	AMA1-C1/Alhydrogel (n=150)	Hiberix (n=150)	AMA1-C1/Alhydrogel (n=142)	Hiberix (n=141)
Pain	[5, 0, 0]	[5, 0, 0]	[2, 0, 0]	[1, 1, 0]
Swelling	[9, 0, 0]	[11, 1, 1]	[4, 0, 0]	[6, 0, 0]
Erythema	[0, 0, 0]	[1, 0, 0]	[0, 0, 0]	[0, 0, 0]

^I No subject had higher than a Grade 3 AE or more than one event after the same vaccination

Table 5

Serious Adverse Events

Subject ID	Sex	Vaccine	Event	Study Day on which AE First Observed	Hospitalization	Relation to Vaccine	Outcome
MV-04-067X	M	Hiberix	Hepatitis A	0	No	Unrelated	Recovered
MV-04-260G	M	AMAI-CI	Complicated Malaria	10	Yes	Unrelated	Recovered
MV-04-209B	M	Hiberix	Hepatitis A	42	No	Unrelated	Recovered
MV-04-130R	M	AMAI-CI	Complicated Malaria	42	Yes	Unrelated	Recovered
MV-04-132T	M	AMAI-CI	Epilepsy	51	Yes	Unlikely*	Recovered
MV-04-331J	M	Hiberix	Hepatitis A	42	Yes	Unrelated	Recovered
MV-04-039S	F	Hiberix	Clinical Malaria	154	No	Unrelated	Recovered
MV-04-109T	M	AMAI-CI	Hepatitis A	42	No	Unrelated	Recovered
MV-04-318V	M	AMAI-CI	Umbilical Hernia	182	Yes	Unrelated	Recovered
MV-04-126M	F	Hiberix	Hepatitis A	213	No	Unrelated	Recovered
MV-04-135W	F	AMAI-CI	Severe Malaria	412	Yes	Unrelated	Recovered
MV-04-173N	M	AMAI-CI	Severe Malaria (presumed)	405	No	Unrelated	Death
MV-04-228X	M	AMAI-CI	Severe Malaria (presumed)	456	No	Unrelated	Death
MV-04-323A	M	AMAI-CI	Severe Malaria	443	Yes	Unrelated	Recovered

* This SAE was reported by the sponsor to the FDA as possibly related.

Table 6
Vaccine impact on *P. falciparum* >3000/μL, clinical malaria, and *P. falciparum* density

	AMA1-C1	Hiberix®	p
Parasitemia >3000/μL ¹	0.0156	0.0136	0.67
Clinical Malaria ¹			
Fever + Parasitemia > 0/μL	0.014	0.012	0.34
Fever + Parasitemia ≥ 3000/μL	0.013	0.012	0.53
Fever + Parasitemia > 10,000/μL	0.013	0.012	0.25
Fever + Parasitemia ≥ 40,754/μL ²	0.000	0.012	0.78
Parasite density			
Median maximum during follow up	50,040/μL	54,161/μL	0.57
Median during clinical malaria	45,270/μL	44,093/μL	0.89
Median mean at monthly visits	2,739/μL	2,722/μL	0.25

¹ Rates shown are median events per day at risk.

² Estimated using method described by Smith et al [35]

Table 7
Vaccine impact on hemoglobin (from vaccination day 42 to day 154)

	AMA1-C1	Hiberix®	p
Median of minimum Hb (g/dL)	8.9	9.2	0.101
Median of mean Hb (g/dL) at monthly visits	10.3	10.4	0.289
Median of mean Hb (g/dL) during clinical episodes	9.95	10.33	0.004
Mean number of episodes with Hb <8.5 g/dL	0.755	0.479	0.029