Phase 1 Dose Escalating, Double-Blind, Randomized Comparator Controlled Trial of the Safety and Immunogenicity of Pfs25M-EPA/AS01 and Pfs230D1M-EPA/AS01 Vaccines, Transmission Blocking Vaccines against *Plasmodium falciparum*, at Full and Fractional Dosing in Adults in Mali

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Sponsored by:
Laboratory of Malaria Immunology and Vaccinology (LMIV)
National Institute of Allergy and Infectious Diseases (NIAID)
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CONFIDENTIAL

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AE	adverse event/adverse experience
AGC	absolute granulocyte count
ALT	alanine transaminase
ANC	absolute neutrophil count
AR	adverse reaction
β-hCG	beta human choriogonadotropin
CBC	complete blood count
CFR	Code of Federal Regulations
cGMP	current Good Manufacturing Practices
CHMI	controlled human malaria infection
CIR	Center for Immunization Research (Johns Hopkins University)
CRF	case report form
CRIMSON	Clinical Research Information Management System of the NIAID
CRP	C-reactive protein
CSO	Clinical Safety Office
CSP	Circumsporozoite protein
DHHS	Department of Health and Human Services
DNA	deoxyribonucleic acid
DSF	direct skin feeds
DSMB	Data Safety Monitoring Board
EC	Ethics committee
ELISA	enzyme-linked immunosorbent assay
ELISPOT	enzyme-linked immunosorbent spot assay
EMA	European Medicines Agency
EPA	ExoProtein A
EPI	Expanded Program of Immunization
FDA	Food and Drug Administration
FMPOS	Faculte de Medecine Pharmacie d'OdontoStomatologie
FWA	Federal Wide Assurance
GCP	Good Clinical Practice
GSK	GlaxoSmithKline
HBsAg	hepatitis B surface antigen
HCV	hepatitis C virus
HIV	human immunodeficiency virus
HPV	human papillomavirus
HRPP	Human Research Protection Program
IB	Investigator's Brochure
IM	intramuscular
IND	Investigational New Drug

List of Abbreviations

IRB	Institutional Review Board
ISM	independent safety monitor
IV	Intravenous therapy
LMIV	Laboratory of Malaria Immunology and Vaccinology (of NIAID)
µg	micrograms
MPL	monophosphoryl lipid
MRTC	Malaria Research and Training Center (Mali)
MVI	Malaria Vaccine Initiative
n	number (typically refers to subjects or participants)
NIAID	National Institute of Allergy and Infectious Diseases, NIH
NIH	National Institutes of Health
NOCI	New Onset of Chronic Illness
OCRPRO	Office of Clinical Research Policy and Regulatory Operations
OHRP	Office for Human Research Protections
OHSR	Office of Human Subjects Research
PBS	peripheral blood mononuclear cell
PCR	Polymerase Chain Reaction
PE	Pre-erythrocytic
Pfs25/Pfs230	Surface antigen of zygotes and ookinetes in the mosquito stage of *Plasmodium falciparum*
PI	Principal Investigator
RNA	ribonucleic acid
RVF	rift valley fever
SAE	serious adverse event/serious adverse experience
SD	standard deviation
SERF	Safety Expedited Report Form
SMFA	standard membrane feeding assay
SOP	Standard Operating Procedure
SRCP	Safety Review and Communication Plan
SUSAR	Serious and Unexpected Suspected Adverse Reaction
TB	tuberculosis
TBA	transmission blocking assay
TBS	TRIS-buffered saline
TBV	transmission blocking vaccine
UP	unanticipated problem
USPHS	U.S. Public Health Service
USTTB	University of Sciences, Techniques & Technologies of Bamako
VIMT	vaccine to interrupt malaria transmission
WBC	white blood cell

List of Abbreviations

WHO	World Health Organization
WMW	Wilcoxon Mann Whitney
WRAIR	Walter Reed Army Institute of Research

PROTOCOL SUMMARY

Full Title:	Phase 1 Dose Escalating, Double-Blind, Randomized Comparator Controlled Trial of the Safety and Immunogenicity of Pfs25M-EPA/AS01 and Pfs230D1M-EPA/AS01 Vaccines, Transmission Blocking Vaccines against *Plasmodium falciparum*, Full and Fractional Dosing in Adults in Mali
Short Title:	Phase 1 Study of Pfs25M-Pfs230D1M/AS01
Clinical Phase:	1
IND Sponsor:	OCRPRO
Conducted by:	Laboratory of Malaria Immunology and Vaccinology (LMIV)/National Institute of Allergy and Infectious Diseases (NIAID)/National Institutes of Health (NIH)
Clinical Sites:	Malaria Research and Training Center (MRTC), University of Sciences, Techniques & Technologies of Bamako (USTTB), Mali, West Africa: Sotuba, Bamako, Mali Bancoumana and surrounding villages, Mali Doneguebougou and surrounding villages, Mali
Principal Investigators:	Patrick Duffy, MD (LMIV/NIAID/NIH) Issaka Sagara MD, MSPH, PhD (MRTC/DEAP/FMPOS)
Sample Size:	N = 305 Vaccinated
Accrual Ceiling:	N = 900; Reconsent: 301 previously enrolled and vaccinated Pilot Study: 65 Main Study: 236
Study Population:	Healthy Malian adults
Accrual Period:	Approximately December 2016 to April 2017 Approximately February 2018 to July 2018 (for re-consent)
Study Design:	**<u>In Sotuba, Bamako, Mali (N=65)</u>** Dose-escalating, open label, randomized, pilot study **<u>Group 1:</u>** Pfs25M-EPA/AS01 (n=15)

• ***Arm 1a*** (n=5), to receive 16 µg Pfs25M-EPA/AS01 on D0, D28, D168

• ***Arm 1b*** (n=10), to receive 47 µg Pfs25M-EPA/AS01 on D0, D28, D168

Group 2: Pfs230D1M-EPA/AS01 (n=15)

• ***Arm 2a*** (n=5), to receive 13 µg Pfs230D1M-EPA/AS01 on D0, D28, D168

• ***Arm 2b*** (n=10), to receive 40 µg Pfs230D1M-EPA/AS01 on D0, D28, D168

Group 3: Pfs25M-EPA/AS01 and Pfs230D1M-EPA/AS01 (n=15)

• ***Arm 3a*** (n=5), to receive 16 µg Pfs25M-EPA/AS01 and 13 µg Pfs230D1M-EPA/AS01 on D0, D28, D168

• ***Arm 3b*** (n=10), to receive 47 µg Pfs25M-EPA/AS01 and 40 µg Pfs230D1M-EPA/AS01 on D0, D28, D168

Group 4: Comparator (n=20)

• ***Arm 4a*** (n=10), to receive ENGERIX-B on D0, D28, and D168

• ***Arm 4b*** (n=10), to receive ENGERIX-B on D0, D28, and D168

In Bancoumana and Doneguebougou, Mali (N=240)

Double-blind, randomized, phase 1 clinical trial

Group 2: Pfs230D1M-EPA/AS01 (n=120)

• ***Arm 2c*** (n=60), to receive 40 µg Pfs230D1M-EPA/AS01 on D0, D28, D168; all subjects will undergo antimalarial drug treatment with Coartem® on D-7 (prior to vaccination #1); 4th vaccination on D476

• ***Arm 2d*** (n=60), to receive 40 µg Pfs230D1M-EPA/AS01 (500 µL TBV + AS01) on D0, D28, then 8 µg Pfs230D1M-EPA/AS01 (100 µL TBV + AS01; ***fractional dose***) on D168; all subjects will undergo antimalarial drug treatment with Coartem® on D-7 (prior to vaccination #1); 4th vaccination on D476

Group 4: Comparators (n=120)

• ***Arm 4c*** (n=120), to receive ENGERIX-B on D0, D28, and D168 (start study with Arm 2c and 2d); all subjects will undergo

antimalarial drug treatment with Coartem® on D-7 (prior to vaccination #1); vaccination with Menactra® on D476

Enrollment will be staggered for additional safety

Study Duration:

Start Date: Approximately December 2016
End Date: Approximately March 2018

Mali study participants will be enrolled for a total of 12 to 16 months (at least 6 month follow-up after the last vaccination) depending on vaccination schedule and arm assignment and timing of screening

Re-enrollment (long term immunogenicity follow-up; 4th vaccination)

Start Date: Approximately February 2018

End Date: Approximately August 2019

Participants who had previously been enrolled in the study (up to 301) will be re-enrolled for an additional 6 to 18 months depending on arm assignment and timing of reconsent.

Study Agent/ Intervention Description:

Pfs25M-EPA/AS01: Pfs25, a surface antigen of zygotes and ookinetes in the mosquito stages of *P. falciparum*, is a lead candidate for developing a malaria transmission blocking vaccine. Recombinant Pfs25M was expressed in *Pichia pastoris (P. pastoris)*. Recombinant ExoProtein A (EPA), a mutant, non-toxic protein corresponding to sequence of EPA of *Pseudomonas aeruginosa (P. aeruginosa)*, was expressed in *Escherichia coli (E. coli)*. Pfs25M and EPA were chemically crosslinked to form Pfs25M-EPA conjugate. $AS01_B$ will be mixed directly with the vialed conjugated Pfs25M for a final formulation of either 16µg conjugated Pfs25M, 15 µg conjugated EPA in AS01 (25µg MPL+ 25µg QS21 liposomal formulation) for Arms 1a, 3a or 47µg conjugated Pfs25M, 45µg conjugated EPA in AS01 for Arms 1b, 3b. All manufacturing processes including recombinant protein production and the chemical conjugation were performed in compliance with cGMP.

Pfs230D1M-EPA/AS01: Pfs230 is a parasite antigen expressed in gametocytes and on the surface of emerging gametes in the mosquito host and is also a TBV target based on observations in

animal studies that the antigen-specific polyclonal and monoclonal antibodies conferred transmission-blocking activities in SMFA (Kaushal, Carter et al. 1983, Williamson, Keister et al. 1995). Recombinant Pfs230D1M was expressed in *P. pastoris* as an approximately 20 kDa fragment containing domain 1. Recombinant EPA, a mutant, non-toxic protein corresponding to sequence of EPA of *P. aeruginosa*, was expressed in *E. coli*. Pfs230D1M and EPA were chemically crosslinked to form Pfs230D1M-EPA conjugate. $AS01_B$ will be mixed directly with the vialed conjugated Pfs230D1M for a final formulation of either 13µg conjugated Pfs230D1M, 10µg conjugated EPA in AS01 (25µg MPL+ 25µg QS21 liposomal formulation) for Arms 2a, 3a or 40µg conjugated Pfs230D1M, 31µg conjugated EPA in AS01 for Arms 2b, 3b, 2c, 2d. The fractional dose (Dose #3) in Arm 2d will be $1/5^{th}$ of the Pfs230D1M dose in AS01 (final formulation of 8µg conjugated Pfs230D1M, 2µg conjugated EPA in AS01 [5µg MPL+ 5µg QS21 liposomal formulation]). All manufacturing processes including recombinant proteins production and the chemical conjugation were performed in compliance with cGMP.

ENGERIX-B (hepatitis B vaccine; recombinant): is a sterile suspension of noninfectious hepatitis B virus surface antigen (HBsAg) for intramuscular administration. It contains purified HBsAg obtained by culturing genetically engineered *Saccharomyces cerevisiae* cells, which carry the surface antigen gene of the hepatitis B virus. Each 1-mL adult dose contains 20 µg of HBsAg adsorbed on 0.5 mg aluminum as aluminum hydroxide. FDA approved for persons 20 years of age and older for a series of 3 doses on a 0-, 1-, 6-month schedule.

Menactra® (meningococcal serogroup A, C, Y, and W-135): is a sterile, intramuscularly administered vaccine that contains *Neisseria meningitidis* serogroup A, C, Y, and W-135 capsular polysaccharide antigens individually conjugated to diphtheria toxoid protein. A single dose (0.5 mL) is recommended for those individuals 18 to 50 years of age and otherwise healthy who are at increased risk for meningococcal disease (e.g., individuals in an epidemic or highly endemic country such as Mali).

Primary Objective:

- To assess safety and reactogenicity of Pfs25M-EPA/AS01, Pfs230D1M-EPA/AS01, and simultaneous administration of Pfs25M-EPA/AS01 and Pfs230D1M-EPA/AS01, including Pfs230D1M-EPA/AS01 at fractional dosing in Malian adults. (All arms)
- To assess safety and reactogenicity of a booster dose of Pfs230D1M-EPA/AS01 at full dosing in Malian adults. (Arms 2c, 2d, 4c)

Secondary Objectives:

- To assess the antibody response to the Pfs25 and Pfs230 protein as measured by ELISA. (All arms)
- To assess the functional antibody response to the Pfs25 and Pfs230 protein as measured by standard membrane feeding assay (SMFA). (All arms except 1a, 2a, 3a)
- To assess the functional antibody response to Pfs230 proteins as measured by direct skin feeds (DSF). (Arms 2c, 2d, 4c only)

Exploratory Objectives:

- To explore cellular and transcriptomic responses to Pfs25 and Pfs230 vaccines when administered alone and in combination. (All arms except 1a, 2a, 3a)
- To explore the impact of co-infections on malaria vaccine responses. (Arms 2c, 2d, 4c only)
- To explore the antibody repertoire of functional antibody responses. (All arms except 1a, 2a, 3a)

Primary Endpoint:

- Incidence of local and systemic adverse events (AEs) and serious adverse events (SAEs) in Malian adults. (All arms)
- Incidence of local and systemic adverse events (AEs) and serious adverse events (SAEs) in Malian adults. (Arms 2c, 2d, 4c)

Secondary Endpoints:

- Anti-Pfs25 IgG levels as measured by ELISA. (All arms)
- Anti-Pfs230 IgG levels as measured by ELISA. (All arms)

- TRA/TBA of induced antibody in SMFA. (All arms except 1a, 2a, 3a)
- Transmission interruption activity measured by DSF. (Arms 2c, 2d, 4c only)

Exploratory Endpoints:

- Cellular immune responses to vaccination. (All arms except 1a, 2a, 3a)
- Whole genome transcriptional profiling. (All arms except 1a, 2a, 3a)
- Antibody levels against recombinant EPA, and other malaria antigens, such as Pfs48/45, expressed during the gametocyte development. (All arms except 1a, 2a, 3a)
- Schistosomiasis detection (in urine). (Arms 2c, 2d, 4c only)
- qPCR for helminth detection (from stool). (Arms 2c, 2d, 4c only)
- Sequence B cell receptor/antibody genes. (All arms except 1a, 2a, 3a)

Précis

A vaccine to interrupt malaria transmission (VIMT) would be a valuable tool for local elimination or eradication of this disease, and may contain components that block transmission to mosquitoes (such as Pfs25 or Pfs230) or that prevent human infection (such as the vaccine RTS,S). Pfs25 and Pfs230, surface antigens of zygotes and ookinetes (and gametocytes for Pfs230) in the mosquito stage of *P. falciparum*, are the lead candidates for a malaria transmission blocking vaccine (TBV). Recombinant Pfs25M and recombinant Pfs230D1M have each been conjugated to *P. aeruginosa* ExoProtein A (EPA) and adjuvanted with AS01. Our ongoing experience with Pfs25M-EPA and Pfs230D1M-EPA in Malian adult trial participants, and the extensive experience with the AS01 adjuvants in African children and adults, justify conducting the first-in-human trial of Pfs25M-EPA and Pfs230D1M-EPA with AS01 in Malian adults. This dose-escalating phase 1 study will determine safety, immunogenicity, and functional activity of these vaccines in Malian adults. Pfs230D1M-EPA in AS01 will be assessed by mosquito feeding assays in Malian adults for evidence that they may reduce the number of malaria transmission events in study subjects.

A total of 305 subjects will be enrolled at multiple sites in Mali, West Africa to receive escalating doses of a malaria transmission blocking vaccine (s): Pfs25M-EPA/AS01, Pfs230D1M-EPA/AS01, or simultaneous administration of Pfs25M-EPA/AS01 and Pfs230D1M-EPA/AS01; or a comparator vaccine (ENGERIX-B). Enrollment within each group will be staggered for additional safety and subjects will only be enrolled into the co-administration group once each individual dose has been administered. Subjects will be followed for at least 6 months after the last vaccination. Safety outcomes will be local and systemic adverse events and serious adverse events. Immunogenicity outcomes will be antibody responses as measured by ELISA against recombinant Pfs25, Pfs230, EPA, CSP, and B cell and T cell responses. Functional activity of the induced antibodies will be assessed in TBV arms by standard membrane feeding assays conducted at the National Institute of Allergy and Infectious Diseases, and activity that interrupts malaria transmission will be measured in the double-blind portion of the study by direct skin feeding assays in Mali.

Subjects in the open label safety cohorts (Arms 1a, 1b, 2a, 2b, 3a, 3b, 4a, 4b) will be offered re-enrollment for follow-up laboratory assessment to explore the duration of immunogenicity and functional activity at approximately 9, 12 months post vaccination. Following scheduled, intentional unblinding, subjects enrolled in Arms 2c, 2d, and 4c will be provided the opportunity to re-enroll for a fourth vaccination (Arm 2c and 2d with 40 μg dose of Pfs230D1M-EPA/AS01; Arm 4c with Menactra®) approximately 1 year post vaccination #3. Subjects in these arms will also be eligible to re-enroll for follow up of duration of immunogenicity and functional activity at approximately 9, 12 months post vaccination if they choose not to enroll to receive the booster vaccination. Subjects will be followed similarly to the previous year for safety, immunogenicity, and functional activity.

1 INTRODUCTION AND RATIONALE

1.1 Background

According to the World Health Organization (WHO), global malaria control efforts have resulted in a reduction in the number of deaths since 2000; however, 438,000 people are estimated to have died due to malaria in 2015, a decline of 48% since 2000.[1] Morbidity and mortality caused by malaria also has significant direct and indirect costs to the economic development of countries in which the disease is endemic.[2] These factors, as well as growing drug resistance of the parasite, widespread resistance of mosquitoes to insecticide, and increased human travel necessitates new approaches to malaria control and eradication. While licensure of the partially protective malaria vaccine (RTS,S 25µg/AS01$_E$; Mosquirix™) seems likely, both the level and duration of protection provided by the vaccine are suboptimal, and RTS,S appears to act by reducing clinical disease rather than preventing infection.[3,4] A vaccine that interrupts malaria transmission (VIMT) would be a valuable resource to add in the fight to eliminate or eradicate this disease.[5,6]

Malaria transmission blocking vaccines (TBVs) aim to induce anti-sporogonic antibodies in the human host. The antibody is subsequently taken up with the blood meal and blocks parasite development in the mosquito, thereby halting transmission to another human host. Pfs25, a surface antigen of zygotes and ookinetes in the mosquito stage, is expressed only in the mosquito host of *P. falciparum.*[7] Pfs25 has long been a lead candidate for a malaria TBV;[8] however, soluble recombinant Pfs25 is poorly immunogenic. LMIV investigators and collaborators have chemically conjugated Pfs25 to ExoProtein A (EPA), a mutant and detoxified protein from *Pseudomonas aeruginosa (P. aeruginosa)*. These conjugates have induced sustained, significantly higher antibody responses in mice, rabbits, and rhesus monkeys than unconjugated Pfs25.[9-11] The conjugate protein, recombinant EPA, is not a component of any licensed vaccines but has been extensively studied as a component of conjugated typhoid and shigellosis vaccines[12-14] and LMIV/MRTC's previous phase 1 TBV studies involving Pfs25H, Pfs25M, and Pfs230D1M formulated with Alhydrogel®.

Pfs25H-EPA, which forms a conjugated nanoparticle to Alhydrogel®, was evaluated in a phase 1 study of US adults (2011 to 2014; NIAID Protocol #11-I-N237) and Malian adults (2013 to 2014; NIAID Protocol #13-I-N109).[15,16] Additionally, Pfs25M-EPA, which no longer contains heterologous amino acids which were present in Pfs25H to facilitate early-stage production, has been recently evaluated in a phase 1 study in US adults (2015) and Malian adults (2015-2016) under NIAID Protocol #15-I-0044. Both have been demonstrated to be a safe, immunogenic vaccines with transmission-blocking activity as assessed by a SMFA. In the Pfs25H-EPA studies, specific anti-Pfs25 antibodies were detected by ELISA in sera from subjects receiving 4 doses of Pfs25H-EPA/Alhydrogel® in US and Mali. With each vaccine dose, antibody responses and associated functional activity increased; however, it was seen in both studies that the

antibody responses, and along with it the functional activity, diminished quickly following vaccination.

Pfs230 is also a TBV target based on observations in animal studies that the antigen-specific polyclonal and monoclonal antibodies conferred transmission-blocking activities in SMFA.[17,18] The full-length Pfs230 precursor of 360 kDa is expressed in gametocytes within erythrocytes, and is processed to become a ~300 kDa mature protein upon translocation to the surface of freshly emerged gametes from erythrocytes.[19] Presence of Pfs230 protein in the human host is consistent with the detection of anti-Pfs230 immunity in malaria-exposed populations, which raised anticipation that a Pfs230-based vaccine may be boosted by natural malaria infection. To facilitate the recombinant protein production, several N-terminal sub-domains within this 300 kDa protein were evaluated and found to be capable of inducing functional antibodies to block transmission in animal studies.[20,21] Based on these findings, using a quality by design strategy, LMIV developed and manufactured a recombinant Pfs230D1M corresponding to amino acid sequence positions #542-#736 of the full-length Pfs230 using *P. pastoris* as a production system. The 20-kDa recombinant Pfs230D1M conjugated to EPA elicited strong transmission-blocking activities in mice, rabbits, and Aotus monkeys. Pfs230D1M-EPA conjugated to Alhydrogel® has been evaluated in a phase 1 study in US adults (2015) and Malian adults (2015-2016) under NIAID Protocol #15-I-0044 and has demonstrated to be safe and immunogenic both in malaria naïve and malaria exposed adults.

Both antigens used in the TBV candidates proposed for this study, Pfs25M and Pfs230D1M, were developed at LMIV and selected for clinical development given their relative conserved sequences against circulating parasites in our targeted study population. Pfs25, which as stated prior is exclusively expressed in the mosquito, is not under immune pressure and consequently the deduced amino acid sequence for 11 Malian *P. falciparum* isolates are identical to the recombinant form of Pfs25 (identified as Pfs25M) with the exception that Pfs25M has had the insertion sequence and membrane anchor deleted, and the putative N-linked glycosylation sites removed (**Figure 1A**). Pfs230, a parasite protein that is expressed during sexual development in the human host as a large protein of approximately 230 kDa, is known to contain various amino acid substitutions throughout the protein; however, the function of these changes is unknown. The recombinant protein Pfs230 domain 1 (also identified as Pfs230D1M) which comprises about 10% of the whole Pfs230 protein is also known to contain minor allelic variants in comparison to NF54 or the 3D7 clone of NF54.[22] A comparative analysis of the deduced amino acid sequence of native Pfs230 domain 1 with the same 11 Malian isolates evaluated above for Pfs25M shows at least a 99% homology including the known N to Q at amino acid position 44 to remove the unique putative N-linked glycosylation site (**Figure 1B**). Two other point mutations were observed within Pfs230D1: G to S at position 64 and K to N at position 120. These same mutations were also observed in an analysis of over two-thousand parasite isolates.[22] In particular, in West Africa, the minor allelic frequency was reported to be 0.111 and 0.339 for G605S and K661N which corresponds to the positions 64 and 120 shown in **Figure 1B**,

respectively. Of note, rabbit antisera raised against Pfs230D1M blocked parasite transmission of a Thailand isolate with the G605S mutation, indicating the presence of this minor allele should not impact efficacy the Pfs230D1M-EPA conjugated vaccine. The impact of the K661N mutation on the biological impact of the vaccine remains to be determined.

Figure 1: Protein alignments of recombinant Pfs25M and Pfs230D1M to their respective native protein or protein fragment

Figure 1A. Pfs25

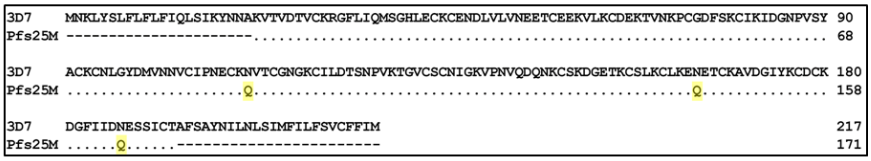


Figure 1B. Pfs230

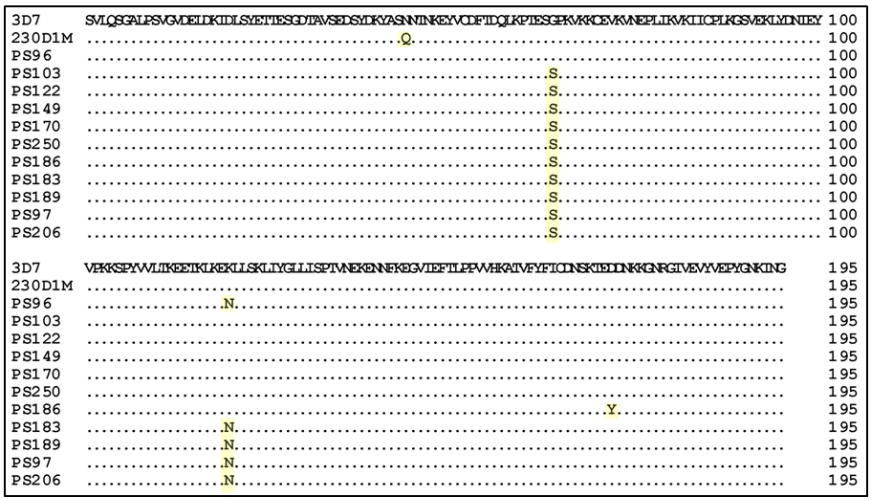


Figure 1A. Deduced amino acid sequence of full-length Pfs25 3D7 allele and the boundaries for recombinant Pfs25M. Eleven additional Malian isolates, identified below, were identical to the 3D7 allele (not shown). **Figure 1B**. Deduced amino acid sequence of Pfs230D1 3D7 and eleven other Malian isolates in addition to the amino acid sequence of recombinant Pfs230D1M (abbreviated as 230D1M). The amino acids highlighted in yellow denote point mutations relative to the 3D7 allele including those mutated N:Q to remove the putative N-linked glycosylation sites i.e., NXS/T.

1.2 Rationale for Study

Our initial rationale for testing the combination of Pfs25- and Pfs230-based vaccines is that we believe this multi-component strategy may improve overall transmission-blocking efficacy. Firstly, Pfs230 is expressed early and Pfs25 is expressed late in sexual development of the parasite. The differential temporal expression may allow dual actions. Secondly, the combination may decrease the proportion of poor responders compared to vaccination with a single agent. Increased transmission-blocking activity was observed when antibodies raised separately against

recombinant Pfs25M and Pfs230D1M were mixed and fed together with a parasite culture to mosquitoes in SMFA. In non human primates, antibodies induced by co-administration of Pfs25M-EPA and Pfs230D1M-EPA also displayed stronger transmission-blocking activities in SMFA than those induced by the individual vaccine candidates.

Given this information, our initial plans for this study were to proceed with a combination vaccine for the double-blind, comparator controlled portion of the study if the Pilot Safety Phase results were reassuring the vaccine at the targeted doses were safe. Following a planned interim group analysis post Vaccination #3 and #4 for protocol #15-I-0044, which looked at antibody responses and functional activity (via SMFA and DSF) of Pfs25M-EPA/Alhydrogel® alone, Pfs230D1M-EPA/ Alhydrogel® alone, Pfs25M-EPA/ Alhydrogel® and Pfs230D1M-EPA/ Alhydrogel® versus a comparator vaccine, the plan for this study has changed given these results. In summary, Pfs25M alone was seen to be inferior to Pfs230 alone or Pfs230 in combination with Pfs25 in regards to functional activity (SMFA and DSF). Thus the conclusion that Pfs25M alone was not recommended for the Main Phase of this study. Pfs230 alone compared to Pfs25-230 in combination, produced similar results in regards to immunogenicity (peak ELISA responses), functional activity (DSF, SMFA), and percentage of responders (detectable antibody responses). Overall there was no statistically significant difference between Pfs230 alone versus Pfs25 and Pfs230 given in combination, though Pfs230 did have a trend to higher overall peak ELISA responses and consistently higher SMFA responses. Given that similar results were obtained in subjects who only received Pfs230 versus those who received Pfs25 and Pfs230 in all identified endpoint assays, the plan is to move forward with a single antigen, Pfs230D1M-EPA/Alhdyrogel®.

In addition to assessing various antigens and combination of antigens, LMIV has been interested in exploring additional vaccine strategies, including alterations in conjugation and change in adjuvant selection in attempt to achieve a higher, prolonged, quality antibody response to provide a long-lived, functional response that can last an entire malaria transmission season. As noted above, the first step in this process was combining LMIV's two leading TBV candidates, Pfs25M and Pfs230D1M, to assess that the combination was safe and did not interfere with each other when administered simultaneously. Having achieved this objective with our current combination study (#15-I-0044), as determined by preclinical studies and an interim analysis completed in the still blinded #15-I-0044 study, the next step was to target improving our antibody responses, and associated functional activity, and durability of responses by changing the adjuvant fromAlhydrogel® to a safe, but more potent adjuvant AS01.

Though AS01 adjuvants are not yet in licensed vaccines, one of the immunostimulant components, monophosphoryl lipid (MPL), is a component of a licensed GSK human papillomavirus vaccine, and the AS01 adjuvant system has been a key component of the Mosquirix™ (RTS,S 25µg/$AS01_E$) and RTS,S/$AS01_B$ (RTS,S 50µg/$AS01_B$) that has been widely tested in children and adults across Africa. Thus, the most logical and safe path forward in our

TBV clinical development plan was to evaluate Pfs25M-EPA and Pfs230D1M-EPA adjuvanted with AS01. Given availability of the adjuvant from our partners GSK and a strong safety profile, $AS01_E$ (25µg MPL+ 25µg QS21 liposomal formulation) rather than $AS01_B$ will be used for the final formulation.

Utilizing GSK's adjuvant AS01 (for this study, AS01 is $AS01_B$ diluted with conjugate/antigen in the formulation to have a *dose equivalent* to to 25µg of MPL and 2 µg of QS21 in a 0.5mL dose), which has shown to induce improved immune responses with $AS01_B$ and $AS01_E$ when compared to Alhydrogel®, while maintaining a strong safety profile, in conjunction with LMIV's leading transmission blocking vaccine candidates, Pfs25M-EPA and Pfs230D1M-EPA, this proposed study attempts to explore the use of Pfs230D1M-EPA/AS01 at full and fractional dosing to determine the safety, immunogenicity, functional activity, and transmission dynamics.

2 Previous Preclinical Experience with Study Agents

In preclinical studies evaluating Pfs25M-EPA and Pfs230D1M-EPA formulated with AS01 adjuvant in rabbits at two-week intervals over a 43-day study interval, both vaccines when administered either alone or in combination were safe, with expected mild, transient injection site reactogenicity and mild, transient alterations in clinical laboratory parameters consistent with immune stimulation following vaccination.

In animal studies conducted at LMIV, AS01 and $AS01_B$ adjuvanted products when compared to Alhydrogel® consistently yielded the highest and longest sustained antibody responses with associated significant functional activity by SMFA (**Figure 2** and **Figure 3**).

Figure 2: Comparison of Transmission Blocking Activity of Pfs25M-EPA/AS01 and Pfs230D1M-EPA/AS01 to Pfs25M-EPA/Alhydrogel® and Pfs230D1M-EPA/Alhydrogel® in CD1 Mice.

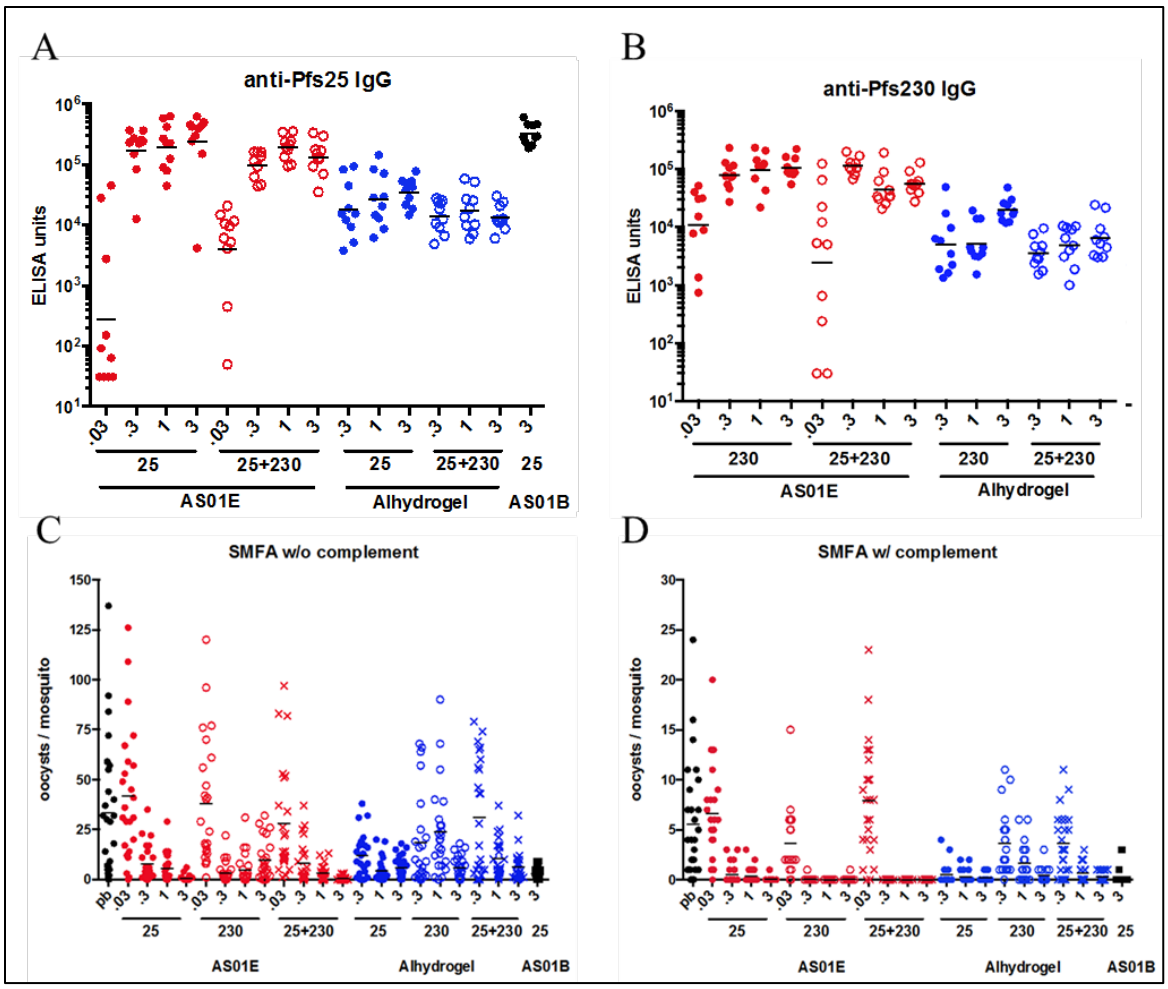
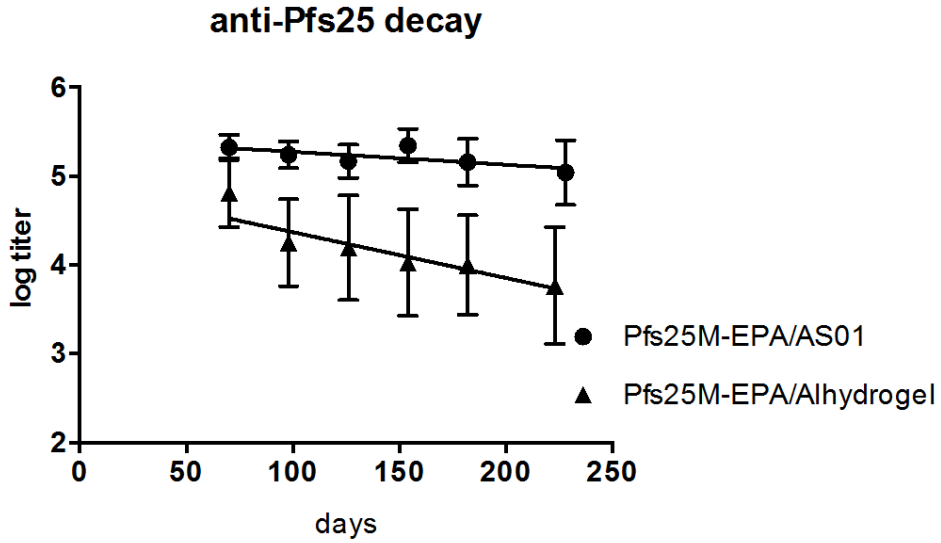


Figure 2A shows antibody responses 14 days following Vaccination #2 to anti-Pfs25 at increasing antigen doses adjuvanted with $AS01_E$, Alhydrogel®, and $AS01_B$ in CD1 mice that either received Pfs25 alone or Pfs25 and Pfs230 vaccinations. **Figure 2B** shows antibody responses 14 days following Vaccination #2 to anti-Pfs230 at increasing antigen doses adjuvanted with $AS01_E$, Alhydrogel®, and $AS01_B$ in CD1 mice that either received Pfs25 alone or Pfs25 and Pfs230 vaccinations. **Figure 2C** and **2D** reports SMFA results without and with complement from sera samples 14 days following Vaccination #2 at increasing antigen doses adjuvanted with $AS01_E$, Alhydrogel®, and $AS01_B$ in CD1 mice that either received Pfs25 alone, Pfs230 alone, or Pfs25 and Pfs230 vaccinations. In **Figure 2A** and **2B**, circles represent antibody level per mouse and black bars indicate geometric mean of antibody levels. In **Figure 2C** and **2D** circles represent individual mosquitoes and black bars indicate geometric mean of oocyst/mosquito. $AS01_B$ and $AS01_E$ in this figure are designations that mean $AS01_B$ was diluted with conjugate/antigen in the formulation to have a *dose equivalent* to 50µg MPL and 50µg QS21 in a 0.5 mL dose ($AS01_B$); and $AS01_E$ is the adjuvant $AS01_B$ diluted to a *dose equivalent* to 25µg of MPL and 25µg of QS21 in a 0.5 mL dose.

Figure 3: Measurement of Pfs25-specific Antibody Decay from Mouse AS01 study Comparing Alhydrogel® versus AS01.



Mean half life for Pfs25M-EPA/Alhydrogel® is 59 days compared to mean half life for Pfs25M-EPA/AS01 is 178 days.

3 Previous Human Experience With Study Agents

3.1 Pfs25-EPA

3.1.1 Pfs25H-EPA/Alhydrogel® in Healthy US Adults

A phase 1 dose escalation study of Pfs25H-EPA/Alhydrogel® was conducted in malaria-naïve adults at the Center for Immunization Research at the Johns Hopkins School of Public Health in Baltimore, Maryland, USA (NIAID Protocol #11-I-N237).[23] The study enrolled 30 subjects as shown in **Table 1**, with Groups 1a and 1b sized for safety and Group 2 sized for safety and immunogenicity.

Table 1: Phase 1 Study of Pfs25H-EPA Conjugates in US Healthy Adults

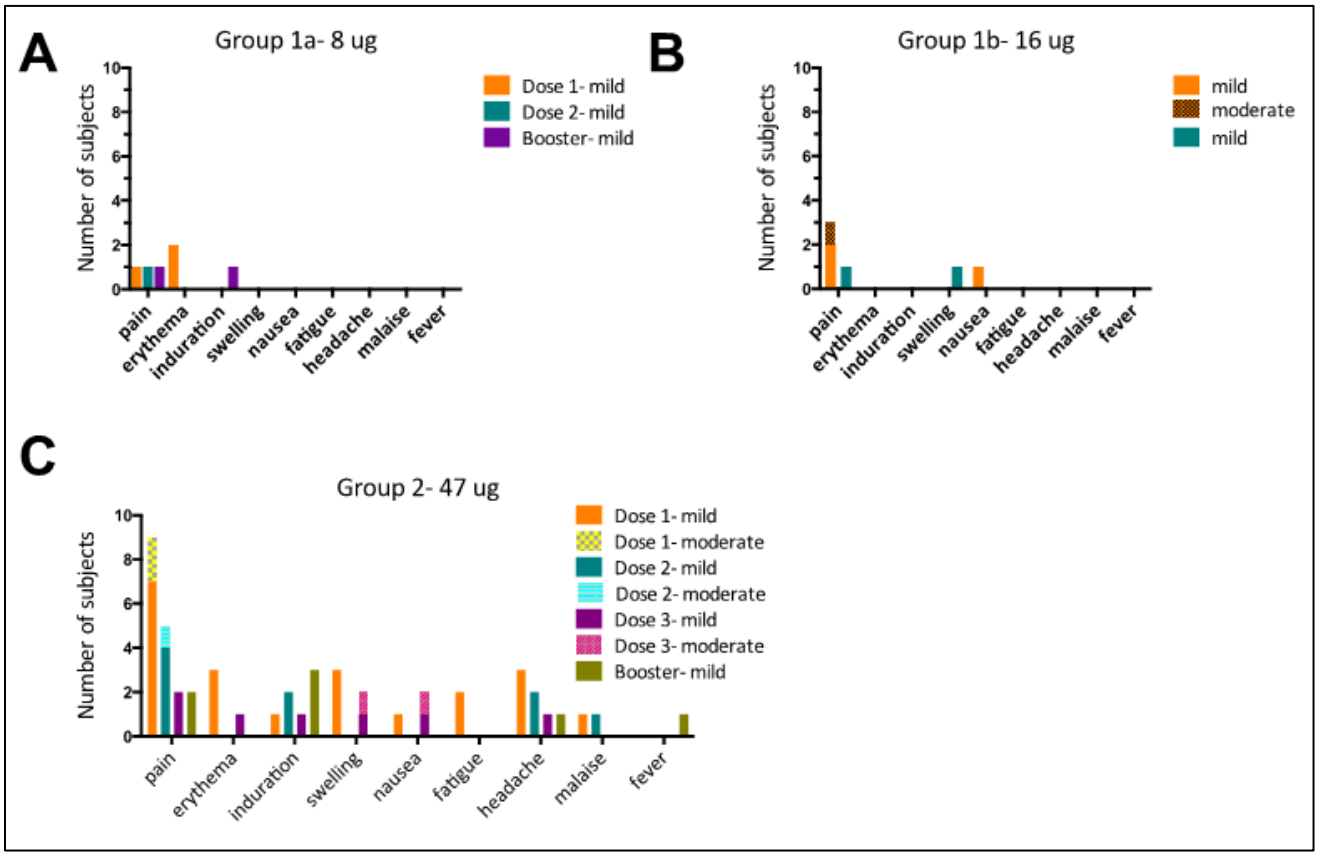
Group	n	Vaccine	Schedule (month)
1a	5	8 µg Pfs25H-EPA/Alhydrogel®	0, 2, optional 10
1b	5	16 µg Pfs25H-EPA/Alhydrogel®	0, 2, optional 10
2	20	47 µg Pfs25H-EPA/Alhydrogel®	0, 2, 4, 10

Eligible subjects (n=15) in the high dose group initially received 3 vaccinations to explore the effect of the third dose on boosting and longevity of antibody responses; however, given the increasing antibody response to each subsequent dose of vaccine, an additional fourth boost was administered to all eligible subjects (n=11) in the high dose group and for 1 high responder in Group 1a.

3.1.1.1 Safety of Pfs25H-EPA/Alhydrogel® in Healthy US Adults

Vaccinations were well-tolerated, with minimal local and systemic reactogenicity.[15,23] There was an increase in the percentage of subjects reporting solicited adverse events (AEs) with each escalating dose, with AEs diminishing with each subsequent dose of vaccine given. No subjects were withdrawn due to related adverse events. A single serious adverse event (SAE) occurred during the course of the study that was unrelated to the vaccine. These findings are summarized in **Figure 4**.

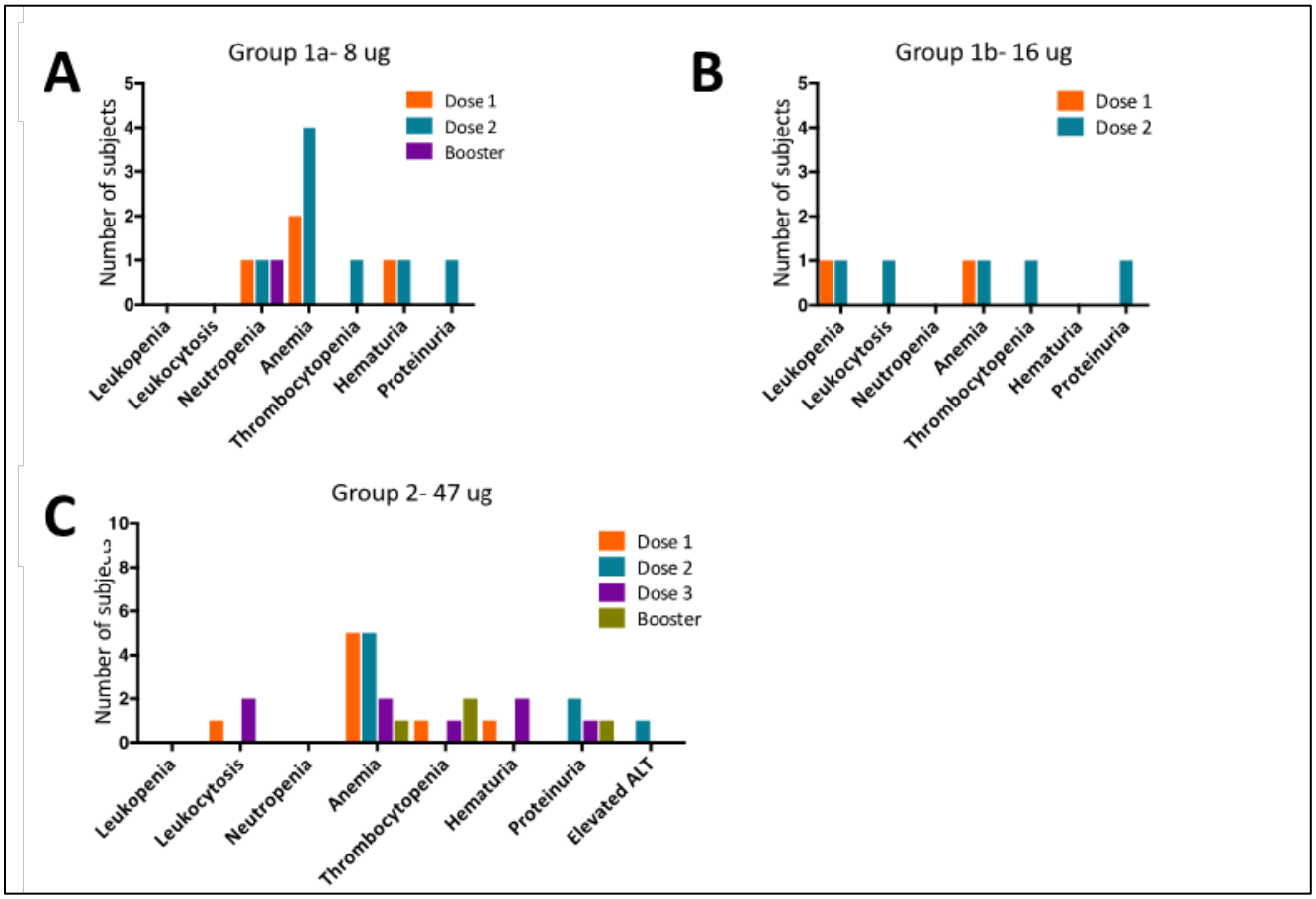
Figure 4: Related Local Injection Site Reactogenicity and Systemic Adverse Events.



All injection site reactogenicity was deemed related to Pfs25H-EPA/Alhydrogel®. Clinical solicited (A-C) adverse events after each vaccination with each dose group are reported in individual graphs: Group 1a (A), 1b (B) and Group 2 (C). All adverse events were mild except when indicated as moderate.

All laboratory adverse events (related and unrelated) are shown in **Figure 5**. While several subjects experienced anemia after vaccination, the majority of these events were judged to be unrelated to vaccination, either because these subjects had low, but normal, hemoglobin levels at baseline (day of first vaccination) or because of an alternate explanation (e.g., blood donation or previously undisclosed history of anemia). An examination of trends in hematologic parameters does not show any pattern related to timing of vaccinations.[15]

Figure 5: Laboratory Adverse Events

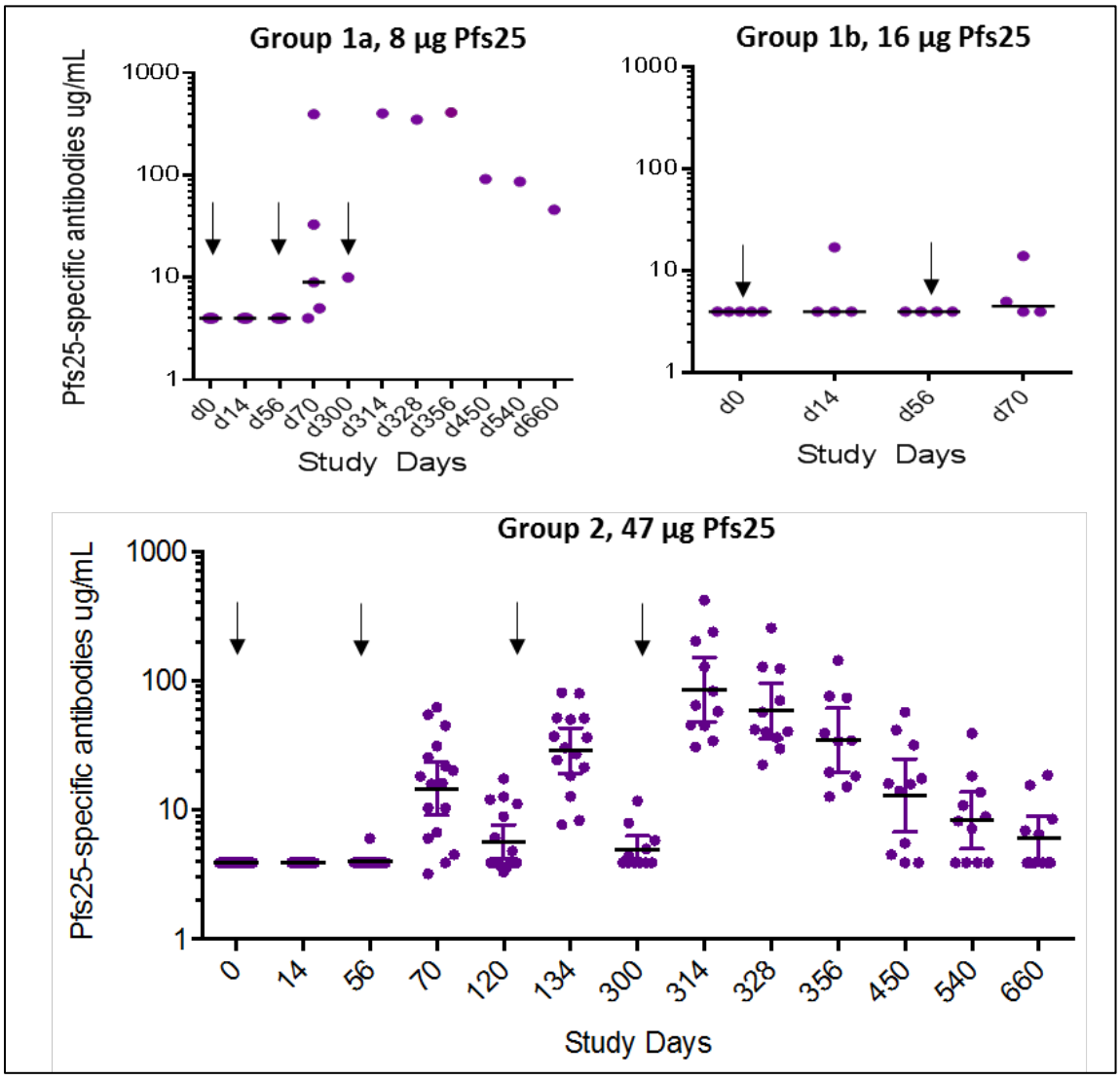


Laboratory (A-C) adverse events after each vaccination with each dose group are reported in individual graphs: Group 1a (A), 1b (B) and Group 2 (C). All adverse events were mild except when indicated as moderate. All neutropenia events occurred in the same subject. All hematuria occurred during menses. All leukopenia events occurred in the same subject. All laboratory adverse events except for a portion of the decreased hemoglobin (n=10) were found by the PI to be unrelated to Pfs25H-EPA/Alhydrogel®

3.1.1.2 Immunogenicity of Pfs25H-EPA/Alhydrogel® in Healthy US Adults

Specific anti-Pfs25 antibodies were detected by ELISA in sera from subjects receiving 2 or 3 doses, and the titers increased further after the booster dose (**Figure 6**).

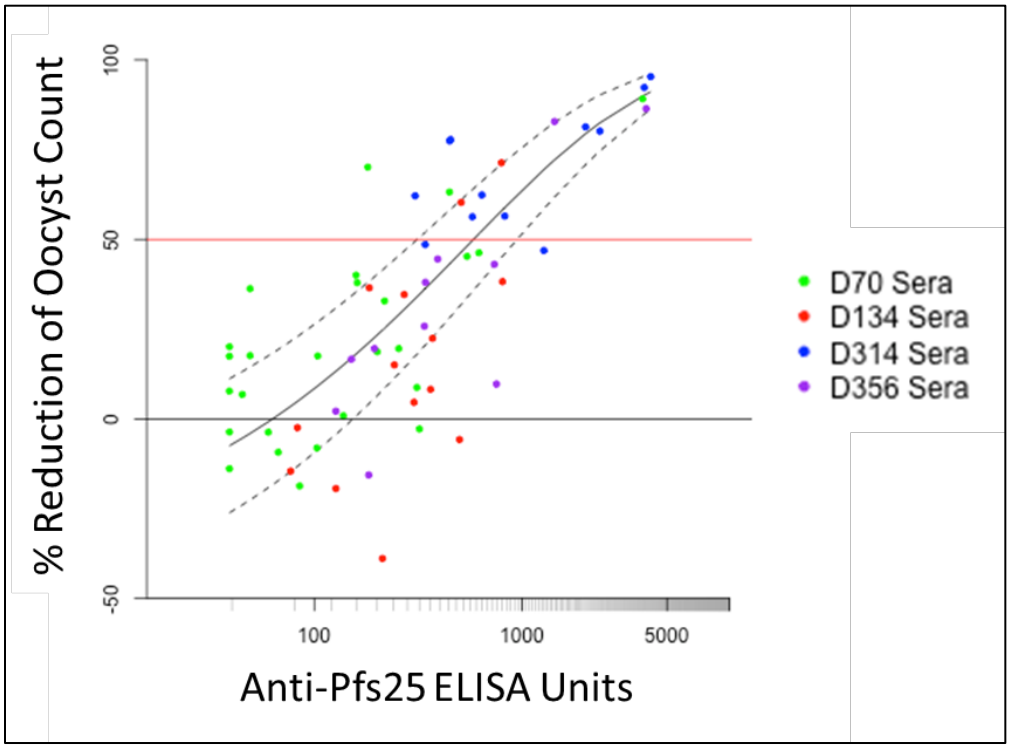
Figure 6: Anti-Pfs25 Antibodies in Volunteers Immunized with Pfs25-EPA/Alhydrogel®



Groups 1a and 1b participants, receiving conjugated proteins comprising of 8µg and 16µg Pfs25H, respectively. Group 2 participants, receiving 47µg Pfs25H. Arrows indicate the day of vaccination. Closed circles represent antibody level and individual participants, and black bars indicate geometric mean of antibody levels. Anti-Pfs25 ELISA units were determined by using a reference standard generated from a previous study in which volunteers were immunized with recombinant Pfs25 formulated Montanide® ISA51. Anti-Pfs25 ELISA units were converted to Pfs25-specific antibodies µg/mL by multiplying a conversion factor (1 EU = 0.104 µg anti-Pfs25-specific IgG) obtained using the same methods described in Cheru et al.[24]

The immunized antisera displayed various levels of transmission reducing activities, measured as reduction of oocyst development in mosquitoes in a standard membrane feeding assay (SMFA). The activity correlated with the antibody titers (**Figure 7**). Following the fourth dose, a large proportion of subjects (9 of 11 vaccinated from the 47µg Group) developed functional antibodies with significant transmission reduction activity above 50% and up to 95%.

Figure 7: Transmission Reducing Activities Induced by Pfs25-EPA/Alhydrogel®

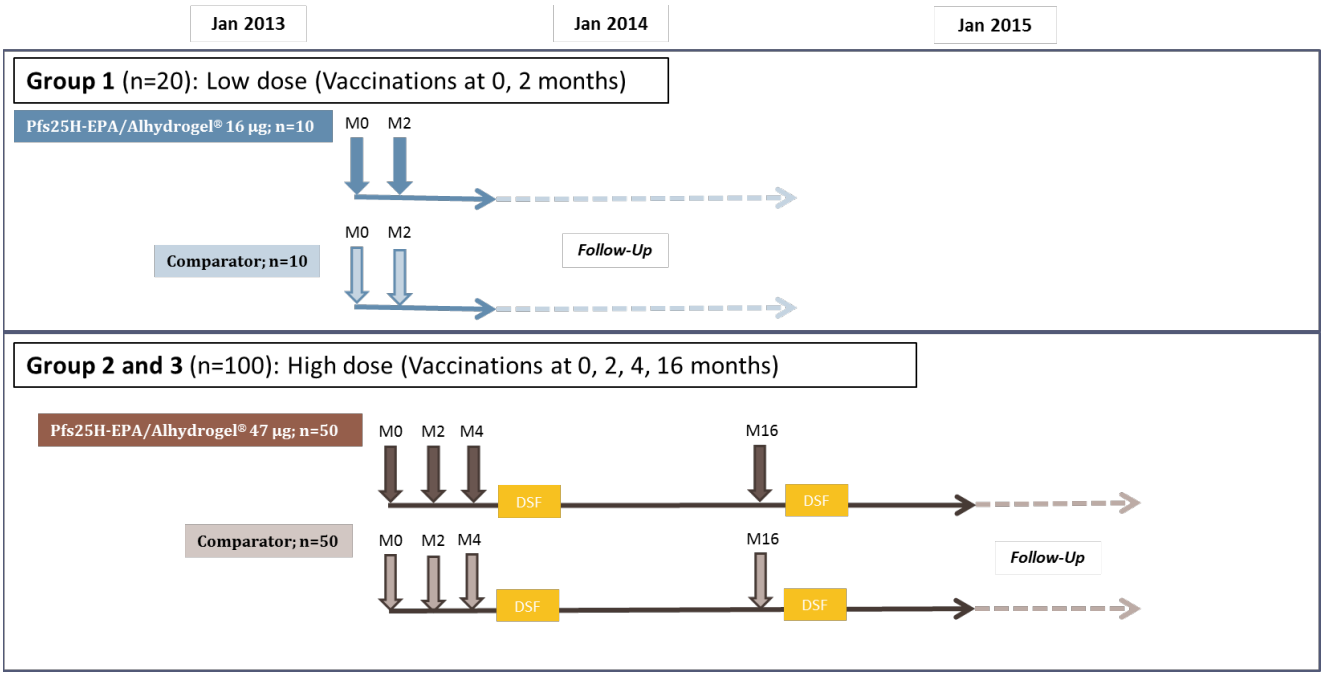


Transmission reducing activity of sera collected 2 weeks after each initial vaccination and 8 weeks after the booster vaccination. Each data point represents the average of two results in the SMFA, and lines indicate estimate EC_{50} and point-wise confidence interval by GEE model. Thick red line indicates the threshold representing significant biological activity (50% TRA). The green, red, blue, and black dots correspond to the data points on D70: 14 days post Vaccination #2; D134: 14 days post Vaccination #3; D314: 14 days post Vaccination #4; D356: 2 months post Vaccination #4.

3.1.2 Pfs25H-EPA/Alhydrogel® in Healthy Malian Adults

A double-blind dose-escalating randomized controlled phase 1 study in malaria exposed adults of the safety and immunogenicity of Pfs25H-EPA/Alhydrogel® was conducted in Bancoumana, Mali. One hundred twenty subjects were enrolled and randomized to receive the low dose (16µg of conjugated Pfs25, n=10), the high dose (47µg of conjugated Pfs25, n=50), or the comparator vaccine (Euvax B for first 3 vaccinations, then Menactra® for the fourth vaccination, n=10 for 2 vaccinations, n=50 for 4 vaccinations) (**Figure 8**). Enrollment within the high dose group was staggered for additional safety. The low dose and the matching comparator cohort (Group 1) received 2 vaccine doses given at 0 and 2 months. For the rest of the subjects, 3 doses were given at a 0, 2, 4 month dosing intervals in 2013, and a fourth (booster) dose at 12 months after the third dose at the start of the subsequent transmission season in 2014. Subjects were followed for at least 6 months following the last vaccination. In total, 82 subjects (40 comparator cohort and 42 Pfs25 high dose cohort) received their fourth vaccination.

Figure 8: Phase 1 Study of Pfs25H-EPA Conjugates in Malian Healthy Adults



Arrows indicate timing of vaccinations. Direct skin feeds (DSF) in 2013 completed only on gametocyte or parasite carriers. DSF in 2014, following receipt of vaccination #4, completed weekly on all eligible subjects starting 2 weeks post dose #4 and continuing for 6 consecutive weeks.

3.1.2.1 Safety of Pfs25H-EPA/Alhydrogel® in Healthy Malian Adults

Overall, vaccinations were well tolerated in both the Pfs25H-EPA/Alhydrogel® and comparator group, with the majority of AEs being mild or moderate, and the most commonly reported adverse events were local site injection pain, clinical malaria, cold, headache, rhinitis, and neutropenia (**Table 2**). Twenty-six (26) Grade 3 AEs were reported during the study, of which all were deemed by the investigator to be not or unlikely related to the vaccine except for a single episode of injection site pain, which was deemed definitely related and occurred in a comparator subject. In total three SAEs (spontaneous abortion, snake bite, post traumatic coma) were reported and all had been determined to be not related to vaccination. No subject was withdrawn or deferred from vaccination secondary to an AE related to the vaccine.

In comparison to the comparator group, those subjects who receive Pfs25H-EPA/Alhydrogel® reported no difference in the number of AEs, systemic or local AEs, laboratory abnormalities, clinical malaria episodes, or unsolicited AEs. Though, overall Pfs25H-EPA/Alhydrogel® vaccinees reported statistically significant more solicited AEs (p=0.01, Fishers Exact test) and related AEs (p=0.019; Fishers Exact test) which increased number of solicited AEs held true when looking at Pfs25H-EPA/Alhydrogel® at the targeted antigen dose of 47µg versus comparator.

Table 2: Summary of Reported Adverse Events in Pfs25H Vaccine Arms, #13-I-N109, Mali Low Dose Cohort (2A) and Mali High Dose Cohort (2B)

2A	16 µg Pfs25-EPA/Alhydrogel			Comparator/ Euvax B		
	Vaccine 1	Vaccine 2	Total	Vaccine 1	Vaccine 2	Total
	N=10	N=9	N=10	N=10	N=10	N=10
Total # AEs	23 (9) 90%	49 (9) 100%	72 (10) 100%	16 (8) 80%	73 (10) 100%	89(10) 100%
Classification						
Local Reactogenicity	3 (2) 20%	2 (2) 22%	5 (3) 30%	3 (2) 20%	4 (3) 30%	7 (4) 40%
Systemic Reactogenicity	1(1)10%	0 (0) 0%	1(1)10%	0 (0) 0%	1 (1) 10%	1 (1) 10%
Laboratory Abnormalities	7 (4) 40%	7 (5) 56%	14 (7) 70%	0 (0) 0%	3 (2) 20%	3 (2) 20%
Unsolicited AEs	12 (7)70%	40(9) 100%	52 (10) 100%	13 (8) 80%	65 (10) 100%	78 (10) 100%
Severity and Relationship						
Grade 1	13 (7) 70%	14 (6) 67%	27 (9) 90%	9 (6) 60%	13 (3) 30%	22 (7) 70%
Related to Pfs25	10 (5) 50%	6 (4) 44%	16 (8) 80%	3 (2) 20%	5 (4) 40%	8 (4) 40%
Grade 2	9 (5) 50%	32 (9) 100%	41 (9) 90%	7 (6) 60%	59 (9) 90%	66 (10) 100%
Related to Pfs25	1 (1)10%	2 (2) 22%	3 (2) 20%	0 (0) 0%	2 (2)20%	2 (2) 20%
Grade 3	1 (1) 10%	3 (2) 22%	4 (3) 30%	0 (0) 0%	1 (1) 10%	1 (1) 10%
Related to Pfs25	0 (0) 0%	0 (0) 0%	0 (0) 0%	0 (0) 0%	0 (0) 0%	0 (0) 0%
Grade 4	0 (0) 0%	0 (0) 0%	0 (0) 0%	0 (0) 0%	0 (0) 0%	0 (0) 0%
SAE	0 (0) 0%	0 (0) 0%	0 (0) 0%	0 (0) 0%	0 (0) 0%	0 (0) 0%

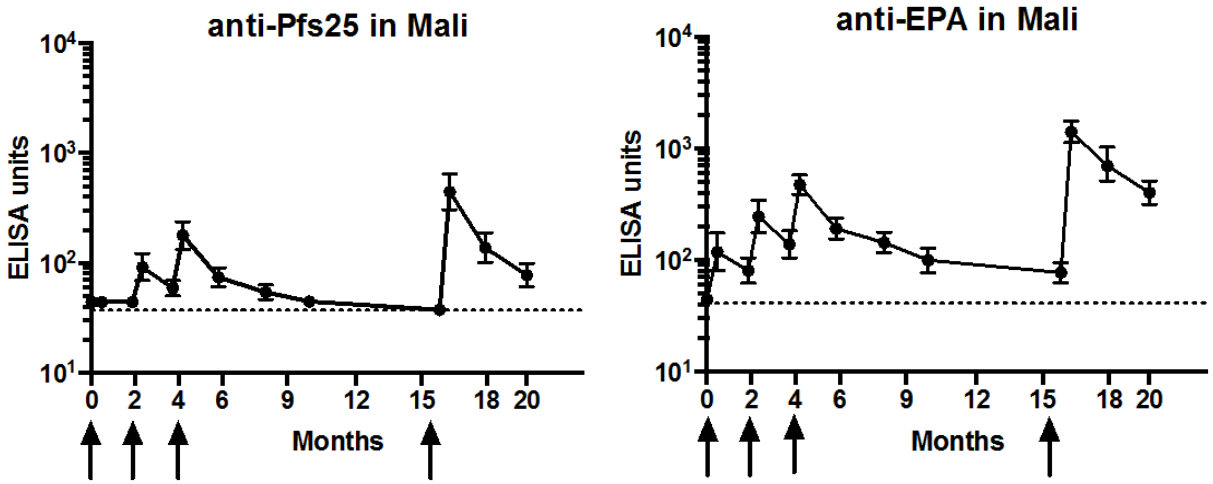
2B	47 µg Pfs25-EPA/Alhydrogel16 ug Pfs25-EPA/Alhydrogel					Comparator/ Euvax B + Menactra				
	Vaccine 1	Vaccine 2	Vaccine 3	Vaccine 4	Total	Vaccine 1	Vaccine 2	Vaccine 3	Vaccine 4	Total
	N=50	N=50	N=45	N=42	N=50	N=50	N=46	N=42	N=40	N=50
Total # AEs	135 (45) 90%	152(36) 72%	368(45)100%	202(42)100%	857 (50) 100%	110 (45) 90%	121 (39) 85%	288(42)100%	161(40)100%	680 (49)98%
Classification										
Local Reactogenicity	22 (21) 42%	28 (26) 52%	17(17)38%	18(18)43%	82 (39) 78%	15 (15) 30%	14(13) 28%	20(20)48%	6(6)15%	55 (31) 62%
Systemic Reactogenicity	11(9)18%	9 (8) 16%	6(5)11%	4(4)10%	29(20)40%	9 (8) 16%	5 (4) 9%	1(1)2%	3(3)8%	18 (12) 24%
Laboratory Abnormalities	22 (18) 36%	30 (19) 38%	31(20)44%	31(18)43%	114 (33) 66%	19(13) 26%	23 (17) 37%	17(13)31%	23(16)40%	82 (31) 62%
Unsolicited AEs	80 (41) 82%	85(40) 80%	314(45)100%	149(40)95%	628 (50) 100%	67 (38) 76%	79 (35) 76%	250(42)100%	129(39)98%	525 (49) 98%
Severity and Relationship										
Grade 1	84 (43) 86%	82 (37) 74%	74(34)76%	43(26)62%	283 (50) 100%	75 (39) 78%	65 (34) 74%	61(30)71%	34(22)55%	235(49) 98%
Related to Pfs25	42 (31) 62%	45(29) 58%	35(25)56%	20(18)43%	142 (47) 94%	39 (27) 54%	25 (20) 43%	31(22)52%	11(9)23%	106 (42) 84%
Grade 2	51 (32) 64%	70(34) 68%	285(45)100%	159(42)100%	565 (49) 98%	34(23) 46%	55 (31)67%	219(42)100%	123(38)95%	431 (45) 90%
Related to Pfs25	7 (6)12%	7 (7) 14%	5(5)11%	11(10)24%	30 (20) 40%	2 (2) 4%	2 (2)4%	2(2)5%	2(2)5%	8 (5) 10%
Grade 3	0 (0) 0%	0 (0) 0%	8 (7) 16%	0 (0) 0%	8 (7) 14%	1 (1) 2%	1(1)2%	8 (6) 14%	3(3)8%	13(10)20%
Related to Pfs25	0 (0) 0%	0 (0) 0%	0 (0) 0%	0 (0) 0%	0 (0) 0%	0 (0) 0%	0 (0) 0%	0 (0) 0%	1(1)3%	1(1)2%
Grade 4	0 (0) 0%	0 (0) 0%	1 (1) 2%	0 (0) 0%	1 (1) 2%	0 (0) 0%	0 (0) 0%	0 (0) 0%	1(1)3%	1(1)2%
SAE	0 (0) 0%	0 (0) 0%	0 (0) 0%	0 (0) 0%	0 (0) 0%	0 (0) 0%	0 (0) 0%	0 (0) 0%	0 (0) 0%	0 (0) 0%

Each field has the format # (X) %, where # is the number of adverse events, X is the number of subjects with one or more episodes of the given event, and % is the number of participants with one or more episodes of the given event divided by the total number of participants who received the study agent (N) multiplied by 100.

3.1.2.2 Immunogenicity of Pfs25H-EPA/Alhydrogel® in Healthy Malian Adults

Immunogenicity outcomes were measured by IgG ELISA against recombinant Pfs25 and EPA (**Figure 9**) and functional activity by SMFA (**Figure 10**) conducted at the NIAID in the U.S. Following each vaccination (0, 2, 4, 16 months), anti-Pfs25 IgG increased with each dose and all but 1 subject responded after receipt of the fourth and final dose (**Figure 9**). Anti-Pfs25 titers correlated with anti-EPA but decayed more rapidly than did anti-EPA after 4th dose (**Figure 9**).

Figure 9: Anti-Pfs25 and Anti-EPA IgG ELISA Titers in Phase 1 Trial of Pfs25H-EPA/Alhydrogel® in Mali



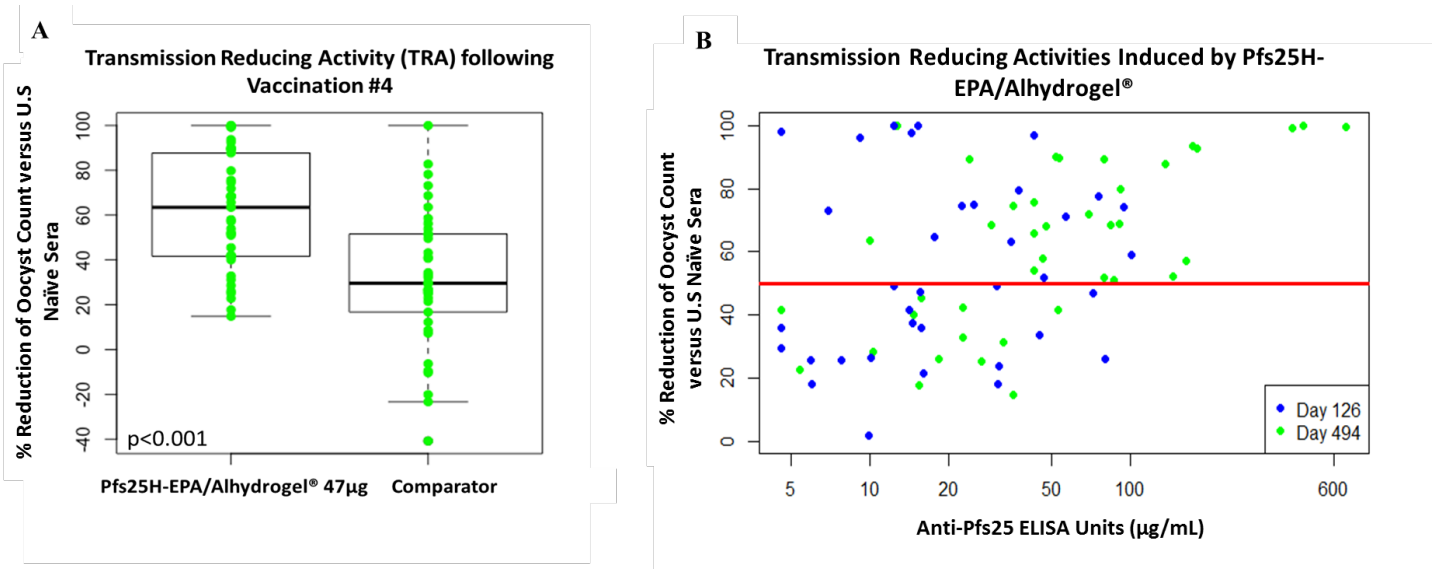
	Anti-Pfs25			Anti-EPA		
After dose:	**2**	**3**	**4**	**2**	**3**	**4**
Geo mean titer	**93.05**	**169.6**	**422.3**	**237.4**	**468.1**	**1406.0**
95% CI	**70-122**	**127-225**	**290-615**	**171-329**	**383-572**	**1124-1759**
Non-responders	**18/45 (40%)**	**5/44 (11.3%)**	**1/41 (1.4%)**	**3/45 (6.7%)**	**1/44 (2.3%)**	**0/41 (0%)**
Geo mean titer of responders	**153.3**	**201.6**	**476.8**	**267.8**	**494.6**	**1406.0**

Arrows indicate the day of vaccination. Only data from Groups 2/3 subjects who received 47µg Pfs25H-EPA/Alhydrogel® vaccination presented. Note all Group 2/3 comparator subjects were below the level of detection for anti-Pfs25 responses at all timepoints. Closed circles represent geometric (geo) mean antibody level and black bars 95% confidence intervals (CI).

Statistically significant functional activity, as measure by SMFA, was not seen after the third dose but was observed after the fourth dose (**Figure 10A**). In general, anti-Pfs25 titers correlated

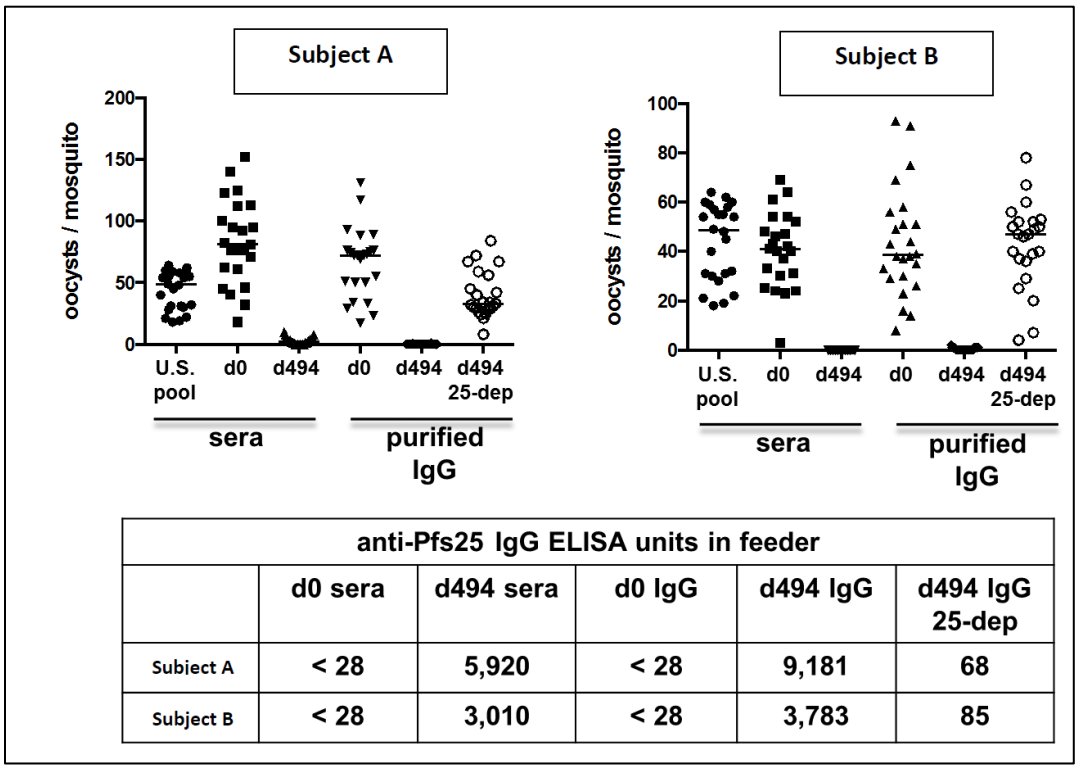
with functional activity (**Figure 10B**), which was assessed to be contained in the IgG fraction directed against Pfs25 (**Figure 11**).

Figure 10: Serum Transmission Blocking Activity after Pfs25H-EPA/Alhydrogel® Dose 4 in Mali.



SMFA of post-dose 4 sera (Day 494) from Pfs25-EPAH/ Alhydrogel® trial in Mali (Group 2/3) shows (**Figure 10A**) increase in serum functional activity in vaccinees over controls and (**Figure 10B**) correlation of antibody titers with serum activity. For **Figure 10A**, each data point represents the average of two results in the SMFA in an individual subject with the thick black line indicating the median. Thick red line indicates the threshold representing significant biological activity (50% TRA). The blue and green dots correspond to the data points on Day 126: 14 days post Vaccination #3; Day 494: 14 days post Vaccination #4. Comparator = Euvax B (for vaccinations #1, 2, 3) and Menactra (for vaccination #4).

Figure 11: Confirmation that Human Transmission Blocking Activity is Mediated by Anti-Pfs25 IgG



anti-Pfs25 IgG ELISA units in feeder					
	d0 sera	d494 sera	d0 IgG	d494 IgG	d494 IgG 25-dep
Subject A	< 28	5,920	< 28	9,181	68
Subject B	< 28	3,010	< 28	3,783	85

SMFA of post-dose 4 sera and IgG from two Pfs25-EPA/Alhydrogel® vaccinees in Mali with relatively high antibody titer (table above) shows high functional activity for serum as well as purified IgG fraction which can be ablated by depletion of Pfs25-specific IgG on an antigen-affinity column.

3.1.3 Pfs25M-EPA/Alhydrogel® in Healthy US Adults

The phase 1 study of the safety and immunogenicity of Pfs230D1M-EPA/Alhydrogel® and Pfs25M-EPA/Alhydrogel® in adults in the US (NIH Clinical Center) and Mali (Bancoumana, Mali) started in December 2014 in the US (NIAID Protocol #15-I-0044; clinicaltrials.gov: NCT02334462). Study enrollment, as seen in **Table 3**, for the US portion of the study (N=35) was to evaluate safety and tolerability of increasing doses and Pfs25M and Pfs230D1M given alone or in combination prior to moving into Mali, West Africa for further safety, immunogenicity, and functional activity evaluation.

Table 3: Subject Enrollment and Vaccinations in US

	Arms	n	Vaccine	Schedule (month)
Pfs25	1a	5	16µg Pfs25M-EPA/Alhydrogel®	0, 1 month
	1b	5	47µg Pfs25M-EPA/Alhydrogel®	
Pfs230	2a	5	5µg Pfs230D1M-EPA/Alhydrogel®	
	2b	5	15µg Pfs230D1M-EPA/Alhydrogel®	
	2c	5	40µg Pfs230D1M-EPA/Alhydrogel®	
Pfs25 + Pfs230	3a	5	16µg Pfs25M-EPA/Alhydrogel® + 15µg Pfs230D1M-EPA/Alhydrogel®	
	3b	5	47µg Pfs25M-EPA/Alhydrogel® + 40µg Pfs230D1M-EPA/Alhydrogel®	

3.1.3.1 Safety of Pfs25M-EPA/Alhydrogel® in Healthy US Adults

Overall vaccinations with Pfs25M-EPA/Alhydrogel® in protocol #15-I-0044 in US adults were well-tolerated with minimal local and systemic reactogenicity (**Table 4**). The majority of the reported AEs were mild (Grade 1; 16µg: 23/27, 85%; 47µg: 21/24, 88%) and solicited. Local reactogenicity was by far the most frequently reported AEs in both the 16µg and 47µg Pfs25M-EPA/Alhydrogel® arms, occurring with an increased frequency of symptoms with increasing dose (16 µg: 60% and 47 µg: 100%). No significant differences were seen between doses with subsequent vaccinations nor with the duration of local symptoms. Solicited systemic symptoms were only reported in two subjects in the low dose arm, 16µg Pfs25M, and only after the first dose. As seen in previous Pfs25 studies, the most commonly noted laboratory abnormality was hemoglobin decreased, which the majority of these events being determined to be unlikely related to vaccination. There were no related Grade 3 AEs reported, and only two Grade 3 AEs (bronchitis, upper respiratory tract infection) were reported during the course of the study. There were no SAEs reported.

Table 4: Summary of Reported Adverse Events in Pfs25M Vaccine Arms, #15-I-0044, US Cohort

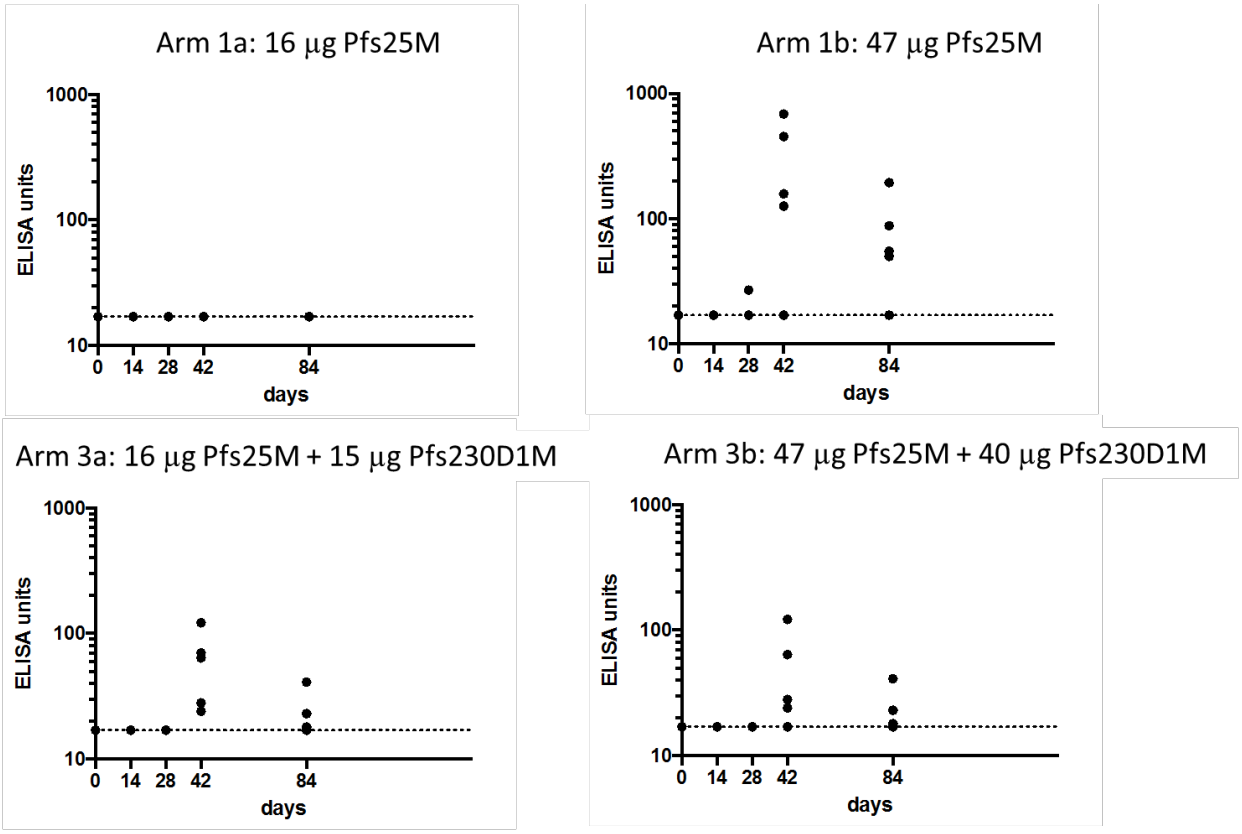
	Pfs25M, 16µg			Pfs25M, 47µg		
	Vaccine 1	Vaccine 2	Total	Vaccine 1	Vaccine 2	Total
	N=5	N=4	N=5	N=5	N=5	N=5
Total # AEs	17 (3) 60%	10 (2) 50%	27 (4) 80%	11 (5) 100%	13 (5) 100%	24 (5) 100%
Classification						
Local Reactogenicity	4 (2) 40%	2 (2) 50%	6 (3) 60%	6 (5) 100%	7 (4) 80%	13 (5) 100%
Systemic Reactogenicity	8 (2) 40%	0 (0) 0%	8 (2) 40%	1 (1) 20%	0 (0) 0%	1 (1) 20%
Laboratory Abnormalities	3 (2) 40%	1 (1) 25%	4 (3) 60%	2 (2) 40%	2 (2) 40%	4 (3) 60%
Unsolicited AEs	2 (2) 40%	7 (1) 25%	9 (3) 60%	2 (2) 40%	4(3)60%	6 (5) 100%
Severity and Relationship						
Grade 1	13 (2) 40%	10 (2) 50%	23 (4) 80%	11 (5) 100%	10 (4) 80%	21 (5) 100%
Pfs25 Related	9 (2) 40%	2 (2) 50%	11 (3) 60%	8 (5) 100%	6(4)80%	14 (5) 100%
Grade 2	4 (2) 40%	0 (0) 0%	4 (2) 40%	0 (0) 0%	1 (1) 20%	1 (1) 20%
Pfs25 Related	2 (2) 40%	0 (0) 0%	2 (2) 40%	0 (0) 0%	0 (0) 0%	0 (0) 0%
Grade 3	0 (0) 0%	0 (0) 0%	0 (0) 0%	0 (0) 0%	2 (2) 40%	2 (2) 40%
Grade 4	0 (0) 0%	0 (0) 0%	0 (0) 0%	0 (0) 0%	0 (0) 0%	0 (0) 0%
SAE	0 (0) 0%	0 (0) 0%	0 (0) 0%	0 (0) 0%	0 (0) 0%	0 (0) 0%

Each field has the format # (X) %, where # is the number of adverse events, X is the number of subjects with one or more episodes of the given event, and % is the number of participants with one or more episodes of the given event divided by the total number of participants who received the study agent (N) multiplied by 100.

3.1.3.2 Immunogenicity of Pfs25M-EPA/Alhydrogel® in Healthy US Adults

As expected, no pre-existing immunity was seen prior to vaccination in US adults for Pfs25 in NIAID protocol #15-I-0044. No subjects responded to the vaccine in Arm 1a (16µg of Pfs25M). The majority of subjects in Arms 1b (47µg of Pfs25M), 3a (16µg of Pfs25M + 15µg of Pfs230D1M), and 3b (47µg of Pfs25M + 40µg of Pfs230D1M) responded to the vaccine following Vaccination #2 (**Figure 12**).

Figure 12: Pfs25–specific antibody responses in US naïve subjects receiving Pfs25M vaccinations (#15-I-0044)

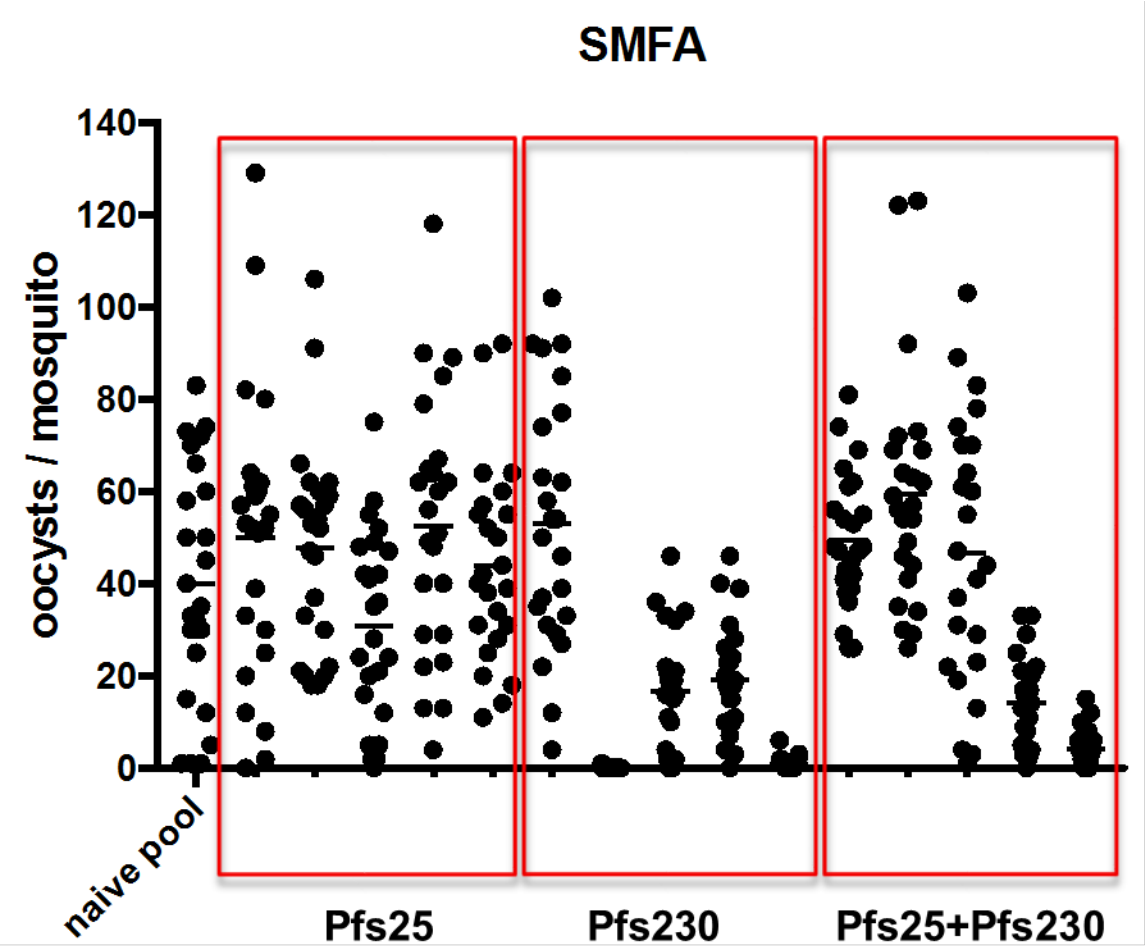


Results presented in ELISA units for each Arm. Vaccinations occurred on Day 0, 28. Day 0 was drawn pre-vaccination, D42 is 14 days post vaccination #2. Each individual data point represents an individual subject anti-Pfs25 ELISA response.

Anti-Pfs25 responses following two doses of Pfs25M-EPA/Alhydrogel® was similar to those seen with Pfs25H-EPA/Alhydrogel® in LMIV's previous study in naïve U.S. adults described in **Section 3.1.1.2** (p=0.43).

Functional activity (as measured by SMFA) related to antibody titers following Vaccination #2 have been assessed in only the highest antigen dose arms at this time. Anti-Pfs25 responses following receipt of Pfs25M, 47µg alone for two doses (0,1 month) were similar to those seen in previous studies with Pfs25H (**Figure 13**).

Figure 13: Pfs25 and Pfs230 Functional Activity (SMFA)



Samples obtained 14 days following receipt of vaccine #2 in the highest antigen dose arms (Pfs25M, 47µg alone, Pfs230D1M, 40µg alone, and Pfs25M, 47µg + Pfs230D1M, 40µg co-administered). Each individual column represents an individual subject. Each individual data point represents a single mosquito dissected.

3.1.4 Pfs25M-EPA/Alhydrogel® in Healthy Malian Adults

The phase 1 study of the safety and immunogenicity of Pfs230D1M-EPA/Alhydrogel® and Pfs25M-EPA/Alhydrogel® (NIAID protocol #15-I-0044) started in April 2015 in Mali and has completed enrollment (**Table 5**).

Cohort 1, which included the single antigen safety Arms A1 (Pfs25M, 16µg), B1 (Pfs230D1M, 15µg), and D1 (Twinrix®), and Cohort 2, which included the co-administration safety Arm C1 (Pfs25M, 16µg + Pfs230D1M, 15µg) and D2 (Twinrix® + normal saline) have completed enrollment, all vaccinations (2 of 2 scheduled at 0, 1 month intervals), and undergone scheduled unblinding per protocol in October 2015.

Cohort 3, which includes increased sample size for immunogenicity and functional activity assessment, included Arms A2 (Pfs25M, 47µg + normal saline), B2 (Pfs230D1M, 40µg + normal saline), C2 (Pfs25M, 47µg + Pfs230D1M, 40µg) and D3 (Twinrix® + normal saline) have completed enrollment, 3 of the 4 scheduled vaccinations (0, 1, 6 months), and undergone 6 weeks of intensive twice a week DSF assessment. Subjects in cohort 3 who are currently active on protocol (171 of 200) are in long term safety follow-up and will receive Vaccination #4 in September 2016, undergo evaluation via DSF following receipt of Vaccination #4, and be unblinded in March/April 2017.

Table 5: Subject Enrollment and Vaccination in Mali

Arm	Dose	Enrolled	Received Vaccination #1 (D0)	Received Vaccination #2 (D28)	Received Vaccination #3 (D168)	Completed Final Study Visit/ On Protocol
Cohort 1 - unblinded						
A1	Pfs25M, 16µg	5	5	5	N/A	5
B1	Pfs230D1M, 15µg	5	5	4	N/A	4
D1	Twinrix®	5	5	5	N/A	4
Cohort 2 - unblinded						
C1	Pfs25M, 16µg + Pfs230D1M, 15µg	5	5	5	N/A	5
D2	Twinrix® + NS	5	5	5	N/A	4
Cohort 3 - blinded						
A2	Pfs25M, 47µg + NS	200	200	190	177	171
B2	Pfs230D1M 40µg + NS					
C2	Pfs25M, 47µg + Pfs230D1M, 40µg					
D3	Twinrix® + NS, then Menactra + NS					

NS = normal saline. N/A = not applicable.

3.1.4.1 Safety of Pfs25M-EPA/Alhydrogel® in Healthy Malian Adults

From the unblinded safety data provided by Arm A1 (low dose of Pfs25M, 16µg), overall vaccinations have been well tolerated with all of the reported AEs in the Pfs25M arm being either mild or moderate (Grade 1 and Grade 2; 19/19, 100%) and with the majority of AEs (14/19; 74%) reported being unsolicited AEs (**Table 6**). The only reported related AEs were injection site pain, which were all Grade 1 and did not increase in frequency of reporting with subsequent vaccination. In comparison to the comparator group, who received Twinrix®, the Pfs25M vaccinees reported overall more AEs (19 versus 11), including increased frequency of

reporting of injection site pain (4 versus 1). However, neither group reported any systemic reactogenicity and only a single laboratory abnormality (Grade 1) was noted following Vaccination #2 in a Pfs25M subject. There were no Grade 3, Grade 4, or SAEs reported in the Pfs25M arm.

Table 6: Summary of Reported Adverse Events in Pfs25M Safety Vaccine Arm versus Comparator, #15-I-0044, Mali Cohort

	Pfs25M, 16µg			Comparator		
	Vaccine 1	Vaccine 2	Total	Vaccine 1	Vaccine 2	Total
	N=5	N=5	N=5	N=5	N=5	N=5
Total # AEs	5 (4) 80%	14 (5) 100%	19 (5) 100%	3 (2) 40%	8 (4) 80%	11 (4) 80%
Classification						
Local Reactogenicity	2 (2) 40%	2 (2) 40%	4 (3) 60%	0 (0) 0%	1 (1) 20%	1 (1) 20%
Systemic Reactogenicity	0 (0) 0%	0 (0) 0%	0 (0) 0%	0 (0) 0%	0 (0) 0%	0 (0) 0%
Laboratory Abnormalities	0 (0) 0%	1 (1) 20%	1 (1) 20%	0 (0) 0%	0 (0) 0%	0 (0) 0%
Unsolicited AEs	3 (2) 40%	11 (5) 100%	14 (5) 100%	3 (2) 40%	7 (4) 80%	10 (4) 80%
Severity and Relationship						
Grade 1	2 (2) 40%	5 (4) 80%	7 (4) 80%	1 (1) 20%	3 (2) 40%	4 (2) 40%
Related to Pfs25	2 (2) 40%	2 (2) 40%	4 (3) 60%	0 (0) 0%	1 (1) 20%	1 (1) 20%
Grade 2	3 (2) 40%	9 (5) 100%	12 (5) 100%	2 (1) 20%	4 (3) 60%	6 (3) 60%
Related to Pfs25	0 (0) 0%	0 (0) 0%	0 (0) 0%	0 (0) 0%	0 (0) 0%	0 (0) 0%
Grade 3	0 (0) 0%	0 (0) 0%	0 (0) 0%	0 (0) 0%	1 (1) 20%	1 (1) 20%
Related to Pfs25	0 (0) 0%	0 (0) 0%	0 (0) 0%	0 (0) 0%	0 (0) 0%	0 (0) 0%
Grade 4	0 (0) 0%	0 (0) 0%	0 (0) 0%	0 (0) 0%	0 (0) 0%	0 (0) 0%
SAE	0 (0) 0%	0 (0) 0%	0 (0) 0%	0 (0) 0%	0 (0) 0%	0 (0) 0%

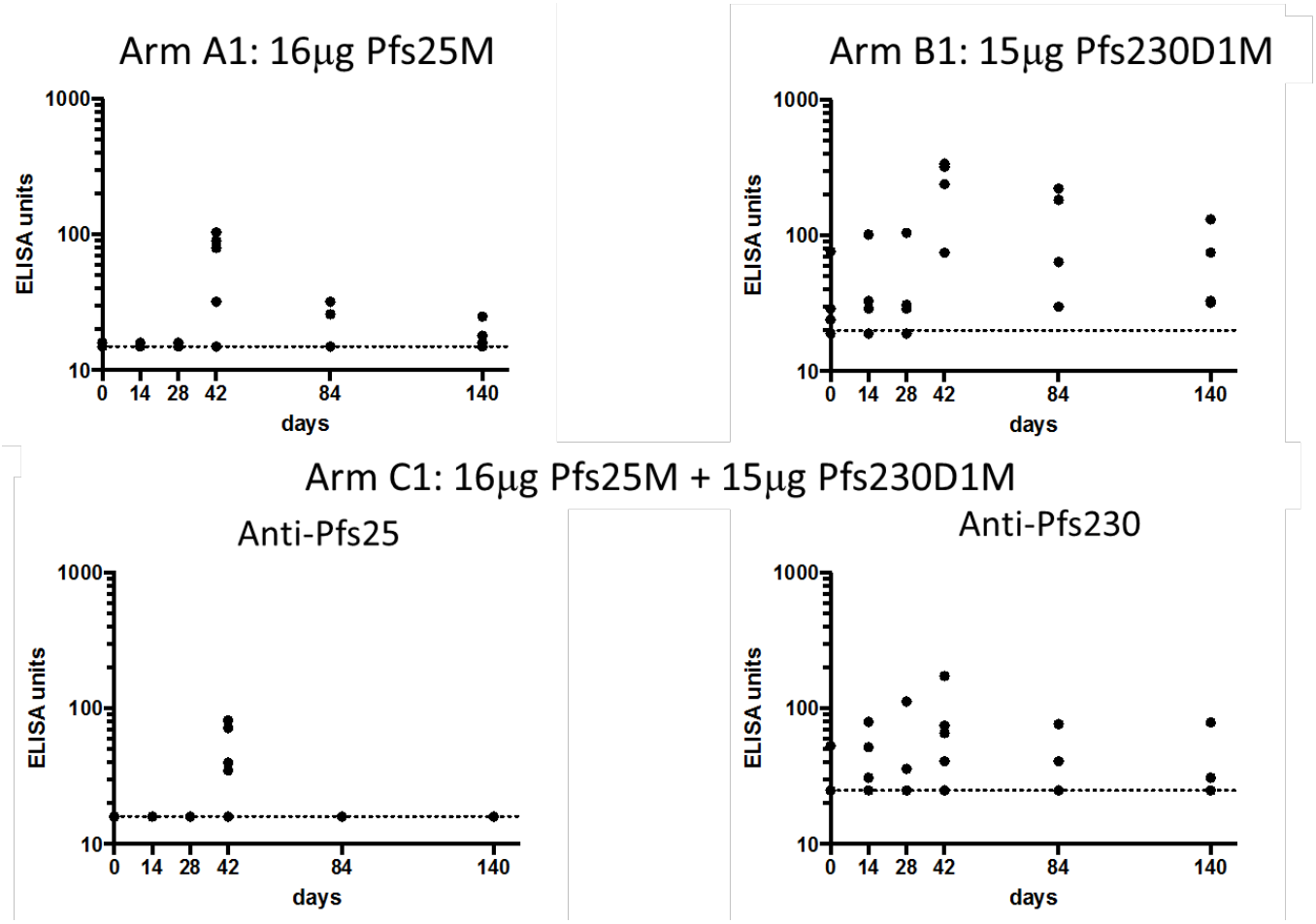
Each field has the format # (X) %, where # is the number of adverse events, X is the number of subjects with one or more episodes of the given event, and % is the number of participants with one or more episodes of the given event divided by the total number of participants who received the study agent (N) multiplied by 100.

Safety analysis of the high dose of Pfs25M-EPA/Alhydrogel®, 47µg of Pfs25M (Arm A2), is in process following unblinding in April 2017. Overall, in the blinded cohort, the majority of reported AEs have been mild (Grade 1; 708/1431; 49%) with the most commonly report AEs being injection site pain, headache, malaria, neutropenia, nasopharyngitis, and rhinitis. The most commonly reported related AEs have been injection site reactogenicity (pain, induration, pruritus, and edema), leukopenia, neutropenia, and headache, which have been all Grade 1 or 2.

3.1.4.2 Immunogenicity of Pfs25M-EPA/Alhydrogel® in Healthy Malian Adults

Evaluation of immunogenicity and functional activity for the low dose, unblinded cohorts (Cohort 1, 2) have been completed (**Figure 14**). As expected, no pre-existing immunity was seen prior to vaccination in Malian adults to Pfs25. The majority of subjects in all Arms (A1: 16µg of Pfs25M; C1: 16µg of Pfs25M + 15µg of Pfs230D1M) responded to the vaccine following Vaccination #2. However, antibody responses to Pfs25 decayed rapidly post vaccination #2, similar to what has been seen previously with Pfs25H.

Figure 14: **Pfs25–specific antibody responses in Malian subjects receiving Low Dose Pfs25M, Pfs230D1M, and Pfs25M + Pfs230D1M (#15-I-0044)**

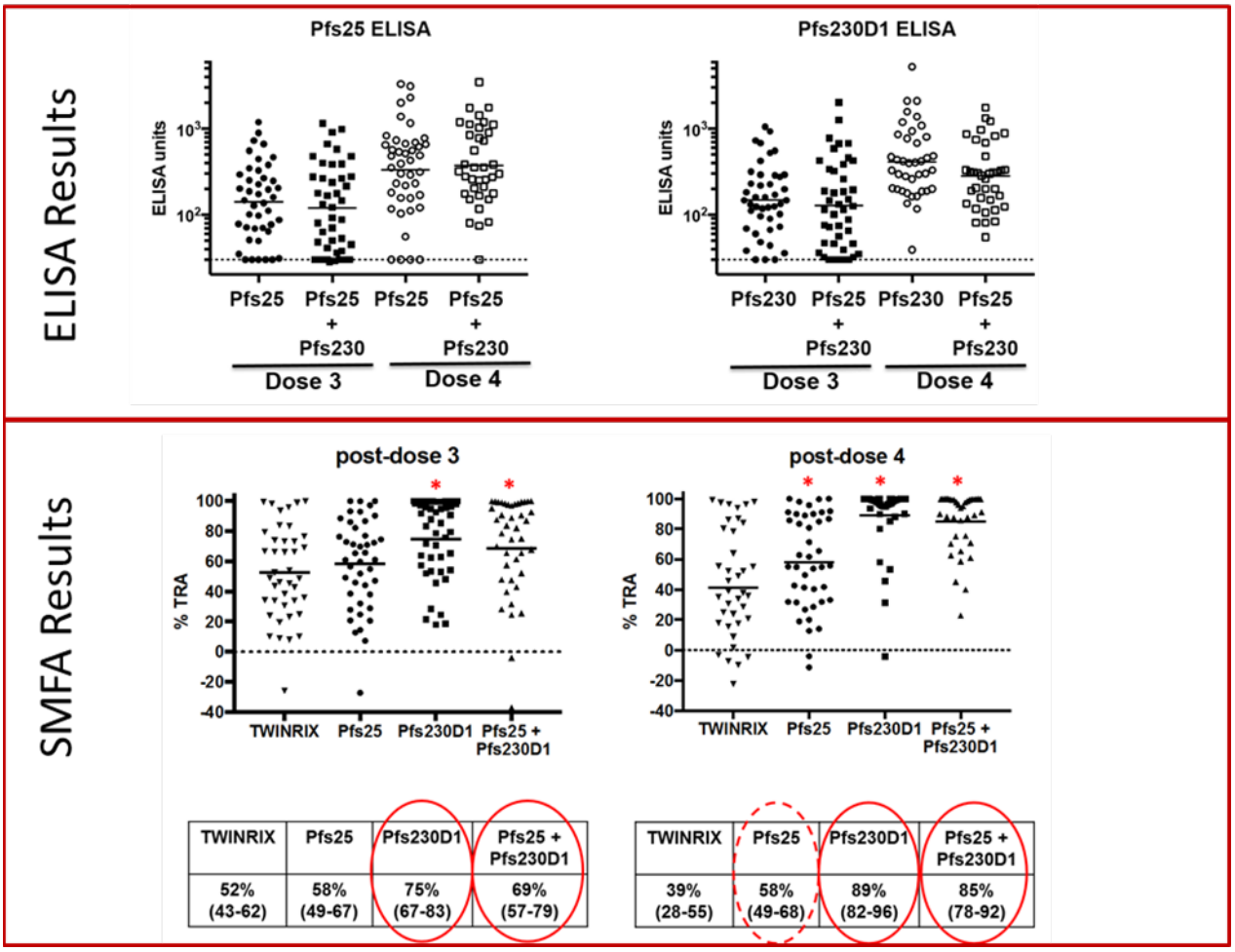


Results presented in ELISA units for each Arm. Vaccinations occurred on Day 0, 28. Day 0 was drawn pre-vaccination, D42 is 14 days post vaccination #2. Each individual data point represents an individual subject anti-Pfs25 (Arms A1, C1) or anti-Pfs230 (Arm B1, C1) ELISA responses.

Evaluation of immunogenicity and functional activity in the targeted dose are in process following unblinding of the study in 2017, though an interim analysis was completed following Vaccination #3 and #4 in the targeted doses (Arm A2: Pfs25M, 47µg; Arm B2: Pfs230D1M, 40µg; Arm C2: Pfs25M, 47µg AND Pfs230D1M, 40µg; Arm D3: comparator, Twinrix®) that assisted in determining the final study plan for this proposed study.

The two planned interim analyses (Jun 2016, Feb 2017) of immunogenicity (ELISA) and functional activity (DSF, SMFA) by Arm were completed after Vaccination #3 and #4 in the larger Main Cohort during the study to inform about proceeding to a booster 4th dose in 2016 and to inform the study design for protocol 17-I-N006 (TBV with AS01). In conclusion, by ELISA and SMFA, Pfs230 induced superior activity to Pfs25, and Pfs230 in combination with Pfs25, was not superior to Pfs230 alone (**Figure 15**).

Figure 15 : Pfs25 anf Pfs230 immunogenicity (ELISA) and functional activity (SMFA) in malaria-exposed Malian adults for 3 and 4 vaccine doses



Samples obtained 14 days following receipt of vaccine #3 (study day 182) and #4 (study day 554) in the highest antigen dose arms (Pfs25M, 47µg alone; Pfs230D1M, 40µg alone; Pfs25M, 47µg + Pfs23D1M, 40µg; TWINRIX [received TWINRIX for first 3 doses, then Menactra® for fourth dose]). Vaccinations received at 0, 1, 6 months (2015) and 18 months (2016). Each individual data points represents an individual ELISA or SMFA results. Black bars represent 95% confidence intervals. Red asterix represent statistical significance when compared to the control (TWINRIX). Tables report the mean TRA (95% confidence intervals) following the third and fourth vaccination.

In 2015, starting 1 week post vaccination #3 and using 60 mosquitoes total, 2005 DSF were performed in 175 individuals, of which 73% participated in all 12 feeds. Fifty-eight (2.9%) of the DSFs resulted in a positive DSF in 18 (10.3%) unique individuals. In 2016, starting 1 week post vaccination #4 and using 30 mosquitoes total, 1843 DSFs were performed in 160 individuals, of which 87% participated in all 12 feeds. Thirty (1.6%) of the DSFs resulted in a positive DSF in 18 (11.3%) unique individuals. Analysis of the DSFs (accounting for all positive DSFs) post vaccination #4 using two models (GEE, GLMM) showed no significant difference in DSF results between Pfs230 alone, Pfs25M + Pfs230 in combination, and the control group. Pfs25M alone, when compared to the control group, in the GEE analyses did show a significant odds ratio of

infection (OR 6.75; 95% CI 1.16-39.35; p=0.034) but this significance was not seen in the GLMM analyses (OR 3.05; 95% CI 0.14-65.89; p=0.633). Further analyses are ongoing to account for possible transmission by other *Plasmodium* species (*P. ovale, P. malariae*), vaccine variants, and the impact of time post vaccination on DSF transmission events.

As noted above, a change was made in the plan for DSF between 2015 and 2016, with a decrease of the number of mosquitoes per each feeding pint (30 to 15). This change was based on modeling with available DSF results from 2011 through 2015. However, in comparing the DSF transmission rates between 2015 and 2016 in the comparator group, there was no change in the percent of unique individuals transmitting overall, but there were substantial differences in the transmission dynamics in the comparator group from 2015 to 2016 as summarized below in **Table 7**. Given this result and the low transmission rate seen in the control arm in 2016 (post Vax #4), as described in Section **14.5**, DSFs will completed twice a week starting one week post vaccination with a total of approximately 60 mosquitoes (30 mosquitoes/cup; 2 cups) for 12 weeks total.

Table 7: **Summary of BS and DSF completed for protocol 15-I-0044 during DSF evaluation period post Vaccination #3 and #4.**

	No. unique subjects with ≥1 DSF completed[A]		No. unique subjects with ≥1 positive DSF[B]		No. DSFs completed over the 6 week period[C]		No. DSFs positive[D]		Median (range) of no. positive DSF per person		Median (range) of no. positive mosquitoes per positive DSF		Percent of positive mosquitoes in the positive DSFs [E]	
	Vax #3	Vax #4	Vax #3	Vax #4	Vax #3	Vax #4	Vax #3	Vax #4	Vax #3	Vax #4	Vax #3	Vax #4	Vax #3	Vax #4
Comparator	43 (24.6%)	39 (24.5%)	6 (9.3%)	4 (10.3%)	497 (24.8%)	452 (24.5%)	18 (3.6%)	4 (0.9%)	2 (1-6)	1 (1)	7 (1-18)	1 (1-7)	34.3%	8.8%
Pfs25 alone	44 (25.1%)	41 (25.9%)	4 (9.1%)	9 (21.4%)	502 (25.0%)	480 (26.0%)	15 (3.0%)	18 (3.7%)	3.5 (2-6)	2 (1-3)	4 (1-16)	2.5 (1-18)	18.6%	63.7%
Pfs230 alone	44 (25.1%)	41 (25.9%)	6 (13.6%)	2 (4.9%)	502 (25.0%)	475 (23.7%)	19 (3.8%)	3 (0.6%)	1.5 (1-7)	1.5 (1-2)	3 (1-32)	6 (2-10)	38.4%	15.9%
Pfs25+230	44 (25.1%)	37 (23.3%)	2 (4.6%)	3 (8.1%)	504 (25.1%)	436 (23.5%)	6 (1.2%)	5 (1.1%)	3 (1-5)	2 (1-2)	4 (1-20)	2 (1-6)	8.7%	11.5%
TOTAL	**175**	**158**	18 (10.3%)	18 (11.3%)	**2005**	**1843**	58 (2.9%)	30 (1.6%)	2 (1-7)	1.5 (1-3)	5.5 (1-32)	2 (1-18)	100.0%	100.0%

A: Number of unique subjects who received vaccination #3 and then #4 and completed at least 1 DSF during the DSF evaluation period.
B: Number of unique subjects who has ≥1 mosquito with ≥1 oocyst seen on dissection. % calculated as # unique subjects with +DSF / # unique subjects in that arm completing a DSF.
C: Total number of DSF and blood smears completed by that arm over the six week period post Vaccination #3 and #4. % calculated as #DSFs completed per arm / total # DSFs completed by the entire cohort (total)
D: % calculated as #positive DSFs by arm / #DSFs completed by that arm
E: % calculated as #positive mosquitoes per arm / # total positive mosquitoes

	No. unique subjects gametocytemic[A]		Total count gametocytemic[B]		No. unique subjects parasitemic[A]		Total count parasitemic[B]	
	Vax #3	Vax #4	Vax #3	Vax #4	Vax #3	Vax #4	Vax #3	Vax #4
Comparator	19 (44.2%)	14 (35.9%)	75 (15.1%)	40 (8.7%)	35 (81.4%)	28 (71.8%)	117 (23.5%)	83 (18.0%)
Pfs25 alone	12 (27.3%)	15 (36.6%)	56 (11.2%)	65 (13.5%)	40 (90.9%)	31 (75.6%)	134 (26.7%)	98 (20.4%)
Pfs230 alone	12 (27.3%)	13 (31.7%)	62 (12.6%)	40 (8.4%)	41 (93.2%)	31 (75.6%)	125 (24.9%)	99 (20.8%)
Pfs25+230	7 (15.9%)	13 (35.1%)	28 (5.6%)	31 (7.1%)	34 (77.3%)	27 (70.3%)	99 (19.6%)	76 (17.4%)
TOTAL	50 (28.6%)	55 (34.8%)	221 (11.0%)	176 (9.5%)	150 (85.7%)	117 (74.1%)	475 (23.7%)	351 (19.3%)

A: Number of unique subjects who had ≥ 1 bloodsmear with ≥ 1 gametocyte or ≥ 1 Pf parasite seen on it over the DSF evaluation period. % calculated as # unique subjects per arm / # unique subjects who received vaccination #3 or #4 and completed at least 1 DSF.
B: Total count is the number of BS with ≥ 1 gametocyte or ≥ 1 Pf parasite seen on it over the DSF evaluation period. % calculated as # positive BS per arm / # BS completed per arm.

3.2 Pfs230-EPA

3.2.1 Pfs230D1M-EPA/Alhydrogel® in Healthy US Adults

Study enrollment, as seen in **Table 3**, for the US portion of study #15-I-0044 (N=35) was to evaluate safety and tolerability of increasing doses and Pfs25M and Pfs230D1M given in combination prior to moving into Mali, West Africa for further safety, immunogenicity, and functional activity evaluation.

3.2.1.1 Safety of Pfs230D1M-EPA/Alhydrogel® in Healthy US Adults

In the US portion of NIAID protocol #15-I-0044, Pfs230D1M vaccinations were well-tolerated, with minimal local and systemic reactogenicity as noted in **Table 8**. The majority of the reported AEs were Grade 1 (5µg: 19/22, 86%; 15µg: 29/34, 85%; 40µg: 24/31, 77%). All related AEs reported were Grade 1, except for a single Grade 2 episode of injection site pain following the first vaccination in the high dose group of 40µg Pfs230D1M. Overall the reported local reactogenicity by vaccination and dose appeared to increase in the frequency of reporting and duration of symptoms with increasing antigen dose of Pfs230, but not with subsequent vaccinations. Solicited systemic symptoms were reported in all dosing arms and did not increase in frequency, severity, nor duration with increasing antigen dose. Various laboratory abnormalities, all Grade 1, were noted across the dosing arms, seven in total in five subjects, but only three were deemed related to vaccination. There were no Grade 3 AEs and no SAEs reported.

Table 8: Summary of Reported Adverse Events in Pfs230D1M Vaccine Arms, #15-I-0044, US Cohort

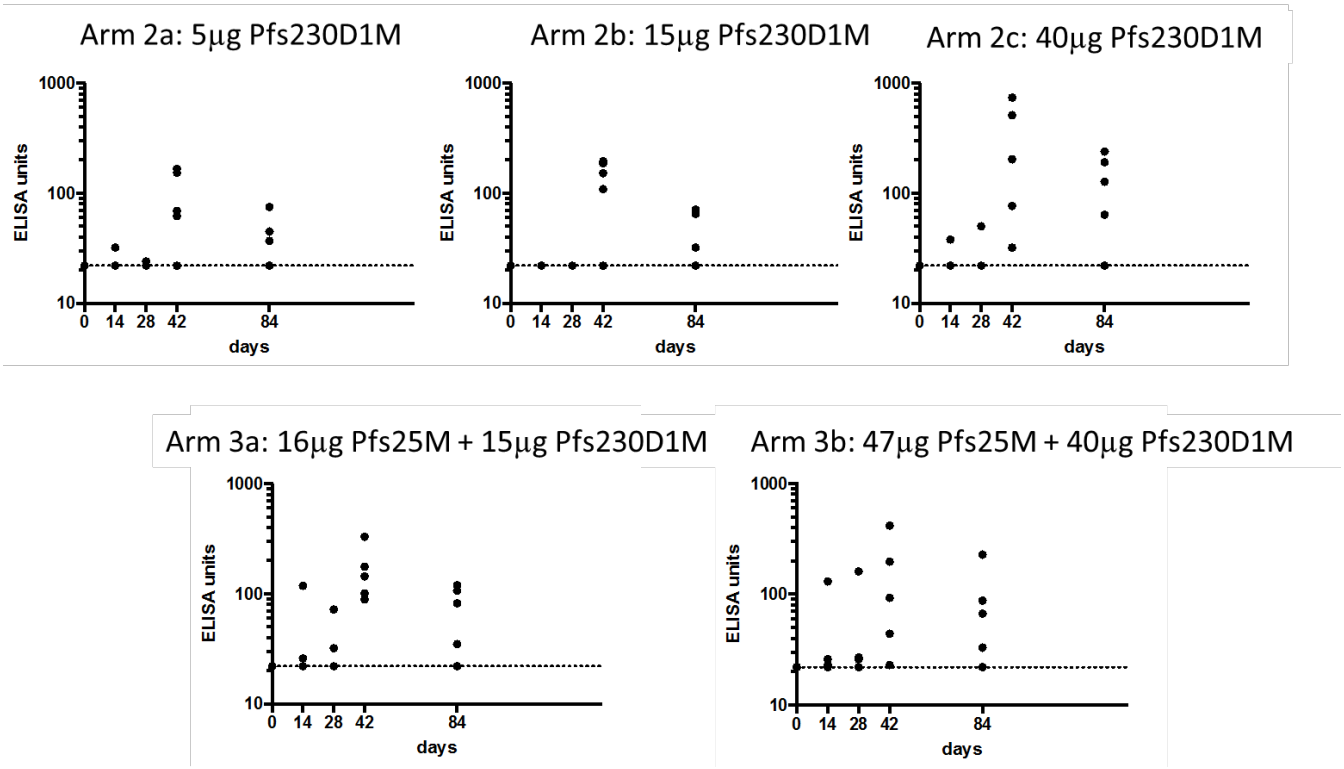
	Pfs230D1M, 5µg			Pfs230D1M, 15µg			Pfs230D1M, 40µg		
	Vaccine 1	Vaccine 2	Total	Vaccine 1	Vaccine 2	Total	Vaccine 1	Vaccine 2	Total
	N=5	N=5	N=5	N=5	N=4	N=5	N=5	N=5	N=5
Total # AEs	8 (4) 80%	14 (5) 100%	22 (5) 100%	10 (5) 100%	24 (4) 100%	34 (5) 100%	19 (5) 100%	12 (5) 100%	31 (5) 100%
Classification									
Local Reactogenicity	2 (2) 40%	4 (4) 80%	6 (4) 80%	5 (4) 80%	4 (3) 75%	9 (4) 80%	7 (5) 100%	4 (4) 80%	11 (5) 100%
Systemic Reactogenicity	1 (1) 20%	1 (1) 20%	2 (2) 40%	3 (2) 40%	6 (2) 50%	9 (3) 60%	6 (3) 60%	0 (0) 0%	6 (3) 60%
Laboratory Abnormalities	0 (0) 0%	2 (2) 40%	2 (2) 40%	1 (1) 20%	0 (0) 0%	1 (1) 20%	3 (1) 20%	1 (1) 20%	4 (2) 40%
Unsolicited AEs	5 (3) 60%	7 (2) 40%	12 (3) 60%	1 (1) 20%	14 (4) 100%	15 (4) 80%	3 (2) 40%	7 (5) 100%	10 (5) 100%
Severity and Relationship									
Grade 1	6 (4) 80%	13 (5) 100%	19 (5) 100%	10 (5) 100%	19 (4) 100%	29 (5) 100%	15 (5) 100%	9 (5) 100%	24 (5) 100%
Pfs230 Related	3 (3) 60%	6 (4) 80%	9 (4) 80%	7 (4) 80%	9 (3) 75%	16 (4) 80%	9 (5) 100%	5 (4) 80%	14 (5) 100%
Grade 2	2 (2) 40%	1 (1) 20%	3 (3) 60%	0 (0) 0%	5 (3) 75%	5 (3) 60%	4 (2) 60%	3 (3) 60%	7 (4) 80%
Pfs230 Related	0 (0) 0%	0 (0) 0%	0 (0) 0%	0 (0) 0%	0 (0) 0%	0 (0) 0%	1 (1) 20%	0(0) 0%	1 (1) 20%
Grade 3	0 (0) 0%	0 (0) 0%	0 (0) 0%	0 (0) 0%	0 (0) 0%	0 (0) 0%	0 (0) 0%	0 (0) 0%	0 (0) 0%
Grade 4	0 (0) 0%	0 (0) 0%	0 (0) 0%	0 (0) 0%	0 (0) 0%	0 (0) 0%	0 (0) 0%	0 (0) 0%	0 (0) 0%
SAE	0 (0) 0%	0 (0) 0%	0 (0) 0%	0 (0) 0%	0 (0) 0%	0 (0) 0%	0 (0) 0%	0 (0) 0%	0 (0) 0%

Each field has the format # (X) %, where # is the number of adverse events, X is the number of subjects with one or more episodes of the given event, and % is the number of participants with one or more episodes of the given event divided by the total number of participants who received the study agent (N) multiplied by 100.

3.2.1.2 Immunogenicity of Pfs230D1M-EPA/Alhydrogel® in Healthy US Adults

As expected and seen with Pfs25 antibody responses, no pre-existing immunity was seen prior to vaccination in US for Pfs230. Unlike Pfs25 responses, at the lower doses, majority of subjects vaccinated did respond to vaccination with Pfs230 alone. The majority of subjects in Arms 2c (40µg of Pfs230D1M), 3a (16µg of Pfs25M + 15µg of Pfs230D1M), and 3b (47µg of Pfs25M + 40µg of Pfs230D1M) responded to the vaccine following Vaccination #2 (see **Figure 16**). There was no obvious interference seen with co-administration of Pfs25M-EPA/Alhydrogel® and Pfs230D1M-EPA/Alhydrogel® versus Pfs230D1M-EPA/Alhydrogel® alone, but these safety arms were not powered to assess interference.

Figure 16: Pfs230–specific antibody responses in US naïve subjects receiving Pfs230D1M vaccinations (#15-I-0044)

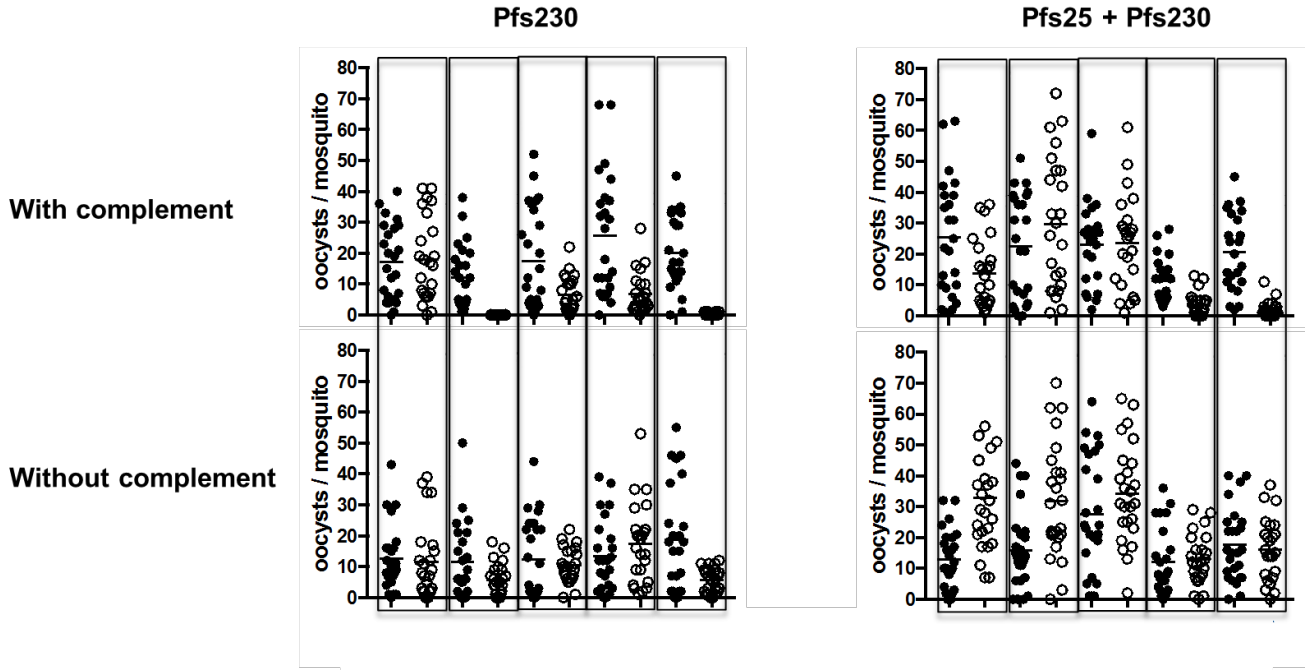


Results presented in ELISA units for each Arm. Vaccinations occurred on Day 0, 28. Day 0 was drawn pre-vaccination, D42 is 14 days post vaccination #2. Each individual data point represents an individual subject anti-Pfs230- ELISA response.

Functional activity by SMFA, in US adults in protocol #15-I-0044, related to antibody titers following Vaccination #2 has only been assessed in the highest antigen dose arms at this time and will be further assessed in the larger Mali cohort once unblinded. A few individuals, after two doses of either Pfs230D1M alone or in combination with Pfs25M, developed appreciable vaccine specific antibody responses which was associated with a reduction in transmission activity (decrease in the number of oocysts/mosquito versus day 0 pool) via SMFA (**Figure 13**)

and for Pfs230 activity, is complement dependent (**Figure 17**). However, the numbers in each arm are too small to be of statistical significance nor were these arms of the study powered for this endpoint.

Figure 17: Pfs230 Functional Activity (SMFA) in the Presence or Absence of Complement



Each individual column represents an individual subject with Day 0 (pre-vaccination #1), Day 42 (14 days post vaccination #2). Each individual data point represents a single mosquito dissected. Top set of figures is the assay completed with complement present, the bottom set of figures is the assay completed without complement present in the assay. Only the highest antigen dose arms (Pfs230D1M, 40µg alone, n=5 and Pfs25M, 47µg + Pfs230D1M, 40µg co-administered, n=5) presented.

3.2.2 Pfs230D1M-EPA/Alhydrogel® in Healthy Malian Adults

Status of protocol #15-I-0044 is already summarized in **Section 3.1.4**.

3.2.2.1 Pfs230D1M-EPA/Alhydrogel® in Healthy Malian Adults

From the unblinded safety data provided by Arm B1 (low dose of Pfs230DM, 15µg), overall vaccinations have been well tolerated with the majority of the reported AEs being mild or moderate (Grade 1 and Grade 2; 14/17, 82%) with very few reported related AEs (headache, n=1; leukopenia, n=1), both of which were Grade 1. The most commonly reported related AEs were injection site pain, neutropenia, and headache, which were all Grade 1. In a single subject, a Grade 3 gastroenteritis was reported with associated Grade 4 laboratory abnormalities (leukocytosis, blood creatinine increased), all deemed unlikely related to vaccination and resolved shortly after resolution of subject's gastroenteritis symptoms. Due to a continued Grade 1 lab abnormality at time of this subject's next scheduled vaccination, it was decided for the

subject's safety to not proceed with Vaccination #2 and to follow them for safety. As had been seen with low dose Pfs25M, in comparison to the comparator group, who received Twinrix®, the Pfs230D1M vaccinees reported overall more AEs (17 versus 11). However, the Pfs230D1M group reported no local reactogenicity and only a single solicited symptom (Grade 1, headache). As noted in **Table 9**, there were more laboratory abnormalities reported in the Pfs230D1M arm, including the Grade 4 as noted above, but of these laboratory abnormalities, only one (Grade 1 leukopenia) was determined possibly related to vaccination. There were no SAEs reported in the Pfs230D1M arm.

Table 9: Summary of Reported Adverse Events in Pfs230D1M Safety Vaccine Arm versus Comparator, #15-I-0044, Mali Cohort

	Pfs230D1M, 15µg			Comparator -- Twinrix®		
	Vaccine 1	Vaccine 2	Total	Vaccine 1	Vaccine 2	Total
	N=5	N=4	N=5	N=5	N=5	N=5
Total # AEs	7 (4) 80%	10 (4) 100%	17 (5) 100%	3 (2) 40%	8 (4) 80%	11 (4) 80%
Classification						
Local Reactogenicity	0 (0) 0%	0 (0) 0%	0 (0) 0%	0 (0) 0%	1 (1) 20%	1 (1) 20%
Systemic Reactogenicity	1 (1) 20%	0 (0) 0%	1 (1) 20%	0 (0) 0%	0 (0) 0%	0 (0) 0%
Laboratory Abnormalities	3 (1) 20%	2 (2) 50%	5 (3) 60%	0 (0) 0%	0 (0) 0%	0 (0) 0%
Unsolicited AEs	3 (3) 60%	8 (2) 50%	11 (5) 100%	3 (2) 40%	7 (4) 80%	10 (4) 80%
Severity and Relationship						
Grade 1	2 (2) 40%	4 (4) 100%	6 (5) 100%	1 (1) 20%	3 (2) 40%	4 (2) 40%
Related to Pfs230	1 (1) 20%	1 (1) 25%	2 (1) 20%	0 (0) 0%	1 (1) 20%	1 (1) 20%
Grade 2	2 (2) 40%	6 (2) 50%	8 (3) 60%	2 (1) 20%	4 (3) 60%	6 (3) 60%
Related to Pfs230	0 (0) 0%	0 (0) 0%	0 (0) 0%	0 (0) 0%	0 (0) 0%	0 (0) 0%
Grade 3	1 (1) 20%	0 (0) 0%	1 (1) 20%	0 (0) 0%	1 (1) 20%	1 (1) 20%
Related to Pfs230	0 (0) 0%	0 (0) 0%	0 (0) 0%	0 (0) 0%	0 (0) 0%	0 (0) 0%
Grade 4	2 (1) 20%	0 (0) 0%	2 (1) 20%	0 (0) 0%	0 (0) 0%	0 (0) 0%
Related to Pfs230	0 (0) 0%	0 (0) 0%	0 (0) 0%	0 (0) 0%	0 (0) 0%	0 (0) 0%
SAE	0 (0) 0%	0 (0) 0%	0 (0) 0%	0 (0) 0%	0 (0) 0%	0 (0) 0%

Each field has the format # (X) %, where # is the number of adverse events, X is the number of subjects with one or more episodes of the given event, and % is the number of participants with one or more episodes of the given event divided by the total number of participants who received the study agent (N) multiplied by 100.

Safety analysis of the high dose of Pfs230D1M-EPA/Alhydrogel®, 40µg of Pfs230D1M, is still blinded. Overall, in the blinded cohort, the majority of reported AEs have been mild (Grade 1; 708/1431; 49%) with the most commonly report AEs being injection site pain, headache, malaria, neutropenia, nasopharyngitis, and rhinitis. The most commonly reported related AEs have been injection site reactogenicity (pain, induration, pruritus, and edema), leukopenia, neutropenia, and headache, which have been all Grade 1 or 2.

3.2.2.2 Immunogenicity of Pfs230D1M-EPA/Alhydrogel® in Healthy Malian Adults

Evaluation of immunogenicity and functional activity for the low dose, unblinded cohorts (Cohort 1, 2) have been completed (**Figure 14**). Pre-existing immunity to Pfs230 was seen prior to vaccination. The majority of subjects in all Arms (B1: 15µg of Pfs230D1M; C1: 16µg of

Pfs25M + 15µg of Pfs230D1M) responded to the vaccine following Vaccination #2. Antibody responses to Pfs230 appeared to persist longer than has been previously seen with Pfs25.

3.3 Pfs25-EPA and Pfs230-EPA

3.3.1 Pfs25M-EPA/Alhydrogel® and Pfs230D1M-EPA/Alhydrogel® in Healthy US Adults

Study enrollment, as seen in **Table 3**, for the US portion of the study (N=35) was to evaluate safety and tolerability of increasing doses and Pfs25M and Pfs230D1M given in combination prior to moving into Mali, West Africa for further safety, immunogenicity, and functional activity evaluation.

3.3.1.1 Safety of Pfs25M-EPA/Alhydrogel® and Pfs230D1M-EPA/Alhydrogel® in Healthy US Adults

Overall vaccinations were well-tolerated with the co-administration of Pfs25 and Pfs230 in US adults in protocol 15-I-0044, with minimal local and systemic reactogenicity as noted in **Table 10**. The majority of the reported AEs were Grade 1 (16µg + 15µg: 27/27, 100%; 16µg + 15µg: 42/44, 95%). All related AEs reported were Grade 1. There were more AEs reported overall given the increased reporting of local reactogenicity per each vaccination received. Overall the reported local reactogenicity by vaccination and dose appeared to increase in the frequency of reporting with increasing doses and was more likely to be attributed to Pfs230 than Pfs25, but not significantly so. However, with increasing antigen dose, the duration of symptoms and severity of symptoms remained unchanged. Solicited systemic symptoms were reported in all dosing arms and did not increase in frequency, severity, nor duration with increasing antigen dose. Various laboratory abnormalities, all Grade 1 and only 1 of 5 reported deemed related to vaccination, were noted across the dosing arms. Hemoglobin decrease was the most commonly reported laboratory abnormality. In the high dose arms, there was one Grade 2 AE (breast disorder) and one Grade 3 AE (systolic hypertension), both determined not related to vaccination, and no SAEs were reported.

Table 10: Summary of Reported Adverse Events in Pfs25 and Pfs230D1M Vaccine Arms, #15-I-0044, US Cohort

	Pfs25M, 16µg AND Pfs230D1M, 15µg			Pfs25M, 47µg AND Pfs230D1M, 40µg		
	Vaccine 1	Vaccine 2	Total	Vaccine 1	Vaccine 2	Total
	N=5	N=5	N=5	N=5	N=5	N=5
Total # AEs	14 (5) 100%	13 (5) 100%	27 (5) 100%	23 (5) 100%	21 (5) 100%	44 (5) 100%
Classification						
Local Reactogenicity	6 (4) 80%	8 (4) 80%	14 (4) 80%	9 (5) 100%	11 (4) 80%	20 (5) 100%
Systemic Reactogenicity	3 (2) 40%	1 (1) 20%	4 (2) 40%	5 (2) 40%	4 (1) 20%	9 (2) 40%
Laboratory Abnormalities	2 (2) 40%	2 (1) 20%	4 (2) 40%	1 (1) 20%	0 (0) 0%	1 (1) 20%
Unsolicited AEs	3 (3) 60%	2 (2) 40%	5 (4) 80%	8 (4) 80%	6 (2) 40%	14 (5) 100%
Severity and Relationship						
Grade 1	14 (5) 100%	13 (5) 100%	27 (5) 100%	22 (5) 100%	20 (5) 100%	42 (5) 100%
Related to Pfs25	4 (2) 40%	4 (4) 80%	8 (4) 80%	9 (5) 100%	7 (3) 60%	16 (5) 100%
Related to Pfs230	6 (4) 80%	4 (3) 80%	10 (4) 80%	9 (5) 100%	8 (4) 80%	17 (5) 100%
Grade 2	0 (0) 0%	0 (0) 0%	0 (0) 0%	0 (0) 0%	1 (1) 20%	1 (1) 20%
Related to Pfs25	0 (0) 0%	0 (0) 0%	0 (0) 0%	0 (0) 0%	0 (0) 0%	0 (0) 0%
Related to Pfs230	0 (0) 0%	0 (0) 0%	0 (0) 0%	0 (0) 0%	0 (0) 0%	0 (0) 0%
Grade 3	0 (0) 0%	0 (0) 0%	0 (0) 0%	1 (1) 20%	0 (0) 0%	1 (1) 20%
Related to Pfs25	0 (0) 0%	0 (0) 0%	0 (0) 0%	0 (0) 0%	0 (0) 0%	0 (0) 0%
Related to Pfs230	0 (0) 0%	0 (0) 0%	0 (0) 0%	0 (0) 0%	0 (0) 0%	0 (0) 0%
Grade 4	0 (0) 0%	0 (0) 0%	0 (0) 0%	0 (0) 0%	0 (0) 0%	0 (0) 0%
SAE	0 (0) 0%	0 (0) 0%	0 (0) 0%	0 (0) 0%	0 (0) 0%	0 (0) 0%

Each field has the format # (X) %, where # is the number of adverse events, X is the number of subjects with one or more episodes of the given event, and % is the number of participants with one or more episodes of the given event divided by the total number of participants who received the study agent (N) multiplied by 100.

Overall there has been no significant difference in reported local or systemic solicited reactogenicity or laboratory abnormalities between Pfs25M-EPA/Alhydrogel® alone, Pfs230D1M-EPA/Alhydrogel® alone, or co-administration of Pfs25M-EPA/Alhydrogel® and Pfs230D1M-EPA/Alhydrogel®.

3.3.1.2 Immunogenicity of Pfs25M-EPA/Alhydrogel® and Pfs230D1M-EPA/Alhydrogel® in Healthy US Adults

As already described in **Sections** 3.1.3.2 and 3.2.1.2, no pre-existing immunity was seen prior to vaccination in US for either Pfs25 or Pfs230. The majority of subjects in the highest antigen co-administration dose arm (Pfs25M, 47µg + Pfs230D1M, 40µg, n=5) responded to the vaccine following Vaccination #2 for both antigens (see **Figure 12**). There was no significant interference seen with co-administration of Pfs25M-EPA/Alhydrogel® and Pfs230D1M-EPA/Alhydrogel® compared to those who received Pfs25 or Pfs230 alone, though overall the antibody responses were slightly lower in the co-administration when compared to the single antigen arms, but as noted above, these safety arms were not powered to assess interference. Additionally, in a few individuals, appreciable functional activity via SMFA was achieved following Vaccination #2, as seen in **Figure 13** and **Figure 17,** which for Pfs230 was complement dependent.

In conclusion, for the US portion of study #15-I-0044, in malaria naïve adults, Pfs25M-EPA/Alhydrogel® given alone or in combination with Pfs230D1M-EPA/Alhydrogel® and

Pfs230D1M-EPA/Alhydrogel® given alone or in combination with Pfs25M-EPA/Alhydrogel® is safe and tolerable. Detectable responses were seen in the majority of vaccinated subjects to Pfs25, 47µg and/or Pfs230, 40µg following receipt of two vaccinations at the targeted dose. Appreciable functional activity, though in a limited number of individuals, is achievable following two doses of Pfs230D1M-EPA/Alhydrogel® or Pfs230D1M-EPA/Alhydrogel® and Pfs25M-EPA/Alhydrogel® at the highest vaccine dose administered.

3.3.2 Pfs25M-EPA/Alhydrogel® and Pfs230D1M-EPA/Alhydrogel® in Healthy Malian Adults

Status of protocol #15-I-0044 is already summarized in **Section 3.1.4**.

3.3.2.1 Safety of Pfs25M-EPA/Alhydrogel® and Pfs230D1M-EPA/Alhydrogel® in Healthy Malian Adults

From the unblinded safety data provided by Arm C1 (low dose of Pfs25M, 16 µg AND Pfs230DM, 15µg), overall vaccinations have been well tolerated with the majority of the reported AEs being mild (Grade 1; 15/23, 65%; **Table 11**). There have been few reported related AEs (majority of which have been injection site pain) and all reported related AEs have been Grade 1. There was a single Grade 3 AE (malaria) reported that occurred over 100 days post vaccination and was not related. There were no Grade 4 AEs or SAEs reported in the Pfs25M and Pfs230D1M arm. In comparison to the comparator group, who received Twinrix® and normal saline, the Pfs25M and Pfs230D1M vaccinees reported overall more AEs (23 versus 17), including increased frequency of reporting injection site pain (6 versus 3) and related AEs (9 versus 4). However, both groups reported few to no systemic reactogenicity, and overall, the comparator arm reported more laboratory abnormalities in comparison to the vaccine group.

Table 11: Summary of Reported Adverse Events in Pfs25M and Pfs230D1M Safety Vaccine Arm versus Comparator, 15-I-0044, Mali Cohort

	Pfs25M, 16µg AND Pfs230D1M, 15µg			Comparator -- Twinrix® + Normal Saline		
	Vaccine 1	Vaccine 2	Total	Vaccine 1	Vaccine 2	Total
	N=5	N=5	N=5	N=5	N=5	N=5
Total # AEs	7 (4) 80%	16 (5) 100%	23 (5) 100%	5 (2) 40%	12 (5) 100%	17 (5) 100%
Classification						
Local Reactogenicity	3 (3) 60%	3 (2) 40%	6 (4) 80%	1 (1) 20%	2 (2) 40%	3 (2) 40%
Systemic Reactogenicity	1 (1) 20%	0 (0) 0%	1 (1) 20%	0 (0) 0%	0 (0) 0%	0 (0) 0%
Laboratory Abnormalities	1 (1) 20%	1 (1) 20%	2 (1) 20%	3 (2) 40%	1 (1) 20%	4 (2) 20%
Unsolicited AEs	2 (2) 40%	12 (5) 100%	15 (5) 100%	1 (1) 20%	9 (4) 80%	10 (4) 80%
Severity and Relationship						
Grade 1	5 (4) 80%	10 (5) 100%	15 (5) 100%	4 (2) 40%	3 (3) 60%	7 (4) 80%
Related to Pfs25 or Pfs230	5 (4) 80%	4 (3) 60%	9 (4) 80%	2 (1) 20%	2 (2) 40%	4 (2) 40%
Grade 2	2 (2) 40%	5 (3) 60%	7 (4) 80%	1 (1) 20%	9 (4) 80%	10 (4) 80%
Related to Pfs25 or Pfs230	0 (0) 0%	0 (0) 0%	0 (0) 0%	0 (0) 0%	0 (0) 0%	0 (0) 0%
Grade 3	0 (0) 0%	1 (1) 20%	1 (1) 20%	0 (0) 0%	0 (0) 0%	0 (0) 0%
Related to Pfs25 or Pfs230	0 (0) 0%	0 (0) 0%	0 (0) 0%	0 (0) 0%	0 (0) 0%	0 (0) 0%
Grade 4	0 (0) 0%	0 (0) 0%	0 (0) 0%	0 (0) 0%	0 (0) 0%	0 (0) 0%
SAE	0 (0) 0%	0 (0) 0%	0 (0) 0%	0 (0) 0%	0 (0) 0%	0 (0) 0%

Each field has the format # (X) %, where # is the number of adverse events, X is the number of subjects with one or more episodes of the given event, and % is the number of participants with one or more episodes of the given event divided by the total number of participants who received the study agent (N) multiplied by 100.

Safety analysis of the high dose of Pfs25M-EPA/Alhydrogel®, 47µg of Pfs25M and high dose of Pfs230D1M-EPA/Alhydrogel, 40µg of Pfs230D1M, is still blinded. Overall the in the blinded cohort, the majority of reported AEs have been mild (Grade 1; 708/1431; 49%) with the most commonly report AEs being injection site pain, headache, malaria, neutropenia, nasopharyngitis, and rhinitis. The most commonly reported related AEs have been injection site reactogenicity (pain, induration, pruritus, and edema), leukopenia, neutropenia, and headache, which have been all Grade 1 or 2.

3.3.2.2 Immunogenicity of Pfs25M-EPA/Alhydrogel® and Pfs230D1M-EPA/Alhydrogel® in Healthy Malian Adults

Evaluation of immunogenicity and functional activity for the low dose, unblinded cohorts (Cohort 1, 2) have been completed (**Figure 14**) and were previously summarized in **Section 3.1.4.2** and **3.2.2.2.**

3.4 Rationale for Fractional Dosing

Dosing sparing by fractional-dose vaccination has been a concept explored in many vaccine fields, including yellow fever, polio, and malaria. In the first ever conducted controlled human malaria infection (CHMI) evaluating vaccines based on the RTS,S antigen, one study group (receiving RTS,S/AS02 at 0, 1, 7-month schedule; WRMAL-003) who received a reduced third immunization dose (fifth of the standard 0.5mL dose) showed high levels of vaccine efficacy

against homologous sporozoite challenge, with six out of seven subjects protected.[25] The 0, 1, 7-month schedule, with a fractional dose delivered as the third immunization which had achieved this high level of protection had never been repeated again until recently (Malaria-071) when the study was reproduced with similar high levels of vaccine efficacy against homologous CHMI seen.[25]

Building off results seen in WRMAL-003, the biological hypothesis going into Malaria-071 was that the delayed fractional dose had allowed a better qualitative immune maturation in the germinal center, with antigen selection of the B cells harboring surface immunoglobulin with highest antigen affinity. Preliminary analyses showed again high efficacy in the fractional dose group (4/30 subjects developed parasitemia; vaccine efficacy: 86.7% [95% CI: 66.8; 94.6; p<0.0001]) compared to standard doses given at 0, 1, 2-months (6/16 subjects developed parasitemia; vaccine efficacy: 62.5% [95% CI: 29.4; 80.1; p=0.0009]). To note all 12 control subjects (no vaccine) developed parasitemia confirming the validity of the malaria challenge. Malaria-071 was not powered to detect superiority of the fractional dosing group over the standard dosing group, but the study did show that protection against *P. falciparum* parasitemia in the fractional dose group represented an increase in VE over the standard doses assesses as a reduction in the proportion of unprotected subjects, of 64.4% [95% CI: -7.9; 88.3], p=0.0741, Fisher's exact; difference in time to parasitemia p=0.0455, logrank). Additionally, in Malaria-071, anti-CS antibody GMCs at all timepoints from 3 weeks to 6 months post-Dose 3 were similar when RTS,S/AS01$_B$ was given at 0, 1, 7- months with a fractional 0.1 mL dose at month 7 (range 75.7-32.8 EU/mL) and when given at 0, 1, 2-months (range 110.1-34.6 EU/mL). Preliminary immunological analyses of serum samples from subjects participating in Malaria-071 suggest that the fractional delayed dose does lead to the production of antibodies with higher antigen avidity as compared to a classical immunization regimen with three full doses.

In the follow-up phase of the Malaria-071 study, subjects who were protected in the initial challenge were randomized to receive a fractional fourth dose or no fourth dose, before being exposed to a second sporozoite challenge approximately six months after the initial challenge. In the absence of a booster dose, efficacy had waned at the time of the rechallenge (subjects from fractional dose group: 42.9% [95% CI: -8.5, 69.9], p=0.1923; subjects from standard dose group: 20.0% [95% CI: -24.0, 48.4], p=0.4545). Boosting with a fractional dose was shown to maintain high protection in subjects in the fractional dose group (90.0% [95% CI: 35.8, 98.4]; p=0.0009). Reduced protection was observed following fractional dose boosting in subjects in the standard dosing group (25.0% [95% CI: -32.1, 57.4]; p=0.4000). The fractional dose boosting was shown to reverse susceptibility to infection in the first challenge to protection against re-challenge in 4 out of 5 subjects who were infected in the primary/challenge phase. The results from the study follow-up phase and the second challenge suggest that there is waning immunity with the fractional schedule too but that the protection can be extended with an additional fractional dose.

These results from the Malaria-071 challenge study may indicate a simple way to improve the functional activity or protective efficacy of a malaria vaccine candidate and requires further assessment in the endemic population under conditions of natural exposure. Building off this observation and similar observations seen in yellow fever and polio vaccination, we plan to assess the impact of a fractional third dose in our TBVs in the field.

3.5 Rationale for Long Term Immunogenicity Follow-up (Pilot/Sotuba) and Evaluation of Fourth Vaccination (Main/Bancoumana-Doneguebougou)

3.5.1 Long Term Immunogenicity Follow-up (Pilot/Sotuba)

The pilot phase (n=65) of this trial (#17-I-N006) completed follow up per protocol in December 2017/January 2018. The pilot group was designed to assess the safety and reactogenicity of increasing dosages of either Pfs25 alone, Pfs230 alone, or Pfs25 co-administered with Pfs230. Safety data, as summarized below, overall indicated that the TBV alone or in combination at low and high dosages were safe and tolerable which allowed the main study group to be enrolled.

3.5.1.1 Safety Results

3.5.1.1.1 Pfs25M-EPA/AS01 alone

Overall vaccinations in Arm 1a (low dose of Pfs25M, 16µg) and Arm 1b (high dose of Pfs25M, 47 µg) have been well tolerated with few adverse events (AEs) reported overall and the majority of which have been mild (Grade 1; Arm 1a: 21/23, 91%; Arm 1b: 24/30, 80%). One Grade 3 AE in Arm 1a (gastroenteritis) has been reported and determined not related to vaccination.

3.5.1.1.2 Pfs230D1M-EPA/AS01 alone

Overall vaccinations in Arm 2a (low dose of Pfs230D1M, 13µg) and Arm 2b (high dose of Pfs230D1M, 40 µg; dose used in the Activity Main Phase of the study) have also been well tolerated. Most of the related AEs reported have been mild (Grade 1; Arm 2a: 23/29, 79%; Arm 2b: 42/51, 82%) with the majority being injection site pain. One subject in Arm 2a and three subjects in Arm 2b have experienced four Grade 3 events (neutropenia, gastritis, respiratory tract infection, malaria), all of which were determined not related to vaccination.

3.5.1.1.3 Pfs25M-EPA/AS01 and Pfs230D1M-EPA/AS01

Overall vaccinations in Arm 3a (low dose of Pfs25M, 16µg and low dose of Pfs230D1M, 13µg) and Arm 3b (high dose of Pfs25M, 47µg and high dose of Pfs230D1M, 40µg), have been well tolerated with majority of AEs reported being mild (Grade 1; Arm 3a: 25/26, 96%; Arm 3b: 57/64, 89%) and with the most commonly reported AEs being local site injection abnormalities (47/90, 52%). To note, the frequency of local reactogenicity reported is increased compared to other Arms, given in these Arms two vaccinations are given at each vaccination time point, one in each arm. Two subjects in Arm 3b reported moderate (Grade 2) injection site pain following receipt of

Vaccination #1, but all other reported local site injection reactions have been Grade 1. No Grade 3 AEs have been reported.

3.5.1.1.4 Engerix-B (comparator)

Overall vaccinations for Arm 4a and Arm 4b (Engerix-B) have been well tolerated. The majority of AEs reported have been mild (Grade 1; 54/67, 81%). A single subject was diagnosed with pulmonary tuberculosis (previously reported SAE; Grade 3; not related to vaccination) and has since completed TB treatment and fully recovered. Two additional Grade 3 AEs have been reported, both malaria AEs, not related to vaccination.

In summary, vaccinations in all Arms of the Safety Pilot Phase thus far have been well tolerated. The most common vaccine related AE seen in the TBV arms have been expected mild local reactogenicity post vaccination that appears on the day of vaccination or one-day post vaccination and resolves within 2-3 days. As expected solicited systemic reactogenicity (such as headache, arthralgia, fatigue) are reported and usually are Grade 1 (mild) and transient in presentation. Laboratory abnormalities appear, with the majority being transient, asymptomatic Grade 1 (mild) and Grade 2 (moderate) neutropenias. In the TBV Arms, a few Grade 3 AEs (5 out of 45 subjects, 11%) have been reported overall (versus 3 out of 20 subjects in the comparator arm, 15%), all of which have been determined by the investigator as not related to study product/procedures. As expected, given the time of year, the number of malaria cases has increased overall post Vaccination #3.

3.5.1.2 Immunogenicity and Functional Activity

In the unblinded Safety Pilot Phase, immunogenicity results (ELISA) have been completed through study day 224 (**Figure 18;** 8 weeks post Vaccination #3) and functional activity (standard membrane feeding assays; SMFA) have been completed through study day 112 (**Figure 19**; 12 weeks post Vaccination #2).

Figure 18. Antibody responses to Pfs25 and Pfs230 at low and high dosage and with single and co-administration dosing obtained 8 weeks post vaccination #3.

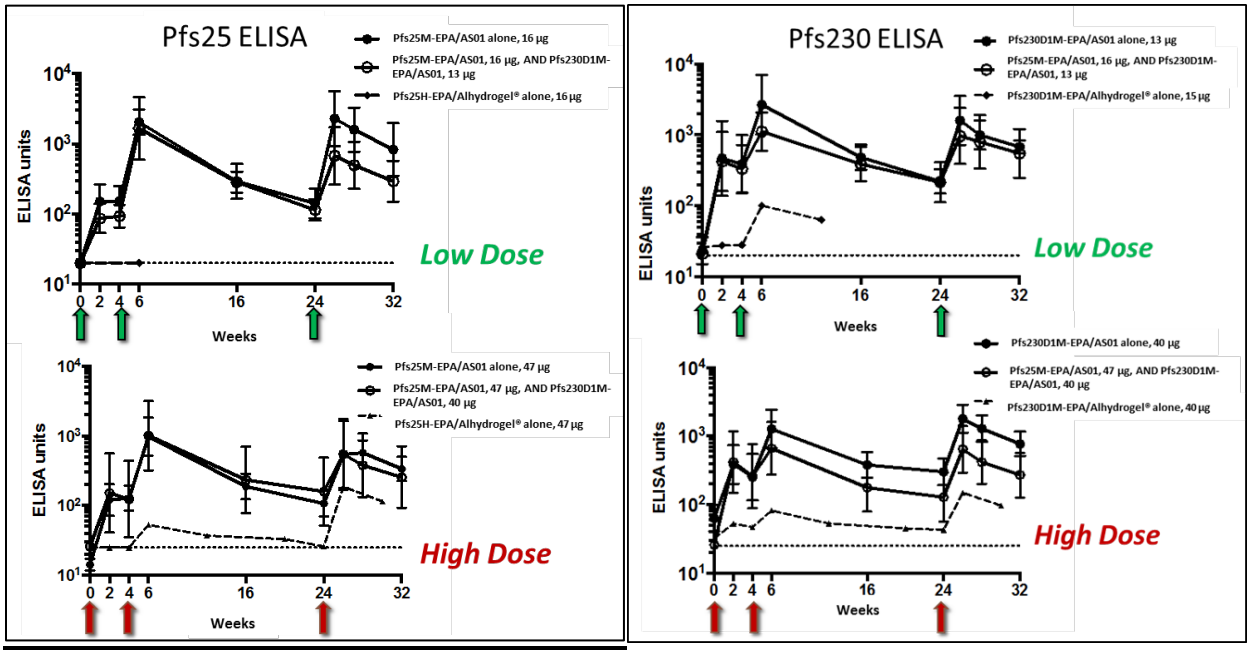


Figure 18. Antibody responses to Pfs25 and Pfs230 at low and high dosage and with single and co-administration dosing. Samples obtained 14 days and then at least monthly following receipt of vaccination. Vaccinations received at 0, 1, 6 months. Each individual data point represents the ELISA geometric mean value with black bars represent 95% confidence intervals. Comparison group (dashed lines) is the same dosage used with the Alhydrogel® adjuvant from NIAID protocol 15-I-0044.

As expected, no pre-existing immunity was seen prior to vaccination in Malian adults to Pfs25. Most subjects in all Arms receiving Pfs25M (Arm 1a: low dose of Pfs25M, 16µg and Arm 1b: high dose of Pfs25M, 47µg and Arm 3a: low dose of Pfs25M, 16µg and low dose of Pfs230D1M, 13µg and Arm 3b: high dose of Pfs25M, 47µg and high dose of Pfs230D1M, 40µg) responded to the vaccine following Vaccination #1, which is significantly different from the lack of antibody responses seen with the same dosage of Pfs25M but with the Alhydrogel® adjuvant. Antibody responses continued to increase with the second vaccination, but were the same or slightly less after the third vaccination. There was no difference in the antibody responses to Pfs25 between Arm 1a versus Arm 1b, nor between those who received the combination of Arm 3a versus Arm 1a and Arm 3b versus Arm 1b, but the overall trend was lower antibody responses to Pfs25 were seen in those individuals that received the combination dose (Arms 3a, 3b) than those who received Pfs25M alone (Arms 1a, 1b).

Consistent with previous findings, a small percentage of subjects who received Pfs230D1M vaccinations had pre-existing antibodies to the Pfs230D1M vaccine antigen. Similar to Pfs25, most subjects in all Arms receiving Pfs230D1M (Arm 2a: low dose of Pfs230D1M, 13µg and Arm 2b: high dose of Pfs230D1M, 40µg and Arm 3a: low dose of Pfs25M, 16µg and low dose of Pfs230D1M, 13µg and Arm 3b: high dose of Pfs25M, 47µg and high dose of Pfs230D1M,

40µg) responded after one vaccination. Antibody responses were similar between those who received the low dose (Arm 2a) and the high dose (Arm 2b) of Pfs230D1M alone. And as seen with Pfs25M, overall lower antibody responses to Pfs230 were seen in those individuals that received the combination dose (Arms 3a, 3b) than those who received Pfs230D1M alone (Arms 2a, 2b).

Standard membrane feeding assays (SMFA) have been completed in Arms 2a and 2b and associated comparator Arms (Arm 4a, 4b) at two timepoints post Vaccination #2 (2 weeks and 12 weeks) to date. As seen in **Figure 19**, significant functional activity, including transmission blocking activity, can be seen in the Pfs230D1M alone Arms at 2 weeks and persisting even at 12 weeks post Vaccination #2. This is a significant improvement from the lack of activity seen with the same TBV product with the Alhydrogel® adjuvant (NIAID protocol #15-I-0044).

Figure 19: Functional activity of Pfs230 vaccinees by SMFA at 2 weeks and 12 weeks post Vaccination #2 in the open label safety cohort.

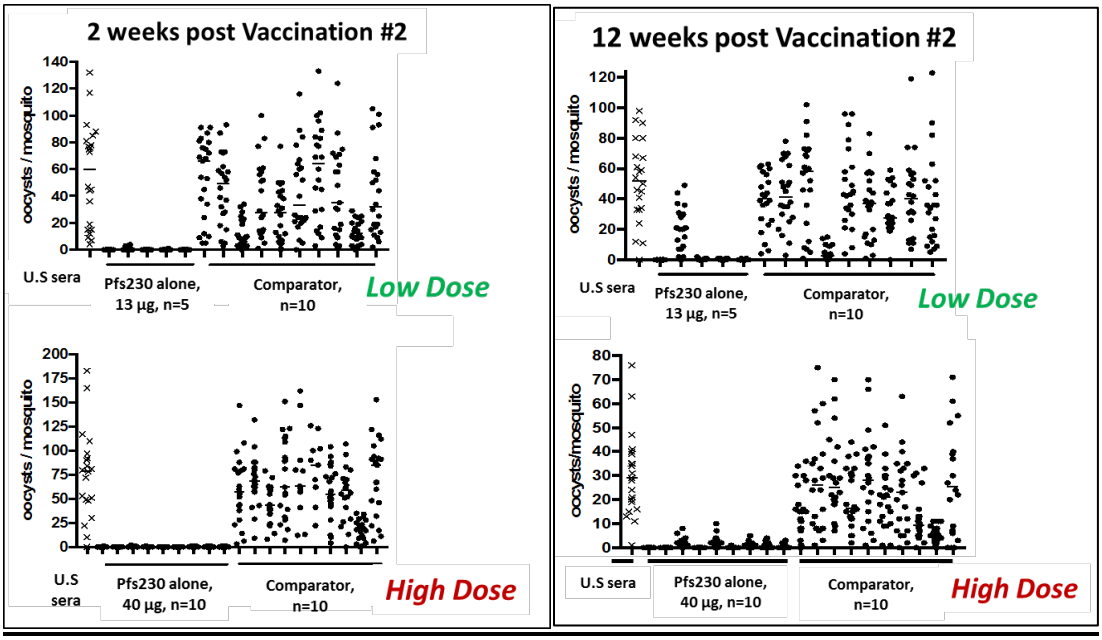


Figure 19. Functional activity of Pfs230 vaccinees by SMFA at 2 weeks and 12 weeks post Vaccination #2 in the open label safety cohort. Samples obtained 14 days and then at least monthly following receipt of vaccination, including at 12 weeks post vaccination #2. Vaccinations received at 0, 1, 6 months. Each individual data point represents an individual mosquito dissection in the SMFAs with the black bars representing the geometric mean oocyst count per mosquito.

Looking at the kinetics of anti-Pfs25 and anti-Pfs230 antibodies, although there is increased durability with the addition of AS01, the rate of decay is unknown. To answer this question, the vaccinated subjects in the pilot arm and some from the main arms will be invited for additional blood draws at 9 and 12 months to evaluate the kinetics of these antibodies.

3.5.2 Evaluation of Fourth Vaccination (Main/Bancoumana-Doneguebougou)

3.5.2.1 Safety

In the blinded Activity Main Phase, most AEs reported have been mild or moderate with most AEs being unrelated to vaccination (1830/2336, 78%). Of the related AEs (506/2336,22%), the majority have been mild (Grade 1; 423/506, 84%), with the most commonly report related AEs being local site reactogenicity (369/506, 73%; Grade 1: 324/369, 88%; Grade 2: 45/369, 12%) and mild (Grade 1) solicited systemic reactogenicity (e.g. headaches, fever, myalgias, arthralgias). Local injection site pain did not appear to increase in severity or frequency with subsequent vaccinations, but following Vaccination #2 the occurrence of other local site reactogenicity (edema, pruritus, impairment of movement, redness) appeared to be reported more frequently, but not post Vaccination #3.

Various laboratory abnormalities have been noted, though most of them have been mild (Grade 1) and transient. A few Grade 2 (moderate) neutropenias and leukopenias have been reported. But no laboratory abnormalities have been reported in the frequency or intensity meeting further evaluation or study halt. As expected, given the time of year, the number of malaria cases has increased overall post Vaccination #3.

A total of thirty-seven Grade 3 AEs have been reported (malaria, snake bite, pyrexia, bronchitis,systolic hypertension,), all determined as not related to vaccination. A single SAE (snake bite) was previously reported and has resolved completely.

In summary, vaccinations have been well tolerated with no identified safety concerns at this time, but safety will be further assessed upon scheduled, intentional unblinding.

3.5.2.2 Immunogenicity and Functional Activity

ELISA and SMFA results are currently pending for the Activity Main Phase, which remains blinded at this time; however, the Pfs230D1M-EPA/AS01 dosing regimen (0, 1, 6 months) and dosage (40µg) assessed in the main study was also evaluated on a smaller scale during the pilot study. As presented in Section 3.5.1.2, immunological data from the pilot group have been encouraging. Results to date have aligned with what was seen in preclinical studies, that adjuvanting with AS01 does lead to significantly higher and more durable antibody responses, as determined by ELISA against Pfs25 and Pfs230 (**Figure 18**), as well as antibody responses developing with fewer vaccine doses.

For a vaccine to play a vital role in blocking malaria transmission, the vaccine will have to be active for more than one transmission season. While increasing the durability of the vaccine by adjuvant is one method, another is boosting at the beginning of a transmission season. In order to explore the effect of boosting, vaccinated participants from the main functional activity arms (Arm 2c and 2d: Pfs230D1M; Arm 4c: comparator) will be invited to receive a 4th vaccination (Arm 2c and 2d: full dose Arm 4c: Menactra®) prior to the next transmission season.

The dose of the fourth vaccination (full versus fractional dosing of Pfs230D1M-EPA/AS01) has been determined after scheduled, intentional unblinding and associated safety review and analysis of anti-Pfs230 ELISA and SMFA results completed in May 2018. There was no significant difference in safety between the Arms. The rates of clinical malaria (Full vs Control p= 0.75; Fractional vs Control p=0.87 and Full vs Fractional p=0.71) and blood smear positivity (Full vs Control p= 0.82; Fractional vs Control p=0.82 and Full vs Fractional p=0.60) were also not clinically significant between the Arms.

A summary of immune responses and functionality results are provided below in **Figure 20** and **Figure 21**. In brief, while there was a significant difference between the full and fractional arms detected by antibody responses to Pfs230 (as seen in **Figure 20** with p=0.00014), functional activity by SMFA showed no significant difference between the two groups (p=0.0597, **Figure 21**). The similarity in SMFA responses may be due to antibody titers remaining elevated above a possible threshold that may provide high levels of TRA at 12 weeks post-vaccination. These levels would be expected to decrease over time allowing for analysis of the difference of the effectiveness of the two doses at later timepoints. With no clearly superior regimen evident after a third vaccination with either a full or a fractional dose of Pfs230, we will plan to assess a full boosting dose in both Arms 2c and 2d. This approach was also recommended in recent discussion with the DSMB.

Figure 20 : Antibody responses to Pfs230 at 12 weeks post-vaccination #3 with either full or fractional dosing

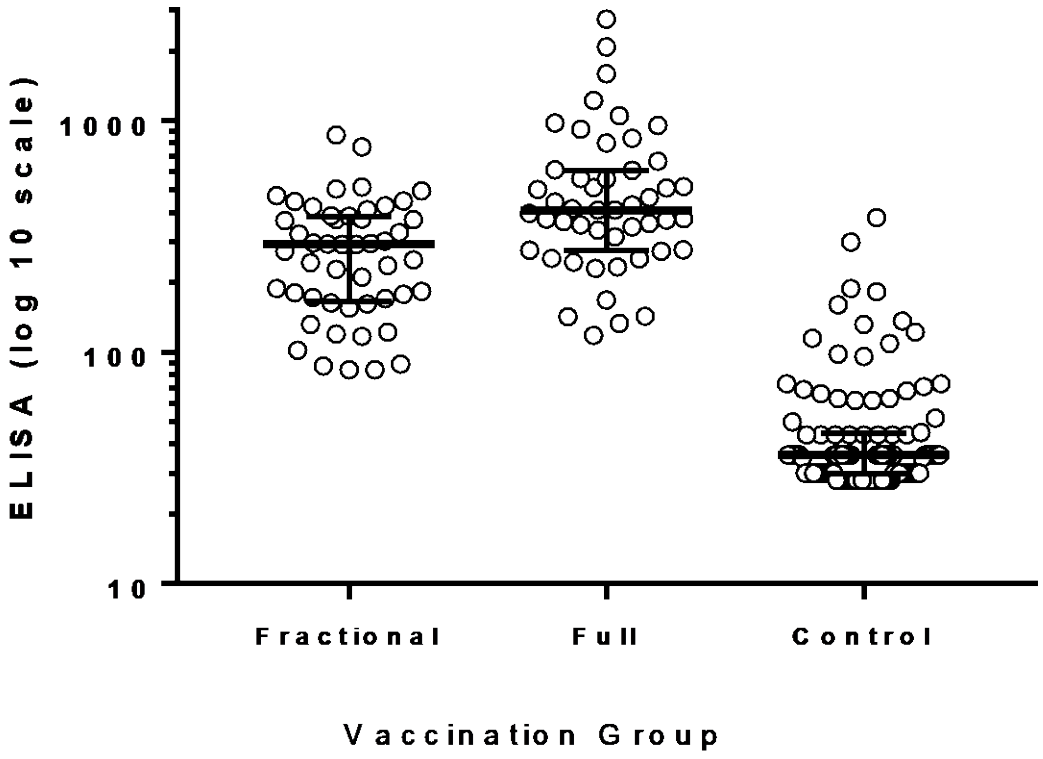


Figure 20: **Antibody responses to Pfs230 at 12 weeks post-vaccination #3 with either full of fractional dosing.** Participants in the fractional dosing group (Arm 2d) received 8µg Pfs230 for their third vaccination, while full dosing (Arm 2c) group received 40µg Pfs230 for their third vaccination. Both groups received 40µg Pfs230 for their first and second vaccinations. Wilcoxon p values for comparisons between arms are as follows: Full vs. Fractional: $p=0.00014$; Full vs. Control: $p<0.0001$; Fractional vs. Control: $p<0.0001$.

Figure 21: Functional activity of Pfs230 by SMFA at 12 weeks post-vaccination #3 with either full or fractional dosing

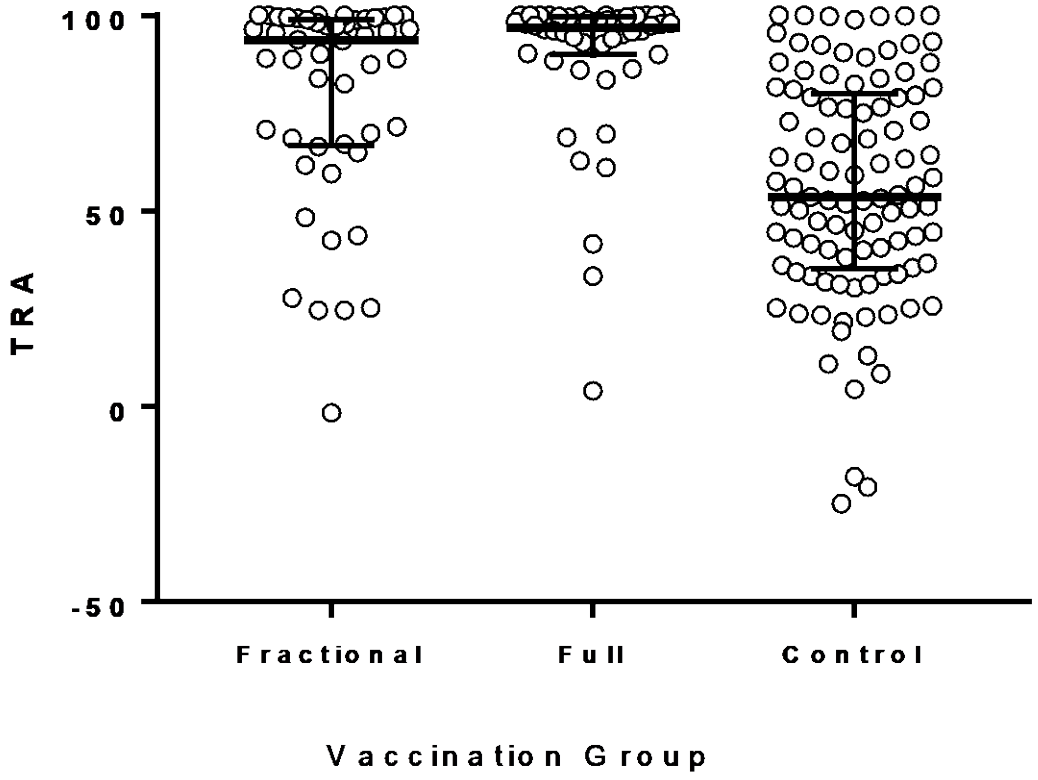


Figure 21: Functional activity of Pfs230 by SMFA at 12 weeks post-vaccination #3 with either full or fractional dosing. Participants in the fractional dosing group (Arm 2d) received 8µg Pfs230 for their third vaccination, while full dosing group (Arm 2d) received 40µg Pfs230 for their third vaccination. Both groups received 40µg Pfs230 for their first and second vaccinations. Wilcoxon p values for comparisons between arms are as follows: Full vs. Fractional: $p=0.0597$; Full vs. Control: $p<0.0001$; Fractional vs. Control: $p<0.0001$.

3.6 Clinical Development Plan

LMIV has a mandate to develop vaccines that will interrupt malaria transmission, and therefore will be useful for malaria elimination campaigns. Vaccines can target either preerythrocytic (PE) malaria parasites to prevent human infection, or can target sexual stage malaria parasites to prevent transmission to mosquitoes (TBV), in order interrupt malaria transmission.

LMIV has the most advanced program for clinical development of TBV. TBV are intended to interrupt malaria transmission by producing antibodies in humans that block parasite development in mosquitoes. The immunological data from a Phase 1 Pfs25H-EPA/Alhydrogel® study in the US and in Mali have shown a consistent increase in Pfs25 antibody response by

ELISA following each subsequent vaccination, but these responses appear short lived. Data from these studies also have shown that increasing antibody titers correlated with increased functional transmission reducing activity in SMFA. Ongoing studies in the US and Mali have thus far determined that adding another transmission blocking target, Pfs230D1M, either alone or in combination with Pfs25M is safe and immunogenic. The immunological data from a Phase 1 Pfs25M-EPA/ Alhydrogel® and Pfs230D1M-EPA/ Alhydrogel® study in the US and Mali has likewise shown a consistent increase in Pfs25 and Pfs230D1M antibody response by ELISA following each subsequent vaccination that correlate with functional activity with SMFA. Specifically, vaccine regimens containing Pfs230 can achieve high functional activity by SMFA, however the duration of this activity is still to be determined. Additionally, preclinical studies of both Pfs25M and Pfs230D1M have shown that formulation with AS01 adjuvant induces significantly higher and longer lived antibody by ELISA titers, and significantly greater transmission blocking activity by SMFA, when compared to the existing formulations in Alhydrogel® (**Figure 2**).

In parallel, we will evaluate the vaccine antigen Pfs230D1M, at full dosing and at fractional dosing, when formulated with AS01 for its ability to induce antibodies that prevent parasite transmission to mosquitoes. Vaccine activity will be assessed by testing sera in SMFA and by direct skin feeds to measure the reduction of parasite transmission in vaccinated individuals versus controls.

If evidence from these initial trials indicate that Pfs230D1M has measurable efficacy/activity, we envision subsequent trial(s) of a pre-erythrocytic vaccine (such as RTS,S/AS01$_B$; adult formulation of Mosquirix™) in combination with Pfs230D1M formulated in AS01 to assess their combined activity for interrupting malaria transmission in naturally exposed populations.

A step forward in this clinical plan is to assess the durability of TBV vaccine responses with the adjuvant AS01. As described in Version 7.0 of the protocol, given the promising immunogenicity and functional activity results from the unblinded pilot study (including the same vaccination regimen and dose utilized in the main study) and the reassuring safety data from the currently blinded main study, the plan is to 1) continue to monitor and evaluated the durability of vaccine specific antibody responses and associated functional activity in both the pilot and main study; 2) assess the safety, immunogenicity, and functional activity over subsequent malaria transmission season following receipt of an additional vaccination.

4 Study Objectives

4.1 Primary Objective

- To assess safety and reactogenicity of Pfs25M-EPA/AS01, Pfs230D1M-EPA/AS01, and simultaneous administration of Pfs25M-EPA/AS01 and Pfs230D1M-EPA/AS01, including Pfs230D1M-EPA/AS01 at fractional dosing in Malian adults. (All arms)

- To assess safety and reactogenicity of a booster dose of Pfs230D1M-EPA/AS01 at full dosing in Malian adults. (Arms 2c, 2d, 4c)

4.2 Secondary Objective

- To assess the antibody response to the Pfs25 and Pfs230 protein as measured by ELISA. (All arms)
- To assess the functional antibody response to the Pfs25 and Pfs230 protein as measured by standard membrane feeding assay (SMFA). (All arms except Arms 1a, 2a, 3a)
- To assess the functional antibody response to Pfs230 protein as measured by direct skin feeds (DSF). (Arms 2c, 2d, 4c only)

4.3 Exploratory Objectives

- To explore cellular and transcriptomic responses to Pfs25 and Pfs230 vaccines when administered alone or in combination. (All arms except 1a, 2a, 3a)
- To explore the impact of co-infections on malaria vaccine responses. (Arms 2c, 2d, 4c only)
- To explore the antibody repertoire of functional antibody responses. (All arms except 1a, 2a, 3a)

5 Study Design

5.1 Overall Design

This is a phase 1 study designed to evaluate safety, reactogenicity, immunogenicity, and transmission-blocking activity (via SMFA)) of Pfs25M-EPA and Pfs230D1M-EPA conjugates formulated on AS01 and transmission-blocking activity (via DSF) of Pfs230D1M-EPA conjugates formulated on AS01 at full and fractional dosing. We will enroll a total of 305 vaccinated subjects to be divided between multiple sites in Mali as shown in **Appendix A** and described below.

In Bamako/Sotuba, Mali (N=65)

Dose-escalating, open label, randomized, pilot study

1. Group 1: Pfs25M-EPA/AS01 (n=15)

• ***Arm 1a*** (n=5), to receive 16 µg Pfs25M-EPA/AS01 on D0, D28, D168

• ***Arm 1b*** (n=10), to receive 47 µg Pfs25M-EPA/AS01 on D0, D28, D168

2. Group 2: Pfs230D1M-EPA/AS01 (n=15)

• ***Arm 2a*** (n=5), to receive 13 µg Pfs230D1M-EPA/AS01 on D0, D28, D168

• ***Arm 2b*** (n=10), to receive 40 µg Pfs230D1M-EPA/AS01 on D0, D28, D168

3. Group 3: Pfs25M-EPA/AS01 and Pfs230D1M-EPA/AS01 (n=15)
• ***Arm 3a*** (n=5), to receive 16 µg Pfs25M-EPA/AS01 and 13 µg Pfs230D1M-EPA/AS01 on D0, D28, D168
• ***Arm 3b*** (n=10), to receive 47 µg Pfs25M-EPA/AS01 and 40 µg Pfs230D1M-EPA/AS01 on D0, D28, D168

4. Group 4: Comparator (n=20)
• ***Arm 4a*** (n=10), to receive ENGERIX-B on D0, D28, and D168
• ***Arm 4b*** (n=10), to receive ENGERIX-B on D0, D28, and D168

In Bancoumana and Doneguebougou, Mali (N=240)
Double-blind, randomized, phase 1 clinical trial

1. Group 2: Pfs230D1M-EPA/AS01 (n=120)
• ***Arm 2c*** (n=60), to receive 40 µg Pfs230D1M-EPA/AS01 on D0, D28, D168, and a fourth dose of 40 µg on D476
• ***Arm 2d*** (n=60), to receive 40 µg Pfs230D1M-EPA/AS01 on D0, D28, then 8 µg Pfs230D1M-EPA/AS01 (*fractional dose*) on D168, and a fourth dose of 40 µg on D476

2. Group 4: Comparators (n=120)
• ***Arm 4c*** (n=120), to receive ENGERIX-B on D0, D28, and D168 (start study with Arm 2c and 2d), and Menactra® on D476.

The multisite study in Mali is designed in a stepwise manner such that each dose of the TBVs is administered in a dose-escalating fashion, and each vaccine (either Pfs25M-EPA/AS01 or Pfs230D1M-EPA/AS01) is administered first as a single vaccination at the target dose before it is administered simultaneously. Additionally, two doses of each vaccine will be administered and assessed for safety as outlined in **Section 12.14.4** and **Appendix A** prior to implementation of the first vaccination in the double blind portion of the study.

5.1.1 Sotuba/Bamako Pilot Study

The open-label, dose-escalating pilot study is designed to evaluate vaccine safety and reactogenicity in healthy Mali adults living in a low malaria transmission setting (Sotuba, a suburb of Bamako, Mali). Subjects enrolled in the initial safety cohort will receive a single dose of a single antigen (either Pfs25M or Pfs230D1M) prior to vaccinating new enrolled subjects who will receive the co-administration of Pfs25M and Pfs230D1M. Additional subjects (n=10 per arm; Arms 1b, 2b, 3b, 4a, 4b) will be enrolled to receive the targeted vaccine dose for further safety and immunogenicity evaluation as outlined in **Appendix A**. Data from subjects enrolled in Arms 1b, 2b, 3b, 4a, and 4b will additionally contribute to functional activity via SMFA analyses

and will be used for exploratory objectives examining the antibody repertoire of functional antibody responses to vaccination. No blinding will be implemented, but comparators will be enrolled to control for variable natural blocking activity that may present during the rainy season and as safety controls. Randomization will occur in Arms 1b, 2b, 3b, 4a, 4b. Arms 1a, 2a, 3a will enroll in the study on an available basis.

5.1.2 Bancoumana and Doneguebougou Main Study

The double-blind, randomized, comparator controlled portion of the study is designed to also evaluate study vaccine safety and reactogenicity in healthy malaria-exposed individuals, but is powered as well to assess for immunogenicity and functional activity via SMFA and DSF.

Following scheduled, intentional unblinding at study day 336, subjects from the main study will be offered re-enrollment into the open label fourth vaccination.

5.2 Study Endpoints

5.2.1 Primary Endpoint

- Incidence of local and systemic AEs and SAEs in Malian adults. (All arms)
- Incidence of local and systemic AEs and SAEs in Malian adults. (Arms 2c, 2d, 4c)

5.2.2 Secondary Endpoints

- Anti-Pfs25 IgG levels as measured by ELISA. (All arms)
- Anti-Pfs230 IgG levels as measured by ELISA. (All arms)
- TRA/TBA of induced antibody in SMFA. (All arms except 1a, 2a, 3a)
- Transmission interruption activity measured by DSF. (Arms 2c, 2d, 4c only)

5.2.3 Exploratory Endpoints

- Cellular immune responses to vaccination. (All arms except 1a, 2a, 3a)
- Whole genome transcriptional profiling. (All arms except 1a, 2a, 3a)
- Antibody levels against recombinant EPA, and other malaria antigens, such as Pfs48/45, expressed during the gametocyte development. (All arms except 1a, 2a, 3a)
- Schistosomiasis detection (in urine). (Arms 2c, 2d, 4c only)
- qPCR for helminth detection (from stool). (Arms 2c, 2d, 4c only)
- Sequence B cell receptor/antibody genes. (All arms except 1a, 2a, 3a)

5.3 Sample Size and Estimated Duration of Study

A total of 305 subjects will be vaccinated in this trial. Note: more subjects may be initially enrolled, receive drug treatment (Coartem®) prior to vaccination, but no more than 305 subjects

will receive at least one vaccine. Each subject will be monitored actively for at least 6 months after the last vaccination, for a total of 12 to 16 months, depending on the vaccination schedule and timing of screening for the study. Up to 900 subjects will be screened to accommodate possible screening failures.

Participants who had previously been enrolled in the study (up to 301) will be re-enrolled for an additional 6 to 18 months depending on arm assignment and timing of reconsent.

Definitions for the purpose of this study:

- Screened – subjects will receive a study identification number when the informed consent is signed and will either be determined as "enrolled" or "screen failures" as noted below.
    - Screening may be completed over the course of multiple visits.
    - Screening, in most cases, will occur within 56 days prior to enrollment into the study
        - If the screening visit is >56 days prior to enrollment, then an updated medical review, physical exam/vital signs, repeat Malaria Comprehension Exam, and repeat laboratory evaluation (inclusive of safety labs, HIV, Hepatitis B, C) will be completed to determine eligibility for enrollment.
- Enrolled – subject will be considered enrolled beginning with the receipt of the first vaccination or at time completion of randomization.
- Randomized – the subjects are considered randomized when they meet the following criteria:
    - Confirmation that the inclusion/exclusion criteria are met.
    - Randomization number assigned. Note, randomization number may be the same as the study identification number.
- Screen Failures – subjects are considered screen failures when they meet 1 of the following criteria after signing consent:
    - Screening results reveal that the subject is ineligible per **Section 6.4** and/or **6.5**.
    - Subject withdraws consent before being vaccinated and/or randomized
- Discontinued – subject are considered discontinued when they meet 1 or more of the following criteria:
    - Subject withdraws consent after being vaccinated and/or randomized.
    - Subject is withdrawn by the PI/Sponsor after being vaccinated and /or randomized.

- Completed -- subjects are considered completed when they complete the final study visit for their arm.
- Re-enrolled – subject will be considered re-enrolled beginning with signing of the re-enrollment consent form.

6 Study Population

6.1 Description of Population and Sites

6.1.1 Rationale for Research Subject Selection

Healthy volunteers will be recruited for this study.

6.2 Clinical Sites

The study will be carried out in collaboration between the LMIV/NIAID/NIH and the MRTC in Bamako, Mali. The study will be conducted by the MRTC at three locations in Mali, West Africa (see **Map 1).** The MRTC staffing at the Mali site is employed by NIAID/USTTB programs and assigned to the Sotuba/Bamako, Bancoumana, and Doneguebougou sites for the duration of the study to provide clinical care and execute the protocol.

Map 1: Map Showing the Three Study Sites

6.2.1.1 Sotuba/Bamako Site

Bamako is the capital of Mali, located on the Niger river and has a population of about 1.7 million people. The district of Bamako is divided into urban (hypoendemic) and periurban (mesoendemic) areas and peripheral villages (hyperendemic). Sotuba is a village located on the outskirts of Bamako on the bank of the Niger River, consisting of ~6,500 inhabitants. Malaria transmission follows the same seasonality as in Bancoumana and Donéguébougou, although entomological inoculation rates are historically much lower. The annual rainfall varies between 800 mm and 1000 mm and occurs from June to October. Many clinical trials (malaria vaccine and drug trials), as well as epidemiological and entomologic malaria studies, have been done in Sotuba. As in Donéguébougou and Bancoumana, the MRTC maintains a medical clinic and laboratory in Sotuba and has been working at this site since 1993.

To note, if any issues arise prohibiting starting the study within Bamako and/or Sotuba, this phase of the study (as outlined in **Appendix A**) can be completed in either Bancoumana or Doneguebougou.

6.2.1.2 Bancoumana and Doneguebougou Site

Bancoumana is located 60 kilometers southwest of Bamako and has a population of about 9,000 people. The site is situated in the south-Sudanian area of Mali. The climate is hot, with daily temperatures ranging from 19°C to 40°C. The annual rainfall varies between 600 mm and 1200 mm and occurs from June to October. Many clinical trials, as well as epidemiological and entomologic malaria studies, have been done in Bancoumana.[26-28]

Doneguebougou is a community located 30 km north of Bamako and has a population of about 2,000, with another 2,000 inhabitants in the surrounding villages. For the purpose of vaccine trials, adequate facilities for conducting an interventional trial have been put in place at Doneguebougou within walking distance to the residents' homes. At Doneguebougou, the malaria transmission is highly seasonal, with the transmission season taking place from June until December. Doneguebougou is situated in a high transmission area, with entomological inoculation rates (determined by human landing catch) as high as 137 to 167 infectious bites per person over 1 transmission season.

Healthy adult subjects will be recruited from the surrounding community and villages, and the demographics of the study population will therefore be representative of that community.

6.3 Recruitment

Community permission will be obtained from village elders and other community members in Sotuba/Bamako, Bancoumana, and Doneguebougou after explanation and discussion of the study at a community meeting (see **Section 15.2.1**). A general announcement inviting household and

family members to the participating clinic to learn about the study will be made at the time of community permission, using local radio or any traditional channel of communication.

6.4 Inclusion Criteria (Initial enrollment and Re-enrollment)

All of the following criteria must be fulfilled for a volunteer to participate in this trial:

1. Age ≥ 18 and ≤ 50 years. (***Note for re-enrollment: ≤ 52 years***)
2. Available for the duration of the trial.
3. Able to provide proof of identity to the satisfaction of the study clinician completing the enrollment process.
4. In good general health and without clinically significant medical history in the opinion of the investigator.
5. Females of childbearing potential must be willing to use reliable contraception (as defined below) from 21 days prior to Study Day 0 (Study Day 476 for re-enrollment) and then until 3 months after last vaccination. (***Arms 2c, 2d, 4c only for re-enrollment)***
   - Reliable methods of birth control include **one** of the following: confirmed pharmacologic contraceptives (parenteral) delivery; intrauterine or implantable device.
   - Reliable methods of birth control include concurrent use of a pharmacologic and a barrier method, i.e. **two** of the following: confirmed pharmacologic contraceptives (oral, transdermal) delivery or vaginal ring **AND** condoms with spermicide or diaphragm with spermicide.
   - Non-childbearing women will also be required to report date of last menstrual period, history of surgical sterility (i.e. tubal ligation, hysterectomy) or premature ovarian insufficiency (POI), and will have a baseline urine or serum pregnancy test performed.
6. Willingness to have blood samples stored for future research.
7. Willingness to undergo DSFs (***Arms 2c, 2d, 4c only***).
8. Known resident of Bancoumana or Doneguebougou or surrounding area or known student or long term resident (more than 1 year) of Bamako/Sotuba, Mali

6.5 Exclusion Criteria (Initial enrollment and Re-enrollment)

A subject will be excluded from participating in this trial if any one of the following criteria is fulfilled:

1. Pregnancy as determined by a positive urine or serum human choriogonadotropin (β-hCG) test (***if female***). (***Arms 2c, 2d, 4c only for re-enrollment)***

   NOTE: Pregnancy is also a criteria for discontinuation of any further dosing or non-safety related interventions for that subject.
2. Currently breast-feeding (***if female***). (***Arms 2c, 2d, 4c only for re-enrollment)***

3. Behavioral, cognitive, or psychiatric disease that in the opinion of the investigator affects the ability of the participant to understand and comply with the study protocol.
4. Hemoglobin, WBC, absolute neutrophils, and platelets outside the local laboratory-defined limits of normal (subjects may be included at the investigator's discretion for 'not clinically significant' values outside of normal range and ≤ Grade 1). (***Arms 2c, 2d, 4c only for re-enrollment)***
5. Alanine transaminase (ALT) or creatinine (Cr) level above the local laboratory-defined upper limit of normal (subjects may be included at the investigator's discretion for 'not clinically significant' values outside of normal range and ≤ Grade 1). (***Arms 2c, 2d, 4c only for re-enrollment)***
6. Infected with human immunodeficiency virus (HIV), hepatitis C virus (HCV), or hepatitis B (HBV). (***Arms 2c, 2d, 4c only for re-enrollment)***
7. Evidence of clinically significant neurologic, cardiac, pulmonary, hepatic, endocrine, rheumatologic, autoimmune, hematological, oncologic, or renal disease by history, physical examination, and/or laboratory studies including urinalysis.
8. History of receiving any investigational product within the past 30 days.
9. Participation or planned participation in a clinical trial with an investigational product prior to completion of the follow up visit 28 days following last vaccination OR planned participation in an investigational vaccine study until the last required protocol visit
10. Subject has had medical, occupational, or family problems as a result of alcohol or illicit drug use during the past 12 months.
11. History of a severe allergic reaction or anaphylaxis. (***Arms 2c, 2d, 4c only for re-enrollment)***
12. Severe asthma, defined as asthma that is unstable or required emergent care, urgent care, hospitalization, or intubation during the past 2 years, or that has required the use of oral or parenteral corticosteroids at any time during the past 2 years. (***Arms 2c, 2d, 4c only for re-enrollment)***
13. Pre-existing autoimmune or antibody-mediated diseases including but not limited to: systemic lupus erythematosus, rheumatoid arthritis, multiple sclerosis, Sjögren's syndrome, or autoimmune thrombocytopenia. (***Arms 2c, 2d, 4c only for re-enrollment)***
14. Known immunodeficiency syndrome. (***Arms 2c, 2d, 4c only for re-enrollment)***
15. Known asplenia or functional asplenia. (***Arms 2c, 2d, 4c only for re-enrollment)***
16. Use of chronic (≥14 days) oral or intravenous corticosteroids (excluding topical or nasal) at immunosuppressive doses (i.e., prednisone >10 mg/day) or immunosuppressive drugs within 30 days of Study Day 0. (***Arms 2c, 2d, 4c only for re-enrollment)***
17. Prior to Study Day 0 and every subsequent vaccination day, receipt of a live vaccine within the past 4 weeks or a killed vaccine within the past 2 weeks. (***Arms 2c, 2d, 4c only for re-enrollment)***

18. Receipt of immunoglobulins and/or blood products within the past 6 months. (***Arms 2c, 2d, 4c only for re-enrollment)***
19. Previous receipt of an investigational malaria vaccine in the last 5 years. (For re-enrollment – does NOT include receipt of Pfs230D1M-EPA/AS01) (***Arms 2c, 2d, 4c only for re-enrollment)***
20. History of severe reaction to mosquito bites (***Arms 2c, 2d, 4c only)***
21. History of allergy to the comparator vaccine (***Initial enrollment: such as latex, yeast, or previous Hepatitis B vaccine***) (***Re-enrollment: Arm 4c only; previous meningococcal vaccine components, diptheria toxoid or CRM_{197} containing vaccine***)
22. History of Guillain-Barre syndrome (***Re-enrollment: Arm 4c only***)
23. Known allergies or contraindications (such as significant cardiac disease; prolonged QTc >450 ms; currently taking medications that may prolong your QTc; serious side effects from Coartem® in the past) to study treatment (Coartem® [artemether/lumefantrine]) (***Arms 2c, 2d, 4c only***) (***Initial enrollment only***)
24. Other condition that in the opinion of the investigator would jeopardize the safety or rights of a participant participating in the trial, interfere with the evaluation of the study objectives, or would render the subject unable to comply with the protocol.

6.6 Justification for Exclusion of Pregnant Women

This study will not enroll pregnant and/or breastfeeding women since the effects of Pfs25M-EPA/AS01 and Pfs230D1M-EPA/AS01 vaccines on the developing human fetus are unknown with the potential for teratogenic or abortifacient effects. Because this is an unknown but potential risk for adverse events in nursing infants secondary to treatment of the mother with Pfs25M-EPA/AS01 and Pfs230D1M-EPA/AS01 women that are breastfeeding will also be excluded from the study.

6.7 Justification for Exclusion of Children

This study will not enroll children, since appropriate dosing or safety have not yet been established in adults.

7 Study Agents

7.1 Transmission Blocking Vaccines (Pfs25M-EPA/AS01, Pfs230D1M-EPA/AS01)

7.1.1 Pfs25M-EPA/AS01

The PpPfs25M and EcEPA lots, both manufactured at Walter Reed Bioproduction facility (Silver Spring, Maryland) in cGMP compliance, were used to manufacture the conjugate. PpPfs25M is a *Pichia*-expressed recombinant Pfs25 with a molecular mass of 18,713 Daltons. EcEPA is an *E. coli*-expressed recombinant protein with molecular mass of 66,975 Daltons. The Pfs25M-EPA conjugate was produced by reaction between thiolated PpPfs25M and maleimide-activated

EcEPA, followed by purification using size-exclusion chromatography. The Pfs25M-EPA conjugate was manufactured at Walter Reed Bioproduction facility in cGMP compliance in January 2015.

The Pfs25M-EPA was formulated as conjugated Pfs25M in 4 mM PBS to a 2X dilution of the high dose (188µg/mL in 0.5mL volume) in cGMP compliance at Walter Reed Bioproduction facility in April 2016 and will be provided as a single use vial. AS01$_B$ adjuvant will also be provided as a single use vial by GSK, with a concentration of 100µg/mL MPL and 100 µg/mL QS21 in a liposomal formulation in a volume of 0.625mL. For each dose of vaccine, the two single-use vials, one of conjugated Pfs25M in 4mM PBS, and one of AS01$_B$, will be mixed 1:1 at bedside. A dilution of the conjugated Pfs25M in 4mM PBS with normal saline will be performed prior to admixing with AS01$_B$ for the 16 µg dose. For the 47µg dose, the AS01$_B$ will be mixed directly with the vialed conjugated Pfs25M in 4mM PBS.

Dosing volumes of 0.5 mL will be prepared for delivery of either 16µg conjugated Pfs25M, 15µg conjugated EPA in diluted AS01$_B$ (25µg MPL+25µg QS21 liposomal formulation); or 47µg conjugated Pfs25M, 4 µg conjugated EPA in diluted AS01$_B$ (25µg MPL+25µg QS21 liposomal formulation). The vaccine will be mixed and drawn up into the syringes directly prior to injection.

7.1.2 Pfs230D1M-EPA/AS01

The PpPfs230D1M and EcEPA lots, were also both manufactured at Walter Reed Bioproduction facility (Silver Spring, Maryland) in cGMP compliance, were used to manufacture the conjugate. PpPfs230D1M is a *Pichia*-expressed recombinant a sub-segment (S542-G736) of Pfs230 with a molecular mass of 21,854 Daltons. EcEPA is an *E. coli*-expressed recombinant protein with molecular mass of 66,975 Daltons. The Pfs230D1M-EPA conjugate was produced by reaction between thiolated PpPfs230D1M and maleimide-activated EcEPA, followed by purification using size-exclusion chromatography. The Pfs230D1M-EPA conjugate was manufactured at Walter Reed Bioproduction facility in cGMP compliance in May 2015.

The Pfs230D1M-EPA was formulated as conjugated Pfs230D1M in 4 mM PBS to a 2X dilution of the high dose (160µg/mL in 0.5 mL volume) in cGMP compliance at Walter Reed Bioproduction facility in April 2016 and will be provided as a single use vial. AS01$_B$ adjuvant will also be provided as a single use vial by GSK, 100µg/mL MPL and 100µg/mL QS21 in a liposomal formulation in a volume of 0.625 mL. For each dose of vaccine, the two single-use vials, one of Conjugated Pfs230D1M in 4mM PBS, and one of AS01$_B$, will be mixed 1:1 at bedside. A dilution of the conjugated Pfs230D1M in 4 mM PBS with normal saline will be performed prior to admixing with AS01$_B$ for the 13µg. For the 40µg dose, the AS01$_B$ will be mixed directly with the vialed conjugated Pfs230D1M in 4 mM PBS. For the 8µg fractional dose, the AS01$_B$ will be mixed with Pfs230D1M directly as noted above for the 40µg dose, but only 1/5th (0.1 mL) of the volume will be administered.

Dosing volumes of 0.5mL will be prepared for delivery of either 13µg conjugated Pfs230D1M, 10µg conjugated EPA in diluted $AS01_B$ (25µg MPL+ 25µg QS21 liposomal formulation); or 40µg conjugated Pfs230D1M, 31µg conjugated EPA in diluted $AS01_B$ (25µg MPL+ 25µg QS21 liposomal formulation). Dosing volume of 0.1mL (fractional dose) will be prepared for delivery of 8µg conjugated Pfs25M, 6µg conjugated EPA in AS01 (5µg MPL+ 5µg QS21 liposomal formulation). The vaccine will be mixed and drawn up into the syringes directly prior to injection.

7.1.3 Disposition and Dispensation

The Pfs25M-EPA and Pfs230D1M-EPA vials will be supplied to the study site pharmacist by the Sponsor or Sponsor Representative. The Sponsor receives the product from Pilot Bioproduction Facility, WRAIR, Silver Spring, Maryland, where the materials were formulated and packaged. Vaccines will be labeled for investigational use only. The $AS01_B$ vials will be supplied by GSK.

7.1.4 Formulation, Packaging, and Labeling

Pfs25M-EPA was manufactured at Walter Reed Bioproduction facility in cGMP compliance in April 2016. Each single-use vial contains 188µg/mL conjugated Pfs25M, 180µg/mL conjugated EPA in 4mM PBS in a volume of 0.5 mL. The vial label reads: 188µg/mL Conjugated Pfs25M in 4mM PBS.

Pfs230D1M-EPA was manufactured at Walter Reed Bioproduction facility in cGMP compliance in April 2016. Each single-use vial contains 160µg/mL conjugated Pfs230D1M, 124µg/mL conjugated EPA in 4mM PBS in a volume of 0.5mL. The vial label reads: 160µg/mL Conjugated Pfs230D1M in 4mM PBS.

AS01 is a liposome-based Adjuvant System containing the immunoenhancers MPL (3-Odesacyl-4'-monophosphoryl lipid A) and QS21 (a saponin molecule purified from the bark extract of Quillaja saponaria Molina tree). There are two formulations in the AS01 family: $AS01_B$ Adjuvant System and $AS01_E$ Adjuvant System. While $AS01_B$ contains 50µg of MPL and 50µg of QS21 per one human dose, $AS01_E$ contains half the quantity of each immunoenhancer, i.e. 25 µg of MPL and 25 µg of QS-21. For this study, the $AS01_B$ adjuvant will be used at a diluted concentration similar to the concentration of $AS01_E$, but not same formulation as $AS01_E$, thus the designation of AS01 or diluted $AS01_B$ throughout the protocol.

7.1.5 Study Agent Storage and Shipping

Pfs25M-EPA and Pfs230D1M-EPA must be stored at -80°C until time of transport to field site. At this time, vaccine can gradually thaw from -80°C to 9°C on day of use. Vaccine can not be refrozen for use after a thaw. $AS01_B$ must be stored at +2°C to +8°C. Vials will be transported and stored at temperature-controlled conditions, as per SOPs. Temperature data loggers will

accompany the vaccines at all times to ensure storage temperatures limits have not been violated. Adjuvant should NOT be frozen at any time. Refrigerator and freezer temperatures will be continuously monitored. Access to study vaccine will be limited to authorized study personnel. Any temperature excursion outside the defined range must be reported to the Sponsor prior to use. The impacted products must not be used and must be stored in quarantine at label temperature conditions until usage approval has been obtained from the Sponsor.

7.1.6 Preparation, Administration, and Dosage of Study Agents

The TBV conjugates are stored at -80C until just before transport to the field for use. Then stored 2-8°C as described in Section **7.1.5**. AS01 adjuvant are stable in the refrigerator at +2°C to +8°C. The final vaccine for administration is obtained by admixing Pfs25M-EPA or Pfs230D1M-EPA with AS01 and has to be injected intra-muscularly within 4 hours of reconstitution. The formulation of the study vaccine is presented in **Table 12**.

Table 12: TBV Formulation

Vaccine	Product Name	Formulation	Volume to be administered	Number of doses	Number of booster dose
TBV/AS01 (Low dose): Arms 1a, 2a, 3a	Pfs25M-EPA (Arm 1a, 3a)	Pfs25M = 16µg EPA = 15µg	0.5 mL	3 doses	0 doses
	Pfs230D1M-EPA (Arm 2a, 3a)	Pfs230D1M = 13µg EPA = 10µg			
	AS01B (Arms 1a, 2a, 3a)	AS01 = 25µg MPL+ 25µg QS21 liposomal formulation			
TBV/AS01 (Targeted dose): Arms 1b, 2b, 3b, 2c, 2d	Pfs25M-EPA (Arms 1b, 3b)	Pfs25M = 47µg EPA = 45µg	0.5 mL	Arm 1b, 3b: 3 doses	0 doses
	Pfs230D1M-EPA (Arms 2b, 3b, 2c, 2d)	Pfs230D1M = 40µg EPA = 31µg		Arm 2b, 2c, 3b: 3 doses Arm 2d: 2 doses	Arms 2c, 2d: 1 dose of 40µg
	AS01B (Arms 1b, 2b, 3b, 2c, 2d)	AS01 = 25µg MPL+ 25µg QS21 liposomal formulation			

TBV/AS01 (fractional dose): Arms 2d only	Pfs230D1M-EPA	Pfs230D1M = 8µg EPA = 6µg	0.1 mL	Arm 2d: 1 dose	
	$AS01_B$	AS01 = 5µg MPL+ 5µg QS21 liposomal formulation			

7.2 Comparator Vaccines

7.2.1 ENGERIX-B (Hepatitis B Vaccine)

ENGERIX-B (Hepatitis B [Recombinant] Vaccine; GlaxoSmithKline) is a sterile suspension for intramuscular administrations that contains inactivated, noninfectious hepatitis B virus surface antigen (HBsAg). The purified HBsAg is obtained by culturing genetically engineered *Saccharomyces cerevisiae* yeast cells, which carry the surface antigen gene of the hepatitis B virus. HBsAg is then adsorbed separately onto aluminum salts. A 1 mL dose of vaccine contains 20 µg of recombinant HBsAg protein, 0.5 mg of aluminum in the form of aluminum hydroxide. The vaccine is manufactured by GlaxoSmithKline. ENGERIX-B is FDA approved for the active immunization against disease caused by all known subtypes of hepatitis B virus in neonates, infants, children, and nonpregnant adults given at 0-, 1-, and 6-month schedule.

A 1 mL dose is recommended for adults aged 20 years or older at a 0-, 1-, and 6-month schedule. While 0.5 mL dose is the package insert recommended dosing for neonates, infants, children, adolescents, and young adults (neonates through 19 years of age), for consistency within the trial and given 1 mL at 0-, 1-, and 6-months is an approved alternate schedule for 11 through 19 years of age, all subjects enrolled in the study (18-50 years old) will receive 1mL ENGERIX-B at 0-, 1-, and 6-months.

Additional information is provided in the ENGERIX-B package insert provided.

For subjects who received a TBV during the study, the comparator vaccine will be offered at no cost to the participants. For Hepatitis B, this may be ENGERIX-B or another FDA approved or WHO certified Hepatitis B containing vaccine, such as TWINRIX.

7.2.2 Menactra®

Menactra® (Sanofi Pasteur) is a sterile, intramuscularly administered vaccine that contains *Neisseria meningitidis* serogroup A, C, Y, and W-135 capsular polysaccharide antigens individually conjugated to diphtheria toxoid protein. No preservative or adjuvant is added during the manufacturing process. Menactra® is FDA approved for active immunization to prevent invasive meningococcal disease caused by *Neisseria meningitidis* serogroups A, C, Y, and W-135 (but does not protect against serotype B) for use in individuals 9 months through 55 years of

age. A single dose (0.5 mL) is recommended for those individuals 18 to 50 years of age and otherwise healthy who are at increased risk for meningococcal disease (e.g., individuals in an epidemic or highly endemic country such as Mali).

Additional information is provided in the Menactra® package insert provided.

Subjects who received a 4th dose of TBV during the study will be offered Menactra® or an equivalent appropriate FDA approved or WHO certified *Neisseria meningitidis* vaccine at no cost to the participants.

7.3 Normal Saline

Sterile isotonic (0.9%) normal saline will be commercially procured in the US and shipped to Mali at ambient temperature. Normal saline will be used for diluting Pfs25M-EPA and Pfs230D1M-EPA prior to formulation with $AS01_B$ for the lower dose groups in Bamako, Mali.

7.4 Administration

Vaccines will be administered as intramuscular injections into the deltoid muscle. Arms may be alternated with successive vaccinations if a single vaccination is given. If simultaneous vaccinations are to be administered (two individual vaccinations at the same time), each vaccine is drawn up and delivered separately. When choosing an arm for the vaccine injection, clinicians should consider whether there is an arm injury, local skin problems such as scaring or rash, or significant tattoo that precludes administering the injection or will interfere with evaluating the arm after injection. In keeping with the MRTC practices and procedures, and good medical practice, acute medical care will be provided to subjects for any immediate allergic reactions or other injury resulting from participation in this research study.

Due to the variance in volume and appearance of the study product (Pfs230D1M-EPA/AS01) in comparison to the control vaccines (ENGERIX-B), opaque tape will be wrapped around the vaccine syringe(s) when being administered in the double blind study in Mali.

7.4.1.1 Contraindications to Vaccination

The following criteria should be checked prior to each immunization and are contraindications to further immunization:

- Hypersensitivity reaction following administration of the study vaccine.
- Positive urine or serum β-hCG test prior to vaccination.

Subjects will be encouraged to remain in the study for safety evaluation of doses already received and completed research visits for immunogenicity, functional activity, and protective efficacy if deemed safe by the PI.

7.4.1.2 Indications for Deferral of Vaccination

If any of the following AEs occurs at the time of the scheduled vaccination, the subject may be either vaccinated at a later date within the allowable visit window as specified in the protocol or withdrawn at the discretion of the Investigator:

- Oral temperature >37.5˚C at the time of vaccination will warrant deferral of immunization until fever resolves (within protocol-defined vaccination window).
- Any other condition that in the opinion of the Investigator poses a threat to the individual if immunized or that may complicate interpretation of the safety of vaccine following immunization.

Such individual(s) will be followed in the clinic until the symptoms resolve or the window for immunization expires. No further vaccination will be performed if the subject does not recover (temperature ≤37.5˚C and/or lack of symptoms) within the vaccination window, though subject, if well may proceed to receive additional vaccines at future vaccination visits at the PI's discretion. The subject will be monitored for safety and immunogenicity for 6 months after their last vaccination, unless the subject has withdrawn consent. If the subject does not receive the second or third vaccination some scheduled safety blood draws may or may not be performed at the discretion of the PI. Blood draws scheduled to measure immune responses and protective efficacy will be obtained if possible.

If the subject meets any of the above criteria for deferral on the day of first immunization, the Investigator may elect to exclude the subject from further participation in the study and that subject may be replaced. If the subject meets any of the above criteria for deferral on the day of second or third immunization, the Investigator may elect to include the subject in future vaccinations (if missed second) or continue to follow them per protocol without additional vaccinations for the remainder of the study. Subjects who miss vaccination #2 or #3 cannot be replaced.

7.5 Coartem®

Artemether/lumefantrine (Coartem®) is a licensed antimalarial in the US and Mali for treatment of uncomplicated malaria. It has an excellent safety profile and is widely used to treat malaria. Subjects who may have any contraindications to the use of these drugs will be excluded at screening. Coartem® will be dosed with food and administered over 3 days for a total of 6 doses, as per package insert and standard adult dosing.

All antimalarial medications used for the study, including Coartem® which is used for pre-emptive treatment of all subjects in Arms 2c, 2d, and 4c prior to Vaccination #1 will be maintained at the study site. Coartem® tablets will be purchased from commercial sources and provided by the MRTC study team to subjects. Drug accountability will be managed by the site clinical team.

7.5.1 Preparation and Administration

Coartem® will be provided as tablets for oral administration. Administration will be by study staff according to dosing parameters.

7.5.2 Storage and Handling

Coartem® tablets will be maintained in the manufacturer's original packaging and stored at the clinic under recommended storage conditions until prepared for dispensing.

7.5.3 Return of Study Product

Final accountability of drug supplies will be performed at the conclusion of the study. Final disposition of any remaining Coartem® will be determined and documented.

7.6 Prohibited Medications and Procedures

Treatment with any of the following medications/procedures could potentially interfere with vaccine-induced immunity and will not be permitted. Use of any of these during the study may exclude a subject from receiving further doses of the study vaccine. However, the subject will be encouraged to remain in the study for safety evaluations.

- Licensed killed vaccines in the two week period prior to and following each vaccination or licensed live vaccines in the four week period prior to and following each vaccination.
- Receipt of immunoglobulins and/or any blood products up to 6 months prior to the first vaccination and for 30 days after administration of the last dose of vaccine.
- Chronic oral or intravenous administration (≥30 days) of immunosuppressive doses of steroids, i.e., prednisone >10 mg per day, immunosuppressants, or other immune-modifying drugs from each day of vaccination to 2 weeks following each vaccination.
- Any investigational drug or investigational vaccine other than the study vaccine during the study period.
- Required surgical removal of the spleen or the development of a hematologic or other disease that would interfere with normal immunity.

Over-the-counter medications, such as acetaminophen or ibuprofen, may be used to help relieve symptoms from vaccination and are not considered prohibited.

Use of antimalarial medications or antibiotics that have antimalarial activity administered during the study period is not exclusionary, but will be documented by clinical staff and will be taken into consideration during data analysis.

7.7 Vaccine Accountability

After administration of a vaccine dose, the single-dose vials of antigen and adjuvant will be accounted for according to the site standard operating procedures and in agreement with the study IND Sponsor for appropriate monitoring.

Accurate inventory and accountability record of vaccine supplies for this study will be maintained by the study site pharmacist (or designee). Partially used vials may not be administered to other subjects.

8 Study Schedule

The study schedule and approximate amounts of blood drawn are summarized in **Appendix B** and detailed in **Appendix C**.

8.1 Screening Procedures

8.1.1 For Initial Enrollment (2016/2017)

The purpose of the screening visit is to determine subject eligibility for study participation. Screening procedures include the informed consent process, Malaria Comprehension Exam, laboratory assessments (completed within 56 days of first vaccination), and clinical assessments. Screening activities can occur over multiple visits if necessary, including the day of enrollment.

In the event that a chronic illness and/or HIV, HBV, or HCV is discovered during the course of screening, long-term treatment and care will not be reimbursed by the study, but referral for continuing care can be provided to subjects.

Per national requirements for reporting communicable diseases, confirmed positive test results for HIV, HBV, and HCV will be reported to the local health department according to applicable laws and appropriate medical referrals initiated.

The following screening evaluations must be completed for all subjects within the 56 days prior to first vaccination:

- Explain the study and informed consent document to the subject.
- Ensure the subject has acknowledged consent by signing or fingerprinting the informed consent document. Ensure that the subject receives a signed copy of the informed consent.
- Ensure the subject has correctly answered ≥80% of the questions (see **Section 15.2.1.2**) on the Malaria Comprehension Exam.
- Elicit a complete medical history, including menstrual and contraceptive history and/or history of surgical sterility for females, and medication use.

- Females of childbearing potential must be willing to use reliable contraception from at least 21 days prior to first vaccination through three months after the last vaccination.
- Administer a complete physical examination, including vital signs (height, weight, blood pressure, temperature, and heart rate).
- HIV pre- and post-test counseling as indicated including follow-up contact with subject to report the results and referral for appropriate medical care if indicated.
- Obtain approximately 10 mL of blood for complete blood count (CBC) with differential and platelet count, ALT, creatinine (Cr), hepatitis B surface antigen, hepatitis C antibody, and HIV antibody.
- Obtain urine (or serum) for pregnancy testing (for females) and urinalysis/urine dipstick for protein and blood.
- Obtain a 12-lead electrocardiogram (EKG) (**for Arms 2c, 2d, and 4c subjects only**)

If screening laboratories are completed within ≤ 2 days prior to Study Day 0, these clinical laboratory values (CBC with differential, ALT, Cr) may be used for Study Day 0 (day of first vaccination) assessments and do not need to be repeated.

8.1.2 Rescreening for Re-enrollment (2018)

The purpose of the re-screening visit is to determine subject eligibility for continued study participation. Screening procedures include the informed consent process, laboratory assessments (for Arm 2c, 2d, 4c only on study day 364) and clinical assessments. Rescreening activities can occur over multiple visits if necessary, including the day of re-enrollment.

For Arms 2c, 2d, 4c, in the event that a chronic illness and/or HIV, HBV, or HCV is discovered during the course of rescreening, long-term treatment and care will not be reimbursed by the study, but referral for continuing care can be provided to subjects.

Per national requirements for reporting communicable diseases, confirmed positive test results for HIV, HBV, and HCV will be reported to the local health department according to applicable laws and appropriate medical referrals initiated.

The following screening evaluations must be completed for all subjects upon re-enrollment into the study:

- Explain the study and informed consent document to the subject.
- Ensure the subject has acknowledged consent by signing or fingerprinting the informed consent document. Ensure that the subject receives a signed copy of the informed consent.
- Elicit a complete medical history, including menstrual and contraceptive history and/or history of surgical sterility for females, and medication use.

- Females of childbearing potential must be willing to use reliable contraception from at least 21 days prior to first vaccination through three months after the last vaccination. (***not required for re-enrollment of the pilot study***)
- Administer a complete physical examination, including vital signs (height, weight, blood pressure, temperature, and heart rate).
- HIV pre- and post-test counseling as indicated including follow-up contact with subject to report the results and referral for appropriate medical care if indicated. (***not required for re-enrollment of the pilot study***)
- Obtain approximately 10 mL of blood for complete blood count (CBC) with differential and platelet count, ALT, creatinine (Cr), hepatitis B surface antigen, hepatitis C antibody, and HIV antibody. (***not required for re-enrollment of the pilot study***)
- Obtain urine (or serum) for pregnancy testing (for females) and urinalysis/urine dipstick for protein and blood. (***not required for re-enrollment of the pilot study***)

Note, for re-enrollment, rescreening procedures will be completed >56 days prior to vaccination #4 as subjects enrolled in this study in Arms 2c, 2d, 4c have already been screened for HIV, Hep B, Hep C prior as well as had clinical labs monitored on multiple occasions over the course of their study participation in the last year. Given the background rate of these diseases in the community, the likelihood of a new infection with HIV, Hep B, Hep C is low. In addition repeat clinical labs will be completed prior to vaccination #4 and those with clinically significant findings will not be vaccinated or will have their vaccination delayed.

8.2 Assignment to Groups

For the dose-escalating, open label, randomized, pilot study in Sotuba/Bamako, the study will be randomized and then enrolled on a first available basis in the following manner:

1. Arms 1a and 2a, n=10; then
2. Arm 3a, n=5; then
3. Arms 1b, 2b, 4a; n=30; then
4. Arms 3b, 4b; n=20

For the field site (Bancoumana and Doneguebougou), the study will be enrolled in the following manner in a double-blind, en-bloc randomization and stratification by village within each of the following groups:

1. Arms 2c, 2d, 4c, n=240

Enrollment and randomization of subjects into the TBV portion of the double blind, randomized study in Bancoumana and Doenguebougou, Mali, as specified above and in **Section 14.6**, will

not occur until at least two vaccinations (Pfs25M and Pfs230D1M) have been administered to the corresponding arms in the Bamako/Sotuba, Mali (see **Appendix A**).

Enrollment occurs with receipt of the first vaccination. Subjects who are screened but not enrolled and/or randomized may be replaced.

8.3 Study Subject Schedule

Day to day study schedule will be completed as outlined in **Appendix B** and **Appendix C**.

9 Study Procedures/Evaluations

9.1 Photographs of Rash or Injection Site Reactions

If a subject develops a rash or injection site reaction, photographs may be taken by the investigators. These photographs will not include the subject's face or any identifying scars, marks, or tattoos.

9.2 Clinical Laboratory Testing

Using standard techniques, the clinical laboratory will perform the following tests:

1. Complete blood count (CBC) plus white blood cell differential and platelet count.
    - The following CBC parameters will be assessed for safety throughout the trial: white blood cell count (WBC), absolute neutrophil count (ANC)/absolute granulocyte count (AGC), hemoglobin (Hgb), and platelet count (plt).
2. Serum creatinine (Cr).
3. Alanine aminotransferase (ALT).
4. HBsAg test (can include rapid diagnostics, ELISA, PCR if indicated).
5. HCV test (can include rapid diagnostics, ELISA, PCR if indicated).
6. HIV test (can include rapid diagnostics, ELISA, Western Blot if indicated).
7. Urine dipstick/Urinalysis (at screening only).
8. Urine and/or serum pregnancy testing (β-hCG).

Schistosomiasis testing (urine microscopic examination for *S. haematobium* and stool PCR for *S. mansoni*) and helminth testing (stool PCR for 8 common gastrointestinal parasite pathogens) will be completed in subjects enrolled in Arms 2c, 2d, 4c on Study Day 0. Test results will be available to the clinical trial sites, and if subjects did not receive the mass drug administration as expected since testing, individuals will be treated.

Urine microscopy for *S. haematobium* will be completed on site and results made available to the study team and subjects in real time. Subjects who test positive for *S. haematobium* will be offered treatment with praziquantel upon diagnosis. Stool results for helminth testing, which are being conducted on a research basis, will be provided retrospectively to the study site once completed, which may be after the completion of the study, and will be handled on a case by case basis for treatment indications of asymptomatic carriage. Positive results from either of these tests (stool or urine) will not be exclusionary for enrollment.

9.3 Malaria Diagnostics

9.3.1 Blood Smears

The gold standard for malaria diagnosis and evaluation of vaccine efficacy endpoints is the detection of malaria parasites on Giemsa-stained thick blood films. Giemsa stained thick and thin films will be examined for asexual and sexual parasites in the MRTC clinical laboratory, as outlined in **Appendix B**. Blood smears are prepared in duplicate according to standard procedures and evaluated by trained study microscopists.

For detection of gametocytemia, each blood smear is read by two independent trained microscopists and is reported per 1,000 WBC. A positive gametocyte read is defined as a single, confirmed gametocyte seen by one reader and confirmed by the other microscopist per 1,000 WBC.

Thick blood smears may be prepared from the blood remaining in the IV cannula, or (at time points when no IV blood collection is planned) from a finger prick or venous blood sample at the subject's request.

Thin/thick blood smears will be used for diagnosis throughout the study.

9.3.1.1 Symptomatic Malaria

Clinical or symptomatic malaria for this study is defined as the presence of asexual *P. falciparum* parasites at any parasitemia with either an axillary temperature of ≥37.5 °C or more of the following symptoms: headache, myalgia, arthralgia, malaise, nausea, dizziness, or abdominal pain and will be reported as an AE.

9.3.2 Rapid Diagnostics

For unscheduled visits and clinical diagnostics, rapid diagnostic tests may be utilized for determination of an acute malarial illness; however, they cannot be used in place of microscopy for determination of parasitemia status for DSF.

9.3.3 Malaria PCR

While detection of parasites on thick blood smears remains the most common primary endpoint in human challenge trials, both PCR- and nucleic acid sequence-based amplification (NASBA)-based methods have been increasingly used to support blood smear data in malaria vaccine trials [29,30]. These research molecular assays have significantly increased sensitivity for detection of *P. falciparum* blood-stage infection approaching 20 parasites/mL, often resulting in diagnosis 2-4 days earlier than by paired thick blood smears. [31-33] Quantification of parasite density by these methods allows evaluation of parasite growth curves for assessing the utility of partially-effective vaccine candidates. LMIV has also developed a research qPCR that detects 18s of *P. falciparum* with a detection limit of at least 500 parasites/mL that will be used during the study for comparison to traditional thick blood smears.

P. falciparum qPCR may be performed from all scheduled visits with a malaria blood smear noted (see **Appendix B**) to capture infections that remain below the detection limit for microscopy. For subject convenience, a finger prick sample can be used for both preparation of the microscopy slide and for DNA preservation, but venous blood draws can also be used.

9.3.4 Malaria Clinical Management

In accordance with the Malian Government treatment guidelines, subjects will be treated with approved anti-malarial treatment, such as artemether/ lumefantrine (Coartem®, 80mg/480 mg per dose) or artesunate/amodiaquine (ASAQ; fixed dose artemisinin based combination therapy) that is indicated and approved for the management of uncomplicated *P. falciparum* malaria. Clinical or symptomatic malaria for this study is defined in **Section 9.3.1.1**.

9.4 Immunologic Laboratory Testing

9.4.1 Antibody Assay

Anti-Pfs25, anti-Pfs230 ELISAs, anti-CSP, and anti-HBsAg will be performed on sera or plasma obtained from immunized subjects at LMIV in Bethesda, Maryland and may also be performed at collaborating laboratories.

For Pfs25 and Pfs230, briefly, microwell plates are coated with antigen solution. Plates are washed with TRIS-buffered saline (TBS) containing Tween-20 (T-TBS) and blocked with TBS containing skim milk powder. After washing with T-TBS, diluted serum samples are added in triplicate and incubated at room temperature for 2 hours. After incubation, unbound antibodies are removed by washing the plates with T-TBS, and alkaline phosphatase-conjugated goat anti-human IgG solution is added to each well and incubated for 2 hours at room temperature. Plates are then washed with T-TBS, followed by adding phosphatase substrate solution to each well; the plates are then covered and incubated for 20 minutes at room temperature for color development. The plates are read immediately at 405 nm with a microplate reader. The optical

density values are used to determine antibody levels by comparing to a standard curve generated from a known positive-control plasma included on each ELISA plate.

Additionally, the magnitude and kinetics of IgG responses may be explored over time post Vaccination #3. Quality of antibody responses may be explored via antibody avidity as well as assessment of antibody subclasses to provide useful insight into the humoral immune response.

9.4.2 B-Cell and T-Cell Assays

Specimens collected for B-cell and T-cell studies will undergo initial processing and cell separation at the clinical site and will be transported to LMIV according to standard procedures.

B-cell studies will be done at LMIV and associated immunology laboratory partners (MVI PATH, Adaptive Biotechnologies, Atreca). The analysis of the generation and maintenance of antigen-specific memory B cells will be carried out to determine if these cells can be elicited and maintained by vaccination. Peripheral blood lymphocytes will be obtained and assayed for the presence of antigen-specific memory B cells, total number of memory B cells, and plasmablast responses using flow cytometry and ELISPOT assays.

T-cell studies will be performed at LMIV. Antigen-specific T-cell responses to vaccination will be determined by ELISPOT and/or intracellular cytokine staining flow cytometry.

Ex vivo studies will be performed at MRTC using whole blood to enumerate various immune subsets (T, B, NK cells) prior to and after each vaccination.

9.4.3 Transcriptional Profiling

Whole genome transcriptional profiling will be performed to explore possible gene expression profiles or pathways that predict optimal responses to vaccination and to determine if innate immune responses are sensitively reflected in the PBMC transcriptome shortly after vaccination. Gene expression profiling following vaccination will allow the predictive capacity of eventual high and low responders, and thus will assist in defining the correlates of protection induced by vaccination.

Transcriptional analyses will be performed on whole blood collected as outlined in **Appendix B**. Blood will be collected via venous puncture and placed in PAXGene tubes to preserve RNA integrity until the RNA is extracted. Specimens will be analyzed at the Research Technologies Branch, NIAID and/or the NIH Intramural Sequencing Center. The molecular profiling encompasses the identification of RNA transcripts present in all humans, which are induced or repressed after each vaccination. This does not represent genetic testing of individuals or their DNA.

9.4.4 Transmission-Blocking Assays/Evaluations

The transmission-blocking assays which will be conducted are summarized in **Table 13**.

Table 13: Transmission Blocking Assays

Assay	Mosquitoes	Test Samples	Site
Standard Membrane-Feeding Assay (SMFA)	Lab strain (*A. stephensi*)	Membrane feeds with lab cultured parasites mixed with test serum/plasma/IgG	LMIV LMVR
Direct Skin Feed (DSF)	MRTC lab colonies (*A. gambiae*)	Direct skin feeds on vaccinees	MRTC

Feeding assays demonstrate biologic activity of transmission-blocking antibodies, and are critical to selection of transmission-blocking vaccine candidates. Subjects will be screened periodically by blood smear (as seen in **Appendix B**) for the presence of asexual parasites and gametocytes. Priority for feeding assays (SMFA and DSF) will be conducted post Vaccination #3 when gametocyte carriage and parasite carriage rates are the highest and antibodies secondary to Pfs230D1M are expected to be peaking post vaccination.

Multiple feeds might be conducted on a single subject at the multiple screening time points, but a single subject will not undergo more than 32 DSFs (inclusive of the twice a week feeds during the intense DSF period following Vaccination #3 and #4) following a vaccination.

9.4.4.1 Standard Membrane-Feeding Assay

Membrane-feeding assays demonstrate biologic activity of transmission-blocking antibody and are critical to selection of vaccine candidates. SMFAs will be performed on blood obtained at baseline and periodically after vaccination as outlined in **Appendix B.** In a SMFA, test serum or plasma obtained from immunized subjects is mixed with parasites from a laboratory culture and the mixture is placed in a feeding cup covered with an artificial membrane. Pre-starved mosquitoes from a laboratory colony are allowed to feed through the membrane. A similar procedure is carried out on a malaria-naïve control serum at the same time using mosquitoes raised from the same laboratory colony. One week after the feed, mosquitoes are dissected and midguts are stained with mercurochrome for the oocyst form of the parasite. The reduction of the proportion of oocyst-laden mosquitoes or the reduction of average oocyst numbers per mosquito compared to mosquitoes fed on the control group demonstrate biologic function of the antibody, and may be predictive of efficacy in the field. SMFA results have been shown to correlate with ELISA antibody titers against Pfs25 in several species.[24] The SMFAs will be conducted at LMIV and LMVR in Rockville, Maryland.

At the time of the direct-feeding assays, venous blood may be collected from each subject as outlined in **Appendix B**. The blood aliquot will be processed immediately for SMFA. The

process will be maintained at approximately 37°C to avoid temperature-induced gametogenesis. SMFA will be conducted at LMIV using laboratory-strain mosquitoes and parasites. Assays will compare feedings with:

- Plasma/sera;
- IgGs purified from the selected plasma/sera, mixed with a malaria-naïve human sera pool (to eliminate non-specific factors which may be present in plasma).

To confirm anti-Pfs25-specific and anti-Pfs230-specific transmission-blocking activities, SMFAs may also be conducted

- using Pfs25-specific and Pfs230-specific IgG purified using affinity chromatography;
- using test plasma/sera that has been depleted of Pfs25-specific and Pfs230-specific antibodies using recombinant Pfs25 and Pfs230 proteins.

9.4.4.2 Direct Skin Feeds

While the SMFA is standardized and reproducible and has been shown to correlate with ELISA antibody titers in several species, the parasite strain, gametocyte load in the feeder, and lab colony mosquitoes are all significantly different from those found under field conditions.[24] DSFs, in which insectary-raised clean mosquitoes are directly fed on infected individuals, we believe may be more likely to be predictive of an intervention's impact on transmission than membrane feeds. Thousands of subjects in Bancoumana or neighboring areas have previously been enrolled in direct-feeding tests without significant adverse effects reported. In 1994 and 1995, Toure and colleagues conducted direct feeds on 72 children aged 2 years or more without any risks identified.[34] From 1996 to 1998, Diallo and colleagues conducted DSFs on 372 children aged 4 to 18 years without any safety problems.[26] In 2002 and 2003, DSFs were carried out in Bancoumana and in a neighboring village on a total of 44 gametocyte carriers aged 6 to 18 years old,[35] also without any reported safety problems.

More recently, under four previously conducted clinical trials (NIAID Protocol #13-I-N109, #11-I-N143, #14-I-N159, #15-I-0044) the study team has performed over 2000 DSFs on subjects older than 5 years since June 2011. All DSF participants were actively followed 24 hours post feed and as many as 12 DSFs over a 2 week period have been conducted. Except for 1 case of definitely related Grade 2 erythema that resolved within 48 hours and 2 episodes of definitely related Grade 1 pruritus, there have been no other expected or unexpected AEs recorded as definitely, probably, or possibly related to the feeding procedures in either study. Additionally, under this protocol (#17-I-N006), 3518 DSFs have been completed with only four pruritus AEs (4/3518, 0.1%) in three subjects have been reported related to the DSFs. All have been mild (Grade 1), all starting on the day of DSF, and all resolved within 2 days. None of the DSF related AEs have met criteria for withdrawal of the subject from participating in future DSFs.

Additionally, under protocol #11-I-N143, multiple DSFs during a 24 hour period have been completed in 18 subjects to date. In these 18 subjects, there have been no reported AEs secondary to having multiple direct skin feeds completed in a short period of time and have been tolerated well by the involved subjects.

For DSFs, 2 feeding pints with approximately 30 pre-starved female mosquitoes in each will be prepared. Each subject will be exposed to the feeding pints for 15-20 minutes. All subjects will be offered a topical antihistamine and/or topical antipruritic to use following the feeds. Systemic medications may also be prescribed for treatment of local or systemic symptoms following DSFs if clinically indicated.

During the time periods outside of the intense twice a week DSF for approximately 12 weeks following Vaccinations #3 attempts to identify and optimize parameters that contribute to DSF variability may be implemented during the conduct of the feeding assays. Subjects will be provided the details of the variation on the DSFs prior to participation and are not required to deviate from the standard DSF (2 feeding pints; approximately 30 pre-starved female mosquitoes per pint; application to the bilateral calves or arms; exposure for 15-20 minutes; feed conducted at subject's convenience, usually dusk or dawn).

These variables include the following:

- Body location (for example, ankle, leg, forearm),
- Number of cups applied (for example, 2 cups or 3 cups),
- Time of day feeds conducted (for example, dawn, dusk, late night),
- Number of feeds per day (up to 2 feeds in a single calendar day),
- Duration of feeds (for example, decreasing time to 10 minutes).

The total number of mosquitoes used for each DSF will be maintained at appropriately 60 mosquitoes total regardless of these variables, and no subject will undergo more than 32 DSFs within any 6 month period post vaccination. Note, if subjects are not willing to undergo twice a week feeds at the time of the DSF, they will not be withdrawn but will be followed per protocol but with periodic DSF or without DSF being completed.

Previously, DSFs started either 1 or 2 weeks post vaccination in order to time the DSFs with expected peak antibody responses. Although antibody responses have significantly improved, in peak and more importantly durability, the question of functional activity no longer is focused on peak time periods, but rather duration of activity, we will still plan to start DSFs 1 week after vaccination #4 in order to assess activity early in the transmission season. DSFs will continue

twice a week for 16 weeks in order to increase timepoints available for analysis to evaluate vaccine efficacy.

Some experiments have already been completed under protocols #13-I-N109 and #11-I-N143 to try to help determine which time of day and body part will maximize positive DSFs. Although there was not a significant difference found between location and time of feed in this small experiment, we did find that feeding at dawn and on the arm were estimated to have the highest counts. The data collected under this protocol will provide additional information about how to optimize/maximize the DSF procedure.

After the feed, surviving mosquitoes will be assessed for infectiousness by microscopy or molecular assays.

9.4.4.2.1 Withdrawal from DSFs

The following criteria will be checked prior to each DSF and are contraindications to DSFs:

- Severe local or systemic reaction to mosquito bites following a previous DSF.
- Positive urine or serum β-hCG test (pregnancy testing results obtained within the last 7-10 days before the DSF are acceptable; see **Appendix B**).
- Acute illness with an oral temperature >37.5˚C at the time of the DSF.
- Any other condition that in the opinion of the Investigator poses a threat to the individual if he/she undergoes DSF or that may complicate interpretation of the safety of DSF.

Recent use of antimalarial medications is not a contraindication to participating in the DSFs. The medication used and time period the medication was taken should be recorded on the subject's source documents and case report forms accordingly.

9.5 Entomological Procedures and Insectary Used in DSF

9.5.1 Mosquito Collection

Mosquitoes may be collected from residences or compounds or communities of study subjects on a periodic basis to assess mosquito infection rates and their relation to the transmission-blocking activity measured in blood samples as described above from consented subjects.

9.5.2 Mosquito Rearing at the Insectary

A laboratory colony of *A gambiae* established from a local catch in 2008 will be used for the DSFs. This colony has been used in the current assay development study, and was demonstrated to have similar susceptibility as the F1 progeny of the wild-caught mosquitoes. The colony has been maintained using blood meals collected under standard procedures of the blood transfusion center from local healthy donors. To ensure that the donor is free of potentially transovarial arbovirus in incubation, the donated blood is only used 5-7 days after the collection and after the

donor is confirmed to remain healthy during this period. Potentially transovarial arbovirus in mosquitoes or in blood meals may be detected by ELISA and PCR methods. However, it is not feasible to conduct these tests routinely on blood donor volunteers due to highly restricted access to positive controls required in these tests, but blood samples from donors and mosquitoes from the colony are tested for RVF on a regular basis in the US.

The insectary in which mosquitoes are reared in Bamako has been in use for more than 15 years. Security is ensured by the use of double doors, which prevents the escape of reared mosquitoes as well as the entry of non-insectary mosquitoes. Mosquitoes are transported to Bancoumana or Doneguebougou in net-sealed feeding cups secured in wooden holders inside a cooler, with wet towels to maintain adequate humidity. After arrival at the assigned insectary in Bancoumana or Doneguebougou, mosquitoes are secured within the transporting containment. The insectary is adequately equipped with rooms with humidifiers which are regularly monitored according to standard insectary procedures, and access to the insectary is limited to study personnel only. After feeds, mosquitoes, still in net-sealed cups, are transported back to the insectary in Bamako. All subsequent handling of mosquitoes will take place in Bamako.

9.5.3 Mosquito Dissection

Mosquitoes are knocked down and then transferred into a Petri dish containing a slightly wet paper towel. Under a dissecting scope and on a slide, each individual specimen is placed in a drop mercurochrome solution. The midgut is pulled and covered with a coverslip. The oocysts are detected under a light microscope. The presence of oocyst and oocyst counts for each specimen will be recorded. A mosquito will be considered positive if ≥1 oocyst is present.

If needed, the head and thorax of the mosquitoes may be processed for ELISA to detect sporozoites and for PCR to identify species and molecular form of the mosquito.

Additional details on mosquito rearing, feeds, and dissection will be described in study SOPs.

9.6 Schistosomiasis and Helminthes Infection Testing

Endemic helminthes infections in sub-Saharan Africa, such as helminth and schistosomiasis infections, are known to elicit a wide range of immunomodulating responses and may impact the efficacy of vaccinations.[36-38] This potential impact of co-infections on vaccine immunogenicity will depend on the prevalence of local helminth and schistosomiasis infections. With the introduction of phase 1 malaria trials into Africa, consideration and initial exploration into the existence of co-infections in vaccinees and the impact on vaccine efficacy and immunogenicity needs to be explored.

To assess the impact of co-infections on immune responses, schistosomiasis testing (may include urine microscopic examination for *S. haematobium* and stool PCR for *S. mansoni;* urine antigen detection) and helminthes testing[39] (stool PCR for 8 common gastrointestinal pathogens) will be

completed on Study Day 0 in Arms 2c, 2d, 4c only. Given these tests are run for research pruposes and retrospectively, if these results are available prior to the final study visit and parasites are detected, and recommended, subjects will be treated with standard treatment and continued in the study.

9.7 Other Laboratory Assays

If there is adequate remaining blood sample available to fulfill the laboratory objectives, other laboratory assays may be performed as follows:

- Malaria rapid diagnostic tests (RDTs) may be used for clinical diagnosis purposes.
- qPCR may be used to detect gametocytes using whole blood collected on the day of DSF and/or positive blood smears.
- Filter paper filled with whole blood, whole blood, or mosquitoes may be used to determine parasite genotype.
- Antibodies against sporozoite, pre-erythrocytic, blood, and sexual stages may be determined by ELISA.
- Mosquito species and molecular forms may be identified by qPCR..[40]
- Hemoglobin variants (Hgb C, S, alpha thalassemia), glucose-6-phosphate dehydrogenase deficiency, and ABO blood group/Rhesus factor may be assessed using high-performance liquid chromatography and/or other standard methodology.
- Human leukocyte antigen genotypes may be evaluated for genetic polymorphisms, allelic and antigenic diversity.
- B cell receptor/antibody genes may be sequenced to explore the antibody repertoire of functional antibody responses.
- Cytokine levels may be evaluated during vaccination and following vaccination.
- Direct membrane feeding assays may be completed following Vaccination #3 to compare to SMFA and DSF results.

Study physicians may ask for additional laboratory exams related to subject care.

10 Research Use of Stored Human Samples, Specimens, or Data

Intended Use: Samples and data collected under this protocol will be used to study malaria and related diseases and possible adverse reactions to vaccination. Genetic testing may be performed in accordance with the genetic testing information that is included in the study informed consent.

Storage: Access to stored research samples will be limited using either a locked room or a locked freezer. Temporary storage of samples collected in Mali, prior to shipment to LMIV, may occur at the Core Immunology Laboratory or the MRTC CAP laboratory. Samples will be stored at the LMIV in Rockville, MD or at LMIV's designated repository, Thermo Scientific,

Rockville, MD, with the exception of retention specimens which may be kept at the MRTC in Mali for quality control. Samples and data will be stored using codes assigned by the investigators or their designees. Data will be kept in password-protected computers. Only investigators or their designees will have access to the samples and data.

Tracking: Samples will be tracked using a sample-tracking software program, e.g., Freezerworks.

Disposition at the Completion of the Protocol: In the future, other investigators (both at the NIH and outside) may wish to study these samples and/or data. In that case, IRB approval must be sought prior to any sharing of samples. Any clinical information shared about the sample with or without patient identifiers would similarly require prior IRB approval.

At the completion of the protocol (termination), samples and data will either be destroyed or, after IRB and FMPOS EC approval, transferred to another existing protocol.

Reporting the Loss or Destruction of Samples/Specimens/Data to the IRB: Any loss or unanticipated destruction of samples (for example, due to freezer malfunction) or data (for example, misplacing a printout of data with identifiers) that meets the definition of Protocol Deviation and/or compromises the scientific integrity of the data collected for the study, will be reported to the NIH IRB and FMPOS EC.

Consent to allow long term storage of study samples is a part of the inclusion criteria for this study. However, if a subject decides following enrollment not to have their samples stored the Principal Investigator or designee will destroy all known remaining samples and report this destruction to the subject and the NIH IRB and FMPOS EC. This decision will not affect the subject's continued participation in this protocol or any other protocols supported by the NIH.

11 Data Sharing Plan

In NIH's and MRTC's view, all data should be considered for data sharing. Data should be made as widely and freely available as possible while safeguarding the privacy of subjects, and protecting confidential and proprietary data. We recognize that the public dissemination of our scientific results can facilitate the creation of collaborative efforts with domestic and international collaborators. Furthermore, we recognize that the proposed project may result in novel ideas for new methods, technologies, and data that could benefit the entire research community. Therefore, final research data will be shared openly and timely in accordance with the most recent NIH guidelines (http://grants.nih.gov/grants/policy/data_sharing/) while being mindful that the confidentiality and privacy of participants in research must be protected at all times. Timelines for distribution of data will vary depending on any required restrictions in accordance with federal and/or institutional policies and guidelines. In general we expect de-identified data will be available through NIH-funded or approved public repository, speaking

engagements and publications, presentations at scientific symposia and seminars. Effort will be made to publish our research findings in scientific journals. All final peer-reviewed manuscripts that arise from this proposal will be submitted to the digital archive PubMed Central. For tools, reagents, data and model organisms generated by the proposed study, pending third parties rights, LMIV/MRTC will transfer materials to outside researchers in both the private and public sectors under a Material Transfer Agreement or Research Collaboration Agreement. Discussions of unpublished ELISA and SMFA data from this trial with collaborators will be conducted under a Confidential Disclosure Agreement.

12 Assessment of Safety

12.1 Documenting, Recording, and Reporting Adverse Events

At each contact with the subject, information regarding adverse events will be elicited by appropriate questioning and examinations and will be:

- immediately documented in the subject's medical record/source document,
- recorded on the Adverse Event Case Report Form (AE CRF) or electronic database, and
- reported as outlined below (e.g., IND Sponsor, IRB, FDA).

A study clinician will be available during the study period and will be available to the study subjects at all times. Should a subject call a study clinician to report an AE, it will be discussed with the PI and documented, recorded, and reported appropriately.

12.2 Definitions

Adverse Event (AE)
An adverse event is any untoward or unfavorable medical occurrence in a human subject, including any abnormal sign (e.g., abnormal physical exam or laboratory finding), symptom, or disease, temporally associated with the subject's participation in the research, whether or not considered related to the research.

Adverse Reaction (AR)
An adverse event that is caused by an investigational agent (drug or biologic).

Suspected Adverse Reaction (SAR)
An adverse event for which there is a reasonable possibility that the investigational agent caused the adverse event. 'Reasonable possibility' means that there is evidence to suggest a causal relationship between the drug and the adverse event. A suspected adverse reaction implies a lesser degree of certainty about causality than adverse reaction which implies a high degree of certainty.

Serious Adverse Event (SAE)

A Serious Adverse Event (SAE) is defined as an AE that results in any of the following outcomes:

- death
- a life threatening (i.e., an immediate threat to life) event
- an inpatient hospitalization or prolongation of an existing hospitalization
- a persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions
- a congenital anomaly/birth defect
- a medically important event*

* Medical and scientific judgment will be exercised in deciding whether expedited reporting is appropriate in other situations, such as important medical events that may not be immediately life threatening or result in death or result in hospitalization but may jeopardize the subject or may require intervention to prevent one of the other outcomes listed above.

Unexpected Adverse Event
An AE is considered unexpected if it is not listed in the Investigator's Brochure or Package Insert (for marketed products) or is not listed at the specificity or severity that has been observed. It is the responsibility of the IND Sponsor to make this determination.

Serious and Unexpected Suspected Adverse Reaction (SUSAR)
A SUSAR is a Suspected Adverse Reaction that is both serious and unexpected.

Unanticipated Problem (UP)
An Unanticipated Problem (UP) is any event, incident, experience, or outcome that is

1. unexpected in terms of nature, severity, or frequency in relation to
    a. the research risks that are described in the IRB-approved research protocol and informed consent document, Investigator's Brochure, or other study documents; and
    b. the characteristics of the subject population being studied; and
2. possibly, probably, or definitely related to participation in the research; and
3. places subjects or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized. (Per the IND Sponsor, an AE with a serious outcome will be considered increased risk.)

Serious Unanticipated Problem (UP)

A UP that meets the definition of a Serious Adverse Event or compromises the safety, welfare or rights of subjects or others.

Unanticipated Problem that is not an Adverse Event (UPnonAE)
An unanticipated problem that does not fit the definition of an adverse event, but which may, in the opinion of the investigator, involve risk to the subject, affect others in the research study, or significantly impact the integrity of research data. Such events would be considered a non-serious UP. For example, we will report occurrences of breaches of confidentiality, accidental destruction of study records, or unaccounted-for study drug.

New Onset of Chronic Illness (NOCI)
The new onset of chronic illness is defined as a diagnosis of a new medical condition that is chronic in nature, including those potentially controllable by medication (e.g., diabetes, asthma). Any NOCI will be recorded in the same manner as unsolicited AEs.

12.3 Investigator Assessment of Adverse Events

If a diagnosis is clinically evident (or subsequently determined), the diagnosis rather than the individual signs and symptoms or lab abnormalities will be recorded as the AE.

All solicited (see **Table 14** below) and unsolicited AEs will be recorded through Day 7 after each vaccination, including injection site reactions, or until resolved. Adverse reactions related to direct skin feeds will be recorded until Day 7 after feeds or until resolved. After that period only unsolicited AEs (including symptomatic malaria), SAEs, UPs, and NOCIs will be recorded. Note that a positive blood smear without associated clinical symptoms will not be reported as an AE.

Table 14: Solicited Adverse Events

Systemic adverse events
Fever (temperature ≥38.0 °C)
Headache
Nausea/Vomiting
Diarrhea
Abdominal pain
Fatigue
Myalgia
Arthralgia
Urticaria
Laboratory adverse events
Hemoglobin – decreased hemoglobin
WBC – leukopenia, leukocytosis
ANC/AGC – decreased neutrophil/granulocyte count

Platelet count – thrombocytopenia
ALT – increased ALT
Creatinine (Cr) – increased Cr
Local reactogenicity
Injection pain/tenderness
Injection erythema/redness
Injection swelling
Injection induration
Injection pruritus
Limitation of arm movement

Additional laboratory abnormalities other than those specified as safety labs in the protocol should be reported as adverse events if they require intervention. Interventions include, but are not limited to, discontinuation of treatment, dose reduction/delay, additional assessments, or concomitant treatment. In addition, any medically important laboratory abnormality may be reported as an adverse event at the discretion of the investigator. This could include a laboratory result for which there is no intervention but the abnormal value suggests a disease or organ toxicity. In addition, similar to solicited AEs, all laboratory AEs as defined in **Appendix D**, will be collected and graded through 7 days after each vaccination or until resolved.

The investigator will evaluate all AEs with respect to Seriousness (criteria listed above), Severity (intensity or grade), and Causality (relationship to study agent and relationship to research) according to the following guidelines.

12.3.1 Severity

Severity of AEs will be assessed by the investigator as described in **Appendix D.** AEs not included in the Appendixes will be graded for severity using the followings definitions as seen in **Table 15**.

Table 15: Definitions for Severity of AE Grading

Severity	Definition
Grade 1 (Mild)	No interference with activity, may use 1 dose of an over the counter medication
Grade 2 (Moderate)	Repeated use of non-narcotic pain reliever > 24 hours or some interference with activity
Grade 3 (Severe)	Activities of daily living limited to <50% of baseline, medical evaluation/therapy required
Grade 4 (Potentially Life-Threatening)	Extreme limitation in activity, significant assistance required; immediate medical

	intervention or therapy required to prevent death
Grade 5	Death

12.3.2 Causality

Causality (likelihood that the event is related to the study agent) will be assessed considering the factors listed under the following categories:

Definitely Related

- reasonable temporal relationship
- follows a known response pattern
- clear evidence to suggest a causal relationship
- there is no alternative etiology

Probably Related

- reasonable temporal relationship
- follows a suspected response pattern (based on similar agents)
- no evidence of a more likely alternative etiology

Possibly Related

- reasonable temporal relationship
- little evidence for a more likely alternative etiology

Unlikely Related

- does not have a reasonable temporal relationship

  OR

- good evidence for a more likely alternative etiology

Not Related

- does not have a temporal relationship

  OR

- definitely due to an alternative etiology

Note: Other factors will also be considered for each causality category when appropriate. Causality assessment is based on available information at the time of the assessment of the AE.

The investigator may revise the causality assessment as additional information becomes available.

The degree of certainty with which an AE can be attributed to administration of the study vaccine will be determined by how well the event can be understood in terms of one or more of the following:

- The event being temporally related with vaccination or reproduced on re-vaccination.
- A reaction of similar nature having previously been observed with this type of vaccine and/or formulation.
- The event having been reported in the literature for similar types of vaccines.
- Whether or not there is another identifiable cause.

All local (injection site) reactions will be considered causally related to vaccination. All malaria cases will be reported as not related to vaccination. Asymptomatic parasitemia (positive blood smears without related malaria clinical symptoms) will not be reported as an AE. Clinical malaria will be reported as an AE.

Reports will further classify AEs as follows:

- Related - all AEs that are assessed as definitely, probably, or possibly related.
- Unrelated - all AEs assessed as unlikely or definitely not related.

When reporting to regulatory authorities and IRBs is needed, AE relationship will be determined as noted above.

12.4 Investigator Reporting Responsibilities to the Sponsor

12.4.1 Adverse Events

Line listings, frequency tables and other summary AE data will be submitted to the IND Sponsor when needed for periodic safety reviews, review of IND annual reports, review of IND safety reports, and preparation of final study reports.

12.4.2 Serious Adverse Events

All SAEs (regardless of relationship and whether or not they are also UPs) will be reported on the Safety Expedited Report Form (SERF) and sent to the Sponsor Clinical Safety Office (CSO) by fax or email attachment. Deaths and immediately life-threatening SAEs will be reported within 1 business day after the clinical site becomes aware of the event. All other SAEs will be reported to the CSO within 3 business days after the site becomes aware of the event.

Sponsor Clinical Safety Office Contact Information:
OCRPRO Clinical Safety Office
5705 Industry Lane
Frederick, MD 21704
Tel: (301) 846-5301
Fax: (301) 846-6224
Email: rchspsafety@mail.nih.gov

The clinical site Investigator in Mali will also notify LMIV PI and the Site Medical Monitor in Mali by email, fax, or telephone within 1 working day of notification of an SAE occurrence.

LMIV Contact Information:
Patrick Duffy, MD
Tel: (301) 761-5089
Fax: (301) 480-1962
Patrick.Duffy@nih.gov

12.4.3 Unanticipated Problems

All UPs that are also adverse events will be reported to the CSO on the NIH Problem Report Form sent by fax or email attachment no later than 7 calendar days of site awareness of the event.

UPs that are not AEs will not be reported to the Sponsor CSO.

12.4.4 Pregnancy

Pregnancy itself is not an AE. However, complications of pregnancies are AEs and may be SAEs. Pertinent obstetrical information for all pregnancies will be reported to the CSO and LMIV via fax or email within 3 business days from the site awareness of the pregnancy. All pregnancies will be reported on the Pregnancy Notification/Outcome Form to the CSO.

Pregnancy outcome data (e.g., delivery outcome, spontaneous, or elective termination of the pregnancy) will be reported to the CSO and LMIV within 3 business days of the site's awareness of the outcome on a protocol-specified form.

In the event of pregnancy, the following steps will be taken:

- Discontinuation of the study agent
- Unblind per the site unblinding procedures
- Withdraw from the study but continue in follow-up for safety
- Report to FMPOS Ethics Committee as an informational item

- Report to NIH IRB at time of Continuing Review
- Report to DSMB, Sponsor Medical Monitor, and Site Medical Monitor
- Advise research subject to notify the obstetrician of study vaccine exposure

12.5 Reporting Procedures to the NIH IRB

12.5.1 Expedited Reporting to the NIH IRB

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Non-compliance: Any actual or suspected non-compliance by any investigator or entity associated with the protocol must be reported by the NIH PI/designee within 7 calendar days of any investigator or individual associated with the protocol first becoming aware, unless otherwise indicated in this policy.

Non-NIH IRB determinations of serious and/or continuing noncompliance about an NIH investigator: If the NIH is relying on a non-NIH IRB and the Reviewing IRB makes a determination of serious and/or continuing non-compliance regarding an NIH investigator, then, even if the determination has already been provided to OHSRP either directly or via the NIH Institutional Official (IO)/designee, the NIH PI/designee must report this in iRIS within 7 calendar days of any member of the research team being notified of the determination by the Reviewing IRB. The NIH PI must provide the OHSRP office of Compliance and Training with documentation from the Reviewing IRB unless this documentation has already been provided directly to the office by the Reviewing IRB or via the IO.

Major Deviation: A deviation must be reported within 7 calendar days of an investigator becoming aware of an actual or suspected deviation. Although protocol deviations are also non-compliance, these should only be reported once as deviations.

Unanticipated Problem (UP): A UP must be reported within 7 calendar days of an investigator becoming aware of the actual or suspected UP.

Death: Any death of a research subject that is possibly, probably or definitely related to the research must be reported within 24 hours of an investigator becoming aware of the death.

New information that might affect the willingness of a subject to enroll or remain in the study should be reported within 7 calendar days of an investigator first becoming aware.

Any suspension or termination of research activities, including holds on new enrollment, placed upon the research by the study sponsor, NIH or IC leadership, or any regulatory agency must be reported within 7 calendar days of an investigator becoming aware.

Investigators must provide the following information to the IRB in summary format at the time of continuing review: Minor protocol deviations; Adverse events and Serious Adverse Events that do not meet the definition of an UP.

Investigators are required to immediately (i.e., no longer than 10 days) report SAEs or UADEs to the study sponsor and, if also an actual or suspected UP, to the IRB within 7 calendar days of an investigator becoming aware.

12.5.2 Annual Reporting to the NIH IRB

All events will be reported to the NIH IRB and FMPOS EC in summary at the time of Continuing Review according to NIH Policy 801.

12.6 Investigator Reporting Responsibilities to the Local IRB

Investigators are responsible for submitting IND Safety Reports and UP summaries that are received from the IND Sponsor to their local IRB/Ethics Committee (FMPOS EC). Investigators must also comply with all local IRB/Ethics Committee (FMPOS EC) reporting requirements, including expedited and annual reporting requirements.

12.7 Follow-up of Adverse Events and Serious Adverse Events

AEs that occur following receipt of a single vaccination are followed until the final outcome is known or until the end of the study follow-up period.

SAEs that have not resolved by the end of the follow-up period will be followed until final outcome is known. If it is not possible to obtain a final outcome for an SAE (e.g., the subject is lost to follow-up), the reason a final outcome could not be obtained will be recorded by the investigator on the AE CRF (if the CRF is still open) and the SERF.

SAEs that occur after the study follow-up period (6 months following the last vaccination) that are reported to and assessed by the Investigator to be possibly, probably, or definitely related must be reported to the CSO as described above.

12.8 Sponsor's Reporting Responsibilities

Serious and unexpected suspected adverse reactions (SUSARs) as defined in 21 CFR 312.32 and determined by the IND Sponsor will be reported to FDA and all participating Investigators as IND Safety Reports.

The IND Sponsor will also submit an IND Annual Report of the progress of the investigation to the FDA as defined in 21 CFR 312.33.

AEs that are also UPs will be summarized by the IND Sponsor and distributed to Investigators.

12.9 Halting Criteria for the Study

The PI will closely monitor study data as they become available and will make determinations regarding the presence and grading of AEs. The AEs will be evaluated with regard to the known complications associated with administration of vaccine components. If a dose of vaccine is considered unacceptably reactogenic (as described in the following criteria), the study will be halted. No new enrollments and no further vaccinations will be administered by the Investigators until reviewed by the DSMB and study IND Sponsor. A report of DSMB recommendations will be submitted to the IRBs. The following criteria will be used to define unacceptable reactogenicity of the malaria vaccine (AEs that are possibly, probably, or definitely related to the vaccine will be considered "related" and will be summarized as such):

1. One or more subjects experience death or any life-threatening SAE, **or**
2. One or more subjects experience an SAE as defined in **Section 12.2** of this protocol that is determined to be possibly, probably, or definitely related to the vaccine, **or**
3. One or more subjects are withdrawn from the study (by investigator or subject request) following a Grade 3 AE that is determined to be possibly, probably, or definitely related to the vaccine, **or**
4. One or more subjects experience a hypersensitivity reaction (e.g. anaphylaxis, diffuse urticaria) that is determined to be possibly, probably, or definitely related to the vaccine, **or**
5. One or more subjects experience any local or solicited AE leading to hospitalization, or fever >40°C (104°F) that is possibly, probably, or definitely related to the vaccine, or necrosiss at the injection site, within the 7 days post vaccination, **or**
6. Ten percent or more of subjects in any dose Arm experience any Grade 3 solicited local AE (lasting 48 hours or more) that is determined to be possibly, probably, or definitely related to the vaccine as defined in this protocol, within the 7 days post vaccination, **or**
7. Ten percent or more of subjects in any dose Arm experience any Grade 3 solicited systemic AE (lasting 48 hours or more) that is determined to be possibly, probably, or definitely related to the vaccine as defined in this protocol, within the 7 days post vaccination, **or**
8. Thirty percent or more of subjects in any dose Arm experience the same Grade 2 or higher laboratory abnormality (see **Appendix D**) or ten percent or more experience the same Grade 3 or higher laboratory abnormality regardless of vaccine relationship, within the 7 days post vaccination, **or**
9. Ten percent or more of subjects in any dose Arm experience the same or similar Grade 3 unsolicited AE that is determined to be possibly, probably, or definitely related to the vaccine as defined in this protocol, **or**
10. Any safety issue that the study PI or IND Sponsor determines should halt the study.

If any of these halting criteria are met, request for an unblinded review by the DSMB may be submitted to determine if the safety issues identified were in the comparator or the test vaccines arm of the study.

The IRBs, the NIAID, the FDA, or other government agencies may discontinue the study at any time. Subsequent review of serious, unexpected, and related AEs by the DSMB or IRB, the IND Sponsor, the FDA, and other regulatory authorities may also result in suspension of further administration of vaccine at the clinical site. The FDA, other regulatory authorities, and the study sponsor(s) retain the authority to suspend additional enrollment and administration of vaccine for the entire study as applicable.

12.9.1 Reporting of Study Halting

If a halting requirement is met, a description of the event(s) or safety issue will be reported by the PI or Site Investigator within 1 business day to the Sponsor CSO by fax or email and reported to the FDA within 3 business days.

The Site Investigator will inform the PI and the local IRB that a halting rule has been met according to their requirements. The IND Sponsor will notify all sites that the study has been halted.

12.9.2 Resumption of a Halted Study

The IND Sponsor, in collaboration with the PI and DSMB will determine if it is safe to resume the study. The IND Sponsor will notify the Site Investigators of this decision. The conditions for resumption of the study will be defined in this notification. The Site Investigators will notify their local IRB(s) of the decision to resume the study.

12.10 Halting Criteria for a Subject

The decision to suspend administration of the study agent(s) for a single subject requires discontinuation of study agent administrated for the study subject(s) until a decision is made whether or not to continue study agent administration.

The halting criteria for a single subject in this study include:

- A subject experiences an SAE regardless of vaccine relationship, **or**
- A subject experiences ≥ 2 or more Grade 3 or greater AEs (solicited local/systemic or unsolicited; lasting 48 hours or more) that are possibly, probably, or definitely related to the study agent, within the 7 days post vaccination, **or**
- A subject experiences ≥ 2 or more Grade 2 or higher laboratory abnormality (see **Appendix D**) or any Grade 3 or higher laboratory abnormality that are possibly, probably, or definitely related to the study agent, within the 7 days post vaccination, **or**

- Any safety issue that the Site Investigator determines should pause administration of the study agent to a single subject or to all subjects in a specific group.

The IND Sponsor, in collaboration with the PI, may also pause for an individual subject or entire group if a safety concern is identified during routine aggregate data analysis.

12.10.1 Reporting of Halting for a Subject

If a pausing requirement is met, a description of the AE(s) or safety issue must be reported by the Site Investigator by fax or email within 2 business day to the Sponsor CSO, PI, the IRB, and DSMB and reported to the FDA within 3 business days

The Site Investigator must inform the PI and the local IRB that a halting rule has been met according to their requirements. The IND Sponsor will notify all sites that the study has been paused.

12.10.2 Resumption of a Halted Subject

The IND Sponsor in collaboration with the PI and the DSMB will determine if it is safe to resume administration of the study agent to the subject/group. The IND Sponsor will notify the Site Investigators of this decision. The Site Investigators will notify their local IRB(s) of the decision to resume administration of the study agent prior to resumption.

12.11 Discontinuation of Study

The NIAID/OCRPRO as the study sponsor, the NIH IRB, and the FDA may terminate this study at any time. Reasons for terminating the study may include, but are not limited to, the following:

- The incidence or severity of an AE in this or other studies indicates a potential health hazard to subjects
- Subject enrollment is unsatisfactory
- Data recording is inaccurate or incomplete
- Investigators do not adhere to the protocol or applicable regulatory guidelines in conducting the study

12.12 Withdrawal of an Individual Subject

A subject will not be considered to have completed the trial if any of the following reasons apply:

1. *Research terminated by Sponsor or Investigator* – applies to the situation where the entire study is terminated by the Sponsor or Investigator, or other regulatory authority for any reason.

2. *Withdrawal of consent* – applies to a subject who withdraws consent to participate in the study for any reason.
3. *Noncompliant with protocol* – applies to a subject who does not comply with protocol-specific visits or evaluations, on a consistent basis, such that adequate follow-up is not possible and the subject's safety would be compromised by continuing in the trial. This also applies to a subject who is lost to follow-up and is not reachable by telephone or other means of communication and cannot be located.
4. *Developed an AE* – applies to a subject who is withdrawn from study due to an AE, serious or otherwise. Any grade 3 or greater AE that is assessed as possibly, probably, or definitely related to vaccination (other than local reactions lasting <72 hours, or systemic reactions lasting <24 hours) will result in withdrawal of the subject from further vaccinations. Subjects may also be withdrawn for any AE that would cause continued participation in the study to not be in the best interest of the subject, as per the investigator's judgment. Any subject who is withdrawn from the study because of an AE related to study agent will be followed for safety until at least resolution of that AE and will be encouraged to remain in the safety evaluation for the duration of the study.
5. *Other* – is used when previous categories do not apply and a written explanation is required.

If a subject withdraws or is withdrawn prior to completion of the study, the reason for this decision will be recorded in the source documents and CRFs. Any subject who has received at least 1 dose of vaccine will be encouraged to remain in the safety evaluation for the duration of the study. The subject's data will be included in the safety and immunogenicity analysis. If a subject fails to complete all planned vaccinations because of an AE or SAE, the subject will be followed until resolution or stabilization of the event. If a subject withdraws, the investigator will make a reasonable effort to determine the reason.

12.12.1 Replacement of Withdrawn Subjects

Subjects who have received at least 1 vaccination and who withdraw or are terminated from the study prior to completion will not be replaced. Subjects withdrawn before the first vaccination may be replaced.

12.13 Unblinding

Intentional, unscheduled unblinding may occur if a subject experiences a SAE that the treating clinician and/or site PI believes warrants unblinding to provide appropriate clinical management of the subject. The request for unblinding may be requested by the site PI or designee, Sponsor, Sponsor Medical Monitor, Independent Medical Monitor, and Data Safety Monitoring Board (DSMB). If non-emergent, all parties should be notified to discuss prior to unblinding taking place. If unblinding is requested, an "Unblinding Report" should be documented per standard operating procedures and submitted to the study statistician for formal unblinding.

If emergency unblinding is required, the PI or designee will contact the study statistician or study pharmacist for unblinding. The Sponsor will be informed within 1 business day that the unblinding was necessary and a submitted "Unblinding Report" will be provided within 2 business days.

Subjects who are unblinded will be encouraged to remain in the study to be followed for safety.

After the time of unblinding, subjects in the experimental vaccine cohort will be offered vaccination with ENGERIX-B, or another FDA-approved Hepatitis B containing vaccine, according to the usual, FDA approved schedule.

Scheduled unblinding for Arms 2c, 2d, 4c will occur on Study Day 336 as already outlined in **Appendix B** and **C**. Scheduled unblinding will be reported to the DSMB at the time of the next meeting.

12.13.1 Unblinding by Group for Interim Analysis

If a follow-up study to explore the proof of concept of a VIMT in the field is proposed for 2018, group unblinding, for statistical analysis of ELISA responses, DSF, and SMFA will occur approximately 3 months following receipt of the final vaccine dose, as long as the individual subject blinding can be maintained (as determined by the unblinded statistician). The data will be reviewed by study staff who have no direct contact with study participants nor conduct clinical assessments. This information will only be used to determine if enough data supports moving forward with a PE/TBV combination study the following year.

12.14 Safety Oversight

12.14.1 Safety Review and Communications Plan

A Safety Review and Communication Plan (SRCP) has been developed for the protocol. The SRCP is an internal communications document between the Principal Investigator and the IND Sponsor Clinical Safety Office (CSO), which delineates the safety oversight responsibilities of the PI, the CSO, and other stakeholders. The SRCP also includes the overall plan for conducting periodic safety surveillance assessments.

12.14.2 Sponsor Medical Monitor

A Medical Monitor, representing the IND Sponsor (OCRPRO), has been appointed for oversight of safety in this clinical study. The Sponsor Medical Monitor will be responsible for performing safety assessments as outlined in the SRCP.

12.14.3Independent Safety Monitor (ISM)

An ISM in Mali will review the study prior to initiation and will be available to advise the Investigators on study-related medical issues and to act as a representative for the welfare of the subjects. The ISM will conduct independent safety monitoring and recommend appropriate action regarding adverse events and other safety issues. The ISM is an expert in the field of oversight of clinical trials conducted in Mali and internal medicine, specifically in the population under study in Mali. The ISM does not have direct involvement in the conduct of the study and does not have other interests with any collaborating pharmaceutical firms or their competitors.

Prior to each ISM review (including DSMB meeting safety reports and at least twice yearly reviews), the PI will provide a safety summary report (similar to the DSMB safety reports). After each ISM review, a recommendation as to whether the study is to be modified, or be terminated will be provided in a summary report to the study PI. If the study is to continue as is, no report will need to be submitted by the ISM, but communication to the PI that the review has been completed (via in person communication, phone, or email). All serious adverse events, all unanticipated problems, and all IND Safety Reports will be reported by the PI to the ISM at the same time they are submitted to the IRB or IND Sponsor. The ISM will be notified immediately if any pausing or halting rule is met and the ISM will provide recommendation for continuation, modification, or termination of the study. The PI will submit the written ISM summary report with the recommendations to their IRB(s) on a biannual basis or more frequently if a safety concern is raised. The PI will also notify the medical monitor if intentional or unintentional unblinding occurs.

12.14.4Data and Safety Monitoring Board

The NIAID Intramural DSMB will review the study prior to initiation and according to the schedule shown below in **Table 16.** The Board may convene additional reviews as necessary. The Board will review the study data to evaluate the safety, efficacy, study progress, and conduct of the study. All SAEs, all UPs, and all IND Safety Reports will be reported by the PI to the DSMB at the same time they are submitted to the IRB or IND Sponsor. The PI will notify the DSMB of any cases of intentional, unscheduled or unintentional unblinding as soon as possible. The PI will notify the Board at the time halting criteria are met and obtain a recommendation concerning continuation, modification, or termination of the study. The PI will submit the written DSMB summary reports with recommendations to the IRB(s).

Table 16: Vaccination Dose Escalation Schedule and DSMB Meeting

Bamako/Sotuba Phase Bancoumana/Doneguebougou Phase

Continuous Time (Weeks)	Vaccination 1 Study Day 0	Vaccination 2 Study Day 28	Vaccination 3 Study Day 168	Vaccination 4 Study Day 476	Feeding Assays	Unblinding
0	**Arm 1a**: 16 µg Pfs25M-EPA/ASO1 (n=5) **Arm 2a**: 13 µg Pfs230D1M-EPA/ASO1 (n=5)					
1	**DSMB Review**					
2	**Arm 1b**: 47 µg Pfs25M-EPA/ASO1 (n=10) **Arm 2b**: 40 µg Pfs230D1M-EPA/ASO1 (n=10) **Arm 3a:** 16 µg Pfs25M-EPA/ASO1 + 13 µg Pfs230D1M-EPA/ASO1 (n=5) **Arm 4a:** Comparator (n=10)					
4	**Arm 3b:** 47 µg Pfs25M-EPA/ASO1 AND 40 µg Pfs230D1M-EPA/ASO1 (n=10) **Arm 4b:** Comparator (n=10)	**Arm 1a**: (n=5) **Arm 2a**: (n=5)				
6		**Arm 1b**: (n=10) **Arm 2b**: (n=10) **Arm 3a:** (n=5) **Arm 4a:** (n=10)				
7	**DSMB Review**					
8		**Arm 3b:** (n=10) **Arm 4b:** (n=10)				
10	**Arm 2c:** 40 µg Pfs230D1M-EPA/ASO1 (n=60) **Arm 2d:** 40 µg Pfs230D1M-EPA/ASO1 fractional dosing regimen (n=60) **Arm 4c**:Comparator (n=120)					
14		**Arm 2c:** (n=60) **Arm 2d:** (n=60) **Arm 4c**: (n=120)				
24			**Arm 1a**: (n=5) **Arm 2a**: (n=5**)**			
26			**Arm 1b**: (n=10) **Arm 2b**: (n=10) **Arm 3a:** (n=5) **Arm 4a:** (n=10)			
28			**Arm 3b:** (n=10) **Arm 4b:** (n=10)			
29	**DSMB Review**					

Continuous Time (Weeks)	Vaccination 1 Study Day 0	Vaccination 2 Study Day 28	Vaccination 3 Study Day 168	Vaccination 4 Study Day 476	Feeding Assays	Unblinding
34			**Arm 2c:** (n=60) **Arm 2d:** (n=60) **Arm 4c**: (n=120)			
35 to 47					**Arm 2c:** (n=60) **Arm 2d:** (n=60) **Arm 4c**: (n=120)	
58						**Arm 2c:** (n=60) **Arm 2d:** (n=60) **Arm 4c:** (n=120)
	DSMB Review					
78				**Arm 2c:** (n=60) **Arm 2d:** (n=60) **Arm 4c**: (n=120)		
82 to 98					**Arm 2c:** (n=60) **Arm 2d:** (n=60) **Arm 4c**: (n=120)	
	DSMB Review					

13 Clinical Monitoring

13.1 Site Monitoring Plan

As per ICH-GCP 5.18 and FDA 21 CFR 312.50, clinical protocols are required to be adequately monitored by the study sponsor. This study monitoring will be conducted according to the "NIAID Intramural Clinical Monitoring Guidelines. Monitors under contract to the NIAID/OCRPRO will visit the clinical research site to monitor aspects of the study in accordance with the appropriate regulations and the approved protocol. The objectives of a monitoring visit will be: 1) to verify the existence of signed informed consent documents and documentation of the informed consent process for each monitored subject; 2) to verify the prompt and accurate recording of all monitored data points, and prompt reporting of all SAEs; 3) to compare abstracted information with individual subjects' records and source documents (subjects' charts, laboratory analyses and test results, physicians' progress notes, nurses' notes, and any other relevant original subject information); and 4) to help ensure investigators are in compliance with the protocol. The monitors also will inspect the clinical site regulatory files to ensure that regulatory requirements (Office for Human Research Protections-OHRP), FDA, and applicable guidelines (ICH-GCP) are being followed. During the monitoring visits, the investigator (and/or designee) and other study personnel will be available to discuss the study progress and monitoring visit.

The investigator (and/or designee) will make study documents (e.g., consent forms) and pertinent hospital or clinical records readily available for inspection by the local IRB, the FDA, the site monitors, and the NIAID staff for confirmation of the study data.

A specific protocol monitoring plan will be discussed with the Principal Investigator and study staff prior to enrollment. The plan will outline the frequency of monitoring visits based on such factors as study enrollment, data collection status and regulatory obligations.

14 Statistical Considerations

14.1 Description of Statistical Methods

This study, like other Phase 1 studies, is exploratory rather than confirmatory; its purpose is to estimate AE rates and patterns of immune responses rather than to test formal statistical hypotheses. Estimates will be presented with their 95% confidence intervals. Descriptive approaches will be used to meet the protocol objectives, as well as formal statistical tests as outlined below. Results will be presented in tabular format, as well as graphically where appropriate.

14.2 Primary Objective

The primary objective of this study is to assess safety and reactogenicity of Pfs25M-EPA/AS01 and Pfs230D1M-EPA/AS01 in Malian adults.

- The frequency of systemic and local AEs will be summarized.
- A line listing of each clinical and laboratory AE classified as local, solicited, or other will be displayed in tables stratified by vaccine allocation.
- AEs will be summarized by severity and relationship to vaccine
- The proportion of subjects with at least 1 AE will be compared by vaccine schedule and/or dose group, and tests will be performed to assess whether these groups differ with respect to these proportions. To evaluate the difference in AEs between the initial vaccination and the subsequent vaccinations, a Wilcoxon signed rank test will be performed, where the response for each subject is the difference between the numbers of AEs in the 7 days following each vaccination. To compare number and severity of AEs between control and vaccine arms, Wilcoxon-Mann-Whitney test (also known as Wilcoxon rank sum test and the Mann-Whitney U test) tests as well as linear regression may be used.
- SAEs occurring within the study period will be listed by relationship to vaccine.

14.3 Secondary Objectives

The secondary objective of this study is to determine the antibody response of both vaccines and their combination as measured by ELISA and transmission-blocking assays as measured by SMFA and DSF and the effect on antibody responses of a third dose.

Anti-Pfs25 antibody will be measured by ELISA on vaccination days, 2 weeks after each vaccination, and periodically until study completion. Antibody responses over time will be shown graphically with geometric mean antibodies and the associated confidence intervals based on the t-distribution applied to the log-transformed responses. The smaller (n=5 and n=10) groups are primarily included for safety, and their ELISA response will only be used in an exploratory nature. They will not be included in the main analysis of the secondary endpoint. We will compare antibody and functional assay responses within Arms over time.

We will also look at differences in antibody decline over time in the vaccination groups.

Graphs will display transmission-blocking assays as a function of ELISA antibody levels.

Preliminary results for the SMFA and ELISA have been ascertained for Day 252 (12 weeks post-dose 3). They have been discussed with the scientific advisory board (SAB) and the data safety monitoring board (DSMB). The conclusion to combine groups for the full booster dose was based on the facts that fractional (1/5 the amount of antigen and adjuvant) dosing (marginally) failed (p=0.0597) to be statistically significantly different than full dosing when looking at TRA readouts from the SMFA while being statistically significantly different in ELISA titer readouts

(p=0.00014). The combined group will be given the full dose in an effort to maximize power to detect a signal. The unblinding occurred as originally planned, with some of the members of the clinical team unblinded later than others as they completed database clean up procedures. Some of the lab teams are still blinded as they are still performing assays. They will be unblinded once these tasks are completed. The DSMB has always received unblinded information throughout the study. The study is being extended for one year in order to assess the kinetics of the fs230 antibody titers with (main phase) and without (pilot phase) a booster vaccination in the second transmission season. The possibility for an enrollment differential between the study arms in the study extension does exist, but is not anticipated due to re-enrollment being very successful in a previous study in this area. The loss to follow-up thus far has not appreciably been affecting one group more than another.

14.4 Exploratory Objectives

We will characterize and compare host immune responses, proteomic profiles, co-infection status, antibody repertoires, and transcriptomes to malarial antigens and to vaccine antigens in African adults prior and after immunization with Pfs230D1M-EPA/AS01 by mixed effects model or Wilcoxon signed rank test depending on the type of immune response. We will also make figures depicting immune response based on the study arm.

We will explore the impact of Pfs230D1M-EPA/AS01 on the genotype and transcriptome profile of parasites isolated from study subjects via standard regression analysis.

Should the study be terminated early, the investigative team will discuss with the DSMB the reason for termination and determine which study questions can be addressed in an unbiased manner with the available data. The available data will be analyzed and interpreted in light of early termination.

14.5 Sample Size and Power Calculations

SAFETY

The arms of five subjects are sized for safety, as the higher dose is expected to be necessary for an adequate immune response. In these arms (Arms 1a, 2a, 3a), 5 subjects each will receive 16 µg of Pfs25M-EPA/AS01 or 13 µg Pfs230D1M-EPA/AS01 or the combination of both. Vaccination arms of 5 subjects gives a probability of at least 0.80 for detecting 1 or more serious or severe AEs that occur with a probability of 0.275 or more per subject.

For each dose level that has a (n=10) in Arms 1b, 2b, 3b, vaccination of 10 subjects gives a probability of at least 0.90 for detecting 1 or more serious or severe AEs that occur with a probability of 0.206 or more per subject.

For each dose level that has a (n=60) in Arms 2c, 2d, and 4c, vaccination of 60 subjects gives a probability of at least 0.90 for detecting 1 or more serious or severe AEs that occur with a probability of 0.038 or more per subject.

If we combine all treated groups in Mali (Arms 1a, 1b, 2a, 2b, 3a, 3b, 2c and 2d), 165 subjects, we have 95% power to detect 1 or more serious or severe AEs that occur with a probability of 0.018 or more per subject. Due to arms having varying doses, combinations and administration villages, this analysis would only be done to catch an AE that transcended these discrepancies.

We will compare all AE event proportions between the control arm and treated arm by Fisher's exact test.

ELISA ANALYSIS

There are several questions of interest based on the antibody response information after the 2^{nd},3rd and for arms 2c, 2d, and 4c, a 4^{th} (booster) doses of the respective vaccines. We are interested in the change in ELISA values from baseline to after a given number of doses of vaccine, and the change in ELISA values between doses. For this we will use Wilcoxon signed rank tests (WSRT) within the 60 subjects receiving a given vaccine regimen. We are also interested in the effect of a given number of vaccinations on the ELISA responses as compared to comparator. This comparison can be made by Wilcoxon Mann Whitney (WMW) test, which accommodates the limit of detection issues that may exist for ELISA results.

The preliminary data from protocol #15-I-0044 on subjects who had each Pfs25 and Pfs230 ELISA measurements after receiving two and three doses of 47µg Pfs25 + 40µg Pfs230 (using Alhydrogel® adjuvant, not AS01 as in this protocol) allow us to estimate the SD of the log transformed Pfs25 ELISA responses at baseline, post vaccination 2 and post vaccination 3. Due to these data still being blinded, we do not report the values for the SD and mean of the log transformed Pfs25 ELISA responses.

Assuming similar values in this trial, there would be over 0.99 power to detect the difference between baseline and post-vaccination 2 and over 0.9 power to detect the difference between post vaccination 2 and post vaccination 3.

In these same preliminary data from protocol #15-I-0044 all control subjects had undetectable levels of Pfs25 ELISA response post 2nd and 3rd vaccination.

Within Arm 2c and using the background information from #15-I-0044, we have greater than 80% power to reject a 2-sided 0.05 level WMW test if the geometric mean Pfs230 ELISA baseline level was 1.6-fold higher geometric mean than the level of detection in the vaccinated group post vaccination 2 (Note: 1.6-fold is lower than what we observed in #15-I-0044, so we treat these numbers as conservative). Given the similarity in SD estimate post vaccination 3, we should have very similar power post vaccination 3 as was calculated for post vaccination 2.

There are three key comparisons regarding ELISA measurements and the booster dose (equivalently dose 4). These include 1) peak titers after dose 4 compared with those following

dose 3; 2) nadir titers at 3 months post dose 4 compared to those post dose 3; and 3) decay rates after dose 4 compared to those after dose 2 and those after dose 3. The power to detect these differences is modest/low due to the expected performance of the booster to perform similarly to the previous doses. However, it is important to note that the primary immunogenicity endpoints will be comparing ELISA titers of each arm 2c and 2d versus the control arm 4c at all time points, which will be powered above 90%.

FUNCTIONAL ASSAYS

SMFA

Using blinded SMFA data from #15-I-0044, a WMWT comparing 60 TRAs of the vaccine arm to 60 TRAs of the control, we anticipate at least 80% power to detect a difference in TRAs of 18% (simulation-based power calculations). If TBAs are used, we will standardize the TBAs to a common target control mean first. The standardization will utilize the estimand that comes from a zero-inflated negative binomial with random effects for feed and container of mosquito. TRAs can be combined across feeds or calculated within feeds as they do not require the same standardization as that of TBAs.

DSF

Using the data from the primary DSF evaluation period of study #13-I-N109 and #15-I-0044 we were able to do power calculations for this study. The power calculations are quite sensitive to the infectivity rate, which varies from year to year. Taking a weighted average of 2014 and 2015 data we estimate infectivity was 14.8% (the percentage of subjects in the control arm that had at least one positive DSF). Using this same % and comparing 108 subjects (this number assumes 10% dropout) of the combined Arms 2c and 2d we will have ~85% power to detect 80% vaccine effect through the (0,1) infective/non-infective way of quantifying each DSF with 32 DSF per person and 30 mosquitoes per cup. The (0,1) infective/non-infective way of quantifying each DSF involves declaring a cup as infective if any of the 30 mosquitoes is infected and conversely non-infective if and only if all 30 mosquitoes are not infected. While combining arms that have full dosing in one and fractional dosing in the other assumes they are having similar effects, this assumption is deemed acceptable in order to power the analysis. If infectivity drops to 10.3%, then the power to detect 80% vaccine effect under same conditions is about 40%. It is important to note that in 2016 only 15 mosquitoes were used per cup and only 12 DSFs per person were conducted. Thus, we are treating the 10.3% infectivity as a “worse case scenario” for the 30 cup / 24 DSFs set-up as proposed in this protocol. A binomial family GEE model will be used to account for repeated measurements.

On average each feed week we had about 3% positivity over all subjects. The (0,1) infective/non-infective way of quantifying DSF is likely an over-simplification of the true system. As well, this way of quantifying the DSF will have lower power when the vaccine is leaky rather than all or nothing.

For the booster (dose 4), the combined Arms 2c and 2d will be evaluated vs. controls as the primary endpoint.

DSF in conjunction with SMFA
As there will be simultaneous blood draws, a unique opportunity to investigate SMFA predicting zero inflated DSF measurements will be pursued. Simple linear regression models will be explored.

14.6 Randomization

See **Section 8.2** for more information.

Enrollment into the Bamako/Sotuba, Mali portion of the study will be randomized as follows:
- Arms 1a and 2a will be enrolled 1-1 with random block based on time of enrollment/randomization;
- Arm 3a will not have a concurrent arm, so there will be no randomization for that arm;
- Arms 1b, 2b and 4a will be enrolled 1-1-1 with the same style of random block based on time of enrollment/ randomization;
- Arms 3b and 4b will be enrolled 1-1 with random block based on time of enrollment/randomization.

Enrollment into the Bancoumana/Doneguebougou, Mali portion of the study will be randomized in random blocks of size no greater than 8 and stratified by village. In order to accomplish this, a list of all enrolled subjects will be given to the study statistician for the first randomization. As subjects that dropout after randomization, and are not vaccinated, cannot be replaced, randomization and first vaccination should occur as close in time as possible to avoid having subjects in the ITT efficacy and safety analyses that have never received vaccination or comparator. Randomization lists are not produced pre-enrollment to reduce the risk of on-site operational error in randomization assignment, which given the stratified nature of the randomization would require management of several lists.

Randomization will occur **between arms** as follows in the Bancoumana/Doneguebougou study:
- Arms 2c, 2d, and 4c will be enrolled 1-1-2 with random block sizes no greater than 8.

Randomization between the arms allows for causal comparisons of ELISA and functional assay responses.

15 Human Subject Protections and Ethical Obligations

This research will be conducted in compliance with the protocol, Good Clinical Practices (GCP), and all applicable regulatory requirements.

15.1 Institutional Review Board

A copy of the protocol, informed consent forms, and other study related information to be completed by subjects, such as questionnaires, diary cards, medical history forms, and any proposed advertising/recruitment materials or letters to the subjects will be submitted to the reviewing IRBs for written approval. The investigator must submit and obtain approval from the IRBs for all subsequent amendments to the protocol, informed consent documents, and other study documentation referenced above. The investigator will be responsible for obtaining IRB approval of the annual Continuing Review throughout the duration of the study. The investigators will notify the reviewing IRBs of protocol violations and SAEs as specified in the relevant sections of the protocol.

15.2 Informed Consent Process

Informed consent is a process where information is presented to enable persons to voluntarily decide whether or not to participate as a research subject. It is an on-going conversation between the human research subject and the researchers which begins before consent is given and continues until the end of the subject's involvement in the research. Discussions about the research will provide essential information about the study and include: purpose, duration, experimental procedures, alternatives, risks and benefits. Subjects will be given the opportunity to ask questions and have them answered.

Consent forms will be approved by all participating IRBs. The subjects will sign the informed consent document prior to undergoing any research procedures. The subjects may withdraw consent at any time throughout the course of the trial. The informed consent process will be documented in the subject’s research chart, as required by 21 CFR 312.62. The informed consent form will be signed (or fingerprinted) and personally dated by the subject and the person who conducted the informed consent discussion. The original signed informed consent form will be retained in the subject’s chart and a signed and dated copy will be provided to the subject. The rights and welfare of the subjects will be protected by emphasizing to them that the quality of their medical care will not be adversely affected if they decline to participate in this study.

15.2.1 Mali Site Community Permission and Individual Informed Consent Process

15.2.1.1 Community Permission

Community permission will be obtained from village elders, family heads, and other community members after explanation and discussion of the study.[41] The community permission process goes through the following steps:

- Study investigators/personnel explain the study to village leaders, including the village chief, family heads, women association, and elders.
- The village leaders then discuss the study with family heads and community members and relay any additional questions or concerns they may have to study personnel.

- The study and the informed consent process are explained in detail to heads of families by study investigators/personnel.

At the time of community permission, the need for both husband and wife to agree to avoid pregnancy for the specified period if a wife chooses to volunteer will also be addressed.

The individual informed consent process and form will be translated into French. The study team conducts careful word-for-word review of the study consent form, and will translate the consent orally into local languages, as the majority of potential study subjects do not read or speak French. Verification that the oral translations are accurate and that the potential subjects understand the contents of the informed consent form will be done by an independent witness who is not a member of the study team. An evaluation checklist is performed to make sure that the study is understood by the subjects before the enrolment.

15.2.1.2 Individual Informed Consent

Local households and families will be invited to come to the study clinic for review of the informed consent, and if the subject agrees to participate, the subject will sign or fingerprint (if illiterate) the consent form.

At the consenting visit, the subject will read the consent form or have it explained in cases of illiteracy. Individuals in each family will be separately consented and not all individuals from a household need to participate.

Subjects will be encouraged to ask questions, and then take a multiple-choice questionnaire (true/false) to evaluate consent comprehension. All incorrect responses will be reviewed with the subject, and he or she must verbalize understanding of all incorrect responses. A score of ≥80% correct is required for enrollment. For subjects scoring less than 80%, study staff may choose to review study details again with subject and reassess comprehension with a repeat Malaria Comprehension Exam. At the discretion of the Investigator, any subject whose comprehension is questionable, regardless of score, may be excluded from enrollment.

The Malaria Comprehension Exam will be translated into French and administered orally in the native dialect in the case of potential subjects who cannot read. Study staff will use incorrect answers from the questionnaire to identify those areas of the informed consent that need further review with subject. This will help ensure that the subject has sufficient understanding before the consent form is signed. The subject may either sign the consent form immediately or later after further consideration. Subjects unable to read will place a fingerprint in the place of a signature. In addition, an independent witness will sign the consent form to attest that the consent was fully explained and all questions were answered.

15.3 Subject Confidentiality

Subjects will not be identified in any publicly released reports of this study. All records will be kept confidential to the extent provided by federal, state, and local law. The study monitors and other authorized representatives of the Sponsor may inspect all documents and records required to be maintained by the Investigator. The investigator will inform the subjects that the above-named representatives will review their study-related records without violating the confidentiality of the subjects. All laboratory specimens, evaluation forms, reports, and other records that leave the site will be identified only by a coded number in order to maintain subject confidentiality. All records will be kept locked and all computer entry and networking programs will be done with coded numbers only. Clinical information will not be released without written permission of the subject, except as necessary for monitoring by IRB, the FDA, the NIAID, the OHRP, or the sponsor's designee.

15.4 Risks

Risks to the subjects are associated with venipuncture, immunization, drug treatment, and DSFs. These risks are outlined below:

15.4.1 Venipuncture

Risks occasionally associated with venipuncture include pain, bruising, bleeding, and infection at the site of venipuncture, lightheadedness, and rarely, syncope.

15.4.2 Intramuscular Immunizations

Possible local vaccine reactions resulting from intramuscular injection include pain, swelling, erythema, induration, limitation of limb movement for several days, lymphadenopathy, or pruritus at the injection site. Systemic reactions such as fever, chills, headache, fatigue, malaise, myalgia, and joint pain may also occur, and may range from mild to severe. These side effect will be monitored, but are generally mild and self limiting.

EPA has been studied in both malaria transmission vaccine studies (as noted with Pfs25H, Pfs25M, Pfs230D1M) and other vaccination studies [12-14,42,43]. The use of EPA has identified no safety issues to date.

As with any vaccine, immediate hypersensitivity reactions including urticaria, anaphylaxis, or other IgE-mediated responses are possible. There is a theoretical possibility of risks about which we have no present knowledge. Subjects will be informed of any such risks should further data become available.

15.4.2.1 Immunization with Pfs25M-EPA/AS01 and/or Pfs230D1M/AS01

As noted in **Section 3.1** (for recent studies for Pfs25H-EPA/Alhydrogel® Pfs25M-EPA/Alhydrogel®) and **Section 3.2** (for recent studies for Pfs230D1M-EPA/Alhydrogel®) and **Section 3.3** (for recent studies for Pfs25M-EPA/Alhydrogel® and Pfs230D1M-EPA/Alhydrogel®), common solicited adverse events include injection site pain, redness, induration, and swelling. Other commonly reported solicited adverse events includes headache, malaise, diarrhea, nausea, and fever.

While several subjects in the US and the Mali studies experienced changes in hemalogic parameters (such as anemia, leukopenia, and neutropenia), some even moderate (Grade 2) in severity, an examination of trends in the US study and the unblinded portion of the Mali study (Group 1) has not shown any pattern deemed related to vaccination. In addition, all neutropenia events that had occurred were not associated with fever and/or subsequent acute infection attributable to the drop in neutrophil counts.

A single subject in protocol #15-I-0044, a healthy 51 year old woman, presented six days following receipt of her fourth vaccination while on study, with an acute onset of hemiplegia. She was diagnosed as having a cerebrovascular accident via CT scan and neurological assessment at the hospital in Bamako, Mali and hospitalized. Her symptoms worsened overnight, and she died the following day. This SAE was reviewed at length by the study team, independent safety monitor, Sponsor medical monitor, NIH IRB, FMPOS EC, and the study's DSMB and was determined unrelated to vaccination. In addition, the SAE was submitted to the FDA for review as an informational item. Which vaccine the subject received during the study (comparator vaccine or test vaccine(s)) remains blinded, but given the severity of the event, at the recommendation of our DSMB, we are informing subjects in this study of the SAE reported in #15-I-0044. At this time we do not believe the arterial or venous occlusion are possible risk factors associated with vaccination, but want to provide as much available information to future volunteers as possible. Which vaccine(s) the subject received during that study will be unblinded in March 2017.

Overall, with the exception of local reactogenicity, such as pain, swelling, erythema, tenderness at the site of injection and systemic symptoms such as low-grade fever and short-term flu-like symptoms: fatigue, myalgia, headache, malaise, MPL-based adjuvants (AS01) have been safe and well tolerated.

15.4.2.2 Immunization with ENGERIX-B

In adults, the most common (≥10%) solicited adverse events include injection site pain and fatigue. Other common reported solicited adverse events (1-10%) in adults includes injection site erythema/induration/swelling, dizziness, and headache. Brief fainting spells and related

symptoms have been reported following vaccination, most often in adolescents, that may result in falls and/or injuries.

For complete safety details, please refer to the package insert provided.

As with any vaccine, hypersensitivity, including severe allergic reaction, to any component of the vaccine may occur. Additionally, the tip caps of the pre-filled syringes contain natural rubber latex, which may cause allergic reactions, thus subjects known to be latex sensitive will be excluded per **Section 6.5**; however, those with an unknown latex sensitivity may be at risk for an allergic reaction.

15.4.3 Immunization with Menactra®

For Menactra® the most common (≥10%) solicited adverse events include injection site pain, redness, induration, and swelling; anorexia and diarrhea. Other common reported solicited adverse events in adults includes headache, fatigue, malaise, and arthralgia. Brief fainting spells and related symptoms have been reported following vaccination, most often in adolescents, that may result in falls and/or injuries.

For complete safety details, please refer to the package insert provided.

15.4.4 Coartem®

Subjects in Arms 2c, 2d and 4c will all be pre-emptively treated prior to Vaccination #1, regardless of their malaria status, with Coartem®, which is a registered, oral, proven, and highly efficacious treatment.

Coartem® has an acceptable safety profile. Individuals who may have any contraindication for the use of this drug (e.g. prolonged QTc or taking other medications that can prolong QTc, history of myocardial infarction) will be excluded at screening. The most common side effects (i.e., >30%) in adults are: headache, anorexia, dizziness, asthenia, arthralgia, and myalgia. Discontinuation of Coartem® due to AE is rare (0.2%) in adults. Rare but serious hypersensitivity reactions (urticarial and angioedema) and skin reactions (bullous eruption) have been reported post marketing. Coartem® should be taken with milk or another fatty food.

Coartem® is a Category C pregnancy drug. Thus all female participants will undergo pregnancy testing prior to receipt of the investigational dose of Coartem® if enrolled in Arms 2c, 2d, or 4c. Also per the package insert, Coartem® may decrease the efficacy of hormonal birth control, so female volunteers who are on hormonal birth control will be counseled about back up pregnancy prevention methods.

For complete safety details, please refer to the package insert provided for Coartem®.

15.4.5 Direct Skin Feeds

Risks associated with mosquito bites include mild to moderate swelling and itching at the site. Participants will be offered symptomatic treatment (i.e., local application of antipruritic agent) if these occur. While malaria and filarial parasites are transmitted through *Anopheline* mosquito bites as part of the parasite life cycle, mosquitoes raised in an insectary that are used for DSFs have never been exposed to human blood (breeding stocks are maintained on human blood as noted in **Section 9.5.2**) and since these parasites are not transovarially (vertically) transmitted in mosquitoes, there is no risk of transmission to participants. O'nyong'nyong virus is transmitted to humans by anopheles mosquitoes, but is not vertically transmitted so does not pose a risk of infection to subjects or the mosquito colony. Anopheline mosquitoes can rarely transmit rift valley fever (RVF) virus, and RVF has been reported to transovarially infect anopheles [44]. RVF is primarily transmitted to humans by direct contact with infected animals, although transmission by mosquitoes can occur, with Aedes mosquitoes being the primary vector. The incubation period is 2-6 days.[45] No outbreaks of RVF have been reported in Mali, and transovarial infection is rare and extremely unlikely to persist for generations (for example, in a lab colony maintained at MRTC or elsewhere). To document the lack of viral contamination, the lab strain and field caught mosquitoes were tested for RVFV using molecular methods. Testing conducted over the last 2 years in Mali on LMIV/MRTC clinical trials have all been negative for RVFV. Because routine tests for this virus are not feasible, we installed a procedure to ensure that blood meals used to rear the mosquitoes are free of the virus (see **Section 9.5.2**).

This study involves direct feeds with *Anopheles* mosquitoes only. *Aedes* and *Culex* mosquitoes transmit viral diseases such as dengue, West Nile, and yellow fever, which may be vertically transmitted in mosquitoes; however no Aedes and Culex mosquitoes are raised in the MRTC insectary, thus transmission of these viral agents to subjects is not possible through feeds conducted as part of this study. As previously described (see **Section 9.4.4.2),** hundreds of subjects in Bancoumana or neighboring areas have been enrolled in DSFs. Except for 1 case of definitely related Grade 2 erythema that were resolved within 48 hours and 2 cases of definitely related local site pruritus at the DSF site (in the same individual), there have been no other expected or unexpected AEs recorded as related (definitely, probably, possibly) to the feeding procedures.

15.4.6 Other risks

Women of reproductive potential will be required to agree to use birth control as outlined in **Section 6.4**. Because this is a research study, women of reproductive potential will be asked to notify the site immediately upon learning of pregnancy during this study and will be tested for pregnancy prior to administration of the vaccine(s) and periodically throughout the course of the study as outlined in **Appendix B**.

15.5 Benefits

Subjects will not receive any direct benefit from participation in this study. It is hoped that information gained in this study will contribute to the development of a safe and effective malaria vaccine.

15.6 Compensation

Subjects will be given in kind (such as rice and/or millet) or cash equivalent, in multiple installments as outlined in **Table 17**, to compensate for the time taken to come to the study clinic for study-related visits. Preferred compensation is in kind, such as rice and/or millet, rather than cash, which had been decided in consultation with village elders, but case-by-case exceptions to receive the cash equivalent have been considered acceptable.

The Mali Ethics Committee recommends compensating the study subject for their time lost for study procedures. The amount equivalent to USD $6 for each scheduled visit with laboratory procedures and equivalent to USD $3 for each scheduled visit without laboratory procedures, and equivalent to USD $6 for DSF visits is therefore proposed to compensate for time lost, since visits with laboratory procedures require more time than visits without and frequent DSF assessments are a significant time commitment to the study on behalf of the subject. The estimated installments will be distributed as outlined in **Table 17**.

Table 17: Estimated Compensation Schedule[1]

Study Activity	US Dollar Equivalent in Rice or Millet Dispensed (Local Currency [CFA])[2]		
	Bamako/Sotuba, low dose – 3 vaccination	Bamako/Sotuba, high dose – 3 vaccinations	Bancoumana/Doneguebougou, high dose and fractional dose – 3 vaccinations
Screening	$6 (= 3000 CFA)		
Completion of Vaccination #1	$24 (= 12000 CFA)	$24 (= 12000 CFA)	$30 (= 16000 CFA)
Completion of Vaccination #2	$39 (= 19500 CFA)	$42 (= 21000 CFA)	$42 (= 21000 CFA)
Completion of Vaccination #3	$30 (= 15000 CFA)	$33 (= 16500 CFA)	$159 (= 79500 CFA)
Safety Follow-up	$24 (= 12000 CFA)	$24 (= 12000 CFA)	$18 (= 9000 CFA)
Duration Follow-up	$12 (= 6000 CFA)	$12 (= 6000 CFA)	$12 (= 6000 CFA)
Completion of Vaccination #4	N/A	N/A	$219 (= 109500 CFA)
Safety Follow-up	N/A	N/A	$24 (= 12000 CFA)
Total	$135 (= 67500 CFA)	$141 (= 70500 CFA)	$510 (= 255000 CFA)

1 Compensation installments may be paid out in 3-6 installments at the clinic's discretion to the subjects during these specified time periods.
2 Assuming currency exchange rate of USD $1 = 500 CFA

16 Data Handling and Record Keeping

16.1 Data Capture and Management

In Mali, study data will be collected on paper CRFs and then put into a study specific DataFax electronic database. Data from CRFs will be collected directly from subjects during study visits and telephone calls, or will be abstracted from subjects' medical records. CRFs will be used as source. Any type of corrections to paper CRFs must be initiated and dated by the person making the correction. All CRFs should be reviewed by the Investigator and signed as required with written signature.

Data entry will be performed by authorized individuals. Corrections to the electronic data systems will be tracked electronically (password protected or through an audit trail) with time,

date, individual making the correction, and what was changed. The Investigator is responsible for assuring that the data collected are complete, accurate, and recorded in a timely manner.

16.2 Types of Data

Source documents include all recordings of observations or notations of clinical activities and all reports and records necessary to confirm the data abstracted for this study. Complete source documentation (laboratory test reports, hospital or medical records, progress notes, observations, etc.) is required for every study subject for the duration of the study. Source documentation will be made available for review or audit by the Sponsors, or their designees and any applicable Federal authorities.

16.3 Retention of Study Records

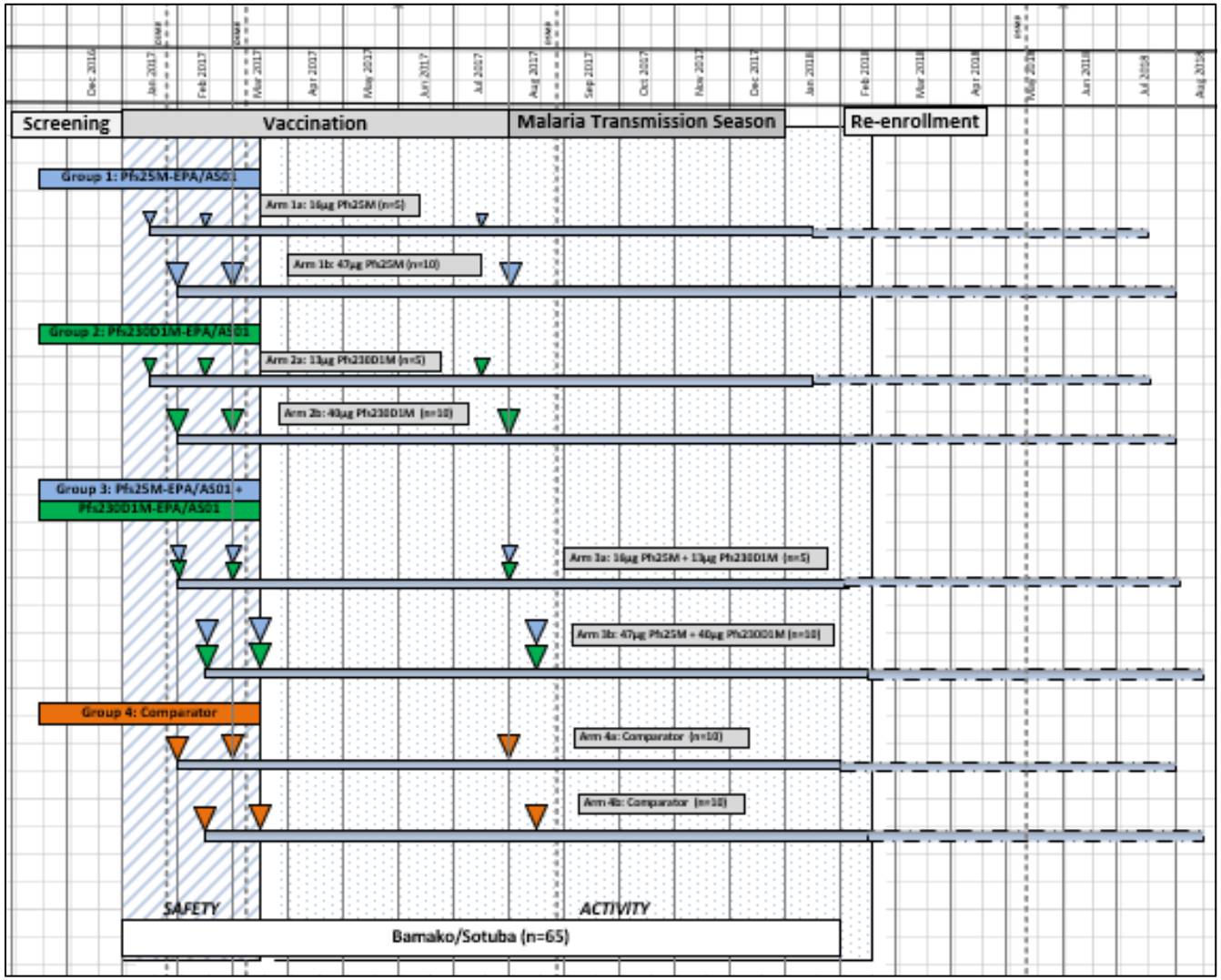
The investigator is responsible for retaining all essential documents listed in the ICH Good Clinical Practice Guideline. Study records will be maintained by the PI for a minimum of 3 years and in compliance with institutional, IRB, state, and federal medical records retention requirements, whichever is longest. All stored records will be kept confidential to the extent required by federal, state, and local law.

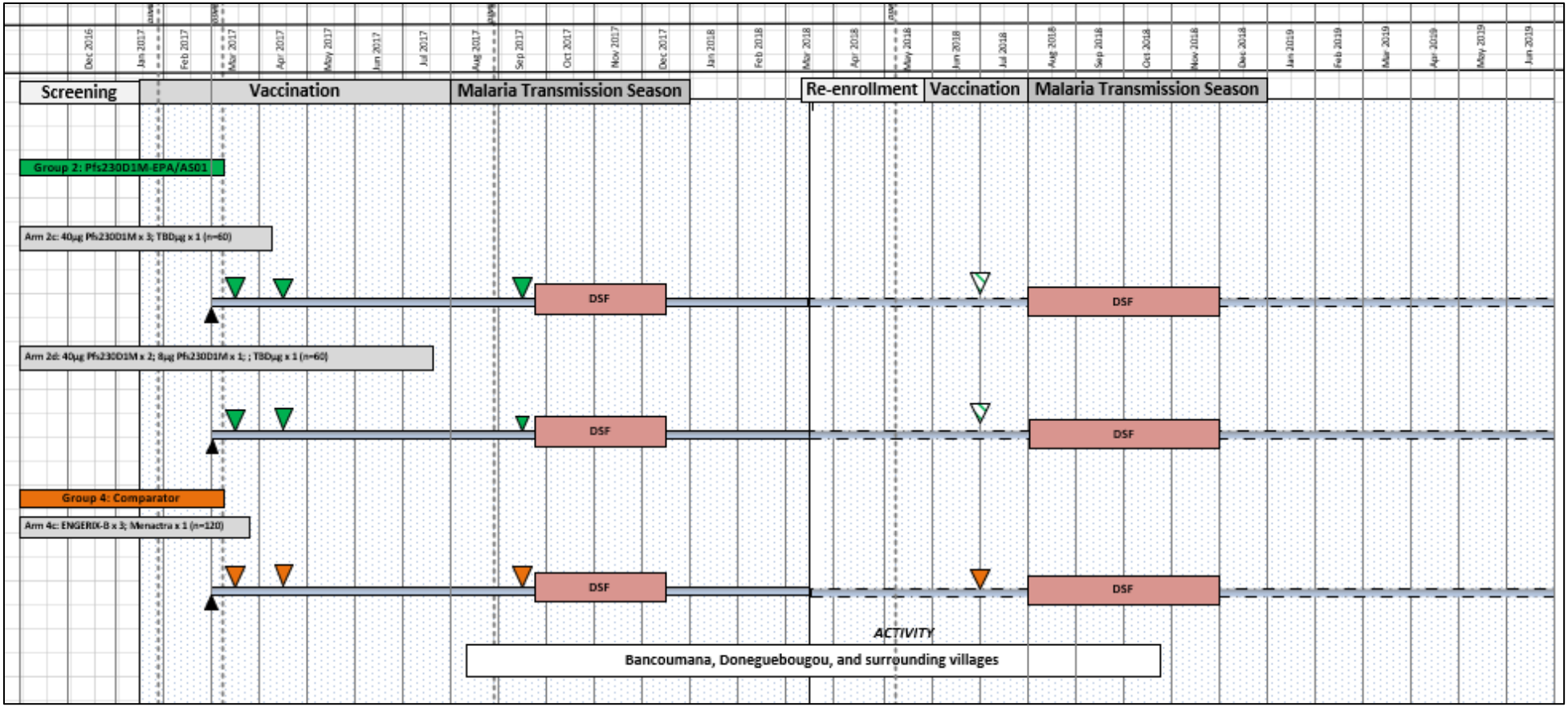
Should the investigator wish to assign the study records to another party and/or move them to another location, the investigator will provide written notification of such intent to OCRPRO/NIAID with the name of the person who will accept responsibility for the transferred records and/or their new location. Destruction or relocation of research records will not proceed without written permission from NIAID/OCRPRO.

16.4 Protocol Revisions

No revisions to this protocol will be permitted without documented approval from the IRBs that granted the original approval for the study. Any change to the protocol will be submitted to the Sponsor and to the participating IRBs as a protocol amendment; changes not affecting risk to subjects may request an expedited review. In the event of a medical emergency, the Investigator shall perform any medical procedures that are deemed medically appropriate and will notify the IND Sponsor of all such occurrences.

Appendix A: Study Schema




Dec 2016
Jan 2017
Feb 2017
Mar 2017
Apr 2017
May 2017
Jun 2017
Jul 2017
Aug 2017
Sep 2017
Oct 2017
Nov 2017
Dec 2017
Jan 2018
Feb 2018
Mar 2018
Apr 2018
May 2018
Jun 2018
Jul 2018
Aug 2018
Sep 2018
Oct 2018
Nov 2018
Dec 2018
Jan 2019
Feb 2019
Mar 2019
Apr 2019
May 2019
Jun 2019
Screening
Vaccination
Malaria Transmission Season
Re-enrollment
Vaccination
Malaria Transmission Season
Group 2: Pfs230D1M-EPA/AS01
Arm 2c: 40µg Pfs230D1M x 3; TBDµg x 1 (n=60)
DSF
DSF
Arm 2d: 40µg Pfs230D1M x 2; 8µg Pfs230D1M x 1; ; TBDµg x 1 (n=60)
DSF
DSF
Group 4: Comparator
Arm 4c: ENGERIX-B x 3; Menactra x 1 (n=120)
DSF
DSF
ACTIVITY
Bancoumana, Doneguebougou, and surrounding villages

Appendix B: Schedule of Procedures/Evaluations

Bamako/Sotuba Arms: 1a, 2a, 3a		Months	Screening	0					1					2	3	4	5	6					7	8	9	10	11	12	15	18
		Visits		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27
Procedures	Blood Volume	Study Day		0	1	3	7	14	28	29	31	35	42	56	84	112	140	168	169	171	175	182	196	224	252	280	308	336	420	532
		Days Post Vac		0	1	3	7	14	0	1	3	7	14	28	56	84	112	0	1	3	7	14	28	56	84	112	140	168	252	364
		Visit Windows (days) (1)			0	±1	±2	±3	±7	0	±1	±2	±3	±7	±14	±21	±21	±21	0	±1	±2	±3	±7	±7	±14	±21	±21	±21	-14/+90	+42
Clinical Procedures																														
Complete medical history/ physical			X																										
Informed consent			X																									X	
Pre-test/Post-test HIV counseling			X																										
Interim clinical evaluation				X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
AE/SAE assessment				X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Pregnancy prevention			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X				
Conmed review			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
VACCINATION				X					X									X												
Laboratory Procedures																														
CBC with differential	EDTA		2	2		2		2	2		2		2					2		2		2								
ALT/ Creatinine	SST		3	3		3		3	3		3		3					3		3		3								
Hepatitis B, C, HIV testing	SST		5																											
Urine dipstick or Urinalysis	Urine Container		X																											
Urine/Serum pregnancy test (females only)	Urine Container or SST		X	X					X									X												
Malaria Blood Smear	CAP/EDTA			0.5				0.5	0.5				0.5			0.5		0.5				0.5	0.5	0.5		0.5		0.5	0.5	0.5
Malaria PCR/Genotyping/ Gametocyte	CAP/EDTA			0.5				0.5	0.5				0.5			0.5		0.5				0.5	0.5	0.5		0.5		0.5	0.5	0.5
Pfs25M and Pfs230 ELISA	SST			10				5	5				5			5		5				5	5	5		5		5	5	5
SMFA (2)																														
Daily blood draw volume in mL			10	16	0	5	0	11	11	0	5	0	11	0	0	6	0	11	0	5	0	11	6	6	0	6	0	6	6	6
Cumulative blood volume in mL			10	26	26	31	31	42	53	53	58	58	69	69	69	75	75	86	86	91	91	102	108	114	114	120	120	126	132	138
1 Visit windows are based off timing of days post the preceding vaccination																														
2 SMFA may be completed on all eligible subjects at Study Day 0 and 182; other SMFA time points indicated above will be completed depending on associated ELISA results at that time point.																														

Note: visits #26 and 27 are the additional blood draw visits.

Appendix B: Schedule of Procedures

Bamako/Sotuba Arms: 1b, 2b, 3b, 4a, 4b		Months		0					1					2	3	4	5	6					7	8	9	10	11	12	15	18
		Visits		1 (2)	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27
Procedures		Study Day	Screening	0	1	3	7	14	28	29	31	35	42	56	84	112	140	168	169	171	175	182	196	224	252	280	308	336	420	532
	Blood Volume	Days Post Vac		0	1	3	7	14	0	1	3	7	14	28	56	84	112	0	1	3	7	14	28	56	84	112	140	168	252	364
		Visit Windows (days) (1)			0	±1	±2	±3	±7	0	±1	±2	±3	±7	±14	±21	±21	±21	0	±1	±2	±3	±7	±7	±14	±21	±21	±21	-14/+90	+42
Clinical Procedures																														
Complete medical history/ physical			X																											
Informed consent			X																									X	
Pre-test/Post-test HIV counseling			X																											
Interim clinical evaluation				X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
AE/SAE assessment				X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Pregnancy prevention			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X					
Conmed review			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
VACCINATION				X					X									X												
Laboratory Procedures																														
CBC with differential	EDTA		2	2		2		2	2		2		2					2		2		2								
ALT/ Creatinine	SST		3	3		3		3	3		3		3					3		3		3								
Hepatitis B, C, HIV testing	SST		5																											
Urine dipstick or Urinalysis	Urine Container		X																											
Urine/Serum pregnancy test (females only)	Urine Container or SST		X	X					X									X												
Malaria Blood Smear	CAP/EDTA			0.5				0.5	0.5				0.5			0.5		0.5				0.5	0.5	0.5		0.5		0.5	0.5	0.5
Malaria PCR/Genotyping/ Gametocyte	CAP/EDTA			0.5				0.5	0.5				0.5			0.5		0.5				0.5	0.5	0.5		0.5		0.5	0.5	0.5
Pfs25M and Pfs230 ELISA	SST			15				10	10				10			10		10				15	10	10		10		10	5	5
SMFA (3)																														
Cellular assays/B cell studies	NaHep			90	40							80									80	30	30					30		
Ex vivo assays (4)				X	X							X									X	X	X					X		
Transcriptional analysis	PAXGene			1	1							1									1	1	1					1		
Daily blood draw volume in mL			10	111	40	5	0	16	16	0	5	80	16	0	0	11	0	16	0	5	80	51	41	11	0	11	0	41	6	6
Cumulative blood volume in mL			10	121	161	166	166	182	198	198	203	283	299	299	299	310	310	326	326	331	411	462	503	514	514	525	525	566	572	578

1 Visit windows are based off timing of days post the preceding vaccination

2 As noted in the protocol, large blood draw on Day 0 may be broken up into two visits due to the large volume of PBMCs requested and to obtain two baseline samples for PBMCs.

3 SMFA will be completed on all eligible subjects at Study Day 0 and 182; other SMFA time points indicated above will be completed depending on associated ELISA results at that time point.

4 *Ex vivo* assays (~0.5 mL) will be completed on most PBMC draw days and completed in Bamako

Note: visits #26 and 27 are the additional blood draw visits

Bancoumana/Donegnebougou Arms: 2c, 2d, 4c		Months			0					1					2	3	4	5	6			7									8								9		10	11	12
		Visits		-7	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44
Procedures	Blood Volume	Study Day	Screening	-7	0	1	3	7	14	28	29	31	35	42	56	84	112	140	168	169	171	175	178	182	185	189	192	196	199	203	206	210	213	217	220	224	227	231	234	238	241	245	248	252	255	280	308	336
		Days post Vac		-7	0	1	3	7	14	0	1	3	7	14	28	56	84	112	0	1	3	7	10	14	17	21	24	28	31	35	38	42	45	49	52	56	59	63	66	70	73	77	80	84	87	112	140	168
		Visit Windows (days) (1)		±7		0	±1	±2	±3	±7	0	±1	±2	±3	±7	±7	±14	±14	±28	0	±1	±2	±2	±2	±2	±2	±2	±2	±2	±2	±2	±2	±2	±2	±2	±2	±2	±2	±2	±2	±2	±2	±2	±2	±14	±21	±21
Clinical Procedures																																															
Laboratory Procedures																																															
CBC with differential	EDTA		2.0	2.0	2.0		2.0		2.0	2.0		2.0		2.0					2.0		2.0			2.0																							
ALT/ Creatinine	SST		3.0	3.0	3.0		3.0		3.0	3.0		3.0		3.0					3.0		3.0			3.0																							
Hepatitis B, C, HIV testing	SST		5.0																																											
Urine dipstick or Urinalysis	Urine Container		X																																										
Hemoglobin Typing	EDTA			X																																										
Urine/Serum pregnancy test (females only)	Urine Container or SST		X	X	X				X										X			X		X		X		X		X		X		X		X		X		X		X				
Schistosomiasis and Helminth Testing	Urine/ Stool Sample				X																																								
Malaria Blood Smear	CAP/EDTA			0.5	0.5				0.5	0.5				0.5			0.5		0.5			0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Malaria PCR/Genotyping/ Gametocyte	CAP/EDTA			0.5	0.5				0.5	0.5				0.5			0.5		0.5			1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0.5	0.5	0.5
Pfs25M and Pfs230 ELISA	SST				10				5	5				5			5		5			5		20				5				5				5					5				5		5	
SMFA (2)																																															
DSF (3)	X																					X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X			
Cellular assays/B cell assays	NaHep			20	40		10					10	20								10	40						40																	40	
Ex vivo assays (4)				X	X		X						X									X						X																	X	
Transcriptional analysis	PAXGene			1	1		1			1		1							1		1	1		1				1				1				1					1				1		1	
Daily blood draw volume in mL			10.0	27.0	57.0	0.0	16.0	0.0	11.0	12.0	0.0	16.0	20.0	11.0	0.0	0.0	6.0	0.0	12.0	0.0	16.0	47.5	1.5	27.5	1.5	1.5	1.5	47.5	1.5	1.5	1.5	7.5	1.5	1.5	1.5	7.5	1.5	1.5	1.5	7.5	1.5	1.5	1.5	7.5	1.5	7.0	1.0	47.0
Cumulative blood volume in mL			10.0	37.0	94.0	94.0	110.0	110.0	121.0	133.0	133.0	149.0	169.0	180.0	180.0	180.0	186.0	186.0	198.0	198.0	214.0	261.5	263.0	290.5	292.0	293.5	295.0	342.5	344.0	345.5	347.0	354.5	356.0	357.5	359.0	366.5	368.0	369.5	371.0	378.5	380.0	381.5	383.0	390.5	392.0	399.0	400.0	447.0

1 Visit windows are based off timing of days post the preceding vaccination

2 SMFA will be completed on all eligible subjects at Study Day 0 and 182; other SMFA time points indicated above will be completed depending on associated ELISA and DSF results at that time point.

3 DSF will be completed twice a week from day 7 post Vaccination #3 through day 87 post Vaccination #3. If insufficient mosquitoes are available for twice a week feeds, once a week feeds will be completed on the following Study Days: 175, 182, 189, 196, 203, 210, 217, 224, 231, 238, 245, 252)

4 Ex vivo assays (~0.5mL) will be completed on most PBMC draw days and completed in Bamako

Appendix B: Schedule of Procedures

Vaccination #4 study procedures

Bancoumana/Donegnebougou Arms: 2c, 2d, 4c		Months	Rescreening (Study Day 462) Visit Window ±70	17									18								19								20								21		22	23	26	29
		Visits		45	46	47	48	49	50	51	52	53	54	55	56	57	58	59	60	61	62	63	64	65	66	67	68	69	70	71	72	73	74	75	76	77	78	79	84	85	86	87
Procedures		Study Day		476	477	479	483	486	490	493	497	501	504	507	511	514	518	521	525	528	532	535	539	542	546	549	553	556	560	563	567	570	574	577	581	584	588	591	616	644	728	812
		Days post Vac		0	1	3	7	10	14	17	21	25	28	31	35	38	42	45	49	52	56	59	63	66	70	73	77	80	84	87	91	94	98	101	105	108	112	115	140	168	252	336
		Visit Windows (days) (1)		±56	0	±1	±2	±2	±2	±2	±2	±2	±2	±2	±2	±2	±2	±2	±2	±2	±2	±2	±2	±2	±2	±2	±2	±2	±2	±2	±2	±2	±2	±2	±2	±2	±2	±2	±2	±21	±21	±21
Clinical Procedures																																									
Complete medical history/ physical			X																																					
Informed consent			X																																					
Pre test/Post test HIV counseling			X																																					
Interim clinical evaluation				X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
AE/SAE assessment			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X				
Pregnancy Prevention			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X				
Conmed review			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
VACCINATION				X																																					
Feeding Evaluation (1)							X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X				
Laboratory Procedures																																									
CBC with differential	EDTA		2.0	2.0		2.0			2.0																																
ALT/ Creatinine	SST		3.0	3.0		3.0			3.0																																
Hepatitis B, C, HIV testing	SST		5.0																																						
Urine dipstick or Urinalysis	Urine Container		X																																						
Hemoglobin Typing	EDTA																																								
Urine/Serum pregnancy test (females only)	Urine Container or SST		X	X			X		X		X		X		X		X		X		X		X		X		X		X		X		X		X					
Malaria Blood Smear	CAP/EDTA		0.5	0.5			0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5	0.5
Malaria PCR/Genotyping/ Gametocyte	CAP/EDTA		0.5	0.5			1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	0.5	0.5	0.5	0.5
Pfs25M and Pfs230 ELISA SMFA (2)	SST		5	5					10				10								5								5								5		10	10	10	10
DSF (3)	X						X		X				X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X				
Cellular assays/B cell assays	NaHep			20		10	20.0						20																											40		40
Ex vivo assays (4)							X						X																											X		X
Transcriptional analysis	PAXGene			1		1	1.0		1.0				1																											1		1
Daily blood draw volume in mL			16.0	32.0	0.0	16.0	22.5	1.5	17.5	1.5	1.5	1.5	32.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	6.5	1.5	1.5	1.5	1.5	1.5	1.5	6.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	6.5	1.5	11.0	52.0	11.0	52.0
Cumulative blood volume in mL (fourth vaccination)			16.0	48.0	48.0	64.0	86.5	88.0	105.5	107.0	108.5	110.0	142.5	144.0	145.5	147.0	148.5	150.0	151.5	153.0	159.5	161.0	162.5	164.0	165.5	167.0	168.5	170.0	176.5	178.0	179.5	181.0	182.5	184.0	185.5	187.0	193.5	195.0	206.0	258.0	269.0	321.0

1 Visit windows are based off timing of days post the preceding vaccination

2 SMFA will be completed on all eligible subjects at Study Day 476 and 490; other SMFA time points indicated above will be completed depending on associated ELISA and DSF results at that time point.

3 DSF will be completed twice a week from day 7 post Vaccination #4 through day 129 post Vaccination #4. If insufficient mosquitoes are available for twice a week feeds, once a week feeds will be completed

4 Ex vivo assays (~0.5mL) will be completed on most PBMC draw days and completed in Bamako

Appendix C: Day to Day Schedule by Arm

Bamako/Sotuba Cohort – Arms 1a, 2a, 3a (3 Vaccination; safety cohort)

Study Day 0 (day of Vaccination #1)

The following evaluations/procedures will be completed before vaccination:

1. Verify that informed consent was obtained.
2. Ensure that all inclusion/exclusion criteria are met.
3. Ensure that CBC, ALT, creatinine, HIV/HCV/HBV, and urine results from screening are within protocol-defined limits (see Exclusion Criteria above) before vaccinating.
4. Perform interim history and physical examination, focusing on any acute complaints.
5. Record vital signs (blood pressure, temperature, and heart rate).
6. During the physical examination, study staff will educate the subject regarding signs and symptoms of potential adverse events (AEs) and indications for use of antipyretics if fever, headache, or malaise occurs.
7. For females, obtain a urine or serum sample for β-hCG testing. Ensure that the test is negative before vaccinating; a positive test will exclude the subject from the trial.
8. For females, ensure agreement and compliance with pregnancy prevention before vaccinating.
9. Obtain approximately 16 mL of blood for CBC with differential and platelet count, ALT, creatinine, malaria blood smear, malaria PCR, and anti-Pfs25M and anti-Pfs230 antibody ELISA.
10. Confirm that all criteria for vaccination have been met according to inclusion/exclusion criteria
11. Confirm continued eligibility to receive vaccination
12. Notify subject of vaccine assignment.
13. Administer the vaccine.

The following evaluations/procedures will be completed following vaccination:

1. Observe the subject for at least 30 minutes after vaccination to evaluate immediate adverse reactions.
2. Record vital signs (blood pressure, temperature, and heart rate).
3. Perform interim history and physical examination, focusing on any acute complaints.
4. Record AEs and concomitant medications, if applicable.

Study Day 1 (±0 days; 1 day after Vaccination #1)

1. Perform basic history and focused physical examination (including injection site), emphasizing examination of any acute complaints.
2. Record vital signs (blood pressure, temperature, and heart rate).
3. Record AEs and concomitant medications, if applicable.

Study Day 3 (±1 day; 3 days after Vaccination #1)

1. Perform basic history and focused physical examination (including injection site), emphasizing examination of any acute complaints.
2. Record vital signs (blood pressure, temperature, and heart rate).
3. Record AEs and concomitant medications, if applicable.

4. Obtain approximately 5 mL of blood for CBC with differential and platelet count, ALT, creatinine.

Study Day 7 (±2 days; 7 days after Vaccination #1)

1. Perform basic history and focused physical examination (including injection site), emphasizing examination of any acute complaints.
2. Record vital signs (blood pressure, temperature, and heart rate).
3. Record AEs and concomitant medications, if applicable.

Study Day 14 (±3 days; 14 days after Vaccination #1)

1. Perform basic history and focused physical examination, emphasizing examination of any acute complaints.
2. Record vital signs (blood pressure, temperature, and heart rate).
3. Record AEs and concomitant medications, if applicable.
4. Obtain approximately 11 mL of blood for CBC with differential and platelet count, ALT, creatinine, malaria blood smear, malaria PCR, and anti-Pfs25M and anti-Pfs230 antibody ELISA.

Study Day 28 (day of Vaccination #2)

The following evaluations/procedures will be completed before vaccination:

1. Perform interim history and physical examination, focusing on any acute complaints.
2. Record vital signs (blood pressure, temperature, and heart rate).
3. During the physical examination, study staff will educate the subject regarding signs and symptoms of potential adverse events (AEs) and indications for use of antipyretics if fever, headache, or malaise occurs.
4. For females, obtain a urine or serum sample for β-hCG testing. Ensure that the test is negative before vaccinating; a positive test will exclude the subject from the trial.
5. For females, ensure agreement and compliance with pregnancy prevention before vaccinating.
6. Obtain approximately 11 mL of blood for CBC with differential and platelet count, ALT, creatinine, malaria blood smear, malaria PCR, anti-Pfs25M and anti-Pfs230 antibody ELISA, and SMFA.
7. Confirm continued eligibility to receive vaccination
8. Administer the vaccine.

The following evaluations/procedures will be completed following vaccination:

1. Observe the subject for at least 30 minutes after vaccination to evaluate immediate adverse reactions.
2. Record vital signs (blood pressure, temperature, and heart rate).
3. Perform interim history and physical examination, focusing on any acute complaints.
4. Record AEs and concomitant medications, if applicable.

Study Day 29 (±0 days; 1 day after Vaccination #2)

1. Perform basic history and focused physical examination (including injection site), emphasizing examination of any acute complaints.
2. Record vital signs (blood pressure, temperature, and heart rate).
3. Record AEs and concomitant medications, if applicable.

Study Day 31 (±1 day; 3 days after Vaccination #2)
1. Perform basic history and focused physical examination (including injection site), emphasizing examination of any acute complaints.
2. Record vital signs (blood pressure, temperature, and heart rate).
3. Record AEs and concomitant medications, if applicable.
4. Obtain approximately 5 mL of blood for CBC with differential and platelet count, ALT, creatinine.

Study Day 35 (±2 days; 7 days after Vaccination #2)
1. Perform basic history and focused physical examination (including injection site), emphasizing examination of any acute complaints.
2. Record vital signs (blood pressure, temperature, and heart rate).
3. Record AEs and concomitant medications, if applicable.

Study Day 42 (±3 days; 14 days after Vaccination #2)
1. Perform basic history and focused physical examination, emphasizing examination of any acute complaints.
2. Record vital signs (blood pressure, temperature, and heart rate).
3. Record AEs and concomitant medications, if applicable.
4. Obtain approximately 11 mL of blood for CBC with differential and platelet count, ALT, creatinine, malaria blood smear, malaria PCR, SMFA, and anti-Pfs25M and anti-Pfs230 antibody ELISA.

Study Day 56 (±7 days; 28 days after Vaccination #2)
1. Perform basic history and focused physical examination, emphasizing examination of any acute complaints.
2. Record vital signs (blood pressure, temperature, and heart rate).
3. Record AEs and concomitant medications, if applicable.

Study Day 84 (±14 days; 56 days after Vaccination #2)
1. Perform basic history and focused physical examination, emphasizing examination of any acute complaints.
2. Record vital signs (blood pressure, temperature, and heart rate).
3. Record AEs and concomitant medications, if applicable.

Study Day 112 (±21 days; 84 days after Vaccination #2)
1. Perform basic history and focused physical examination, emphasizing examination of any acute complaints.
2. Record vital signs (blood pressure, temperature, and heart rate).
3. Record AEs and concomitant medications, if applicable.
4. Obtain approximately 6 mL of blood for malaria blood smear, malaria PCR, SMFA, and anti-Pfs25M and anti-Pfs230 antibody ELISA.

Study Day 140 (±21 days; 112 days after Vaccination #2)
1. Perform basic history and focused physical examination, emphasizing examination of any acute complaints.

2. Record vital signs (blood pressure, temperature, and heart rate).
3. Record AEs and concomitant medications, if applicable.

Study Day 168 (day of Vaccination #3)

The following evaluations/procedures will be completed before vaccination:

1. Perform interim history and physical examination, focusing on any acute complaints.
2. Record vital signs (blood pressure, temperature, and heart rate).
3. During the physical examination, study staff will educate the subject regarding signs and symptoms of potential adverse events (AEs) and indications for use of antipyretics if fever, headache, or malaise occurs.
4. For females, obtain a urine or serum sample for β-hCG testing. Ensure that the test is negative before vaccinating; a positive test will exclude the subject from the trial.
5. For females, ensure agreement and compliance with pregnancy prevention before vaccinating.
6. Obtain approximately 11 mL of blood for CBC with differential and platelet count, ALT, creatinine, malaria blood smear, malaria PCR, anti-Pfs25M and anti-Pfs230 antibody ELISA, and SMFA.
7. Confirm continued eligibility to receive vaccination
8. Administer the vaccine.

The following evaluations/procedures will be completed following vaccination:

1. Observe the subject for at least 30 minutes after vaccination to evaluate immediate adverse reactions.
2. Record vital signs (blood pressure, temperature, and heart rate).
3. Perform interim history and physical examination, focusing on any acute complaints.
4. Record AEs and concomitant medications, if applicable.

Study Day 169 (±0 days; 1 day after Vaccination #3)

1. Perform basic history and focused physical examination (including injection site), emphasizing examination of any acute complaints.
2. Record vital signs (blood pressure, temperature, and heart rate).
3. Record AEs and concomitant medications, if applicable.

Study Day 171 (±1 day; 3 days after Vaccination #3)

1. Perform basic history and focused physical examination (including injection site), emphasizing examination of any acute complaints.
2. Record vital signs (blood pressure, temperature, and heart rate).
3. Record AEs and concomitant medications, if applicable.
4. Obtain approximately 5 mL of blood for CBC with differential and platelet count, ALT, creatinine.

Study Day 175 (±2 days; 7 days after Vaccination #3)

1. Perform basic history and focused physical examination (including injection site), emphasizing examination of any acute complaints.
2. Record vital signs (blood pressure, temperature, and heart rate).
3. Record AEs and concomitant medications, if applicable.

Study Day 182 (±3 days; 14 days after Vaccination #3)

1. Perform basic history and focused physical examination, emphasizing examination of any acute complaints.
2. Record vital signs (blood pressure, temperature, and heart rate).
3. Record AEs and concomitant medications, if applicable.
4. Obtain approximately 11 mL of blood for CBC with differential and platelet count, ALT, creatinine, malaria blood smear, malaria PCR, SMFA, and anti-Pfs25M and anti-Pfs230 antibody ELISA.

Study Day 196 (±7 days; 28 days after Vaccination #3)

1. Perform basic history and focused physical examination, emphasizing examination of any acute complaints.
2. Record vital signs (blood pressure, temperature, and heart rate).
3. Record AEs and concomitant medications, if applicable.
4. Obtain approximately 6 mL of blood for malaria blood smear, malaria PCR, SMFA, anti-Pfs25M and anti-Pfs230 antibody ELISA.

Study Day 224 (±7 days; 56 days after Vaccination #3)

1. Perform basic history and focused physical examination, emphasizing examination of any acute complaints.
2. Record vital signs (blood pressure, temperature, and heart rate).
3. Record AEs and concomitant medications, if applicable.
4. Obtain approximately 6 mL of blood for malaria blood smear, malaria PCR, SMFA, and anti-Pfs25M and anti-Pfs230 antibody ELISA.

Study Day 252 (±14 days; 84 days after Vaccination #3)

1. Perform basic history and focused physical examination, emphasizing examination of any acute complaints.
2. Record vital signs (blood pressure, temperature, and heart rate).
3. Record AEs and concomitant medications, if applicable.

Study Day 280 (±21 days; 112 days after Vaccination #3)

1. Perform basic history and focused physical examination, emphasizing examination of any acute complaints.
2. Record vital signs (blood pressure, temperature, and heart rate).
3. Record AEs and concomitant medications, if applicable.
4. Obtain approximately 6 mL of blood for malaria blood smear, malaria PCR, SMFA, and anti-Pfs25M and anti-Pfs230 antibody ELISA.

Study Day 308 (±21 days; 140 days after Vaccination #3)

1. Perform basic history and focused physical examination, emphasizing examination of any acute complaints.
2. Record vital signs (blood pressure, temperature, and heart rate).
3. Record AEs and concomitant medications, if applicable.

Study Day 336 (±21 days; 168 days after Vaccination #3)

1. Perform basic history and focused physical examination, emphasizing examination of any acute complaints.
2. Record vital signs (blood pressure, temperature, and heart rate).
3. Record AEs and concomitant medications, if applicable.
4. Obtain approximately 6 mL of blood for malaria blood smear, malaria PCR, SMFA, and anti-Pfs25M and anti-Pfs230 antibody ELISA.

Re-enrollment visits:

Study Day 420 (-14/+90 days; 252 days after Vaccination #3);Re-screening

1. Explain the study and informed consent document to the subject.
2. Ensure the subject has acknowledged consent by signing or fingerprinting the informed consent document. Ensure that the subject receives a signed copy of the informed consent.
3. Perform basic history and focused physical examination, emphasizing examination of any acute complaints.
4. Record vital signs (blood pressure, temperature, and heart rate).
5. Record AEs and concomitant medications, if applicable.
6. Obtain approximately 6 mL of blood for blood for malaria blood smear, malaria PCR, SMFA, and anti-Pfs230 antibody ELISA,

Study Day 532 (+42 days; 364 days after Vaccination #3)

1. Perform basic history and focused physical examination, emphasizing examination of any acute complaints.
2. Record vital signs (blood pressure, temperature, and heart rate).
3. Record AEs and concomitant medications, if applicable.
4. Obtain approximately 6 mL of blood for blood for malaria blood smear, malaria PCR, SMFA, and anti-Pfs230 antibody ELISA
5. Offer ENGERIX-B (or other approved Hepatitis B vaccine) to those subjects that received TBV(s) during the study.

Bamako/Sotuba Cohort – Arms 1b, 2b, 3b, 4a, 4b (3 Vaccinations; safety cohort)

Study Day 0 (day of Vaccination #1)

The following evaluations/procedures will be completed before vaccination:

1. Verify that informed consent was obtained.
2. Ensure that all inclusion/exclusion criteria are met.
3. Ensure that CBC, ALT, creatinine, HIV/HCV/HBV, and urine results from screening are within protocol-defined limits (see Exclusion Criteria above) before vaccinating.
4. Perform interim history and physical examination, focusing on any acute complaints.
5. Record vital signs (blood pressure, temperature, and heart rate).
6. During the physical examination, study staff will educate the subject regarding signs and symptoms of potential adverse events (AEs) and indications for use of antipyretics if fever, headache, or malaise occurs.
7. For females, obtain a urine or serum sample for β-hCG testing. Ensure that the test is negative before vaccinating; a positive test will exclude the subject from the trial.
8. For females, ensure agreement and compliance with pregnancy prevention before vaccinating.
9. Obtain approximately 111 mL of blood for CBC with differential and platelet count, ALT, creatinine, malaria blood smear, malaria PCR, anti-Pfs25M and anti-Pfs230 antibody ELISA, standard membrane feeding assay (SMFA), cellular assays, *ex vivo* assays, transcriptional assays, and B cell studies.
   NOTE: This initial blood draw prior to vaccination, given requested large volume of PBMCs, can be broken up into two visits prior to vaccination.
10. Confirm that all criteria for vaccination have been met according to inclusion/exclusion criteria
11. Confirm continued eligibility to receive vaccination
12. Notify subject of vaccine assignment.
13. Administer the vaccine.

The following evaluations/procedures will be completed following vaccination:

1. Observe the subject for at least 30 minutes after vaccination to evaluate immediate adverse reactions.
2. Record vital signs (blood pressure, temperature, and heart rate).
3. Perform interim history and physical examination, focusing on any acute complaints.
4. Record AEs and concomitant medications, if applicable.

Study Day 1 (±0 days; 1 day after Vaccination #1)

1. Perform basic history and focused physical examination (including injection site), emphasizing examination of any acute complaints.
2. Record vital signs (blood pressure, temperature, and heart rate).
3. Record AEs and concomitant medications, if applicable.
4. Obtain approximately 40 mL of blood for cellular assays, *ex vivo* assays, transcriptional assays, and B cell studies.

Study Day 3 (±1 day; 3 days after Vaccination #1)

1. Perform basic history and focused physical examination (including injection site), emphasizing examination of any acute complaints.
2. Record vital signs (blood pressure, temperature, and heart rate).
3. Record AEs and concomitant medications, if applicable.
4. Obtain approximately 5 mL of blood for CBC with differential and platelet count, ALT, creatinine.

Study Day 7 (±2 days; 7 days after Vaccination #1)

1. Perform basic history and focused physical examination (including injection site), emphasizing examination of any acute complaints.
2. Record vital signs (blood pressure, temperature, and heart rate).
3. Record AEs and concomitant medications, if applicable.

Study Day 14 (±3 days; 14 days after Vaccination #1)

1. Perform basic history and focused physical examination, emphasizing examination of any acute complaints.
2. Record vital signs (blood pressure, temperature, and heart rate).
3. Record AEs and concomitant medications, if applicable.
4. Obtain approximately 16 mL of blood for CBC with differential and platelet count, ALT, creatinine, malaria blood smear, malaria PCR, and anti-Pfs25M and anti-Pfs230 antibody ELISA, and SMFA.

Study Day 28 (day of Vaccination #2)

The following evaluations/procedures will be completed before vaccination:

1. Perform interim history and physical examination, focusing on any acute complaints.
2. Record vital signs (blood pressure, temperature, and heart rate).
3. During the physical examination, study staff will educate the subject regarding signs and symptoms of potential adverse events (AEs) and indications for use of antipyretics if fever, headache, or malaise occurs.
4. For females, obtain a urine or serum sample for β-hCG testing. Ensure that the test is negative before vaccinating; a positive test will exclude the subject from the trial.
5. For females, ensure agreement and compliance with pregnancy prevention before vaccinating.
6. Obtain approximately 16 mL of blood for CBC with differential and platelet count, ALT, creatinine, malaria blood smear, malaria PCR, anti-Pfs25M and anti-Pfs230 antibody ELISA, and standard membrane feeding assay (SMFA).
7. Confirm continued eligibility to receive vaccination
8. Administer the vaccine.

The following evaluations/procedures will be completed following vaccination:

1. Observe the subject for at least 30 minutes after vaccination to evaluate immediate adverse reactions.
2. Record vital signs (blood pressure, temperature, and heart rate).
3. Perform interim history and physical examination, focusing on any acute complaints.
4. Record AEs and concomitant medications, if applicable.

Study Day 29 (±0 days; 1 day after Vaccination #2)

1. Perform basic history and focused physical examination (including injection site), emphasizing examination of any acute complaints.
2. Record vital signs (blood pressure, temperature, and heart rate).
3. Record AEs and concomitant medications, if applicable.

Study Day 31 (±1 day; 3 days after Vaccination #2)

1. Perform basic history and focused physical examination (including injection site), emphasizing examination of any acute complaints.
2. Record vital signs (blood pressure, temperature, and heart rate).
3. Record AEs and concomitant medications, if applicable.
4. Obtain approximately 5 mL of blood for CBC with differential and platelet count, ALT, creatinine.

Study Day 35 (±2 days; 7 days after Vaccination #2)

1. Perform basic history and focused physical examination (including injection site), emphasizing examination of any acute complaints.
2. Record vital signs (blood pressure, temperature, and heart rate).
3. Record AEs and concomitant medications, if applicable.
4. Obtain approximately 80 mL of blood for cellular assays, *ex vivo* assays, transcriptional assays, and B cell studies.

Study Day 42 (±3 days; 14 days after Vaccination #2)

1. Perform basic history and focused physical examination, emphasizing examination of any acute complaints.
2. Record vital signs (blood pressure, temperature, and heart rate).
3. Record AEs and concomitant medications, if applicable.
4. Obtain approximately 16 mL of blood for CBC with differential and platelet count, ALT, creatinine, malaria blood smear, malaria PCR, SMFA, and anti-Pfs25M and anti-Pfs230 antibody ELISA.

Study Day 56 (±7 days; 28 days after Vaccination #2)

1. Perform basic history and focused physical examination, emphasizing examination of any acute complaints.
2. Record vital signs (blood pressure, temperature, and heart rate).
3. Record AEs and concomitant medications, if applicable.

Study Day 84 (±14 days; 56 days after Vaccination #2)

1. Perform basic history and focused physical examination, emphasizing examination of any acute complaints.
2. Record vital signs (blood pressure, temperature, and heart rate).
3. Record AEs and concomitant medications, if applicable.

Study Day 112 (±21 days; 84 days after Vaccination #2)

1. Perform basic history and focused physical examination, emphasizing examination of any acute complaints.
2. Record vital signs (blood pressure, temperature, and heart rate).

3. Record AEs and concomitant medications, if applicable.
4. Obtain approximately 11 mL of blood for malaria blood smear, malaria PCR, SMFA, and anti-Pfs25M and anti-Pfs230 antibody ELISA.

Study Day 140 (±21 days; 112 days after Vaccination #2)

1. Perform basic history and focused physical examination, emphasizing examination of any acute complaints.
2. Record vital signs (blood pressure, temperature, and heart rate).
3. Record AEs and concomitant medications, if applicable.

Study Day 168 (day of Vaccination #3)

The following evaluations/procedures will be completed before vaccination:

1. Perform interim history and physical examination, focusing on any acute complaints.
2. Record vital signs (blood pressure, temperature, and heart rate).
3. During the physical examination, study staff will educate the subject regarding signs and symptoms of potential adverse events (AEs) and indications for use of antipyretics if fever, headache, or malaise occurs.
4. For females, obtain a urine or serum sample for β-hCG testing. Ensure that the test is negative before vaccinating; a positive test will exclude the subject from the trial.
5. For females, ensure agreement and compliance with pregnancy prevention before vaccinating.
6. Obtain approximately 16 mL of blood for CBC with differential and platelet count, ALT, creatinine, malaria blood smear, malaria PCR, anti-Pfs25M and anti-Pfs230 antibody ELISA, SMFA.
7. Confirm continued eligibility to receive vaccination
8. Administer the vaccine.

The following evaluations/procedures will be completed following vaccination:

1. Observe the subject for at least 30 minutes after vaccination to evaluate immediate adverse reactions.
2. Record vital signs (blood pressure, temperature, and heart rate).
3. Perform interim history and physical examination, focusing on any acute complaints.
4. Record AEs and concomitant medications, if applicable.

Study Day 169 (±0 days; 1 day after Vaccination #3)

1. Perform basic history and focused physical examination (including injection site), emphasizing examination of any acute complaints.
2. Record vital signs (blood pressure, temperature, and heart rate).
3. Record AEs and concomitant medications, if applicable.

Study Day 171 (±1 day; 3 days after Vaccination #3)

1. Perform basic history and focused physical examination (including injection site), emphasizing examination of any acute complaints.
2. Record vital signs (blood pressure, temperature, and heart rate).
3. Record AEs and concomitant medications, if applicable.

4. Obtain approximately 5 mL of blood for CBC with differential and platelet count, ALT, creatinine.

Study Day 175 (±2 days; 7 days after Vaccination #3)

1. Perform basic history and focused physical examination (including injection site), emphasizing examination of any acute complaints.
2. Record vital signs (blood pressure, temperature, and heart rate).
3. Record AEs and concomitant medications, if applicable.
4. Obtain approximately 80 mL of blood for cellular assays, *ex vivo* assays, transcriptional assays, and B cell studies.

Study Day 182 (±3 days; 14 days after Vaccination #3)

1. Perform basic history and focused physical examination, emphasizing examination of any acute complaints.
2. Record vital signs (blood pressure, temperature, and heart rate).
3. Record AEs and concomitant medications, if applicable.
4. Obtain approximately 51 mL of blood for CBC with differential and platelet count, ALT, creatinine, malaria blood smear, malaria PCR, anti-Pfs25M and anti-Pfs230 antibody ELISA, SMFA, and cellular assays, *ex vivo* assays, transcriptional assays, and B cell studies.

Study Day 196 (±7 days; 28 days after Vaccination #3)

1. Perform basic history and focused physical examination, emphasizing examination of any acute complaints.
2. Record vital signs (blood pressure, temperature, and heart rate).
3. Record AEs and concomitant medications, if applicable.
4. Obtain approximately 41 mL of blood for malaria blood smear, malaria PCR, SMFA, anti-Pfs25M and anti-Pfs230 antibody ELISA, and cellular assays, *ex vivo* assays, transcriptional assays, and B cell studies

Study Day 224 (±7 days; 56 days after Vaccination #3)

1. Perform basic history and focused physical examination, emphasizing examination of any acute complaints.
2. Record vital signs (blood pressure, temperature, and heart rate).
3. Record AEs and concomitant medications, if applicable.
4. Obtain approximately 11 mL of blood for malaria blood smear, malaria PCR, SMFA, and anti-Pfs25M and anti-Pfs230 antibody ELISA.

Study Day 252 (±14 days; 84 days after Vaccination #3)

1. Perform basic history and focused physical examination, emphasizing examination of any acute complaints.
2. Record vital signs (blood pressure, temperature, and heart rate).
3. Record AEs and concomitant medications, if applicable.

Study Day 280 (±21 days; 112 days after Vaccination #3)

1. Perform basic history and focused physical examination, emphasizing examination of any acute complaints.
2. Record vital signs (blood pressure, temperature, and heart rate).

3. Record AEs and concomitant medications, if applicable.
4. Obtain approximately 11 mL of blood for malaria blood smear, malaria PCR, SMFA, and anti-Pfs25M and anti-Pfs230 antibody ELISA.

Study Day 308 (±21 days; 140 days after Vaccination #3)

1. Perform basic history and focused physical examination, emphasizing examination of any acute complaints.
2. Record vital signs (blood pressure, temperature, and heart rate).
3. Record AEs and concomitant medications, if applicable.

Study Day 336 (±21 days; 168 days after Vaccination #3)

1. Perform basic history and focused physical examination, emphasizing examination of any acute complaints.
2. Record vital signs (blood pressure, temperature, and heart rate).
3. Record AEs and concomitant medications, if applicable.
4. Obtain approximately 41 mL of blood for malaria blood smear, malaria PCR, SMFA, anti-Pfs25M and anti-Pfs230 antibody ELISA, and cellular assays and B cell studies.

Re-enrollment visits:

Study Day 420 (-14 /+90 days; 252 days after Vaccination #3);Re-screening

1. Explain the study and informed consent document to the subject.
2. Ensure the subject has acknowledged consent by signing or fingerprinting the informed consent document. Ensure that the subject receives a signed copy of the informed consent.
3. Perform basic history and focused physical examination emphasizing examination of any acute complaints.
4. Record vital signs (blood pressure, temperature, and heart rate).
5. Record AEs and concomitant medications, if applicable.
6. Obtain approximately 6 mL of blood for blood for malaria blood smear, malaria PCR, SMFA, and anti-Pfs230 antibody ELISA,

Study Day 532 (+42 days; 364 days after Vaccination #3)

1. Perform basic history and focused physical examination emphasizing examination of any acute complaints.
2. Record vital signs (blood pressure, temperature, and heart rate).
3. Record AEs and concomitant medications, if applicable.
4. Obtain approximately 6 mL of blood for blood for malaria blood smear, malaria PCR, SMFA, and anti-Pfs230 antibody ELISA
5. Offer ENGERIX-B (or other approved Hepatitis B vaccine) to those subjects that received TBV(s) during the study.

Bancoumana and Doneguebougou Cohort (TBV at full and fractional dosing versus Comparator) – Arms 2c, 2d, 4c (3 Vaccinations)

Study Day -7 (±7 day; Drug Treatment Visit; -7 prior to Vaccination #1)

1. Perform basic history and focused physical examination, emphasizing examination of any acute complaints.
2. Record vital signs (blood pressure, temperature, and heart rate).
3. Record AEs and concomitant medications, if applicable.
4. For females, obtain a urine or serum sample for β-hCG testing. Ensure that the test is negative before vaccinating; a positive test will exclude the subject from the trial.
5. For females, ensure agreement and compliance with pregnancy prevention before vaccinating.
6. Obtain approximately 27 mL of blood for CBC with differential and platelet count, ALT, creatinine, hemoglobin typing, malaria blood smear, malaria PCR, cellular assays and B cell assays, and transcriptional analysis.
7. Administer Coartem (6 dosing timepoints per package insert)

Study Day 0 (day of Vaccination #1)

The following evaluations/procedures will be completed before vaccination:

1. Verify that informed consent was obtained.
2. Ensure that all inclusion/exclusion criteria are met.
3. Ensure that CBC, ALT, creatinine, HIV/HCV/HBV, and urine results from screening are within protocol-defined limits (see Exclusion Criteria above) before vaccinating.
4. Perform interim history and physical examination, focusing on any acute complaints.
5. Record vital signs (blood pressure, temperature, and heart rate).
6. During the physical examination, study staff will educate the subject regarding signs and symptoms of potential adverse events (AEs) and indications for use of antipyretics if fever, headache, or malaise occurs.
7. Obtain urine sample for schistosomiasis testing
8. For females, obtain a urine or serum sample for β-hCG testing. Ensure that the test is negative before vaccinating; a positive test will exclude the subject from the trial.
9. For females, ensure agreement and compliance with pregnancy prevention before vaccinating.
10. Obtain stool sample for helminth testing (**Note:** This can be completed within +/- 7 days of Study Day 0 if unable to provide on Study Day 0)
11. Obtain approximately 57 mL of blood for CBC with differential and platelet count, ALT, creatinine, malaria blood smear, malaria PCR, anti-Pfs230 antibody ELISA, cellular assays, *ex vivo* assays, B cell assays, and transcriptional analysis.
12. Confirm that all criteria for vaccination have been met according to inclusion/exclusion criteria
13. Confirm continued eligibility to receive vaccination
14. Assign study ID number from randomization list
15. Administer the vaccine.

The following evaluations/procedures will be completed following vaccination:

1. Observe the subject for at least 30 minutes after vaccination to evaluate immediate adverse reactions.
2. Record vital signs (blood pressure, temperature, and heart rate).
3. Perform interim history and physical examination, focusing on any acute complaints.
4. Record AEs and concomitant medications, if applicable.

Study Day 1 (±0 days; 1 day after Vaccination #1)

1. Perform basic history and focused physical examination (including injection site), emphasizing examination of any acute complaints.
2. Record vital signs (blood pressure, temperature, and heart rate).
3. Record AEs and concomitant medications, if applicable.

Study Day 3 (±1 day; 3 days after Vaccination #1)

1. Perform basic history and focused physical examination (including injection site), emphasizing examination of any acute complaints.
2. Record vital signs (blood pressure, temperature, and heart rate).
3. Record AEs and concomitant medications, if applicable.
4. Obtain approximately 16 mL of blood for CBC with differential and platelet count, ALT, creatinine, ex vivo assays, cellular assays, *ex vivo* assays, and B cell assays, and transcriptional analysis.

Study Day 7 (±2 days; 7 days after Vaccination #1)

1. Perform basic history and focused physical examination (including injection site), emphasizing examination of any acute complaints.
2. Record vital signs (blood pressure, temperature, and heart rate).
3. Record AEs and concomitant medications, if applicable.

Study Day 14 (±3 days; 14 days after Vaccination #1)

1. Perform basic history and focused physical examination, emphasizing examination of any acute complaints.
2. Record vital signs (blood pressure, temperature, and heart rate).
3. Record AEs and concomitant medications, if applicable.
4. Obtain approximately 11 mL of blood for CBC with differential and platelet count, ALT, creatinine, malaria blood smear, malaria PCR, and anti-Pfs230 antibody ELISA.

Study Day 28 (day of Vaccination #2)

The following evaluations/procedures will be completed before vaccination:

1. Perform interim history and physical examination, focusing on any acute complaints.
2. Record vital signs (blood pressure, temperature, and heart rate).
3. During the physical examination, study staff will educate the subject regarding signs and symptoms of potential adverse events (AEs) and indications for use of antipyretics if fever, headache, or malaise occurs.
4. For females, obtain a urine or serum sample for β-hCG testing. Ensure that the test is negative before vaccinating; a positive test will exclude the subject from the trial.
5. For females, ensure agreement and compliance with pregnancy prevention before vaccinating.

6. Obtain approximately 12 mL of blood for CBC with differential and platelet count, ALT, creatinine, malaria blood smear, malaria PCR, anti-Pfs230 antibody ELISA, and transcriptional analysis.
7. Confirm continued eligibility to receive vaccination
8. Confirm subject identification and study ID number
9. Administer the vaccine.

The following evaluations/procedures will be completed following vaccination:
1. Observe the subject for at least 30 minutes after vaccination to evaluate immediate adverse reactions.
2. Record vital signs (blood pressure, temperature, and heart rate).
3. Perform interim history and physical examination, focusing on any acute complaints.
4. Record AEs and concomitant medications, if applicable.

Study Day 29 (±0 days; 1 day after Vaccination #2)
1. Perform basic history and focused physical examination (including injection site), emphasizing examination of any acute complaints.
2. Record vital signs (blood pressure, temperature, and heart rate).
3. Record AEs and concomitant medications, if applicable.

Study Day 31 (±1 day; 3 days after Vaccination #2)
1. Perform basic history and focused physical examination (including injection site), emphasizing examination of any acute complaints.
2. Record vital signs (blood pressure, temperature, and heart rate).
3. Record AEs and concomitant medications, if applicable.
4. Obtain approximately 16 mL of blood for CBC with differential and platelet count, ALT, creatinine, cellular assays, B cell assays, and transcriptional analysis.

Study Day 35 (±2 days; 7 days after Vaccination #2)
1. Perform basic history and focused physical examination (including injection site), emphasizing examination of any acute complaints.
2. Record vital signs (blood pressure, temperature, and heart rate).
3. Record AEs and concomitant medications, if applicable.
4. Obtain approximately 20 mL of blood for cellular assays, *ex vivo* assays, B cell assays.

Study Day 42 (±3 days; 14 days after Vaccination #2)
1. Perform basic history and focused physical examination, emphasizing examination of any acute complaints.
2. Record vital signs (blood pressure, temperature, and heart rate).
3. Record AEs and concomitant medications, if applicable.
4. Obtain approximately 11 mL of blood for CBC with differential and platelet count, ALT, creatinine, malaria blood smear, malaria PCR, and anti-Pfs230 antibody ELISA.
5. If gametocytemic, subject may be offered to participate in experimental hut.

Study Day 56 (±7 days; 28 days after Vaccination #2)
1. Perform basic history and focused physical examination, emphasizing examination of any acute complaints.

2. Record vital signs (blood pressure, temperature, and heart rate).
3. Record AEs and concomitant medications, if applicable.

Study Day 84 (±7 days; 56 days after Vaccination #2)

1. Perform basic history and focused physical examination, emphasizing examination of any acute complaints.
2. Record vital signs (blood pressure, temperature, and heart rate).
3. Record AEs and concomitant medications, if applicable.

Study Day 112 (±14 days; 84 days after Vaccination #2)

1. Perform basic history and focused physical examination, emphasizing examination of any acute complaints.
2. Record vital signs (blood pressure, temperature, and heart rate).
3. Record AEs and concomitant medications, if applicable.
4. Obtain approximately 6 mL of blood for malaria blood smear, malaria PCR, and anti-Pfs230 antibody ELISA.
5. If gametocytemic, subject may be offered to participate in experimental hut.

Study Day 140 (±14 days; 112 days after Vaccination #2)

1. Perform basic history and focused physical examination, emphasizing examination of any acute complaints.
2. Record vital signs (blood pressure, temperature, and heart rate).
3. Record AEs and concomitant medications, if applicable.

Study Day 168 (day of Vaccination #3)

The following evaluations/procedures will be completed before vaccination:

1. Perform interim history and physical examination, focusing on any acute complaints.
2. Record vital signs (blood pressure, temperature, and heart rate).
3. During the physical examination, study staff will educate the subject regarding signs and symptoms of potential adverse events (AEs) and indications for use of antipyretics if fever, headache, or malaise occurs.
4. For females, obtain a urine or serum sample for β-hCG testing. Ensure that the test is negative before vaccinating; a positive test will exclude the subject from the trial.
5. For females, ensure agreement and compliance with pregnancy prevention before vaccinating.
6. Obtain approximately 12 mL of blood for CBC with differential and platelet count, ALT, creatinine, malaria blood smear, malaria PCR, anti-Pfs230 antibody ELISA, and transcriptional analysis.
7. Confirm continued eligibility to receive vaccination
8. Administer the vaccine.

The following evaluations/procedures will be completed following vaccination:

1. Observe the subject for at least 30 minutes after vaccination to evaluate immediate adverse reactions.
2. Record vital signs (blood pressure, temperature, and heart rate).

3. Perform interim history and physical examination, focusing on any acute complaints.
4. Record AEs and concomitant medications, if applicable.

Study Day 169 (±0 days; 1 day after Vaccination #3)

1. Perform basic history and focused physical examination (including injection site), emphasizing examination of any acute complaints.
2. Record vital signs (blood pressure, temperature, and heart rate).
3. Record AEs and concomitant medications, if applicable.

Study Day 171 (±1 day; 3 days after Vaccination #3)

1. Perform basic history and focused physical examination (including injection site), emphasizing examination of any acute complaints.
2. Record vital signs (blood pressure, temperature, and heart rate).
3. Record AEs and concomitant medications, if applicable.
4. Obtain approximately 16 mL of blood for CBC with differential and platelet count, ALT, creatinine, cellular assays and B cell assays, and transcriptional analysis.

Study Day 175 (±2 days; 7 days after Vaccination #3)

1. Perform basic history and focused physical examination, emphasizing examination of any acute complaints.
2. Record vital signs (blood pressure, temperature, and heart rate).
3. Record AEs and concomitant medications, if applicable.
4. Obtain approximately 48 mL of blood for hemoglobin, malaria blood smear, malaria PCR, and anti-Pfs230 antibody ELISA, cellular assays, *ex vivo* assays, B cells assays, and transcriptional analysis.
5. For females, obtain a urine or serum sample for β-hCG testing. Ensure that the test is negative before completing feed.
6. Feeding evaluations (DSF) will also be performed at this visit.
    a. Confirm eligible for DSF

Study Day 178 (±2 days; 10 days after Vaccination #3)

1. Perform basic history and focused physical examination, emphasizing examination of any acute complaints.
2. Record vital signs (blood pressure, temperature, and heart rate).
3. Record AEs and concomitant medications, if applicable.
4. Obtain approximately 2 mL of blood for malaria blood smear, and malaria PCR.
5. Feeding evaluations (DSF) will also be performed at this visit.
    a. Confirm eligible for DSF

Study Day 182 (±2 days; 14 days after Vaccination #3)

1. Perform basic history and focused physical examination, emphasizing examination of any acute complaints.
2. Record vital signs (blood pressure, temperature, and heart rate).
3. Record AEs and concomitant medications, if applicable.
4. Obtain approximately 28 mL of blood for CBC with differential and platelet count, ALT, creatinine, malaria blood smear, malaria PCR, SMFA, and anti-Pfs25M and anti-Pfs230 antibody ELISA.

5. For females, obtain a urine or serum sample for β-hCG testing. Ensure that the test is negative before completing feed.
6. Feeding evaluations (DSF) will also be performed at this visit.
    a) Confirm eligible for DSF

Study Day 185 (±2 days; 17 days after Vaccination #3)

1. Perform basic history and focused physical examination, emphasizing examination of any acute complaints.
2. Record vital signs (blood pressure, temperature, and heart rate).
3. Record AEs and concomitant medications, if applicable.
4. Obtain approximately 2 mL of blood for malaria blood smear, and malaria PCR.
5. Feeding evaluations (DSF) will also be performed at this visit.
    a. Confirm eligible for DSF

Study Day 189 (±2 days; 21 days after Vaccination #3)

1. Perform basic history and focused physical examination, emphasizing examination of any acute complaints.
2. Record vital signs (blood pressure, temperature, and heart rate).
3. Record AEs and concomitant medications, if applicable.
4. Obtain approximately 2 mL of blood for malaria blood smear, and malaria PCR.
5. For females, obtain a urine or serum sample for β-hCG testing. Ensure that the test is negative before completing feed.
6. Feeding evaluations (DSF) will also be performed at this visit.
    a. Confirm eligible for DSF

Study Day 192 (±2 days; 24 days after Vaccination #3)

1. Perform basic history and focused physical examination, emphasizing examination of any acute complaints.
2. Record vital signs (blood pressure, temperature, and heart rate).
3. Record AEs and concomitant medications, if applicable.
4. Obtain approximately 2 mL of blood for malaria blood smear and malaria PCR.
5. Feeding evaluations (DSF) will also be performed at this visit.
    a. Confirm eligible for DSF

Study Day 196 (±2 days; 28 days after Vaccination #3)

1. Perform basic history and focused physical examination, emphasizing examination of any acute complaints.
2. Record vital signs (blood pressure, temperature, and heart rate).
3. Record AEs and concomitant medications, if applicable.
4. Obtain approximately 48 mL of blood for malaria blood smear, malaria PCR, SMFA, cellular assays, *ex vivo* assays, B cells assays and anti-Pfs230 antibody ELISA and transcriptional analysis.
5. For females, obtain a urine or serum sample for β-hCG testing. Ensure that the test is negative before completing feed.
6. Feeding evaluations (DSF) will also be performed at this visit.
    a. Confirm eligible for DSF

Study Day 199 (±2 days; 31 days after Vaccination #3)

1. Perform basic history and focused physical examination, emphasizing examination of any acute complaints.
2. Record vital signs (blood pressure, temperature, and heart rate).
3. Record AEs and concomitant medications, if applicable.
4. Obtain approximately 2 mL of blood for malaria blood smear and malaria PCR.
5. Feeding evaluations (DSF) will also be performed at this visit.
    a. Confirm eligible for DSF

Study Day 203 (±2 days; 35 days after Vaccination #3)

1. Perform basic history and focused physical examination, emphasizing examination of any acute complaints.
2. Record vital signs (blood pressure, temperature, and heart rate).
3. Record AEs and concomitant medications, if applicable.
4. Obtain approximately 2 mL of blood for malaria blood smear and malaria PCR.
5. For females, obtain a urine or serum sample for β-hCG testing. Ensure that the test is negative before completing feed.
6. Feeding evaluations (DSF) will also be performed at this visit.
    a. Confirm eligible for DSF

Study Day 206 (±2 days; 38 days after Vaccination #3)

1. Perform basic history and focused physical examination, emphasizing examination of any acute complaints.
2. Record vital signs (blood pressure, temperature, and heart rate).
3. Record AEs and concomitant medications, if applicable.
4. Obtain approximately 2 mL of blood for malaria blood smear and malaria PCR.
5. Feeding evaluations (DSF) will also be performed at this visit.
    a. Confirm eligible for DSF

Study Day 210 (±2 days; 42 days after Vaccination #3)

1. Perform basic history and focused physical examination, emphasizing examination of any acute complaints.
2. Record vital signs (blood pressure, temperature, and heart rate).
3. Record AEs and concomitant medications, if applicable.
4. Obtain approximately 8 mL of blood for malaria blood smear, malaria PCR, SMFA, and anti-Pfs230 antibody ELISA, and transcriptional analysis.
5. For females, obtain a urine or serum sample for β-hCG testing. Ensure that the test is negative before completing feed.
6. Feeding evaluations (DSF) will also be performed at this visit.
    a. Confirm eligible for DSF

Study Day 213 (±2 days; 45 days after Vaccination #3)

1. Perform basic history and focused physical examination, emphasizing examination of any acute complaints.
2. Record vital signs (blood pressure, temperature, and heart rate).
3. Record AEs and concomitant medications, if applicable.
4. Obtain approximately 2 mL of blood for malaria blood smear and malaria PCR.

5. Feeding evaluations (DSF) will also be performed at this visit.
    a. Confirm eligible for DSF

Study Day 217 (±2 days; 49 days after Vaccination #3)

1. Perform basic history and focused physical examination, emphasizing examination of any acute complaints.
2. Record vital signs (blood pressure, temperature, and heart rate).
3. Record AEs and concomitant medications, if applicable.
4. Obtain approximately 2 mL of blood for malaria blood smear and malaria PCR.
5. For females, obtain a urine or serum sample for β-hCG testing. Ensure that the test is negative before completing feed.
6. Feeding evaluations (DSF) will also be performed at this visit.
    a. Confirm eligible for DSF

Study Day 220 (±2 days; 52 days after Vaccination #3)

1. Perform basic history and focused physical examination, emphasizing examination of any acute complaints.
2. Record vital signs (blood pressure, temperature, and heart rate).
3. Record AEs and concomitant medications, if applicable.
4. Obtain approximately 2 mL of blood for malaria blood smear and malaria PCR.
5. Feeding evaluations (DSF) will also be performed at this visit.
    a. Confirm eligible for DSF

Study Day 224 (±2 days; 56 days after Vaccination #3)

1. Perform basic history and focused physical examination, emphasizing examination of any acute complaints.
2. Record vital signs (blood pressure, temperature, and heart rate).
3. Record AEs and concomitant medications, if applicable.
4. Obtain approximately 8 mL of blood for malaria blood smear, malaria PCR, SMFA, and anti-Pfs230 antibody ELISA, and transcriptional analysis.
5. For females, obtain a urine or serum sample for β-hCG testing. Ensure that the test is negative before completing feed.
6. Feeding evaluations (DSF) will also be performed at this visit.
    a. Confirm eligible for DSF

Study Day 227 (±2 days; 59 days after Vaccination #3)

1. Perform basic history and focused physical examination, emphasizing examination of any acute complaints.
2. Record vital signs (blood pressure, temperature, and heart rate).
3. Record AEs and concomitant medications, if applicable.
4. Obtain approximately 2 mL of blood for malaria blood smear and malaria PCR.
5. Feeding evaluations (DSF) will also be performed at this visit.
    a. Confirm eligible for DSF

Study Day 231 (±2 days; 63 days after Vaccination #3)

1. Perform basic history and focused physical examination, emphasizing examination of any acute complaints.

2. Record vital signs (blood pressure, temperature, and heart rate).
3. Record AEs and concomitant medications, if applicable.
4. Obtain approximately 2 mL of blood for malaria blood smear and malaria PCR.
5. Feeding evaluations (DSF) will also be performed at this visit.
    a. Confirm eligible for DSF

Study Day 234 (±2 days; 66 days after Vaccination #3)

1. Perform basic history and focused physical examination, emphasizing examination of any acute complaints.
2. Record vital signs (blood pressure, temperature, and heart rate).
3. Record AEs and concomitant medications, if applicable.
4. Obtain approximately 2 mL of blood for malaria blood smear and malaria PCR.
5. Feeding evaluations (DSF) will also be performed at this visit.
    a. Confirm eligible for DSF

Study Day 238 (±2 days; 70 days after Vaccination #3)

1. Perform basic history and focused physical examination, emphasizing examination of any acute complaints.
2. Record vital signs (blood pressure, temperature, and heart rate).
3. Record AEs and concomitant medications, if applicable.
4. Obtain approximately 8 mL of blood for malaria blood smear, malaria PCR, SMFA, and anti-Pfs230 antibody ELISA, and transcriptional analysis..
5. For females, obtain a urine or serum sample for β-hCG testing. Ensure that the test is negative before completing feed.
6. Feeding evaluations (DSF) will also be performed at this visit.
    a. Confirm eligible for DSF

Study Day 241 (±2 days; 73 days after Vaccination #3)

1. Perform basic history and focused physical examination, emphasizing examination of any acute complaints.
2. Record vital signs (blood pressure, temperature, and heart rate).
3. Record AEs and concomitant medications, if applicable.
4. Obtain approximately 2 mL of blood for malaria blood smear and malaria PCR.
5. Feeding evaluations (DSF) will also be performed at this visit.
    a. Confirm eligible for DSF

Study Day 245 (±2 days; 77 days after Vaccination #3)

1. Perform basic history and focused physical examination, emphasizing examination of any acute complaints.
2. Record vital signs (blood pressure, temperature, and heart rate).
3. Record AEs and concomitant medications, if applicable.
4. Obtain approximately 2 mL of blood for malaria blood smear and malaria PCR.
5. Feeding evaluations (DSF) will also be performed at this visit.
    a. Confirm eligible for DSF

Study Day 248 (±2 days; 80 days after Vaccination #3)

1. Perform basic history and focused physical examination, emphasizing examination of any acute complaints.
2. Record vital signs (blood pressure, temperature, and heart rate).
3. Record AEs and concomitant medications, if applicable.
4. Obtain approximately 2 mL of blood for malaria blood smear and malaria PCR.
5. Feeding evaluations (DSF) will also be performed at this visit.
    a. Confirm eligible for DSF

Study Day 252 (±2 days; 84 days after Vaccination #3)

1. Perform basic history and focused physical examination, emphasizing examination of any acute complaints.
2. Record vital signs (blood pressure, temperature, and heart rate).
3. Record AEs and concomitant medications, if applicable.
4. Obtain approximately 8 mL of blood for malaria blood smear, malaria PCR, SMFA, and anti-Pfs230 antibody ELISA, and transcriptional analysis.
5. For females, obtain a urine or serum sample for β-hCG testing. Ensure that the test is negative before completing feed.
6. Feeding evaluations (DSF) will also be performed at this visit.
    a. Confirm eligible for DSF

Study Day 255 (±2 days; 87 days after Vaccination #3)

1. Perform basic history and focused physical examination, emphasizing examination of any acute complaints.
2. Record vital signs (blood pressure, temperature, and heart rate).
3. Record AEs and concomitant medications, if applicable.
4. Obtain approximately 2 mL of blood for malaria blood smear and malaria PCR.
5. Feeding evaluations (DSF) will also be performed at this visit.
    a) Confirm eligible for DSF

Study Day 280 (±14 days; 112 days after Vaccination #3)

1. Perform basic history and focused physical examination, emphasizing examination of any acute complaints.
2. Record vital signs (blood pressure, temperature, and heart rate).
3. Record AEs and concomitant medications, if applicable.
4. Obtain approximately 7 mL of blood for malaria blood smear, malaria PCR, SMFA, and anti-Pfs230 antibody ELISA.

Study Day 308 (±21 days; 140 days after Vaccination #3)

1. Perform basic history and focused physical examination, emphasizing examination of any acute complaints.
2. Record vital signs (blood pressure, temperature, and heart rate).
3. Record AEs and concomitant medications, if applicable.
4. Obtain approximately 1 mL of blood for malaria blood smear and malaria PCR.

Study Day 336 (±21 days; 168 days after Vaccination #3)

1. Perform basic history and focused physical examination, emphasizing examination of any acute complaints.

2. Record vital signs (blood pressure, temperature, and heart rate).
3. Record AEs and concomitant medications, if applicable.
4. Obtain approximately 47 mL of blood for malaria blood smear, malaria PCR, SMFA, and anti-Pfs230 antibody ELISA, and cellular assays, *ex vivo* assays, B cell assays and transcriptional analysis.
5. Scheduled unblinding of all subjects
    a. Offer ENGERIX-B (or other approved Hepatitis B vaccine) to those subjects that received TBV(s) during the study.

Re-enrollment visits

Study Day 462 +/-70 days (294 days after Vaccination #3);Re-screening

1. Explain the study and informed consent document to the subject.
2. Ensure the subject has acknowledged consent by signing or fingerprinting the informed consent document. Ensure that the subject receives a signed copy of the informed consent.
3. Elicit a complete medical history, including menstrual and contraceptive history and/or history of surgical sterility for females, and medication use.
4. Females of childbearing potential must be willing to use reliable contraception from at least 21 days prior to fourth vaccination through three months after the fourth vaccination.
5. Administer a complete physical examination, including vital signs (height, weight, blood pressure, temperature, and heart rate).
6. HIV pre- and post-test counseling as indicated including follow-up contact with subject to report the results and referral for appropriate medical care if indicated.
7. Obtain approximately 16 mL of blood for complete blood count (CBC) with differential and platelet count, ALT, creatinine (Cr), hepatitis B surface antigen, hepatitis C antibody, HIV antibody, malaria blood smear, malaria PCR, SMFA, and anti-Pfs230 antibody ELISA.
8. Obtain urine (or serum) for pregnancy testing (for females) and urinalysis/urine dipstick for protein and blood.

Study Day 476 ±56 days (308 days after Vaccination #3; Day of Vaccination #4)

The following evaluations/procedures will be completed before vaccination:

1. Verify that informed consent was obtained.
2. Ensure that all inclusion/exclusion criteria are met.
3. Ensure that CBC, ALT, creatinine, HIV/HCV/HBV, and urine results from re-screening are within protocol-defined limits (see Exclusion Criteria above) before vaccinating.
4. Perform interim history and physical examination, focusing on any acute complaints.
5. Record vital signs (blood pressure, temperature, and heart rate).
6. During the physical examination, study staff will educate the subject regarding signs and symptoms of potential adverse events (AEs) and indications for use of antipyretics if fever, headache, or malaise occurs.
7. For females, obtain a urine or serum sample for β-hCG testing. Ensure that the test is negative before vaccinating; a positive test will exclude the subject from the trial.
8. For females, ensure agreement and compliance with pregnancy prevention before vaccinating.

9. Obtain approximately 32 mL of blood for malaria blood smear, malaria PCR, SMFA, and anti-Pfs230 antibody ELISA, and cellular assays, *ex vivo* assays, B cell assays and transcriptional analysis.
10. Confirm that all criteria for vaccination have been met according to inclusion/exclusion criteria, including reviewing safety labs prior to vaccination.
11. Confirm continued eligibility to receive vaccination
12. Administer the vaccine.

The following evaluations/procedures will be completed following vaccination:
1. Observe the subject for at least 30 minutes after vaccination to evaluate immediate adverse reactions.
2. Record vital signs (blood pressure, temperature, and heart rate).
3. Perform interim history and physical examination, focusing on any acute complaints.
4. Record AEs and concomitant medications, if applicable.

Study Day 477 ±0 days (1 day after Vaccination #4)
1. Perform basic history and focused physical examination (including injection site), emphasizing examination of any acute complaints.
2. Record vital signs (blood pressure, temperature, and heart rate).
3. Record AEs and concomitant medications, if applicable.

Study Day 479 ±1 day (3 days after Vaccination #4)
1. Perform basic history and focused physical examination (including injection site), emphasizing examination of any acute complaints.
2. Record vital signs (blood pressure, temperature, and heart rate).
3. Record AEs and concomitant medications, if applicable.
4. Obtain approximately 16 mL of blood for CBC with differential and platelet count, ALT, creatinine, cellular assays and B cell assays, and transcriptional analysis.

Study Day 483 ±2 days (7 days after Vaccination #4)
1. Perform basic history and focused physical examination, emphasizing examination of any acute complaints.
2. Record vital signs (blood pressure, temperature, and heart rate).
3. Record AEs and concomitant medications, if applicable.
4. Obtain approximately 23 mL of blood for cellular assays, *ex vivo* assays, B cells assays, and transcriptional analysis.
5. For females, obtain a urine or serum sample for β-hCG testing. Ensure that the test is negative before completing feed.
6. Feeding evaluations (DSF) will also be performed at this visit.
    a) Confirm eligible for DSF

Study Day 486 ±2 days (10 days after Vaccination #4)
1. Perform basic history and focused physical examination, emphasizing examination of any acute complaints.
2. Record vital signs (blood pressure, temperature, and heart rate).
3. Record AEs and concomitant medications, if applicable.
4. Obtain approximately 2 mL of blood for malaria blood smear and malaria PCR.

5. Feeding evaluations (DSF) will also be performed at this visit.
    a. Confirm eligible for DSF

Study Day 490 ±3 days (14 days after Vaccination #4)

1. Perform basic history and focused physical examination, emphasizing examination of any acute complaints.
2. Record vital signs (blood pressure, temperature, and heart rate).
3. Record AEs and concomitant medications, if applicable.
4. Obtain approximately 17 mL of blood for malaria blood smear, malaria PCR, CBC with differential and platelet count, ALT, creatinine, SMFA, and anti-Pfs230 antibody ELISA and transcriptional analysis.
5. For females, obtain a urine or serum sample for β-hCG testing. Ensure that the test is negative before completing feed.
6. Feeding evaluations (DSF) will also be performed at this visit.
    a. Confirm eligible for DSF

Study Day 493 ±2 days (17 days after Vaccination #4)

1. Perform basic history and focused physical examination, emphasizing examination of any acute complaints.
2. Record vital signs (blood pressure, temperature, and heart rate).
3. Record AEs and concomitant medications, if applicable.
4. Obtain approximately 2 mL of blood for malaria blood smear and malaria PCR.
5. Feeding evaluations (DSF) will also be performed at this visit.
    a. Confirm eligible for DSF

Study Day 497 ±2 days (21 days after Vaccination #4)

1. Perform basic history and focused physical examination, emphasizing examination of any acute complaints.
2. Record vital signs (blood pressure, temperature, and heart rate).
3. Record AEs and concomitant medications, if applicable.
4. Obtain approximately 2 mL of blood for malaria blood smear and malaria PCR.
5. For females, obtain a urine or serum sample for β-hCG testing. Ensure that the test is negative before completing feed.
6. Feeding evaluations (DSF) will also be performed at this visit.
    a. Confirm eligible for DSF

Study Day 501 ±2 days (25 days after Vaccination #4)

1. Perform basic history and focused physical examination, emphasizing examination of any acute complaints.
2. Record vital signs (blood pressure, temperature, and heart rate).
3. Record AEs and concomitant medications, if applicable.
4. Obtain approximately 2 mL of blood for malaria blood smear and malaria PCR.
5. Feeding evaluations (DSF) will also be performed at this visit.
    a. Confirm eligible for DSF

Study Day 504±2 days (28 days after Vaccination #4)

1. Perform basic history and focused physical examination, emphasizing examination of any acute complaints.
2. Record vital signs (blood pressure, temperature, and heart rate).
3. Record AEs and concomitant medications, if applicable.
4. Obtain approximately 33 mL of blood for malaria blood smear, malaria PCR, SMFA, and anti-Pfs230 antibody ELISA, and cellular assays, *ex vivo* assays, B cell assays and transcriptional analysis.
5. For females, obtain a urine or serum sample for β-hCG testing. Ensure that the test is negative before completing feed.
6. Feeding evaluations (DSF) will also be performed at this visit.
    a. Confirm eligible for DSF

Study Day 507 ±2 days (31 days after Vaccination #4)

1. Perform basic history and focused physical examination, emphasizing examination of any acute complaints.
2. Record vital signs (blood pressure, temperature, and heart rate).
3. Record AEs and concomitant medications, if applicable.
4. Obtain approximately 2 mL of blood for malaria blood smear, and malaria PCR.
5. Feeding evaluations (DSF) will also be performed at this visit.
    a. Confirm eligible for DSF

Study Day 511 ±2 days (35 days after Vaccination #4)

1. Perform basic history and focused physical examination, emphasizing examination of any acute complaints.
2. Record vital signs (blood pressure, temperature, and heart rate).
3. Record AEs and concomitant medications, if applicable.
4. Obtain approximately 2 mL of blood for malaria blood smear, and malaria PCR.
5. For females, obtain a urine or serum sample for β-hCG testing. Ensure that the test is negative before completing feed.
6. Feeding evaluations (DSF) will also be performed at this visit.
    a. Confirm eligible for DSF

Study Day 514 ±2 days (38 days after Vaccination #4)

1. Perform basic history and focused physical examination, emphasizing examination of any acute complaints.
2. Record vital signs (blood pressure, temperature, and heart rate).
3. Record AEs and concomitant medications, if applicable.
4. Obtain approximately 2 mL of blood for malaria blood smear and malaria PCR.
5. Feeding evaluations (DSF) will also be performed at this visit.
    a. Confirm eligible for DSF

Study Day 518 ±2 days (42 days after Vaccination #4)

1. Perform basic history and focused physical examination, emphasizing examination of any acute complaints.
2. Record vital signs (blood pressure, temperature, and heart rate).
3. Record AEs and concomitant medications, if applicable.

4. Obtain approximately 2 mL of blood for malaria blood smear and malaria PCR.
5. For females, obtain a urine or serum sample for β-hCG testing. Ensure that the test is negative before completing feed.
6. Feeding evaluations (DSF) will also be performed at this visit.
    a. Confirm eligible for DSF

Study Day 521 ±2 days (45 days after Vaccination #4)

1. Perform basic history and focused physical examination, emphasizing examination of any acute complaints.
2. Record vital signs (blood pressure, temperature, and heart rate).
3. Record AEs and concomitant medications, if applicable.
4. Obtain approximately 2 mL of blood for malaria blood smear and malaria PCR.
5. Feeding evaluations (DSF) will also be performed at this visit.
    a. Confirm eligible for DSF

Study Day 525 ±2 days (49 days after Vaccination #4)

1. Perform basic history and focused physical examination, emphasizing examination of any acute complaints.
2. Record vital signs (blood pressure, temperature, and heart rate).
3. Record AEs and concomitant medications, if applicable.
4. Obtain approximately 2 mL of blood for malaria blood smear and malaria PCR.
5. For females, obtain a urine or serum sample for β-hCG testing. Ensure that the test is negative before completing feed.
6. Feeding evaluations (DSF) will also be performed at this visit.
    a. Confirm eligible for DSF

Study Day 528 ±2 days (52 days after Vaccination #4)

1. Perform basic history and focused physical examination, emphasizing examination of any acute complaints.
2. Record vital signs (blood pressure, temperature, and heart rate).
3. Record AEs and concomitant medications, if applicable.
4. Obtain approximately 2 mL of blood for malaria blood smear and malaria PCR.
5. Feeding evaluations (DSF) will also be performed at this visit.
    a. Confirm eligible for DSF

Study Day 532±2 days (56 days after Vaccination #4)

1. Perform basic history and focused physical examination, emphasizing examination of any acute complaints.
2. Record vital signs (blood pressure, temperature, and heart rate).
3. Record AEs and concomitant medications, if applicable.
4. Obtain approximately 7 mL of blood for malaria blood smear, malaria PCR, SMFA, and anti-Pfs230 antibody ELISA
5. For females, obtain a urine or serum sample for β-hCG testing. Ensure that the test is negative before completing feed.
6. Feeding evaluations (DSF) will also be performed at this visit.
    a. Confirm eligible for DSF

Study Day 535 ±2 days (59 days after Vaccination #4)

1. Perform basic history and focused physical examination, emphasizing examination of any acute complaints.
2. Record vital signs (blood pressure, temperature, and heart rate).
3. Record AEs and concomitant medications, if applicable.
4. Obtain approximately 2 mL of blood for malaria blood smear and malaria PCR.
5. Feeding evaluations (DSF) will also be performed at this visit.
    a. Confirm eligible for DSF

Study Day 539 ±2 days (63 days after Vaccination #4)

1. Perform basic history and focused physical examination, emphasizing examination of any acute complaints.
2. Record vital signs (blood pressure, temperature, and heart rate).
3. Record AEs and concomitant medications, if applicable.
4. Obtain approximately 2 mL of blood for malaria blood smear and malaria PCR.
5. For females, obtain a urine or serum sample for β-hCG testing. Ensure that the test is negative before completing feed.
6. Feeding evaluations (DSF) will also be performed at this visit.
    a. Confirm eligible for DSF

Study Day 542 ±2 days (66 days after Vaccination #4)

1. Perform basic history and focused physical examination, emphasizing examination of any acute complaints.
2. Record vital signs (blood pressure, temperature, and heart rate).
3. Record AEs and concomitant medications, if applicable.
4. Obtain approximately 2 mL of blood for malaria blood smear and malaria PCR.
5. Feeding evaluations (DSF) will also be performed at this visit.
    a. Confirm eligible for DSF

Study Day 546 ±2 days (70 days after Vaccination #4)

1. Perform basic history and focused physical examination, emphasizing examination of any acute complaints.
2. Record vital signs (blood pressure, temperature, and heart rate).
3. Record AEs and concomitant medications, if applicable.
4. Obtain approximately 2 mL of blood for malaria blood smear and malaria PCR.
5. For females, obtain a urine or serum sample for β-hCG testing. Ensure that the test is negative before completing feed.
6. Feeding evaluations (DSF) will also be performed at this visit.
    a. Confirm eligible for DSF

Study Day 549 ±2 days (73 days after Vaccination #4)

1. Perform basic history and focused physical examination, emphasizing examination of any acute complaints.
2. Record vital signs (blood pressure, temperature, and heart rate).
3. Record AEs and concomitant medications, if applicable.
4. Obtain approximately 2 mL of blood for malaria blood smear and malaria PCR.
5. Feeding evaluations (DSF) will also be performed at this visit.

a. Confirm eligible for DSF

Study Day 553 ±2 days (77 days after Vaccination #4)

1. Perform basic history and focused physical examination, emphasizing examination of any acute complaints.
2. Record vital signs (blood pressure, temperature, and heart rate).
3. Record AEs and concomitant medications, if applicable.
4. Obtain approximately 2 mL of blood for malaria blood smear and malaria PCR.
5. For females, obtain a urine or serum sample for β-hCG testing. Ensure that the test is negative before completing feed.
6. Feeding evaluations (DSF) will also be performed at this visit.
    a. Confirm eligible for DSF

Study Day 556 ±2 days (80 days after Vaccination #4)

1. Perform basic history and focused physical examination, emphasizing examination of any acute complaints.
2. Record vital signs (blood pressure, temperature, and heart rate).
3. Record AEs and concomitant medications, if applicable.
4. Obtain approximately 2 mL of blood for malaria blood smear and malaria PCR.
5. Feeding evaluations (DSF) will also be performed at this visit.
    a. Confirm eligible for DSF

Study Day 560 ±2 days (84 days after Vaccination #4)

1. Perform basic history and focused physical examination, emphasizing examination of any acute complaints.
2. Record vital signs (blood pressure, temperature, and heart rate).
3. Record AEs and concomitant medications, if applicable.
4. Obtain approximately 7 mL of blood for malaria blood smear and malaria PCR, SMFA, and anti-Pfs230 antibody ELISA.
5. For females, obtain a urine or serum sample for β-hCG testing. Ensure that the test is negative before completing feed.
6. Feeding evaluations (DSF) will also be performed at this visit.
    a. Confirm eligible for DSF

Study Day 563 ±2 days (87 days after Vaccination #4)

1. Perform basic history and focused physical examination, emphasizing examination of any acute complaints.
2. Record vital signs (blood pressure, temperature, and heart rate).
3. Record AEs and concomitant medications, if applicable.
4. Obtain approximately 2 mL of blood for malaria blood smear and malaria PCR.
5. Feeding evaluations (DSF) will also be performed at this visit.
    a. Confirm eligible for DSF

Study Day 567 ±2 days; (91 days after Vaccination #4)

1. Perform basic history and focused physical examination, emphasizing examination of any acute complaints.

2. Record vital signs (blood pressure, temperature, and heart rate).
3. Record AEs and concomitant medications, if applicable.
4. Obtain approximately 2 mL of blood for malaria blood smear and malaria PCR.
5. For females, obtain a urine or serum sample for β-hCG testing. Ensure that the test is negative before completing feed.
6. Feeding evaluations (DSF) will also be performed at this visit.
    a. Confirm eligible for DSF

Study Day 570 ±2 days; (94 days after Vaccination #4)

1. Perform basic history and focused physical examination, emphasizing examination of any acute complaints.
2. Record vital signs (blood pressure, temperature, and heart rate).
3. Record AEs and concomitant medications, if applicable.
4. Obtain approximately 2 mL of blood for malaria blood smear and malaria PCR.
5. Feeding evaluations (DSF) will also be performed at this visit.
    a. Confirm eligible for DSF

Study Day 574 ±2 days; (98 days after Vaccination #4)

1. Perform basic history and focused physical examination, emphasizing examination of any acute complaints.
2. Record vital signs (blood pressure, temperature, and heart rate).
3. Record AEs and concomitant medications, if applicable.
4. Obtain approximately 2 mL of blood for malaria blood smear and malaria PCR.
5. For females, obtain a urine or serum sample for β-hCG testing. Ensure that the test is negative before completing feed.
6. Feeding evaluations (DSF) will also be performed at this visit.
    a. Confirm eligible for DSF

Study Day 577 ±2 days; (101 days after Vaccination #4)

1. Perform basic history and focused physical examination, emphasizing examination of any acute complaints.
2. Record vital signs (blood pressure, temperature, and heart rate).
3. Record AEs and concomitant medications, if applicable.
4. Obtain approximately 2 mL of blood for malaria blood smear and malaria PCR.
5. Feeding evaluations (DSF) will also be performed at this visit.
    a. Confirm eligible for DSF

Study Day 581 ±2 days; (105 days after Vaccination #4)

1. Perform basic history and focused physical examination, emphasizing examination of any acute complaints.
2. Record vital signs (blood pressure, temperature, and heart rate).
3. Record AEs and concomitant medications, if applicable.
4. Obtain approximately 2 mL of blood for malaria blood smear and malaria PCR.
5. For females, obtain a urine or serum sample for β-hCG testing. Ensure that the test is negative before completing feed.
6. Feeding evaluations (DSF) will also be performed at this visit.

a. Confirm eligible for DSF

Study Day 584 ±2 days; (108 days after Vaccination #4)

1. Perform basic history and focused physical examination, emphasizing examination of any acute complaints.
2. Record vital signs (blood pressure, temperature, and heart rate).
3. Record AEs and concomitant medications, if applicable.
4. Obtain approximately 2 mL of blood for malaria blood smear and malaria PCR.
5. Feeding evaluations (DSF) will also be performed at this visit.
    a. Confirm eligible for DSF

Study Day 588 ±2 days; (112 days after Vaccination #4)

1. Perform basic history and focused physical examination, emphasizing examination of any acute complaints.
2. Record vital signs (blood pressure, temperature, and heart rate).
3. Record AEs and concomitant medications, if applicable.
4. For females, obtain a urine or serum sample for β-hCG testing. Ensure that the test is negative before completing feed.
5. Obtain approximately 7mL of blood for malaria blood smear and malaria PCR, SMFA, and anti-Pfs230 antibody ELISA.
6. Feeding evaluations (DSF) will also be performed at this visit.
    a. Confirm eligible for DSF

Study Day 591 ±2 days; (115 days after Vaccination #4)

1. Perform basic history and focused physical examination, emphasizing examination of any acute complaints.
2. Record vital signs (blood pressure, temperature, and heart rate).
3. Record AEs and concomitant medications, if applicable.
4. Obtain approximately 2 mL of blood for malaria blood smear and malaria PCR.
5. Feeding evaluations (DSF) will also be performed at this visit.
    a. Confirm eligible for DSF

Study Day 616 ±2 days; (140 days after Vaccination #4)

1. Perform basic history and focused physical examination, emphasizing examination of any acute complaints.
2. Record vital signs (blood pressure, temperature, and heart rate).
3. Record AEs and concomitant medications, if applicable.
4. Obtain approximately 11 mL of blood for malaria blood smear and malaria PCR, SMFA, and anti-Pfs230 antibody ELISA.

Study Day 644 ±21 days; (168 days after Vaccination #4)

1. Perform basic history and focused physical examination, emphasizing examination of any acute complaints.

2. Record vital signs (blood pressure, temperature, and heart rate).
3. Record AEs and concomitant medications, if applicable.
4. Obtain approximately 52 mL of blood for malaria blood smear, malaria PCR, SMFA, and anti-Pfs230 antibody ELISA, cellular assays, *ex vivo* assays, B cells assays and transcriptional analysis.

Study Day 728 ±21 days; (252 days after Vaccination #4)

1. Perform basic history and focused physical examination, emphasizing examination of any acute complaints.
2. Record vital signs (blood pressure, temperature, and heart rate).
3. Record AEs and concomitant medications, if applicable.
4. Obtain approximately 11 mL of blood for malaria blood smear and malaria PCR, SMFA, and anti-Pfs230 antibody ELISA.

Study Day 812±21 days; (336 days after Vaccination #4)

1. Perform basic history and focused physical examination, emphasizing examination of any acute complaints.
2. Record vital signs (blood pressure, temperature, and heart rate).
3. Record AEs and concomitant medications, if applicable.
4. Obtain approximately 52 mL of blood for malaria blood smear, malaria PCR, SMFA, and anti-Pfs230 antibody ELISA, cellular assays, *ex vivo* assays, B cells assays and transcriptional analysis.
5. Offer Menactra or equivalent vaccine

Appendix D: Toxicity Table

Local Reactogenicity Grading[1]

Local Reaction to Injectable Product	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)
Pain	Does not interfere with activity	Repeated use of non-narcotic pain reliever > 24 hours or interferes with activity	Any use of narcotic pain reliever or prevents daily activity	Emergency room (ER) visit or hospitalization
Tenderness	Mild discomfort to touch	Discomfort with movement	Significant discomfort at rest	ER visit or hospitalization
Erythema/Redness[2]	2.5 – 5 cm	5.1 – 10 cm	> 10 cm	Necrosis or exfoliative dermatitis
Induration/Swelling[3]	2.5 – 5 cm and does not interfere with activity	5.1 – 10 cm or interferes with activity	> 10 cm or prevents daily activity	Necrosis
Pruritus at Site	Does not interfere with activity	Repeated use of medication > 24 hours or interferes with activity	Prevents daily activity	ER visit or hospitalization
Limitation of Arm Movement	Does not interfere with activity	Repeated use of medication > 24 hours or interferes with activity	Prevents daily activity	ER visit or hospitalization

[1] The definitions provided in the table are taken from the FDA Guidance for Industry "Toxicity Grading Scale for Healthy Adults and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials" dated September 2007.
[2] In addition to grading the measured local reaction at the greatest single diameter, the measurement should be recorded as a continuous variable.
[2] Induration/Swelling should be evaluated and graded using the functional scale as well as the actual measurement.

Vital Sign AE Grading[1]

Vital Signs[2]	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)
Fever[3] (°C) **(°F)**	38.0 – 38.4 100.4 – 101.1	38.5 – 38.9 101.2 – 102.0	39.0 – 40 102.1 – 104	> 40 > 104
Tachycardia - beats per minute	101 – 115	116 – 130	> 130	ER visit or hospitalization for arrhythmia
Bradycardia - beats per Minute[4]	50 – 54	45 – 49	< 45	ER visit or hospitalization for arrhythmia
Hypertension (systolic) - mm Hg	141 – 150	151 – 155	> 155	ER visit or hospitalization for malignant hypertension
Hypertension (diastolic) - mm Hg	91 – 95	96 – 100	> 100	ER visit or hospitalization for malignant hypertension
Hypotension (systolic) – mm Hg	85 – 89	80 – 84	< 80	ER visit or hospitalization for hypotensive shock

[1] The definitions provided in the table are taken from the FDA Guidance for Industry "Toxicity Grading Scale for Healthy Adults and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials" dated September 2007.
[2] Subject should be at rest for all vital sign measurements.
[2] Oral temperature; no recent hot or cold beverages or smoking.
[3] When resting heart rate is between 60 – 100 beats per minute. Use clinical judgment when characterizing bradycardia among some healthy subject populations, for example, conditioned athletes.

Systemic AE Grading[1]

Systemic AEs	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)
Diarrhea	2 – 3 loose stools or < 400 gms/24 hours	4 – 5 stools or 400 – 800 gms/24 hours	6 or more watery stools or > 800gms/24 hours or requires outpatient IV hydration	ER visit or hospitalization
Headache	No interference with activity	Repeated use of non-narcotic pain reliever > 24 hours or some interference with activity	Significant; any use of narcotic pain reliever or prevents daily activity	ER visit or hospitalization
Malaise	No interference with activity	Some interference with activity	Significant; prevents daily activity	ER visit or hospitalization
Myalgia	No interference with activity	Some interference with activity	Significant; prevents daily activity	ER visit or hospitalization
Arthralgia	No interference with activity	Some interference with activity	Significant; prevents daily activity	ER visit or hospitalization
Nausea/ Vomiting	No interference with activity or 1 – 2 episodes/24 hours	Some interference with activity or > 2 episodes/24 hours	Prevents daily activity, requires outpatient IV hydration	ER visit or hospitalization for hypotensive shock
Urticaria	No interference with activity	Requiring PO or topical treatment > 24 hours or IV medications or steroids for ≤24 hours	Requiring IV medication or steroids for >24 hours	ER visit or hospitalization

[1] The definitions provided in the table are taken from the FDA Guidance for Industry "Toxicity Grading Scale for Healthy Adults and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials" dated September 2007.

Mali Laboratory AE Grading

Hematology/Chemistry

Hematology and Biochemistry values[1, 2]	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)
Hemoglobin (Male) - gm/dL	9.5 – 10.5	8.0 – 9.4	6.5 – 7.9	< 6.5 and / or requiring transfusion
Hemoglobin (Female) gm/dL	8.0 – 9.0	7.0 – 7.9	6.0 – 6.9	< 6 and /or requiring transfusion
WBC Increase – 10^3/µL	11.5 – 15.0	15.1 – 20.0	20.1 – 25.0	> 25.0
WBC Decrease - 10^3/µL	2.5 – 3.3	1.5 – 2.4	1.0 – 1.4	< 1.0
Neutrophil/Granulocyte Decrease[3] - 10^3/µL	0.80 – 1.00	0.50 – 0.79	< 0.50	< 0.50 with fever
Platelets Decreased – 10^3/µL	100 – 115	70 – 99	25 – 69	< 25
Creatinine (Male) µmol/L	132.60 – 159.11	159.12 – 185.63	185.64 – 221.00	> 221.00 and requires dialysis
Creatinine (Female) µmol/L	97.42 – 116.94	116.95 – 136.44	136.45 – 162.43	> 162.43 and requires dialysis
Liver Function Tests – ALT U/L	46.0 – 106.5	106.6 – 209.0	209.1 – 410.0	> 410.0

[1] The laboratory values provided in the table are taken from the FDA Guidance for Industry "Toxicity Grading Scale for Healthy Adults and Adolescent Volunteers Enrolled in Preventive Vaccine Clinical Trials" dated September 2007.The laboratory values provided in the referenced tables serve as guidelines and have been adapted for institutional normal parameters (see below). Institutional normal reference ranges should be provided to demonstrate that they are appropriate.

[2]The clinical signs or symptoms associated with laboratory abnormalities might result in characterization of the laboratory abnormalities as Potentially Life Threatening (Grade 4). For example, a low sodium value that falls within a grade 3 parameter should be recorded as a grade 4 hyponatremia event if the subject had a new seizure associated with the low sodium value.

[3] Note, neutropenias are graded and followed, but based on previous experience in African populations, should be interpreted with caution since lower values are more frequently observed in people of African descent [46,47].

Mali Adult: Institutional Normal Parameters

Chemistry

Serum[1]	Reference Range
Creatinine (Female) - µmol/L	< 72
Creatinine (Male) - µmol/L	48 – 98
ALT – U/L	< 41

[1]The laboratory values provided in the table are based on Bancoumana, Malian adults (age 18-45 years old)

Hematology

Hematology[1]	Reference Range
Hemoglobin (Female) - gm/dL	9.1 – 13.8
Hemoglobin (Male) - gm/dL	10.8 – 15.8
WBC – 10^3/µL	3.6 – 9.0
Absolute Neutrophil/Granulocyte Count - 10^3/µL	1.3 – 4.4
Absolute Lymphocyte Count - 10^3/µL	1.3 – 4.4
Platelet Count (Female)- 10^3/µL	144 – 413
Platelet Count (Male)- 10^3/µL	114 – 335

[1]The laboratory values provided in the table are based on Bancoumana, Malian adults (age 18-45 years old)

Urine Dip/Urinalysis

Urine[1]	Reference Ranges
Protein	None or Trace
Blood (microscopic) – red blood cells per high power field (RBC/HPF)	None or Trace < 5

[1]The laboratory values provided in the table are based on Bancoumana, Malian adults (age 18-45 years old)

Appendix E: References

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