Safety, Immunogenicity and Efficacy of Pfs230D1M-EPA/AS01 Vaccine, a Transmission Blocking Vaccine against *Plasmodium falciparum*, in an Age De-Escalation Trial of Children and a Family Compound Trial in Mali

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List of Abbreviations

AE	adverse event
ACIP	Advisory Committee on Immunization Practices
AGC	absolute granulocyte count
AL	artemether/lumefantrine
ALT	alanine transaminase
ANC	absolute neutrophil count
AR	adverse reaction
AS01	Adjuvant System AS01
β-hCG	beta human choriogonadotropin
BS	blood smear
CBC w/diff	complete blood count with differential
CFR	Code of Federal Regulations
cGMP	current Good Manufacturing Practices
Cr	creatinine
CRF	case report form
CSO	Clinical Safety Office
DEAP	Epidemiology Department of Parasitic Diseases (FMPOS/USTTB)
DSF	direct skin feeds
DSMB	Data and Safety Monitoring Board
EC	ethics committee
EKG	electrocardiogram
ELISA	enzyme-linked immunosorbent assay
EPA	ExoProtein A
ER	emergency room
FDA	Food and Drug Administration
FMPOS	Faculté de Médecine Pharmacie d'OdontoStomatologie
GCP	Good Clinical Practice
GEE	generalized estimating equation
GSK	GlaxoSmithKline
HIV	human immunodeficiency virus
HRPP	Human Research Protection Program
ICH	International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use
Ig	immunoglobulin
IM	intramuscular
IND	Investigational New Drug application
IRB	institutional review board
ISM	independent safety monitor
IV	intravenous

LMIV	Laboratory of Malaria Immunology and Vaccinology (of NIAID)
LMVR	Laboratory of Malaria and Vector Research
µg	micrograms
MPL	monophosphoryl lipid
MRTC	Malaria Research and Training Center (Mali)
n	number (typically refers to subjects)
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
OHSRP	Office of Human Subjects Research Protections
PBS	phosphate-buffered saline
PCR	polymerase chain reaction
Pfs25/Pfs230	surface antigens of zygotes and ookinetes in the mosquito stage of *Plasmodium falciparum*
PI	principal investigator
qPCR	quantitative polymerase chain reaction
RVF	Rift Valley fever
SAE	serious adverse event
SAR	suspected adverse reaction
SD	standard deviation
SERF	Safety Expedited Report Form
SMC	seasonal malaria chemoprophylaxis
SMFA	standard membrane feeding assay
SOP	standard operating procedure
SRCP	Safety Review and Communications Plan
SUSAR	serious and unexpected suspected adverse reaction
TBA	transmission-blocking activity
TBS	TRIS-buffered saline
TBV	transmission-blocking vaccine
TRA	transmission-reducing activity
T-TBS	TRIS-buffered saline containing Tween-20
UP	unanticipated problem
UPnonAE	unanticipated problem that is not an adverse event
USD	United States dollar
USTTB	University of Sciences, Techniques, & Technologies of Bamako
VIMT	vaccine to interrupt malaria transmission
VU	Vaccine unit
WBC	white blood cell
WHO	World Health Organization

WMW	Wilcoxon-Mann-Whitney test
WSRT	Wilcoxon signed rank test

Protocol Summary

Short Title:	Pfs230D1M Age De-escalation and Family Compound Trial
Clinical Phase:	2
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 Doneguebougou and surrounding villages, Mali, West Africa
Principal Investigators:	Patrick Duffy, MD (LMIV/NIAID/NIH) Issaka Sagara MD, MSPH, PhD (MRTC/DEAP/FMPOS)
Study Agent/ Intervention Description:	Pfs230D1M-EPA/AS01, 40 μg, or matched comparator vaccine, administered via intramuscular (IM) injection on days 0, 28, 56, 392/448. **Pfs230D1M-EPA/AS01:** recombinant Pfs230 domain 1 (a subdomain of a surface antigen of gametocytes, gametes, and zygotes, in the mosquito stage of *Plasmodium falciparum*) conjugated to a recombinant *Pseudomonas aeruginosa* ExoProtein A (EPA) and adjuvanted with Adjuvant System $AS01_B$ diluted to equivalent $AS01_E$ concentration directly before administration. Dosed on days 0, 28, 56, 392/448 **<u>Comparator Vaccines</u>** **HAVRIX:** inactivated hepatitis A viral vaccine adsorbed on aluminum hydroxide. HAVRIX is indicated for active immunization against disease caused by hepatitis A virus (HAV). HAVRIX is approved for use in persons 12 months of age and older with 2 doses (0, 6 months). Dosed on days 0 and 392/448. **TYPHIM Vi:** polysaccharide vaccine containing the cell surface Vi polysaccharide extracted from *Salmonella enterica serovar Typhi, S typhi* Ty2 strains; non-adjuvanted. TYPHIM Vi is indicated for active immunization against typhoid fever in subjects

	2 years of age and over and is given as a single dose. Dosed on day 28. **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 2 years of age and older who are otherwise healthy and are at increased risk for meningococcal disease (e.g., individuals in an epidemic or highly endemic country such as Mali).
Sample Size:	N~1960 (approximately 137 family compounds/vaccine units) N=60 (pilot) N~1500 (main, vaccine) N~400 (main, no vaccine)
Accrual Ceiling:	N=2380 N=80 (pilot) N=1800 (main, vaccine) N=500 (main, no vaccine)
Accrual Period:	March 2019-September 2019 (primary series) June 2020-October 2020 (4^{th} vaccination; continuation on study)
Study Duration:	**Start Date:** Approximately March 2019 **End Date:** Approximately October 2021 Study participants will be enrolled for a total of approximately 26 to 28 months (for up to 12 months following the last vaccination) depending on vaccination schedule and arm assignment and timing of screening
Study Population:	Pilot: Malian children 5-18 years of age Main (vaccine): Malians 5 years of age and older Main (no vaccine): Malian children 1-4 years of age
Study Design:	Two phase study: pilot, main ***Pilot*** Age de-escalating, double-blind, randomized comparator controlled; pilot groups will join main at the time of vaccine #3 **Group 1:** 9 to 18 years of age (n=30)

- ***Arm 1a*** (n=15), to receive 40 µg of Pfs230D1M-EPA/AS01 on days 0, 28, 126, 448; receipt of artemether/lumefantrine (AL) on day -7
- ***Arm 1b*** (n=15), to receive HAVRIX (day 0, 448), TYPHIM Vi (day 28), Menactra (day 126); receipt of AL on day -7

Group 2: 5 to 8 years of age (n=30)

- ***Arm 2a*** (n=15), to receive 40 µg of Pfs230D1M-EPA/AS01 on days 0, 28, 126, 448; receipt of AL on day -7,112, 441
- ***Arm 2b*** (n=15), to receive HAVRIX (day 0, 448), TYPHIM Vi (day 28), Menactra (day 126); receipt of AL on day -7,112, 441

Main

Double-blind, family/compound-randomized comparator-controlled trial

Group 3 (n~1500): Pfs230D1M-EPA/AS01 or comparator vaccine

- ***Arm 3a*****:** 5-8 years of age to receive 40 µg of Pfs230D1M-EPA/AS01 on days 0, 28, 56, 392; receipt of AL on day -7, 42, 385
- ***Arm 3b:*** 5-8 years of age to receive HAVRIX (day 0, 392), TYPHIM Vi (day 28), Menactra (day 56); receipt of AL on day -7, 42, 385
- ***Arm 3c*****:** 9-18 years of age to receive 40 µg of Pfs230D1M-EPA/AS01 on days 0, 28, 56, 392; receipt of AL on day -7
- ***Arm 3d:*** 9-18 years of age to receive HAVRIX (day 0, 392), TYPHIM Vi (day 28), Menactra (day 56); receipt of AL on day -7
- ***Arm 3e*****:** 19 years of age or older to receive 40 µg of Pfs230D1M-EPA/AS01 on days 0, 28, 56, 392; receipt of AL on day -7
- ***Arm 3f*****:** 19 years of age or older to receive HAVRIX (day 0, 392), TYPHIM Vi (day 28), Menactra (day 56); receipt of AL on day -7

Group 4 (n~400): AL treatment only; no vaccination (parasitemia)

- ***Arm 4a***: 1-4 years of age residing with/in same families/compounds as *Arms 1a, 2a, 3a, 3c, 3e (Pfs230D1M-EPA/AS01 compounds)* receipt of AL on day -14, 385
- ***Arm 4b***: 1-4 years of age residing with/in same families/compounds as *Arms 1b, 2b, 3b, 3d, 3f (comparator compounds)* receipt of AL on day -14, 385

NOTE: each family compound/vaccine unit will be randomized as a whole, and all identified subjects eligible and enrolled from that family compound will be assigned that determination (Pfs230D1M-EPA/AS01 or comparator)

Pilot Study Objectives:

Primary objectives:

- To assess safety and reactogenicity of administration of Pfs230D1M-EPA/AS01
- To assess vaccine activity of Pfs230D1M-EPA/AS01 against *P. falciparum* transmission by direct skin feeding assay (DSF) (Arms 1a/1b only) after receipt of 3rd and 4th vaccination and analyzed with the main group

Secondary objectives:

- To assess vaccine efficacy of Pfs230D1M-EPA/AS01 against *P. falciparum* infection measured by blood smear after receipt of 3rd and 4th vaccination and analyzed with the main group
- To assess immunogenicity as measured by enzyme-linked immunosorbent assay (ELISA) titer response to Pfs230D1M
- To assess functional antibody response by standard membrane feeding assay (SMFA) to Pfs230D1M

Exploratory objectives:

- To explore cellular and humoral responses to Pfs230D1M
- To identify host and parasite factors associated with transmission
- To track transmission of parasites from human to human using highly variant gene fragments
- To explore the impact of human movement on transmission and parasitemia

Pilot Study Endpoints:

Primary endpoints:

- Incidence of local and systemic adverse events (AEs) and serious adverse events (SAEs)
- *P. falciparum* functional activity as measured by DSF (Arms 1a/1b only) after receipt of 3rd and 4th vaccination and analyzed with the main group

Secondary endpoints:

- *P. falciparum* asexual parasitemia as measured by blood smears after receipt of 3rd and 4th vaccination and analyzed with the main group
- Anti-Pfs230D1M immunoglobulin (Ig) G levels as measured by ELISA
- Transmission-reducing activity (TRA)/Transmission-blocking activity (TBA) of induced antibody in SMFA

Exploratory endpoints:

- Cellular immune responses and antibody repertoire of functional antibody responses to vaccination
- Measuring hemoglobin, hemoglobin variants, levels of parasitemia/gametocytemia, OMICS analyses (proteomics, genomics), clinical malaria infection and treatment
- Sequences of highly variant parasite gene fragments
- Evaluation of congregate movement measurements by GPS tracking on functional activity as measured by DSF and *P. falciparum* asexual parasitemia as measured by blood smears (subgroup from Arms 1a/1b only)

Main Study Objectives:

Primary objective:

- To assess vaccine activity of Pfs230D1M-EPA/AS01 against *P. falciparum* transmission by direct skin feeding assay (DSF) (Arms 3c/3d only)

Secondary objectives:

- To assess vaccine efficacy of Pfs230D1M-EPA/AS01 against *P. falciparum* infection measured by blood smear (All Arms except Arms 3e/3f)
- To assess safety and reactogenicity of administration of Pfs230D1M-EPA/AS01 (All Arms except 4a/4b)

- To assess immunogenicity as measured by enzyme-linked immunosorbent assay (ELISA) titer response to Pfs230D1M (All Arms except Arms 4a/4b)
- To assess functional antibody response by standard membrane feeding assay (SMFA) to Pfs230D1M (All Arms except Arms 4a/4b)

Exploratory objectives:

- To explore cellular and humoral responses to Pfs230D1M (All Arms except Arms 4a/4b)
- To identify host and parasite factors associated with transmission
- To track transmission of parasites from human to human using highly variant gene fragments
- To explore the impact of human movement on transmission and parasitemia

Main Study Endpoints:

Primary endpoints:

- *P. falciparum* functional activity as measured by DSF (Arms 3c/3d)

Secondary endpoints:

- *P. falciparum* asexual parasitemia as measured by blood smears (All Arms except Arms 3e/3f)
- Incidence of local and systemic AEs and SAEs (All Arms except Arms 4a/4b)
- Anti-Pfs230D1M immunoglobulin (Ig) G levels as measured by ELISA (All Arms except Arms 4a/4b)
- Transmission-reducing activity (TRA)/Transmission-blocking activity (TBA) of induced antibody in SMFA (All Arms except Arms 4a/4b)

Exploratory endpoints:

- Cellular immune responses and antibody repertoire of functional antibody responses to vaccination (All Arms except Arms 4a/4b)
- Measuring hemoglobin, hemoglobin variants, levels of parasitemia/gametocytemia, OMICS analyses (proteomics, genomics), clinical malaria infection and treatment
- Sequences of highly variant parasite gene fragments
- Evaluation of congregate movement measurements by GPS tracking on functional activity as measured by DSF and *P.*

falciparum asexual parasitemia as measured by blood smears (subgroup from Arms 3c/3d only)

Précis

A vaccine to interrupt malaria transmission (VIMT), targeting disruption of both human and mosquito transmission, would be a valuable tool for local elimination or eradication of this disease. One strategy to design a VIMT is using components that block transmission of malaria to mosquitoes, such as Pfs230. Pfs230, a surface antigen of intracellular gametocytes, as well as extracellular gametes and zygotes in the mosquito stage of *Plasmodium falciparum*, is currently the leading candidate in clinical trials for a malaria transmission-blocking vaccine (TBV). Recombinant Pfs230D1M has been conjugated to a recombinant *Pseudomonas aeruginosa* ExoProtein A (EPA) and adjuvanted with AS01. When formulated in AS01, results from a recent first-in-human trial demonstrated that Pfs230-EPA induces functional transmission-reducing, and in a significant proportion of vaccinees, transmission-blocking serum activity that can be measured for months, the vaccine is well-tolerated and safe in adults, and our recent natural history data clearly indicate that children play a disproportionate role in malaria transmission. The next step in the development of Pfs230D1M-EPA as a TBV is therefore to conduct an age de-escalation trial to ensure that the vaccine is safe to administer to children and then to conduct a community clinical trial to assess efficacy in family groups.

This Phase 2 study will first determine safety and tolerability of Pfs230D1M-EPA/AS01 in healthy Malian children of decreasing ages: 9-18 years old, followed by 5-8 years old. A total of 60 subjects will be enrolled in Doneguebougou, Mali, West Africa. Children will be recruited from compounds/family that have agreed to participate in the main phase of the study and will enroll in a staggered manner to receive either Pfs230D1M-EPA/AS01 vaccine or comparator as assigned by their compound block randomization. Prior to receipt of vaccination #1, all subjects will receive a full treatment course of artemether/lumefantrine (AL). Safety and tolerability will be monitored and reported as local and systemic adverse events (AEs) and serious adverse events (SAEs) and reviewed by DSMB, sponsor, medical monitors, and study team prior to proceeding with enrollment of the main phase.

If there are no safety concerns, in a staggered manner, the main phase will begin enrollment of approximately 137 compounds/vaccine units (~1500 vaccinees + ~400 under 5 years of age for parasite surveillance). Children enrolled during the pilot safety phase will join their main phase compounds/family for vaccination #3. Prior to receipt of first vaccination, all subjects will receive a full treatment course of AL. All vaccinated subjects will be monitored for safety and tolerability. Immunogenicity outcomes will be antibody responses as measured by enzyme-linked immunosorbent assay (ELISA) against recombinant Pfs230D1M. Functional activity of the induced antibodies will be assessed by standard membrane feeding assays in select samples. Vaccine activity will be measured in children 9-18 years of age who will undergo direct skin feeds (DSF) starting 2 weeks post vaccination #3 for a total of 8 DSFs.

Prior to scheduled last vaccination in members of the compound/family, children 1-4 years of age and vaccinated children 5-8 years of age will receive a full treatment course of AL prior to the expected start of the transmission season and will then be followed every 2 weeks by blood smear (BS) along with all vaccinated children. Children 9-18 years of age will also be assessed for vaccine efficacy, but as a separate analysis from those 1-8 years of age.

In Year 2, those who received vaccination during Year 1 (5 years of age and older at enrollment), if eligible and still on study, will receive a single fourth vaccination per their vaccine unit (VU) blinded arm assignment. No new individuals will be enrolled. Again, children 1-4 years of age and vaccinated children 5-8 years of age will receive a full treatment course of AL prior to the expected start of the transmission season and will then be followed every 2 weeks by BS along with all vaccinated children. Children 9-18 years of age will also be assessed for vaccine efficacy against parasitemia, but as a separate analysis from those 1-8 years of age. Immunogenicity outcomes will be antibody responses as measured by ELISA against recombinant Pfs230D1M. Functional activity of the induced antibodies will be assessed by standard membrane feeding assays in select samples. Vaccine activity will be measured in children 9-18 years of age who will undergo DSF starting 2 weeks post vaccination #4 for a total of 10 DSFs.

1 Background Information and Scientific Rationale

1.1 Background Information

According to the World Health Organization (WHO), global malaria control efforts have resulted in a reduction in the number of deaths since 2000. About 445,000 people are estimated to have died due to malaria in 2016, a decline of 37% since 2010 in the WHO regions of Africa.[1] However, the progress in malaria control has stalled, with no reductions in the number of malaria cases worldwide in the past 2 years, per WHO World Malaria Reports in 2017 and 2018. 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—necessitate new approaches to malaria elimination. A vaccine to interrupt malaria transmission (VIMT), targeting disruption of parasite transmission through both human and mosquito, would be a valuable additional resource in the fight to eliminate this disease.[3,4]

Transmission blocking vaccines (TBVs) induce anti-sporogonic antibodies that disrupt parasite transmission to the mosquito, thereby halting transmission to another human host. Pfs230, a parasite protein expressed by gametocytes in the human stage of *P. falciparum*, and a surface antigen of gametes and zygotes in the mosquito stage, is a target of polyclonal and monoclonal antibodies with transmission-blocking activity (TBA) in standard membrane feeding assay (SMFA.)[5,6,7] The full-length Pfs230 precursor of 360 kDa is expressed in gametocytes within erythrocytes, and is processed to become an approximately 300-kDa mature protein upon translocation to the surface of freshly emerged gametes from erythrocytes.[8] Malaria-exposed populations acquire antibody against Pfs230, which suggests that a Pfs230-based vaccine may be boosted by natural malaria infection.

1.1.1 Development of the Study Agent, Pfs230D1M-EPA/AS01

The recombinant protein Pfs230 domain 1 (Pfs230D1M) was developed at Laboratory of Malaria Immunology and Vaccinology (LMIV) and selected for clinical development. Pfs230 contains various amino acid substitutions throughout the protein; however, the function of these changes is unknown. Recombinant Pfs230D1M, which comprises about 10% of the whole Pfs230 protein, contains minor allelic variants.[7] A comparative analysis with 11 Malian isolates shows two point mutations (G to S at position 64 and K to N at position 120) as well as the known N-to-Q substitution at amino acid position 44 that LMIV engineered to remove the unique putative N-linked glycosylation site (**Figure 1**). These same mutations were also observed in an analysis of over 2,000 parasite isolates.[7] In West Africa, the minor allelic frequency was reported to be 0.111 and 0.339 for G605S and K661N, respectively, shown **Figure 1**. Of note, rabbit antisera raised against Pfs230D1M blocked parasite transmission of a Thailand isolate with the G605S

mutation, suggesting efficacy of Pfs230D1M-EPA against the variant. The biological impact of the K661N mutation remains to be determined.

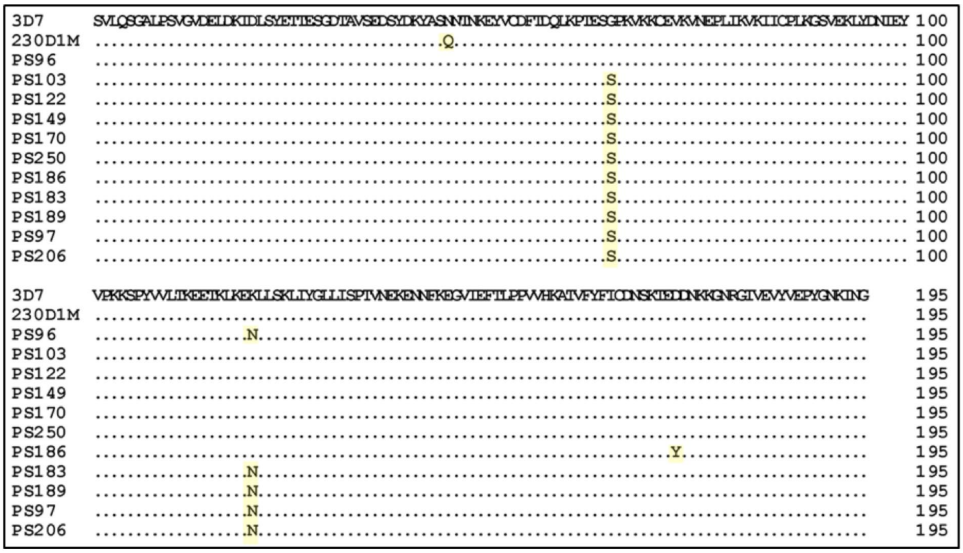


Figure 1: Protein Alignments of Recombinant Pfs230D1M to its Respective Native Protein or Protein Fragment.

Deduced amino acid sequence of Pfs230D1 3D7 and 11 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).

Several N-terminal sub-domains within the 300-kDa protein were previously evaluated and found to induce functional antibodies to block transmission in animal studies.[9,10] 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 with *Pichia pastoris* as the production system.

LMIV investigators chemically conjugated Pfs230D1M to EPA, a recombinant mutant and detoxified protein from *Pseudomonas aeruginosa*. EPA is not a component of any licensed vaccines but has been extensively studied as a component of conjugated typhoid and shigellosis vaccines[11-13] and LMIV/MRTC's previous phase 1 TBV studies involving Pfs25H, Pfs25M, and Pfs230D1M formulated with Alhydrogel or AS01 elicited strong TBAs in mice, rabbits, and *Aotus* monkeys. Pfs230D1M-EPA formulated in Alhydrogel has been evaluated in a Phase 1 study in US adults (2015) and Malian adults (2015-2016) under National Institute of Allergy and Infectious Diseases (NIAID) Protocol #15-I-0044 and was demonstrated to be safe and immunogenic both in malaria-naïve and malaria-exposed adults (see **Section 3.1**).

Pfs230D1M-EPA formulated in the more potent adjuvant AS01 has now completed recent evaluation over two years in the ongoing NIAID Protocol #17-I-N006 with initial results

supporting higher potency (enzyme-linked immunosorbent assay [ELISA] titers and antibody activity) for AS01 (see **Section 3.2**). AS01 is a liposome-based adjuvant system containing the immune-enhancers: MPL (3-O-desacyl-4'-monophosphoryl lipid A) and QS21 (a saponin molecule purified from the bark extract of *Quillaja saponaria* Molina tree). There are 2 concentrations in the AS01 family, the $AS01_B$ Adjuvant System and $AS01_E$ Adjuvant System; this study will use $AS01_B$ diluted 1:1 with conjugated Pfs230D1M to deliver an $AS01_E$ dose concentration. Further discussion on clinical studies of the study vaccine is provided below (**Section 3**).

1.2 Rationale for Study

The ongoing Phase 1 study of Pfs230D1M-EPA/AS01 in adults (NIAID Protocol #17-I-N006) indicates that the vaccine is safe, tolerable, immunogenic, and provides high and durable transmission-blocking activity that can be increased with a fourth dose for another season. These initial results suggest that Pfs230D1M-EPA/AS01 can be employed in a community wide trial to evaluate its efficacy to reduce malaria infections in the community across two malaria seasons.

1.2.1 Rationale for Study Design: Vaccination of School-Aged Children

The major determinants of an individual's contribution to onward malaria transmission are infectivity and exposure to mosquitoes. Schoolchildren are in general the most infectious age group, represent ~35% of the population, and are more often bitten by mosquitoes than toddlers. A recent study performed in Burkina Faso and Kenya confirms that after accounting for baseline infectiousness (measured by mosquito feeding assays), real-life mosquito exposure (by using molecular typing of blood meals) and demography, schoolchildren's contribution to transmission is comparable to adults.[14] In particular, in that study, the authors estimated that between 40 – 75% of mosquito infections originate from schoolchildren.

Our studies of malaria transmission (NIAID Protocol #17-I-N180) using Direct Skin Feeding (DSF) assays (cups of mosquitoes are fed directly on study participant skin to quantify transmission) demonstrate that Malian schoolchildren disproportionately transmit parasites to mosquitoes (**Figure 2**). In DSF studies performed from March to August 2018, >95% of transmission events among the general Malian population were observed in children >4 years of age. For this reason, the impact of the malaria vaccine on transmission in this age group is particularly important to understand. Further, trials will need to vaccinate school-age children in order to interrupt malaria transmission and demonstrate efficacy (reduction in malaria infection incidence in the vaccinated group).

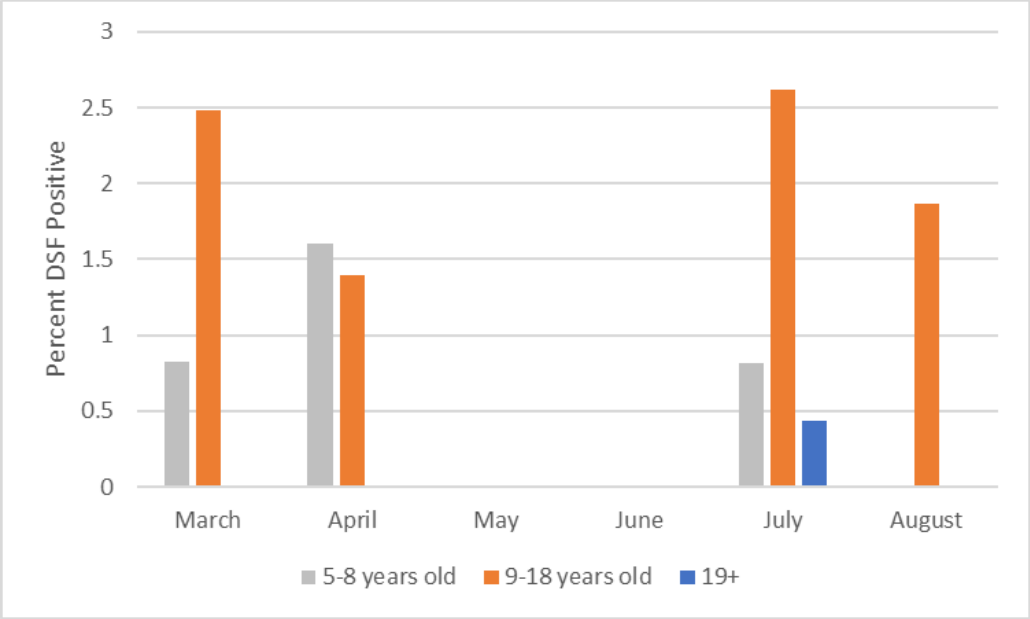


Figure 2: Frequency of Positive DSF Assays by Month and Age in Bancoumana, Mali (NIAID Protocol #17-I-N180).

Malian children 5-18 years of age disproportionately transmit in DSF assays, being responsible for 27 of the 28 transmission events

Taking the vaccine activity measured via SMFA in Protocol #17-I-N006, in preliminary modeling studies (see **Figure 3**) Pfs230D1M-EPA/AS01, as a single malaria elimination tool, is estimated to reduce clinical malaria incidence by ~60% across a malaria season if administered to 80% of the target population.

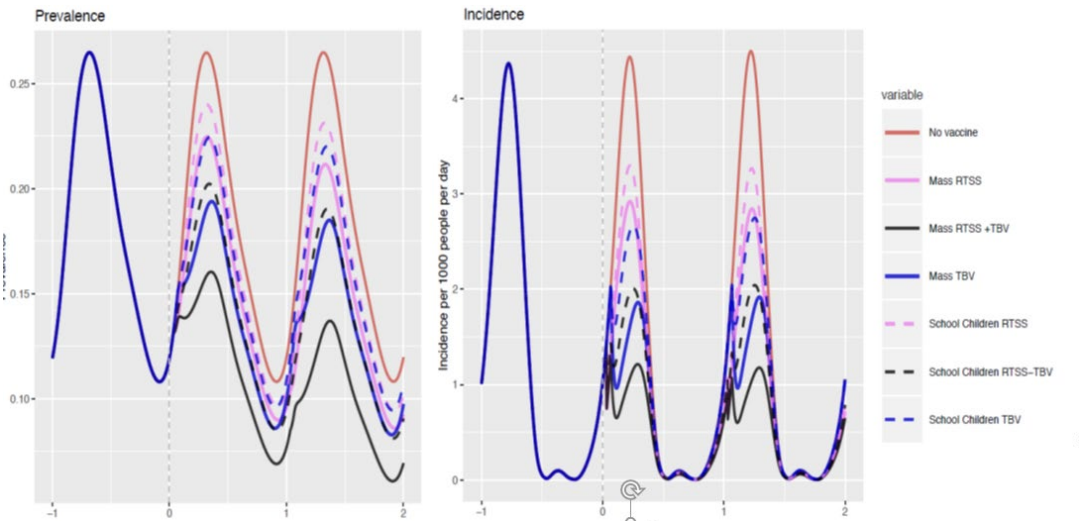


Figure 3: Modeling estimated vaccine effects on prevalence and clinical incidence of *P. falciparum* malaria.

Solid line is mass distribution (80% coverage); dashed line targets children <17 years of age. Transmission intensity driven by rainfall data from Bamako region. VE estimates from unpublished Pfs230-EPA/AS01 Phase 1 trial. Predictions will change with refined VE, study population data.

Underpinning these plots is a developed mathematical model of malaria transmission.[15] It is fitted to data on *Plasmodium falciparum* malaria prevalence and clinical incidence from across Africa, covering a range of transmission settings. In the model, a population develops immunity according to its level of exposure to the parasite. The model also allows for heterogeneity of exposure within the population (i.e. some individuals are bitten more often than others). The model also tracks the mosquito population (see supplement for Griffin et al paper[15]): adult female mosquitoes can be susceptible, latently infected, or infectious.

The model has been used to estimate the impact of certain interventions, such as bed nets, indoor residual spraying and the RTS,S vaccine. Our aim was to take provisional data from the pilot study of Pfs230D1M-EPA/AS01 (presented at ASTMH 2017) to obtain a rough profile of the vaccine, describing the antibody dynamics & vaccine efficacy as a function of antibody titer (see **Figure 4**). For the latter, a Hill function relation was assumed, using the same 'shape parameters' as fitted to detailed RTS,S data. Essentially, we assume that the high transmission-reducing activity (TRA) observed for high antibody titers translates to high TBA (**Figure 4B**), which then wanes over time, as antibody levels decrease.

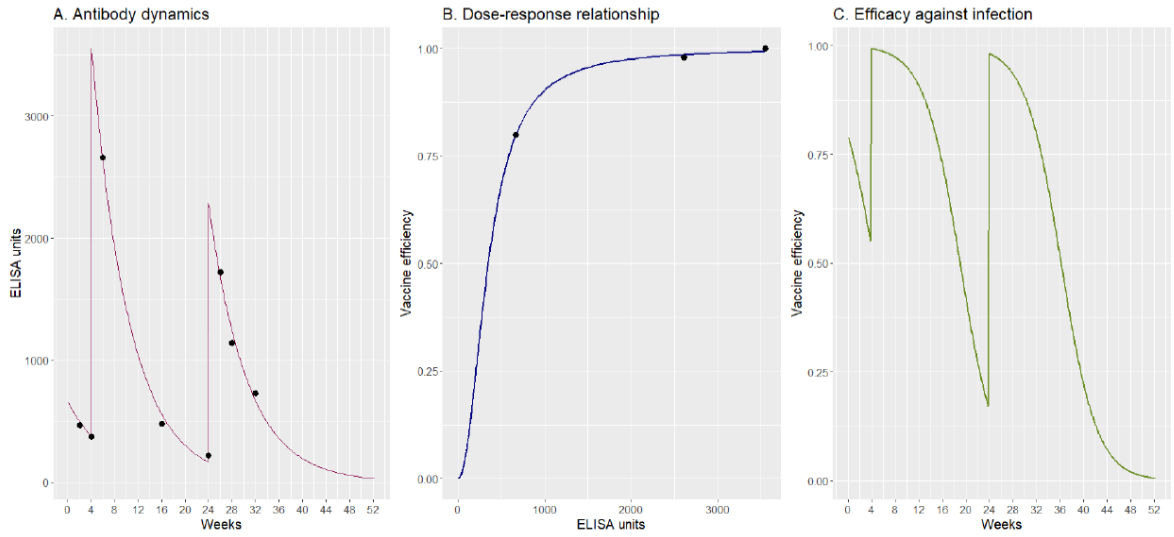


Figure 4: Antibody dynamics and association with vaccine efficacy.

Black dots denote the data points from preliminary Phase I results. (A) Antibody dynamics predicted by the model after TBV vaccination schedule with two doses once per month and a booster dose 6 months after the first dose. (B) Estimated dose response relationship between antibody titers and vaccine efficiency after the second TBV dose. (C) Vaccine efficacy estimation over time based on the model predictions from panel A and dose response curve from panel B.

This vaccine efficacy is useful to reduce the infectiousness (to mosquitoes) of those individuals in the model who are infected. The model is run with different scenarios for who receives the vaccine, to see how reducing the force of infection from humans to mosquitoes reduces subsequent human infections. A number of scenarios were considered, as described in the **Figure 4** caption above. We looked at the impact of TBV alone, and delivered along with the RTS,S vaccine.

In all scenarios, we assumed 80% vaccine coverage in the population targeted in the intervention. We assumed that everyone who receives the vaccine received all doses.

An important limitation of this model is that it does not account for spatial details. When an individual in the model is infected, the previous human host for those parasites could be any infected individual in the population, i.e. there is no sense in which one person is 'near' to another, or 'far away' from a third person. For example, in this model, it is inconsequential if people don't sleep in their compound, whereas in reality this could be quite important.

This model underscores how important it is to test this vaccine by administering it to school-age (5 years of age and older) children and to adults in a small community setting (family household) and then assessing its safety, functional immunogenicity, and efficacy for reducing malaria infections within that community.

These results, together with evidence that transmission is local (as described in **Section 1.2.2**), suggest that for each malaria transmission unit, which corresponds to individual households, inclusion of schoolchildren in transmission-blocking intervention trials will directly impact infection incidence where they live, including their own risk of future infection in the same transmission season.

1.2.2 Rationale for Study Design: Family Compound and Requirement of Broad Vaccine Coverage

We believe TBV clinical trials will need to vaccinate individuals in groups (or "clusters") in order to interrupt malaria transmission and demonstrate efficacy (i.e. efficacy to reduce malaria infection incidence in the vaccinated group). Vaccinating at the family compound level may be sufficient to achieve the effect, because clustering of malaria infection may occur at the family compound level. The hypothesized mechanism for compound infection clustering relates to the activity of female Anopheles mosquitoes, which after blood-feeding and then ovipositing in the nearest breeding site (water source), return to the same or neighboring houses, especially when there are nearby breeding sites.

Clustering of infections is also the basis of the widely used strategy of reactive case detection, which involves testing and treatment of individuals living in the same or neighboring households of passively-detected clinical cases. The efficiency of this strategy in identifying infections corroborates the notion that infection in a household depends mostly on the infection status of its members.[16]

The following evidence provides support that a malaria transmission unit corresponds to a household or at most neighboring households:

- There is clustering of malaria infections within individual households[17]
- *Anopheles* mosquitoes generally disperse less than 2 km[18]
- Household-level variation in sporozoite infection rates have been observed, independent of mosquito numbers[19]
- There are reports of non-*P. falciparum* malaria household clustering in areas with *P. falciparum* transmission[20]
- Mathematical models that account for mosquito oviposition suggest that distribution of breeding sites is a determinant of locality of transmission[21]

First, individuals living in households with passively detected cases are more likely to be infected, compared to control households with no cases.[17] Of note, the odds ratio of infection is increased in the households where the cases live compared to the neighboring households, and is also increased but to a lesser extent in neighboring households compared to randomly selected households. This pattern suggests that infection clustering at the household level is due to a

limited range of transmission for individual mosquitoes, and not due to other factors that would not explain the weaker association in neighboring households, such as multiple interrupted feeding episodes from the same mosquito or host genetics.

Micro-epidemiological analysis in coastal Kenya confirms this pattern, and single-household clusters have been identified in that region.[22]

Pertinent to our proposed trial, published analyses of longitudinal data from Mali show the presence of single-household clusters of transmission that lasted several months, further highlighting the narrow range of transmission for individual mosquitoes.[20] When analyzed in relation to season, clustering was observed within individual or a few adjacent compounds (i.e., households) in the rainy season (**panels A and B, Figure 5 below**), while clustering was extended over a larger number of compounds after the rains or during the dry season (**panels C and D**). This is consistent with mosquitoes staying within individual compounds during the rains when oviposition/breeding sites are relatively ubiquitous, and then with mosquitoes dispersing more widely after the rains have ended when breeding sites become sparser.

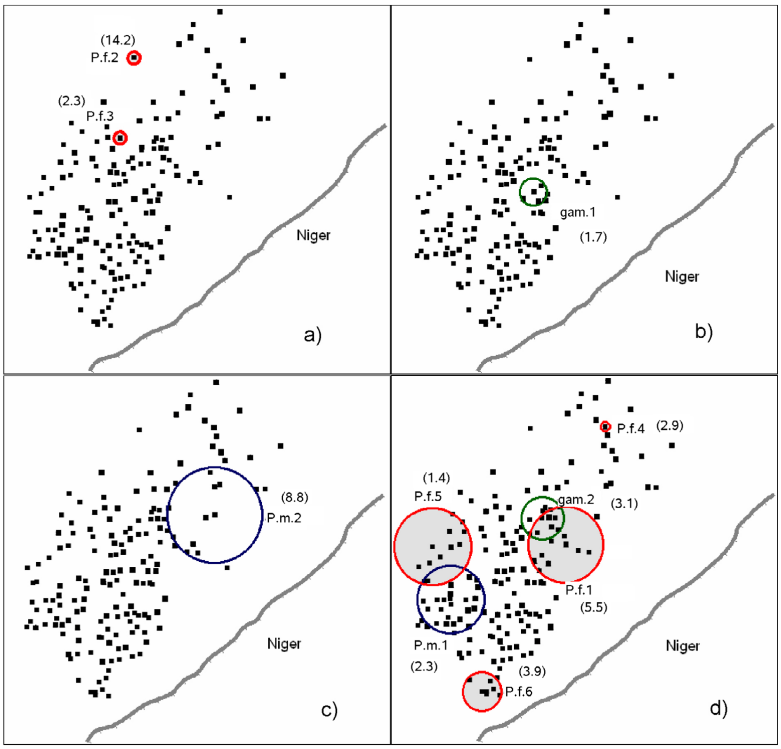


Figure 5: Spatial and temporal locations of infection clusters[20]:

In this figure, temporal changes in the spatial scale of transmission in Bancoumana village, Mali, are shown. (a) October 1996, (b) October 1997, (c) December 1998, (d) May 2000. Dots represent households. Rate ratios are presented in brackets near each cluster. P.f.: cluster of *P. falciparum* infections (in red). gam: cluster of *P. falciparum* gametocyte carriages (in green). P.m.: cluster of *P. malariae* infections (in blue). The 4 time frames were

selected such that all the clusters were represented. For each of the 4 time frames, the x- and y-axis represent GPS coordinates. In the panels on the top (**5a** and **5b**), clusters of transmission that consist of single or a few households are highlighted; these plots present data from the rainy season. After the rainy season subsides (panels **5c** and **5d**), clustering of infection extended through larger geographical areas, which is consistent with greater dispersal of mosquitoes as described in the arguments presented in this protocol.

These patterns of infection clustering are consistent with entomological observations that, although long-distance mosquito dispersal has been observed, most mosquito movements are thought to be limited to a radius of less than 2 km,[18] and may be more limited when sites for oviposition are nearby and readily accessible. Differences in household-level prevalence of infection in mosquitoes are also evidence of locality of transmission, as there is no evidence that infected mosquitoes' movements would be systematically different from uninfected mosquitoes, and biased towards particular households. Data from a recent study performed in Burkina Faso showed that infection (sporozoite) rates in bloodfed mosquitoes that had head-thorax tested by polymerase chain reaction (PCR) vary between households independently of the number of mosquitoes,[19] which supports the hypothesis that mosquitoes tend to return to the same house after feeding and ovipositing.

Describing parasite genetic similarity at different spatial scales is another way of assessing the scale of transmission. Although these data are limited at the micro-epidemiological level, the distribution of non-falciparum malaria parasites in areas with predominant falciparum transmission is informative in this respect. In Mali, non-falciparum species are more likely to occur in a small fraction of households, where their household-level prevalence is high (see **Figure 5** above), suggesting that these non-falciparum parasites, transmitted by the same vectors as *Plasmodium falciparum*, circulate locally, i.e. individuals in these households are infected by other members of the same household. Clustering data supports the idea that parasites circulate locally within households.

Finally, mathematical models that have explicitly incorporated mosquito oviposition suggest that the distribution of breeding sites, whether they are productive or not, are an important determinant of the scale of transmission, i.e. the average distance between the locations where individual mosquitoes acquire infection and where they feed after full parasite development.[21] In this respect, locality of transmission is likely to be more important in households near permanent breeding sites during the dry season, and throughout villages during the rainy season, since even temporary breeding sites can support oviposition and during this period it is more likely that mosquitoes find a suitable oviposition site near the house where they feed.

The prominent role that children play in malaria transmission, as previously described in **Section 1.2.1**, combined with this evidence that transmission is local, suggests that for each malaria transmission unit, which corresponds to individual family compounds, inclusion of children 5 years and above in transmission-blocking intervention trials will impact the incidence

of malaria infections for all individuals residing in the family compound, including young unvaccinated children as well as vaccinated children and adults.

1.2.3 Rationale for Study Design: Vaccine Dose and Regimen

The vaccination dose and schedule were selected, and subsequently adjusted as described below in **Section 1.2.3.1**, based on initial results of the ongoing Phase 1 trial of Pfs230D1M-EPA/AS01 and another investigational TBV, Pfs25M-EPA/AS01, in Malian adults (NIAID Protocol #17-I-N006). The main phase of this study (#17-I-N006) evaluated Pfs230 antibody titers and function using a dose of 40 micrograms (µg) administered on study days 0, 28, 168 days, which is the same dose proposed to be used in this study in both children and adults.

Preliminary analysis of antibodies against Pfs230 titers and function indicate that significantly high transmission reducing activity is observed for at least 12 weeks post the second dose compared to the activity observed in controls (**Figure 16**). However, it is only after receipt of the third dose when the transmission blocking activity becomes significantly higher compared to controls, indicating the benefit of a 3-dose regimen (**Figure 17**).

Given this background, we plan to conduct an age de-escalating trial for Pfs230D1M-EPA/AS01 in 2 different age groups: ages 9 to 18 years, and 5 to 8 years. Subjects will be recruited from compounds that have undergone initial consenting and screening and of which approximately 80% of the compound/vaccine unit has agreed to participate and appears eligible to participate. In each group, subjects will receive three doses at 0, 28, 56 days of either 40 µg Pfs230D1M-EPA/AS01 or comparator vaccine. Safety data will be gathered, monitored, reviewed, and reported throughout the trial in order to proceed with further enrollment. Periodic blood collections will also be used to assess immunogenicity and functional activity of Pfs230D1M-EPA/AS01 over the course of the study. Subjects will be followed through at least six months after the last vaccination and will join their family compound during the main phase around vaccination #3.

Upon determining that Pfs230D1M-EPA/AS01 is safe in children 5-18 years of age, all eligible residents 5 years of age and older in identified participating family compounds/vaccine units will be enrolled for vaccine administration to assess safety and efficacy. Subjects will receive study vaccine or comparator vaccine according to the family compound assignment at 0, 28, and 56 days. Safety outcomes will be monitored and reported as local and systemic adverse events and serious adverse events (SAEs). Vaccine efficacy will be assessed by blood smear (BS) microscopy for detection of new *P. falciparum* infections in children 1-8 years of age living in the compound as well as 9-18 years of age. Vaccine activity will be assessed in children 9-18 years of age by DSF assays whereby laboratory-raised mosquitoes are fed on study participants' skin.

Our previous studies indicate that treatment with artemether-lumefantrine (AL) can reduce the rate of positive DSF assays for 4 or more weeks. For this reason, children 9-18 years of age who will undergo DSF to assess vaccine activity will not receive AL treatment before their 3rd or 4th vaccine dose. Since they are not receiving treatment before the transmission season, we will explore their reduced risk of infection as compared to controls on the basis of the proportion of positive BS.

We hope that the results of this study will help us determine the safety and tolerability of Pfs230D1M-EPA/AS01 in Malian children and to assess its functional activity as well as its efficacy to prevent *P. falciparum* infection.

1.2.3.1 Rationale for Change in Vaccine Dosing Schedule

1.2.3.1.1 Malaria Transmission Dynamics in Mali

The two epidemiological motivations for this change are: (i) expected temporal variation in locality of malaria transmission and (ii) changes in the number of human-to-mosquito transmission events over the course of a transmission season.

1.2.3.1.1.1 Local, Seasonal Transmission

There is evidence, that includes different types of studies (described in the **Section of 1.2.2**), that malaria transmission occurs at a very local scale, [18-20] that is, that the distance between the locations where mosquitoes acquire infection and where they feed after *falciparum* parasite sporogonic development, potentially inoculating sporozoites in uninfected individuals, is on average small. This key epidemiological observation provided the basis for the selection of compounds as units of randomization and analysis in this clinical trial, as the impact of transmission blocking vaccines is likely to be most evident at this scale.

One of the determinants of the spatial scale in which malaria transmission occurs is the distribution of mosquito breeding sites. [21,23] Between blood meals, *Anopheles* mosquitoes lay eggs on water bodies, and the distance traveled for oviposition will depend on the presence of nearby breeding sites. During the rainy season, in areas with endemic malaria transmission, including in the village where this vaccine trial is being performed, the number of temporary breeding sites and their larval productivity increases, as observed in entomological studies across different setting[24,25] and respective colleagues observed that the frequency of aquatic habitats and their stability were influenced by rainfall.[26,27] This more widespread distribution of potential oviposition sites implies a high likelihood that there will be nearby breeding sites for mosquitoes ovipositing after feeding in study compounds/vaccine units. It is thus during this period that circulation of parasites within vaccine units (which may be one or more compounds) is expected to be highest (i.e. infected mosquitoes biting individuals living in the same compound/vaccine unit where they acquired their infection). As explained in detail in **Section 1.2.2**, there is

evidence from epidemiological studies in Mali that the degree of locality of malaria transmission varies seasonally (**Figure 5**).

The primary endpoint for this trial and a measure of vaccine activity is the frequency of parasite transmission events measured by direct skin feed (DSF) assays in older children. As positive DSF assays are infrequent, maximizing antibody titers throughout the time period when positive DSF assays are most frequent will increase our power for detecting vaccine activity. A secondary endpoint of this trial and a measure of vaccine efficacy is the prevalence of malaria parasites in children living in the randomization units (*P. falciparum* asexual parasitemia measured by blood smears). Since malaria transmission is local during the rainy season, it is during this period that the vaccine, randomized to entire compounds, will have a more important effect on compound-level human infection prevalence. For this reason, we propose to modify the schedule of vaccine dosing so that antibodies induced by the vaccine reach sufficiently high levels when direct benefit to vaccinated units is more likely.

1.2.3.1.1.2 Changes in Human-to-Mosquito Transmission Events

The optimal dosing schedule of transmission blocking vaccines with regards to their impact on infection numbers in mosquito populations requires that vaccine activity, measured by reduction in mosquito infection probability after blood feeding, reaches maximum levels during the periods when human-to-mosquito transmission is epidemiologically more important, that is, when most transmission events from humans to mosquitoes occur (**Figure 6**) In areas with seasonal transmission, two different processes determine malaria infection status in *Anopheles* mosquitoes: probability of transmission of parasites from humans in individual mosquito blood meals and age distribution of mosquito populations. While it has been argued that at the end of the transmission season the prevalence of malaria infection in mosquitoes is explained by the latter, mosquito age demographics, and the presence of, on average, older mosquitoes[28] (as a direct consequence of the cumulative risk of infection, for mosquitoes, during the entire transmission season), human *falciparum* transmission to uninfected mosquitoes is essential throughout the wet season. Preventing mosquito infection at the start and peak of the rainy season is thus more likely to have an effect on infection levels compared to preventing human-to-mosquito transmission at the end of the wet season.

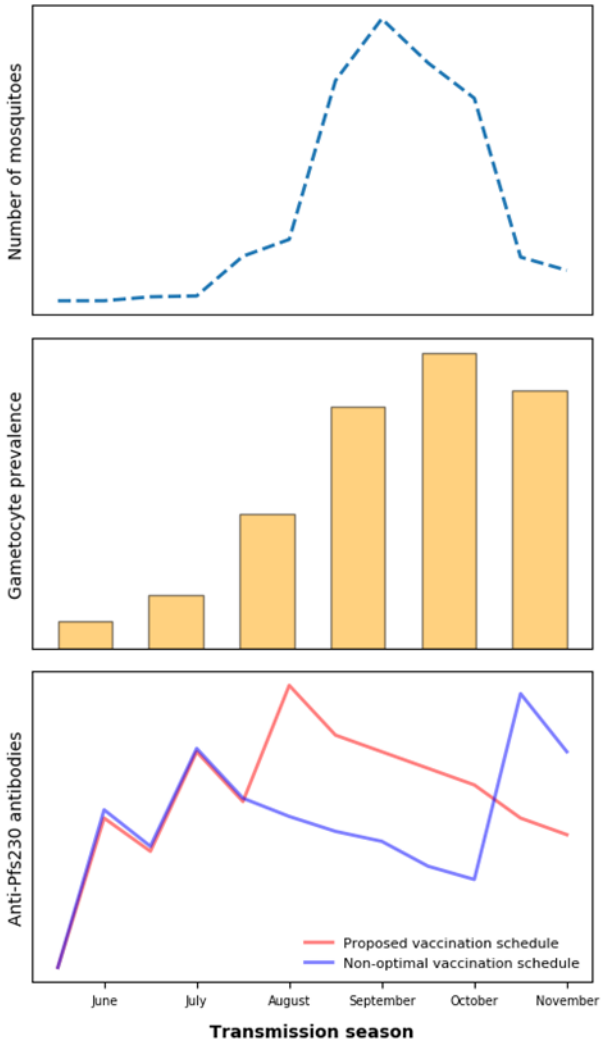


Figure 6. Impact of number of Anopheles and gametocyte prevalence on number of human-to-mosquito transmission events.

This figure illustrates temporal changes in two major determinants of the number of human-to-mosquito transmission events: number of *Anopheles* mosquitoes in a village and gametocyte prevalence, which is, for example, directly linked to infection prevalence. In the top panel, numbers of mosquitoes collected in the village of Bancoumana, Mali, with indoor live catches are shown (data from 2018). The second panel presents gametocyte prevalence in the villages of Bancoumana and Doneguebougou in 2018; note that parasitological data are not available for Doneguebougou before September. The third panel illustrates Pfs230-targeted immune responses with vaccine dosing on days 0, 28 and 56; the data presented is from experiments involving mice immunization, and here it is assumed that the vaccination would start in June. With the vaccination schedule proposed in this amendment, higher antibody levels will be present in individuals living in vaccinated randomization units during the time period when most transmission events occur. The bottom panel shows unpublished data from a clinical trial undertaken in the study area,

Our data in both animal and human studies have shown that anti-Pfs230 ELISA titers correlate well with serum functional activity that blocks parasite transmission to mosquitoes, hence we seek to maximize the antibody levels and thereby 1) block parasite transmission during the time

window when within-household transmission may be maximal; 2) block parasite transmission during the time window when the frequency of positive DSF may be maximal. By changing the dosing schedule so that the second dose of vaccine is given early August and the third dose of the vaccine is given in early September 2019, we expect to induce high transmission reducing and blocking activities during peak transmission season, which will maximize the impact of the vaccine on parasite circulation within households *in natura* and on parasite transmission in the DSF assay. Indeed, as mentioned in the **Section 3.2.2** of the study protocol, preliminary findings of a pilot vaccine trial indicate that Pfs230D1M-EPA/AS01 transmission reducing activity, as measured in standard membrane feeding assays, is statistically significantly higher 3 months after the 3rd dose compared to 3 months after the 2nd dose. Hence, giving the third vaccine dose one month rather than 5 months after the second dose will allow us to maximize antibody titers (and we therefore expect serum functional activity) during the periods of maximal intra-household transmission and of most frequent DSF transmission events.

These two factors, the expected relative increase in within-randomization unit parasite circulation during the rainy season and the proportionally high number of human-to-mosquito transmission events in the same period, implies that by administering a third dose of the Pfs230D1M-EPA/AS01 vaccine on study day 56 rather than on day 168, transmission blocking antibody levels will be sustainably high when the effect of the vaccine on prospective risk of human malaria infection would provide maximal benefit to the vaccinated units.

1.2.3.1.2 Supporting Safety and Immunological Data from RTS,S/AS01$_E$ Experience

In addition to these epidemiological arguments, there is evidence, from a malaria vaccine clinical study that used the same adjuvant as this trial (AS01$_E$) that a vaccine schedule of 0, 1, 2 months is both safe and immunogenic.

In a Phase 2 pediatric trial of RTS,S, a malaria vaccine using the same adjuvant (AS01$_E$), that is being used in this protocol with Pfs230D1M-EPA, various vaccine dosing regimens were compared (0, 1 versus 0, 1, 2 versus 0, 1, 7 months) with the primary outcomes measured being the occurrence of SAEs until 10 months post dose 1.[29] For the vaccine dosing regimens that included three vaccinations (0, 1, 2 months versus 0, 1, 7 months), Owusu-Agyei and colleagues observed similar numbers of AEs between the two vaccination regimens (**Figure 7**). The proportion of study participants that developed pain at the injection site, swelling or fever were similar in the two vaccine dosing schedules as well. The occurrence of at least one SAE, at 10 months post first vaccination and 19 months post vaccination, were also similar in the 0, 1, 2 month vaccination regimen arm (14.4%, 7.9-23.4; 24.4%, 16.0-34.6) versus the 0, 1, 7 month vaccination regimen arm (15.6%, 8.8-24.7; 18.9%, 11.4-28.5).[29]

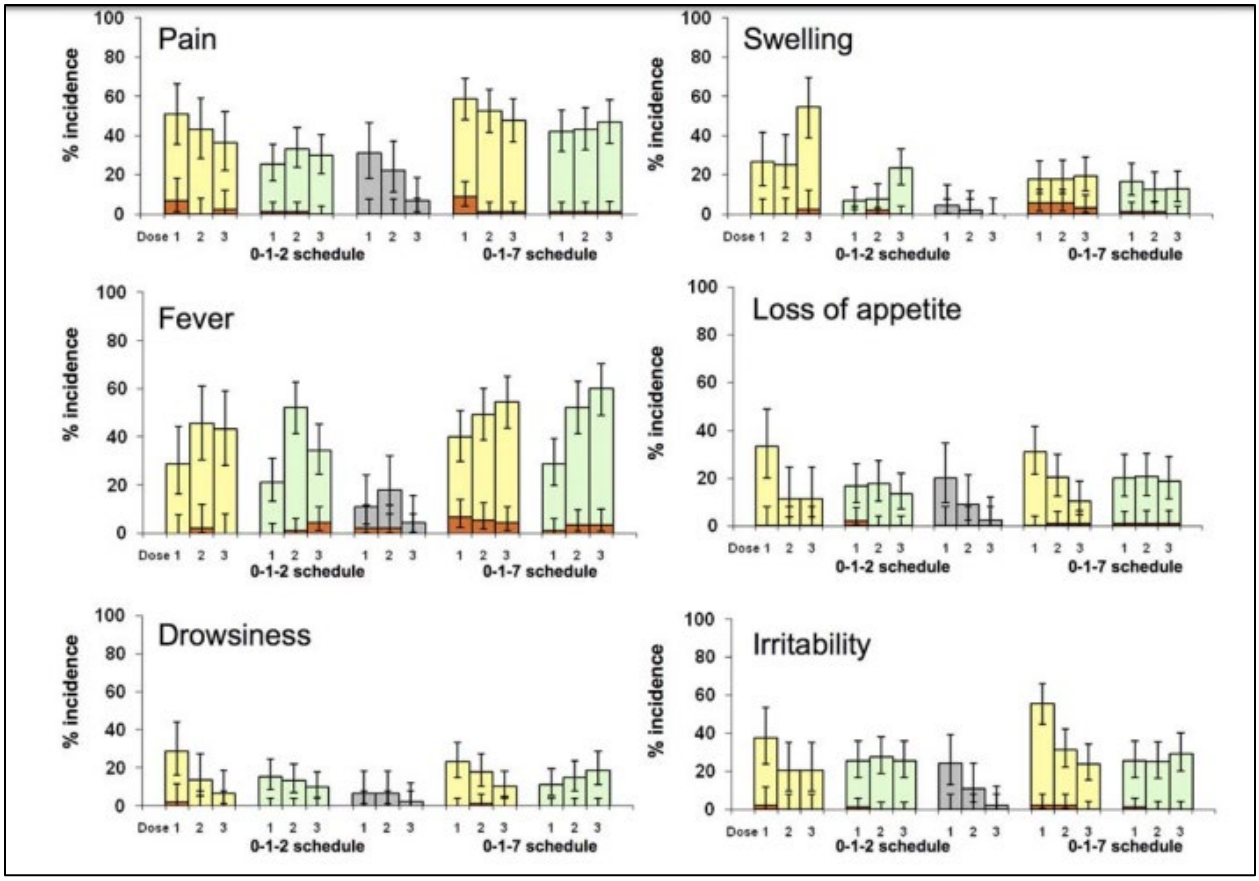


Figure 7. Percentage of solicited events post dose 1, 2, and 3 RTS,S (total vaccinated cohort).

Yellow = RTS,S/AS02$_D$; Green = RTS,S/AS01$_E$;l; Grey = Rabies vaccine; Orange = Grade 3; Bars represent 95% confidence intervals. Adapted from Owusu-Agyei, S et al.[29]

In that study, antibody levels against circumsporozoite protein reached higher peak levels when the third dose of the vaccine was administered a month after the second dose (0, 1, 2 months) compared to dosing of the same vaccine at 0, 1, 7 months (**Table 1**). The highest anti-CS GMTS seen in the study, comparing all regimens and adjuvants, was seen in the RTS,S/AS01$_E$ 0, 1, 2, month schedule (month 3 = 632 EU/mL, 95% CI 554, 720; compared with month 8 in the 0, 1, 7 month schedule = 373 EU/mL, 95% CI 311, 447).

Table 1. GMTs and sAUC for anti-CSP antibodies and GMTs for anti-HBs antibodies.
Adapted from Owusu-Agyei, S et al.[29]

Schedule	0,1-month		0,1,2-month			0,1,7-month	
	RTS,S/AS02$_D$	RTS,S/AS01$_E$	RTS,S/AS02$_D$	RTS,S/AS01$_E$	Rabies	RTS,S/AS02$_D$	RTS,S/AS01$_E$
Anti-CS (EU/mL); GMTS [95% CI]							
SCR	0.3 [0.3, 0.4]	0.3 [0.3, 0.4]	0.3 [0.2, 0.3]	0.3 [0.3, 0.4]	0.3 [0.3, 0.4]	0.3 [0.3, 0.3]	0.3 [0.3, 0.4]
M2	318 [269, 377]	483 [395, 591]					
M3	-	-	367 [293, 459]	632 [554, 720]	0.4 [0.3, 0.6]	-	-
M7	35 [26, 46]	53 [41, 68]	78 [58, 106]	162 [134, 196]	0.3 [0.3, 0.4]	26 [20, 34]	51 [40, 64]
M8	-	-	-	-	-	272 [219, 339]	373 [311, 447]
M10	20 [15, 27]	32 [23, 40]	43 [32, 60]	102 [83, 125]	0.3 [0.3, 0.4]	120 [92, 156]	167 [140, 198]
M19	10 [7,14]	15 [11,21]	20 [14, 29]	46 [37, 57]	0.5 [0.3, 0.7]	44 [33, 58]	51 [40, 66]
Anti-CS sAUC; Gmean [95% CI]							
sAUC7	181 [151, 218]	269 [218, 332]	225 [182, 279]	371 [324, 426]	-	166* [139, 198]	249* [204, 302]
sAUC19	83 [69, 101]	124 [101, 153]	113 [90, 143]	200 [173, 232]	-	141 [116, 173]	203 [172, 240]
Anti-HBs (mIU/mL); GMTS [95% CI]							
SCR	101 [68, 149]	108 [74, 158]	109 [61, 195]	82 [61, 111]	108 [64, 183]	88 [61, 128]	90 [61, 133]
M2	17043 [10467, 27751]	15107 [9508, 24001]					
M3			30000 [18799, 47874]	34935 [25178, 48474]	122 [63, 235]		
M8						96754 [72062, 129908]	103225 [83035, 128324]
M19	3510 [2398, 5137]	4478 [3155, 6357]	5112 [3350, 7803]	7106 [5161, 9784]	114 [58, 227]	17191 [12529, 23589]	13386 [9661, 18548]

SCR = screening visit; sAUC 7/19 = standardised area under the curve over the vaccination period to Month 7/Month 19.
*sAUC7 here represents a period during which only the first two vaccine doses were given, as for the 0,1-month schedule.

1.2.4 Rationale for Study Design: Inclusion of Children Under 5 Years of Age

Children under 5 years of age are excluded from vaccination during this study because there are insufficient data regarding dosing or AEs from our trials in adults to judge the potential risk in children in this age group. Some evidence suggests an increased risk of febrile seizures with receipt of AS01 from RTS,S/AS01E studies in young children; since febrile seizures in the general population are most likely to occur in children under the age of 5 years, children under 5 years old are excluded from vaccination in this study.

In addition, Malian children under 5 years of age may make only a modest contribution to malaria transmission in the community. Recent studies suggest that mosquitoes feed infrequently on under-5 African children (compared to school age children or adults), hence their contribution to malaria transmission may be as low as 2-4% at some sites.[14] Further, Malian children now receive monthly antimalarial treatments with long acting agents (termed Seasonal Malaria Chemoprevention or SMC), which limits their rates of gametocytemia, further reducing their contribution to malaria transmission. Thus, under-5 children are not a critical target demographic for vaccination.

Notably, under-5 children are an important target to assess TBV efficacy even though they are not an important target for vaccination per se. Although under-5 children receive mosquito bites

infrequently, they have a high rate of infection in Mali as well as any African site, presumably because they lack immunity to prevent infection even when bitten by a single infectious mosquito. In our studies of Malian children who receive monthly SMC, we observed that 58% of children develop a patent parasitemia based on BS performed at the time of the monthly SMC treatments.

2 Previous Preclinical Experience with Pfs230D1M-EPA/AS01

In preclinical studies evaluating Pfs230D1M-EPA formulated with AS01 adjuvant in rabbits at two-week intervals over a 43-day study interval, the vaccine was determined to be 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 8**).

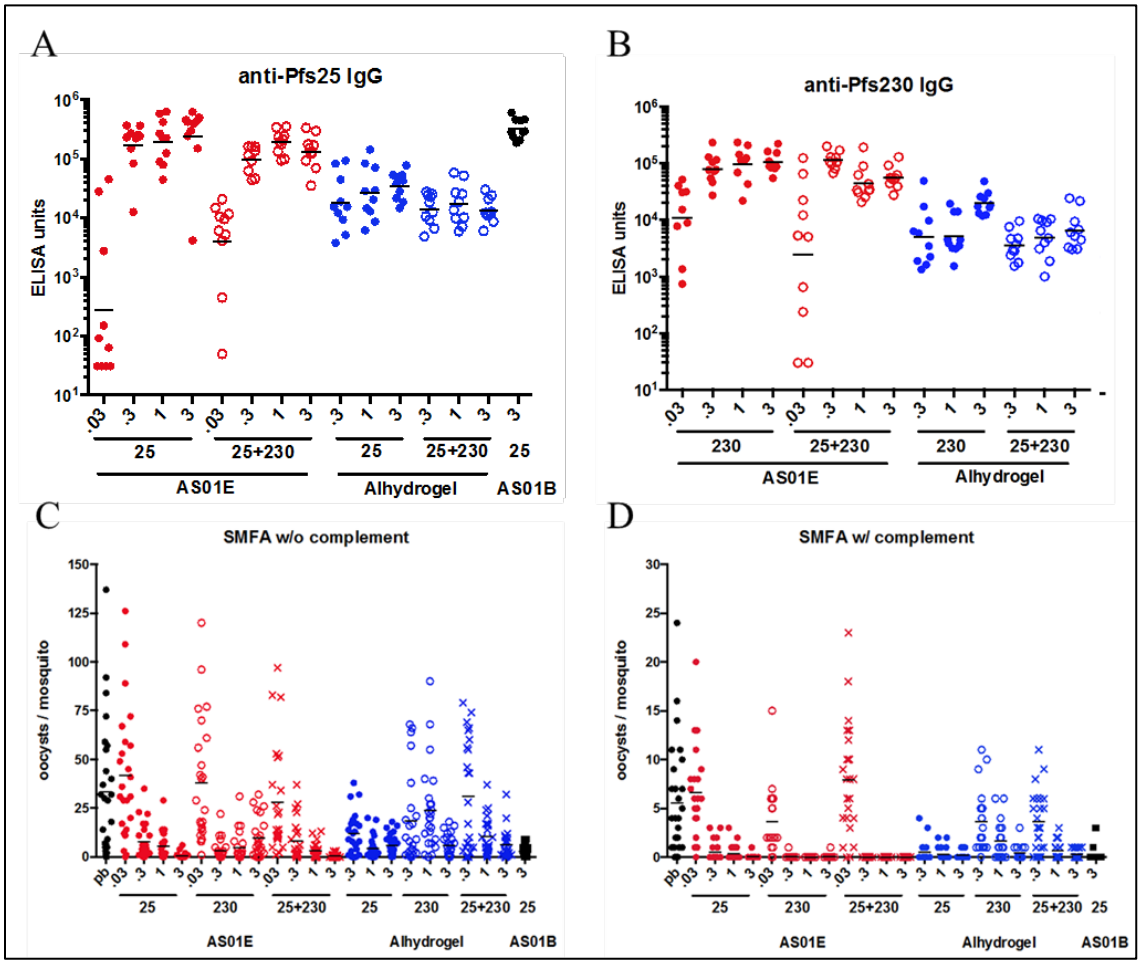


Figure 8: Comparison of Transmission-Blocking Activity of Pfs25M-EPA/AS01 and Pfs230D1M-EPA/AS01 to Pfs25M-EPA/Alhydrogel and Pfs230D1M-EPA/Alhydrogel in CD1 Mice.

A and B show antibody responses 14 days following Vaccination #2 to anti-Pfs25 (A) and anti-Pfs230 (B) immunoglobulin G (IgG) 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. Circles represent antibody level per mouse and black bars indicate geometric mean of antibody levels. C and D report standard membrane feeding assay (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 C and D, 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 50 µg monophosphoryl lipid (MPL) and 50 µg QS21 in a 0.5 mL dose; and $AS01_E$ is the adjuvant $AS01_B$ diluted to a *dose equivalent* to 25 µg of MPL and 25 µg of QS21 in a 0.5 mL dose.

3 Previous Human Experience with Pfs230-EPA

3.1 Pfs230D1M-EPA/Alhydrogel in Healthy Adults (#15-I-0044)

A Phase 1 dose-escalating study evaluating the safety, tolerability, immunogenicity, and functional activity of Pfs230D1M-EPA adjuvanted with Alhydrogel was conducted in 2014-2017 in both the U.S. and Bancoumana, Mali (NIAID Protocol #15-I-0044; clinicaltrials.gov: NCT02334462). Another TBV candidate, Pfs25M-EPA/Alhydrogel, was also assessed as a standalone vaccine and also co-administered with Pfs230D1M-EPA/Alhydrogel (**Table 2**).

Table 2. NIAID Protocol #15-I-0044 Enrollment and Vaccinations.

	US	Mali		
	n	n	Vaccine	Schedule (month)
Pfs25	5	5	16 µg Pfs25M-EPA/Alhydrogel	0, 1, 6[A], 18[A] months
	5	50	47 µg Pfs25M-EPA/Alhydrogel [A]	
Pfs230	5	0	5 µg Pfs230D1M-EPA/Alhydrogel	
	5	5	15 µg Pfs230D1M-EPA/Alhydrogel	
	5	50	40 µg Pfs230D1M-EPA/Alhydrogel [A]	
Pfs25 + Pfs230	5	5	16 µg Pfs25M-EPA/Alhydrogel + 15 µg Pfs230D1M-EPA/Alhydrogel	
	5	50	47 µg Pfs25M-EPA/Alhydrogel + 40 µg Pfs230D1M-EPA/Alhydrogel [A]	
Comparator	0	60	TWINRIX, Menactra [A]	

[A] Arms that received the full 4 dose regimen (initial series + booster) during the vaccine activity phase (main) of the Mali study.

3.1.1 Safety of Pfs230D1M-EPA/Alhydrogel in Healthy Adults

In both the U.S. and Mali safety dose-escalation of Pfs230D1M-EPA/Alhydrogel, vaccinations at increasing doses were well-tolerated, with minimal local and systemic reactogenicity. The majority of the reported AEs were mild (Grade 1) or moderate (Grade 2). 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, the majority being Grade 1, were noted across the dosing arms, and when compared to a control vaccine in Mali, Pfs230 appeared to have an increase in abnormal laboratory values, though only one was deemed related to Pfs230 vaccination. In a single Pfs230 vaccinated subject in Mali, a Grade 3 gastroenteritis was reported with associated Grade 4 laboratory abnormalities (leukocytosis, blood creatinine increased), all deemed unlikely related to vaccination and all of which resolved shortly after resolution of the gastroenteritis symptoms. Other than the one noted above, there were no Grade 3 or 4 AEs. No SAEs were reported in the Pfs230 vaccinated arms.

Safety analysis of the high dose (40 μg) of Pfs230D1M-EPA/Alhydrogel showed that many reported AEs have been mild (Grade 1; 708/1431; 49%), with the most commonly reported AEs being injection site pain, headache, malaria, neutropenia, nasopharyngitis, and rhinitis. The most commonly reported related AEs were injection site reactogenicity (pain, induration, pruritus, and edema), leukopenia, neutropenia, and headache, which have been all Grade 1 or 2. Most reported related AEs were injection site pain, which did not increase in frequency with subsequent vaccination. Overall, in comparison to the comparator arms, Pfs230D1M vaccinees, alone or in combination, reported more related AEs, the majority of which were Grade 1 or 2 local reactogenicity. During the study period, 3 serious adverse events were reported in the high dose arms; Pfs230D1M: a snake bite; Pfs230D1M: peritonsillar abscess; Pfs25M+Pfs230D1M: cerebrovascular accident which resulted in death - all were determined to be unrelated to vaccination. All of these subjects completed the 4 vaccinations. No participants were removed from study participation due to a related AE of any severity. The cerebrovascular accident which resulted in death case was a healthy 51-year-old woman enrolled in a trial conducted from 2014 to 2016 who developed symptoms of a stroke approximately a week after receiving her fourth and last vaccination on study. She died the following day as a result of the stroke. This serious event was reviewed by Sponsor, Institutional Review Board (IRB), Faculté de Médecine Pharmacie d'OdontoStomatologie (FMPOS) Ethics Committee (EC), DSMB, and U.S. Food and Drug Administration (FDA) and was determined unrelated to the vaccine she received.

As mentioned above, Protocol #15-I-0044 also evaluated the use of Pfs230D1M-EPA in combination (co-administration at separate sites) with Pfs25M-EPA at 2 different dose regimens: Pfs25M 16 μg + Pfs230D1M 15 μg and Pfs25M 47 μg + Pfs230D1M 40 μg. In general, there

were more AEs reported overall given the increased reporting of local reactogenicity per each vaccination received (2 versus 1). 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.

Overall, there has been no significant difference in reported local or systemic solicited reactogenicity or laboratory abnormalities between Pfs230D1M-EPA/Alhydrogel alone, Pfs25M-EPA/Alhydrogel alone, or co-administration of Pfs25M-EPA/Alhydrogel and Pfs230D1M-EPA/Alhydrogel. In conclusion, in malaria-naïve and malaria-exposed adults, Pfs25M-EPA/Alhydrogel administered alone, Pfs230D1M-EPA/Alhydrogel administered alone, or co-administration of Pfs25M-EPA/Alhydrogel and Pfs230D1M-EPA/Alhydrogel is safe and tolerable.

3.1.2 Immunogenicity and Functional Activity of Pfs230D1M-EPA/Alhydrogel in Healthy Adults

Pfs230D1M induced antibody responses in most US vaccinees (**Figure 9C-E**) and resulted in high transmission blocking activity in 2/5 individuals (100%, 98% transmission-reducing activity [TRA]) and significant transmission reducing activity (73%, 62% TRA) in 2 others (**Figure 10A**) after just 2 vaccine doses. In the Pfs25M + Pfs230D1M combination group, antibody responses were similar to the individual antigen arms (**Figure 9F-I**) and 2 individuals had appreciable functional activity after 2 vaccinations (one individual had 90% and one had 68% (**Figure 10A**). The activity correlated well with anti-Pfs230D1M titers, demonstrating that the functional activity is due to the vaccine (**Figure 10C**). Pfs230D1M functional activity dependency on complement was confirmed with the Pf230D1M immune sera samples, as heat-inactivated sera markedly reduced inhibitory activity from these individuals (**Figure 11**).

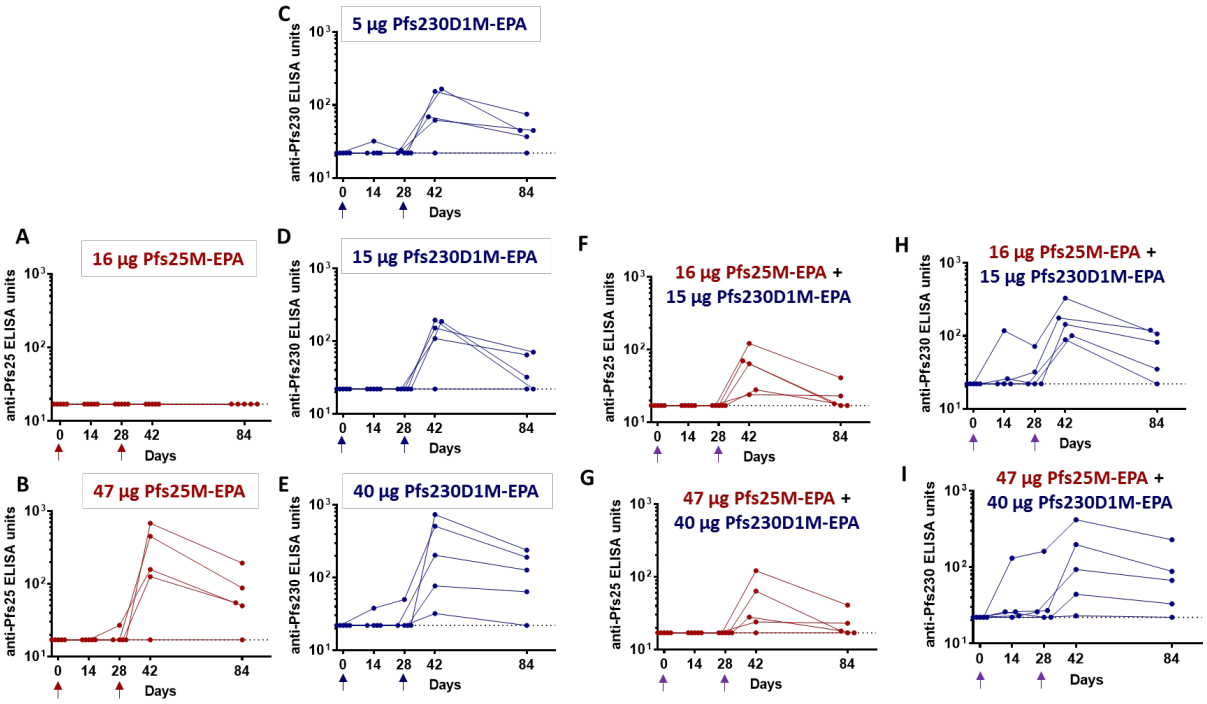


Figure 9: Pfs230-specific Antibody Responses in Subjects Receiving Pfs230D1M, U.S. Cohort (#15-I-0044).

Results presented in enzyme-linked immunosorbent assay (ELISA) units for each arm. Vaccinations occurred on Days 0, 28. Day 0 was drawn pre-vaccination; Day 42 is 14 days post Vaccination #2. Each individual datapoint represents an individual subject anti-Pfs230 ELISA response.

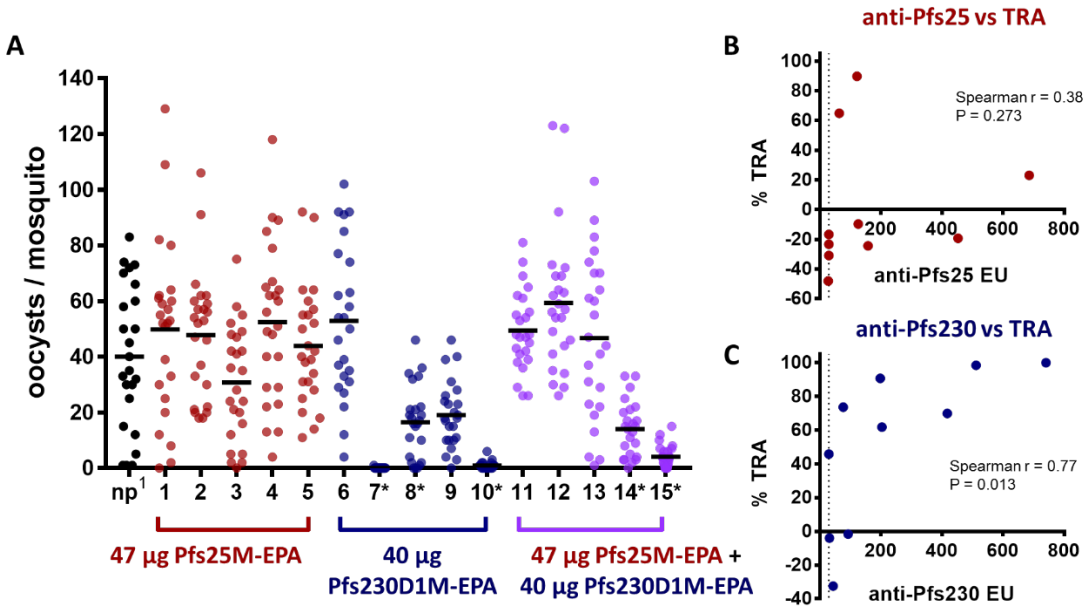


Figure 10: Pfs25 and Pfs230 Functional Activity by Standard Membrane Feeding Assay (SMFA).

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

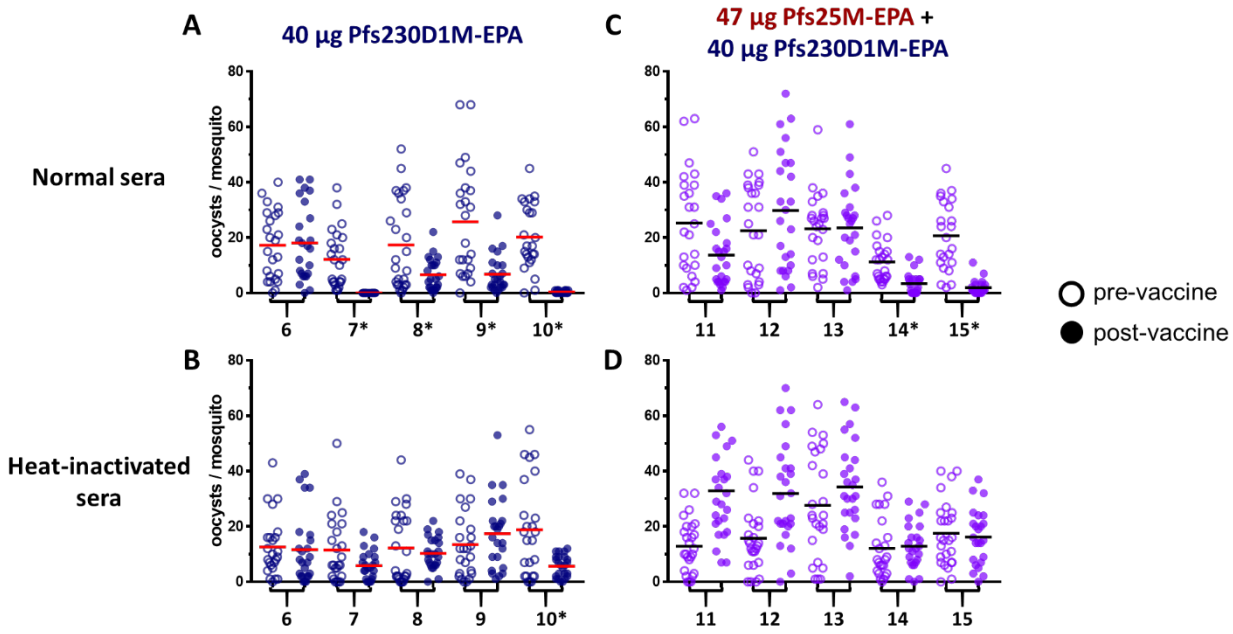


Figure 11: Pfs230 Functional Activity by Standard Membrane Feeding Assay in the Presence or Absence of Complement.

Each individual column represents assay results for an individual subject using Day 0 (before Vaccination #1) and Day 42 (14 days post Vaccination #2) serum samples. Each individual datapoint 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 40 µg alone, n=5, and Pfs25M 47 µg + Pfs230D1M 40 µg co-administered, n=5) are presented.

Evaluation of immunogenicity by ELISA in healthy Mali adults showed a few individuals did have pre-existing baseline responses to Pfs230. The majority of vaccinated subjects developed responses to Pfs25 or Pfs230 following 2 doses of vaccine (**Figure 12**). When comparing Pfs25M-EPA to Pfs230D1M-EPA in Alhydrogel during the main phase of the Mali study, we found that Pfs230 alone compared to Pfs230 and Pfs25 in combination produced similar results in regard to immunogenicity (peak ELISA responses; **Figure 12**), functional activity (SMFA**;** **Figure 13**), and percentage of responders (detectable antibody responses).

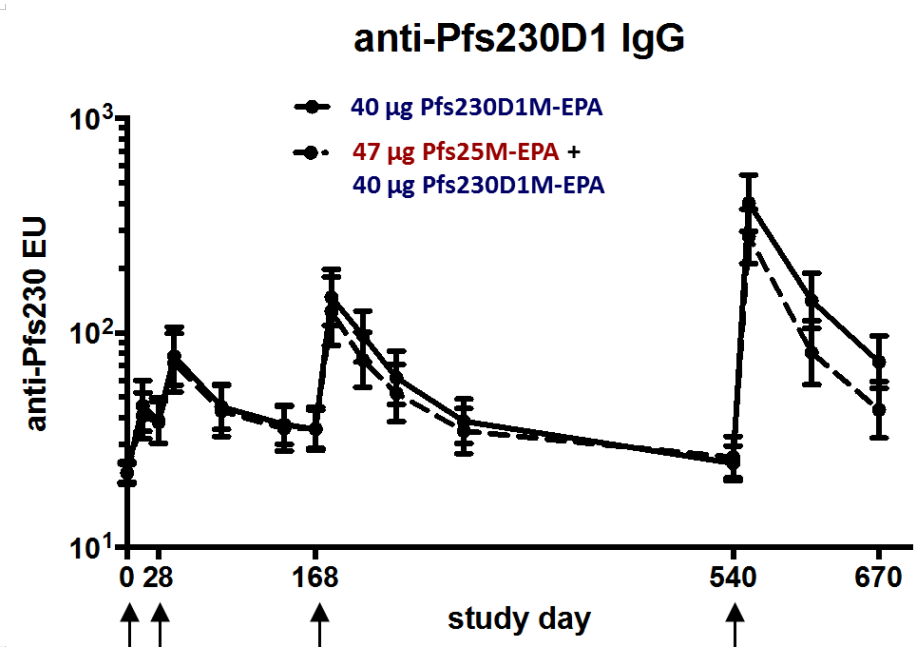
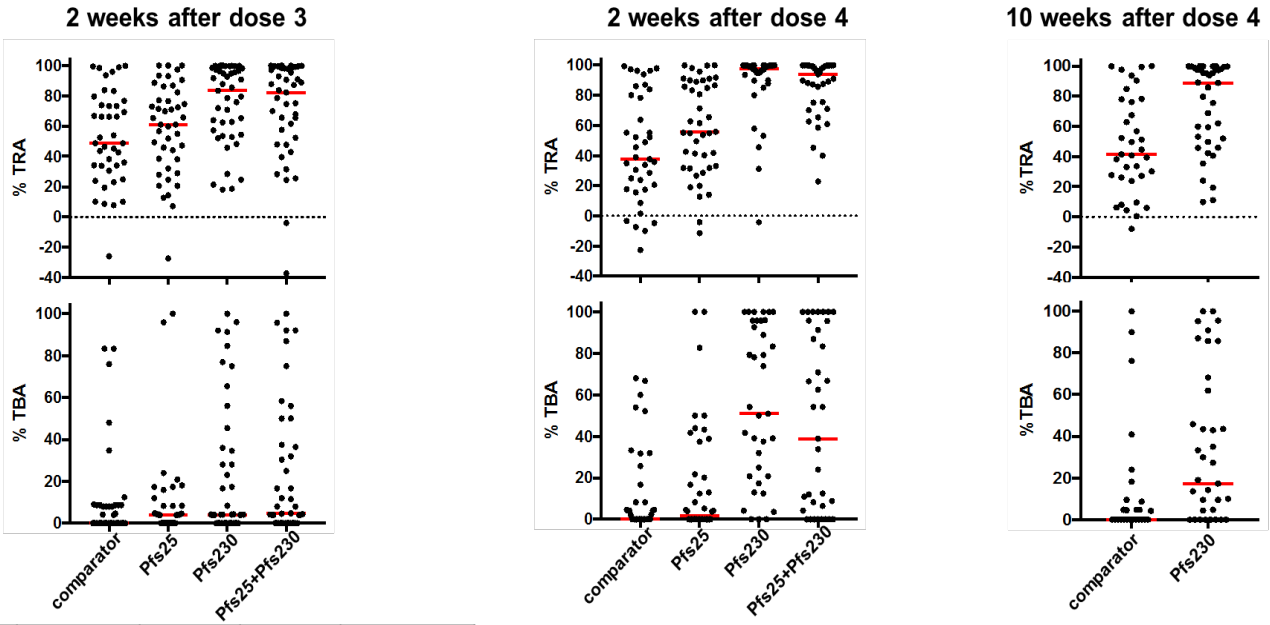


Figure 12: Pfs230-specific Antibody Responses in Subjects Receiving Pfs230D1M, 40 µg in Mali (#15-I-0044).

Results presented in enzyme-linked immunosorbent assay (ELISA) units for each arm. Vaccinations occurred on Days 0, 28, 168, 540. Day 0 was drawn pre-vaccination; Each individual datapoint represents an individual subject anti-Pfs230 ELISA response.



	comparator	Pfs25	Pfs230	Pfs25+Pfs230
TRA mean	52.5	58.2	74.5	68.6
TRA median	48.8	61.1	83.3	82.2
TBA mean	10.5	9.2	23.5	23.8
TBA median	0	4.1	4.2	4.8

comparator	Pfs25	Pfs230	Pfs25+Pfs230
41.4	58.2	88.9	85.0
37.8	55.5	97.6	93.9
11.6	16.1	53.7	45.4
0	1.9	51	38.9

comparator	Pfs230
47.8	73.4
41.5	88.9
10.1	32.3
0	17.4

Figure 13: Pfs230 Functional Activity by Standard Membrane Feeding Assay in Mali, #15-I-0044.

Each individual column represents assay results (calculated TRA, TBA) for an Arm of the Main Phase in Mali. Each individual datapoint represents a single subject's TRA or TBA for that time point. Vaccinations were received on 0, 28, 168, 540 and samples for SMFA taken 2 weeks post dose 3 and 4 as well as 10 weeks post dose 4.

Overall, there was no statistically significant difference between Pfs230 alone versus Pfs230 and Pfs25 given in combination, though Pfs230 alone did have a trend to higher overall peak ELISA responses and consistently higher SMFA responses.

3.2 Pfs230D1M-EPA/AS01 in Healthy Malian Adults (#17-I-N006)

The double-blind comparator-controlled Phase 1 trial of Pfs230D1M-EPA/AS01 in Malian adults (NIAID Protocol #17-I-N006) evaluated Pfs230D1M-EPA/AS01 at escalating doses of 13 µg and 40 µg administered on a schedule of 0, 1, and 6 months in a pilot study. Once safety was reviewed, the main phase started and looked at Pfs230D1M 40 µg at full (3-doses of 40 µg at 0, 1, and 6 months) and fractional dosing (2 vaccinations at 0 and 1 month of 40 µg Pfs230D1M/AS01 and third vaccination at 6 months of 1/5 of the full dose) and then ~12 months post last vaccination all received Pfs230D1M 40 µg at full or 4 vaccinations of Engerix-B + Menactra® (controls).

3.2.1 Safety of Pfs230D1M-EPA/AS01 in Healthy Adults (Primary Series)

In the pilot phase of the study, there was a staggered dose escalation of Pfs230D1M and Pfs25M, given individually and in combination. In the Pfs230D1M arms alone, vaccinations with the low dose of Pfs230D1M (13 µg) and high dose of Pfs230D1M (40 µg) were overall well-tolerated. Most of the related AEs reported have been mild (Grade 1) (13 µg: 23/29, 79%) and (40 µg: 42/51, 82%), with the majority being injection site pain. One subject in the low dose arm and 3 subjects in the high dose arm experienced a total of 4 Grade 3 events (neutropenia, gastritis, respiratory tract infection, and malaria), all of which were determined not related to vaccination.

When Pfs25M and Pfs230D1M were combined, the vaccinations were also safe and well tolerated with the most commonly reported AEs being local injection site abnormalities. The frequency of local reactogenicity reported is increased compared to other Arms, given that in these Arms, 2 vaccinations are given at each vaccination time point, 1 in each arm. No Grade 3 AEs have been reported.

The rate of AE reporting in subjects receiving Pfs230 were similar to what was seen in the comparator Arm that received Engerix-B. Overall, the most common vaccine-related AE seen in the TBV arms have been expected mild local reactogenicity post vaccination that appeared on the day of vaccination or one day post vaccination and resolved within 2-3 days. As expected, solicited systemic reactogenicity (such as headache, arthralgia, fatigue) were reported and usually were Grade 1 (mild) and transient in presentation.

Laboratory abnormalities were also observed, 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%) were reported overall (versus 3 out of 20 subjects in the comparator arm, 15%), all of which were determined not related to study product/procedures.

As the high dose (40 µg) of the Pfs230D1M/AS01 was determined safe and tolerable in the pilot study, it was selected to be used for the main phase of the study.

In the full dose Arm (n=56), overall vaccinations were well tolerated. There was a total of 383 AEs, 252 (66%) of which were mild (Grade 1) AEs. The most common Grade 1 AE (95/252; 38%) was injection site pain. Other common Grade 1 AEs were headache and malaria. There was a total of 127 (33%) Grade 2 AEs. Of these, only 24/127 (19% of Grade 2 AEs; 6% of total AEs) were designated possibly, probably or definitely related to the study procedures. Of the related Grade 2 AEs, 16/24 (67%) were injection site pain. Other related Grade 2 AEs were 2 episodes each of fever, headache, arthralgias and neutropenia. Of the 103/127 unrelated Grade 2 AEs, the most common were malaria, respiratory infections and headache. There were 4 Grade 3 AEs of fever, hypertension, malaria and bronchitis that were not related to study procedures.

In the fractional dose Arm (n=61) alone, overall vaccinations were well tolerated. There was a total of 342 AEs, 190 (55%) of which were mild (Grade 1) AEs. Similar to the full dose Arm, the most common Grade 1 AE (78/190; 41%) was injection site pain. Notably, fewer episodes (16/78, 21%) of injection site pain were reported after the third vaccination in which the participant received a fractional dose of the vaccine. Other common Grade 1 AEs were headache and malaria. There was a total of 150 (44%) Grade 2 AEs, of these only 20/150 (13% of Grade 2 AEs; 6% of total AEs) were designated possibly, probably or definitely related to the study procedures. Of the related Grade 2 AEs, 12/20 (60%) were injection site pain. Other related Grade 2 AEs were headache and one episode of increased total white blood cell count. Of the 130/150 (87%) unrelated Grade 2 AEs, the most common were malaria, headache, respiratory infections and dental caries. There were 2 Grade 3 AEs of malaria that were not related to study procedures.

In the comparison, the control Arm, which received Engerix-B (n=119), overall vaccinations were well tolerated. There was a total of 524 AEs, 268 (51%) of which were mild (Grade 1) AEs. In comparison to the full and fractional dose Arms, Grade 1 injection site pain occurred at a much lower rate of 39/268; 15%. Other most common Grade 1 AEs in an almost equal distribution were headache, malaria and respiratory infections. There was a total of 247 (47%) Grade 2 AEs, of these only 15/247 (6% of Grade 2 AEs; 3% of total AEs) were designated possibly, probably or definitely related to the study procedures. Of the related Grade 2 AEs, 3/15 (20%) were injection site pain. Other related Grade 2 AEs were headache (6/15; 40%), abdominal pain, fatigue and neutropenia. Of the 232/247 (94%) unrelated Grade 2 AEs, similar to full and fractional arms as expected, the most common were malaria, headache, respiratory infections and dental caries. There were 9 Grade 3 AEs, 7/9 were of malaria, and 1 episode each of systolic hypertension and snake bite that were not related to study procedures. These AEs are summarized in **Table 3** below. A few laboratory abnormalities were also observed during the follow up period, with the majority being transient, asymptomatic Grade 1 (mild) and Grade 2 (moderate) neutropenias and leukopenias. There were no Grade 3 laboratory abnormalities noted (**Table 4**).

Table 3. Summary of Total AEs and Comparing Most Common AEs Among the Study Arms

	Arm 2c Pfs230/AS01 full dose N=56	**Arm 2d** Pfs230/AS01 fractional dose N=60	**Arm 4c** Engerix-B N=120	**Total** N= 236
Total # AEs	383 (55) 98%	342 (59) 98%	524 (113) 94%	1249 (227) 96%
Classification				
Local Reactogenicity	111 (51) 91%*	90 (48) 80%*	42 (31) 26%	243 (130) 55%
Systemic Reactogenicity	10 (9) 16%*	4 (3) 5%	2 (2) 2%	16 (14) 6%
Laboratory Abnormalities	14 (13) 25%*	3 (3) 5%	16 (13) 11%	33 (29) 12%
Unsolicited AEs	188 (51) 91%	200 (54) 90%	408 (110) 92%	796 (215) 91%
Severity and Relationship				
Grade 1				
Injection Site Pain	95 (47) 84%*	78 (43) 72%*	39 (29) 24%	212 (119) 50%
Headache	20 (14) 25%*	13 (13) 22%*	6 (5) 4%	39 (32) 14%
Pyrexia	8 (7) 13%*	4 (3) 5%	2 (2) 2%	14 (12) 5%
Grade 2				
Injection Site Pain	16 (14) 25%*	12 (11) 18%*	3 (2) 2%	31 (27) 11%
Headache	2 (2) 4%	7 (7) 12%	6 (5) 4%	15 (14) 6%
Pyrexia	2 (2) 4%	0	0	2 (2) 1%
Grade 3				
Injection Site Pain	0 (0)	0 (0)	0 (0)	0 (0)
Headache	0 (0)	0 (0)	0 (0)	0 (0)
Pyrexia	0 (0)	0 (0)	0 (0)	0 (0)
Grade 4				
Injection Site Pain	0 (0)	0 (0)	0 (0)	0 (0)
Headache	0 (0)	0 (0)	0 (0)	0 (0)
Pyrexia	0 (0)	0 (0)	0 (0)	0 (0)

1) X(X)X% = absolute number of AE (number of subjects experiencing AEs) Percentage of subjects with AEs

2) * indicates a significant difference between the vaccine arm and the control by Fisher's Exact test, $p<0.05$

In closer analysis of solicited local and systemic reactogenicity (occurring within 7 days of vaccination), there were some significant differences between the vaccine and control arms. Participants in the full dose arm experienced significantly more Grade 1 injection site pain ($p= 0.02$), than the fractional dose arm. Grade 1 injection site pain was reported in the full dose arm in 95/168 (57%) doses, fractional dose arm in 78/180 (43%) doses and control arm in 39/360 (11%) doses. Reports of Grade 1 headaches were significantly greater in the full dose arm than the control ($p< 0.0001$), and similarly were greater in the fractional arm versus the control arm ($p= 0.0019$), there was no significant difference between full and fractional arms ($p= 0.15$). Pyrexia was

reported more frequently in the full dose arm than the control arm (p=0.01), but the fractional dose was not significantly different from the control (p=0.1), and there were no significant differences in pyrexia between the full and fractional dose arms.

The most common Grade 2 AEs were headache and injection site pain. There were no significant differences between arms for Grade 2 reports of headache. Grade 2 injection site pain was significantly greater in the full and fractional dose arms compared to the control, p<0.01 and p= 0.01, respectively.

Although minor differences can be seen between the full and fractional dose arms, the dosing for both arms was similar for the first and second vaccination as previously described. The dosing difference only occurred during the third vaccination. Thus, we can dissect the AEs further by dose if we look exclusively at the post-dose 3 solicited AEs. Grade 1 AEs for headache were reported in 5/56 (9%) full dose participants, 1/60 (2%) fractional dose participants and 2/120 (2%) control participants; these were not significantly different by arm. Grade 1 injection site pain was reported in 27/56 (48%) full dose participants, 16/60 (27%) fractional arm participants and 5/120 (4%) control participants. Grade 1 injection site pain was significantly more frequent in the full dose arm (p<0.01) and fractional dose arm (p<0.01) compared to the control. Grade 2 injection site pain was significantly different between the full and control arms (p=0.01), but was not significantly different between the fractional and control arms (p=0.26). Thus, in performing this further analysis, it is possible to deduce that there is a direct relationship between dosage and injection site pain (**Table 4**).

Table 4. Rates of Solicited Local and Systemic Adverse Reactions of Interest Within Seven Days of Receiving Vaccine

	Pfs230/AS01 full dose (N=56)			Pfs230/AS01 fractional dose (N=61)			Engerix-B (N=119)		
	Dose 1	Dose 2	Dose 3	Dose 1	Dose 2	Dose 3	Dose 1	Dose 2	Dose 3
Grade 1									
ABDOMINAL PAIN	-	-	1(1) 2%	-	-	-	-	-	-
ALANINE AMINOTRANSFERASE INCREASED	-	-	-	-	-	-	1 (1) 1%	-	-
ARTHRALGIA	1 (1) 2%	3 (3) 5%	1(1) 2%	-	2 (2) 3%	-	-	-	-
BLOOD CREATININE INCREASED	-	-	-	-	-	-	-	1 (1) 1%	-
CHILLS	-	-	1(1) 2%	-	-	-	-	-	-
FATIGUE	-	1 (1) 2%	-	-	1 (1) 2%	-	-	-	-
HEADACHE	5 (5) 9%	10 (10) 18%	5 (5) 9%	2 (2) 3%	10 (10) 17%	1 (1) 2%	3 (3) 3%	1 (1) 1%	2 (2) 2%
HEMOGLOBIN DECREASED	-	-	-	-	1 (1) 2%	-	-	-	-
INJECTION SITE MOVEMENT IMPAIRMENT	-	3 (3) 5%	-	-	1 (1) 2%	-	-	-	-
INJECTION SITE OEDEMA	-	2 (2) 4%	-	-	-	-	-	-	-
INJECTION SITE PAIN	37 (37) 66%	31 (31) 55%	27 (27) 48%	39 (39) 65%	23 (23) 38%	16 (16) 27%	21 (21) 18%	13 (13) 11%	5 (5) 4%
INJECTION SITE PRURITUS	-	-	-	-	-	-	-	1 (1) 1%	-
LEUKOPENIA	2 (2) 4%	-	3 (3) 5%	-	-	-	1 (1) 1%	3 (3) 3%	2 (2) 2%
MYALGIA	-	4 (4) 7%	-	-	-	-	-	1 (1) 1%	-
NEUTROPENIA	1 (1) 2%	3 (3) 5%	2 (2) 4%	-	1 (1) 2%	-	-	3 (3) 3%	2 (2) 2%
PAIN	-	-	1 (1) 2%	-	-	-	-	-	-
PYREXIA	1 (1) 2%	6 (6) 11%	1 (1) 2%	1 (1) 2%	3 (3) 5%	-	1 (1) 1%	1 (1) 1%	-
THROMBOCYTOPENIA	-	-	1 (1) 2%	-	-	-	-	1 (1) 1%	-
URTICARIA	-	-	-	-	1 (1) 2%	-	-	-	-
Grade 2									
ABDOMINAL PAIN	-	-	-	-	-	-	1 (1) 1%	-	-
ARTHRALGIA	2 (2) 4%	-	-	-	-	-	-	-	-
FATIGUE	-	-	-	-	-	-	-	1(1) 1%	-
HEADACHE	-	2 (2) 4%	-	1 (1) 2%	3 (3) 5%	2 (2) 3%	4 (4) 3%	2 (2) 2%	-
HYPERLEUKOCYTOSIS	-	-	-	1 (1) 2%	-		-	-	-
INJECTION SITE PAIN	2 (2) 4%	9 (9) 16%	5 (5) 9%	3 (3) 5%	7 (7) 12%	2 (2) 3%	1 (1) 1%	1 (1) 1%	1 (1) 1%
NEUTROPENIA	-	1 (1) 2%	-	-	-	-	-	-	1 (1) 1%
PYREXIA	-	2 (2) 4%	-	-	-	-	-	-	-
Grade 1 Total	47	63	43	42	43	17	27	25	11
Grade 2 Total	4	14	5	5	10	4	6	4	2
Grade 3 and 4 Total	0	0	0	0	0	0	0	0	0
TOTAL	**51**	**77**	**48**	**47**	**53**	**21**	**33**	**29**	**13**

1) X(X)X% = absolute number of AE (number of subjects experiencing AEs) % of subjects with AEs; 2) " – " is not observed

3.2.2 Safety of Pfs230D1M-EPA/AS01 in Healthy Adults (Year 2, 4th Dose)

The following year (2018) a fourth dose was provided to all eligible participants with those randomized to Pfs230D1M arms (full and fractional dosing) receiving a single Pfs230D1M 40 μg full dose (n=82) and those in the control arm receiving a single vaccination with Menactra® (controls; n=80) ~12 months post dose 3.

The fourth dose of Pfs230D1M-EPA/AS01 was well tolerated; similar to the prior Pfs230D1M doses with a total of 363 AEs; most of which were mild (Grade 1 AEs; 160/363, 44%). The most commonly reported Grade 1 AE was injection site pain, which was observed in about half of Pfs230D1M vaccinees (full dosing: 22/39, 56%; fractional dosing: 20/42, 48%).

A total of 197 Grade 2 AEs (54%) were reported in Pfs230D1M vaccinees. Of these only 11/197 (6%) were designated possibly, probably or definitely related to vaccination. Of the related Grade 2 AEs, as expected injection site pain (7/11, 63%) was most commonly reported, followed by headache (2/11, 18%), fatigue (1/11, 9%) and injection site movement impairment (1/11, 9%). There were six Grade 3 AEs reported (fever, malaria, sinobronchitis and systolic hypertension) that were not related to vaccination.

In the comparator arm (Menactra®), a total of 305 AEs were reported, of which 117 (38%) were Grade 1. The most commonly reported Grade 1 AEs were headache and malaria both with (20/117, 17%), followed by injection site pain and rhinitis with (10/117, 8%). A total of 185 (60%) Grade 2 AEs were reported. Of the related Grade 2 AEs (n=6) injection site pain (3/6, 50%) was most commonly reported, followed by injection site movement impairment (2/6, 33%), and headache (1/6, 17%). There were 3 grade 3 AEs (dysentery and malaria) that were not related to vaccination.

Table 5. Rates of Solicited Local and Systemic Adverse Reactions of Interest Within Seven Days of Receiving Vaccine.

	BOOSTER PHASE	
	Booster Pfs230/AS01 full (Arm 2c + 2d)	Menactra® (Arm 4c)
	N= 82‡	N= 80
Total # AEs	363 (78) 95%	305 (80) 100%
Classification		
Local Reactogenicity	53 (49) 60%*	17 (14) 18%
Systemic Reactogenicity	29 (22) 27%*	4 (4) 5%
Laboratory Abnormalities	5 (4) 5%	6 (5) 6%
Unsolicited AEs	276 (74) 90%	278 (76) 95%
Severity and Relationship		
Grade 1		
Injection Site Pain	42 (42) 51%*	10 (10) 13%
Headache	17 (16) 20%*	0 (0) 0%
Pyrexia (38-38.4°C)	0 (0) 0%	0 (0) 0%
Grade 2		
Injection Site Pain	7 (7) 9%	3 (3) 4%
Headache	3 (3) 4%	2 (2) 3%
Pyrexia (38.5-38.9°C)	0 (0) 0%	0 (0) 0%

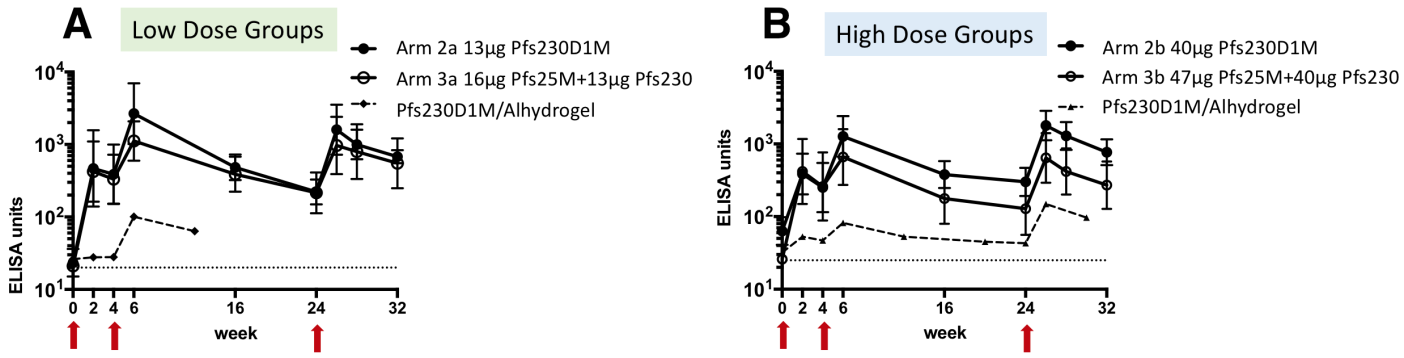
X(X)X% = absolute number of AE (number of subjects experiencing AEs) Percentage of subjects with AEs; 2) * indicates a significant difference between the vaccine arm and the control by Fisher's Exact test, $p<0.05$

Analysis of the solicited local and systemic reactogenicity (occurring within 7 days of 4th vaccination) showed there were some significant differences between the Pfs230D1M vaccine and control arms (**Table 5**). Participants in the Pfs230D1M booster arm experienced significantly more Grade 1 injection site pain ($p <0.0001$) and headache ($p <0.0001$) than the comparator arm. Reports of Grade 2 injection site pain was also reported more frequently in the Pfs230D1M arm but was not significantly different from the comparator ($p=0.3285$). Similarly Grade 2 headaches were not reported significantly differently from the control arm ($p=1$). There was no statistical difference in occurrence of fevers seen between the two arms.

3.2.3 Immunogenicity and Functional Activity (SMFA) of Pfs230D1M-EPA/AS01 in Healthy Adults (Primary Series + 4th Dose; Year 1 + 2)

Vaccination induced detectable antibody titers 2 weeks after the first dose, which increased further after dose 2, but the peak after a third dose was not significantly higher (**Figure 14**). There were no differences in titers between the low and high vaccine doses. Antibody function was assessed 2 weeks and 12 weeks after dose 2 (**Figure 15**). Anti-Pfs230 was sufficient to induce 100% TRA 2 weeks post-dose 2, which was still >90% 12 weeks post-dose 2. In contrast,

anti-Pfs25 did not exceed 80% TRA weeks post-dose 2. The combination of Pfs25 and Pfs230 was not superior to Pfs230 alone for inducing functional serum activity.



geomean anti-Pfs230D1M titers				
	wk 2	wk 6	wk 24	wk 26
arm 2a	467	2,661	221	1,603
arm 3a	424	1,117	215	968
Alhydrogel	30	100	--	--

geomean anti-Pfs230D1M titers				
	wk 2	wk 6	wk 24	wk 26
arm 2b	385	1,271	300	1,975
arm 3b	418	661	129	643
Alhydrogel	*53*	*82*	*43*	*149*

Figure 14. Antibody Responses to Pfs230D1M-EPA/AS01 after Low Dose and High Dose Vaccinations of Adults in Sotuba, Mali during Pilot Phase trial.

Response assessed by enzyme-linked immunosorbent assay (ELISA). (A) shows antibody responses of Malian adults after administration of low-dose vaccinations of Pfs230D1M-EPA/AS01 (either 13 µg Pfs230D1M alone or 16 µg Pfs230D1M-EPA/AS01 plus Pfs25M) at various time points out to 6 months. (B) shows antibody responses after administration of high-dose vaccinations of Pfs230D1M-EPA/AS01 (either 40 µg Pfs230D1M alone or 47 µg Pfs230D1M-EPA/AS01 plus Pfs25M) at various timepoints out to 6 months. Red arrows indicate immunization administrations at 0, 1, and 6 months. Dotted lines represent antibody titers to Alhydrogel-adjuvanted Pfs230D1M-EPA obtained in previous studies.

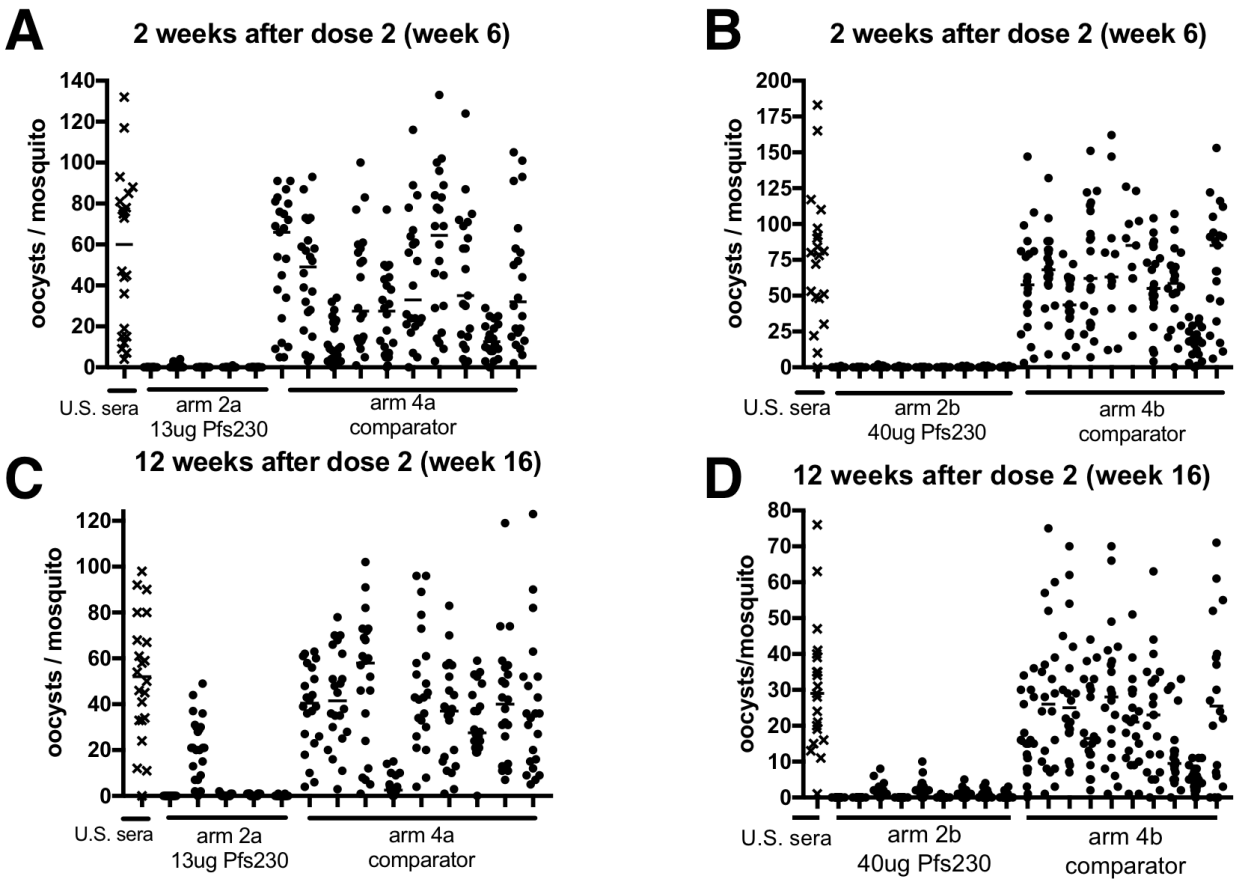


Figure 15. Antibody Function by Standard Membrane Feeding Assay to Pfs230D1M-EPA/AS01 after Low Dose and High Dose Vaccinations of Adults in Sotuba, Mali during Pilot Phase trial.

(A) shows numbers of malaria oocysts recovered per mosquito after being fed on blood collected from Malian adults 2 weeks after the second administration of low-dose (13 µg) Pfs230D1M-EPA/AS01 vaccine with a control of malaria-naïve sera collected from volunteers in the U.S. and a comparison arm after immunization with ENERGIX-B, a hepatitis B vaccine. (B) similarly shows results after high-dose (40 µg) vaccination with Pfs230D1M-EPA/AS01. (C) and (D) show results at 12 weeks after the second administration of low-dose and high-dose vaccinations, respectively.

As was seen with the pilot phase, higher antibody titers were seen with AS01 adjuvant compared with what has been observed previously with other adjuvants. A significantly higher antibody titer measured by ELISA against Pfs230 was observed in both the full and fractional dose arms compared to the control arm after only 2 doses given at 0 and 1 months ($p<0.01$ in both; **Figure 16**) Antibody titers were also assessed after the 3rd vaccination, and as expected, the full and fractional dose arms had significantly higher titers compared to control arms ($p<0.01$ in both). Interestingly, although there was a trend in the full dose arm, the antibody titer in both full and fractional arms were not significantly higher after the 3rd vaccination compared to 2nd vaccination ($p=0.20$ for full dose group; $p=>0.99$ for fractional dose group; **Figure 16**). Notably,

Pfs230D1 titers were significantly higher in the full versus fractional dosing regimen at 12 weeks post-dose 3.

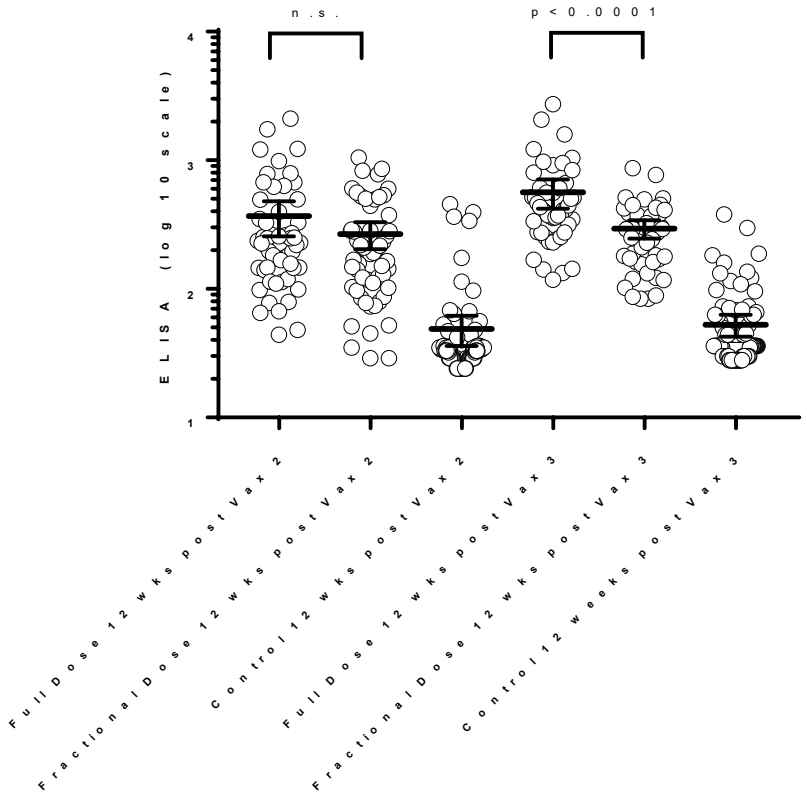


Figure 16. Antibodies against Pfs230D1M measured by ELISA 12 weeks post 2nd and 3rd vaccination in Bancoumana/Doneguebougou, Mali during Main Phase trial.

Note*:* Error bars are median and 95% CI.

Immune responses, as defined by antibody titers and functional activity in SMFA (standard membrane feeding assay), have been also assessed post vaccination #4. Higher Pfs230 antibody responses were seen post Vaccination #4 than post Vaccination #3 in both the original full Pfs230 dose and fractional Pfs230 dose arms (**Figure 17**)**.**

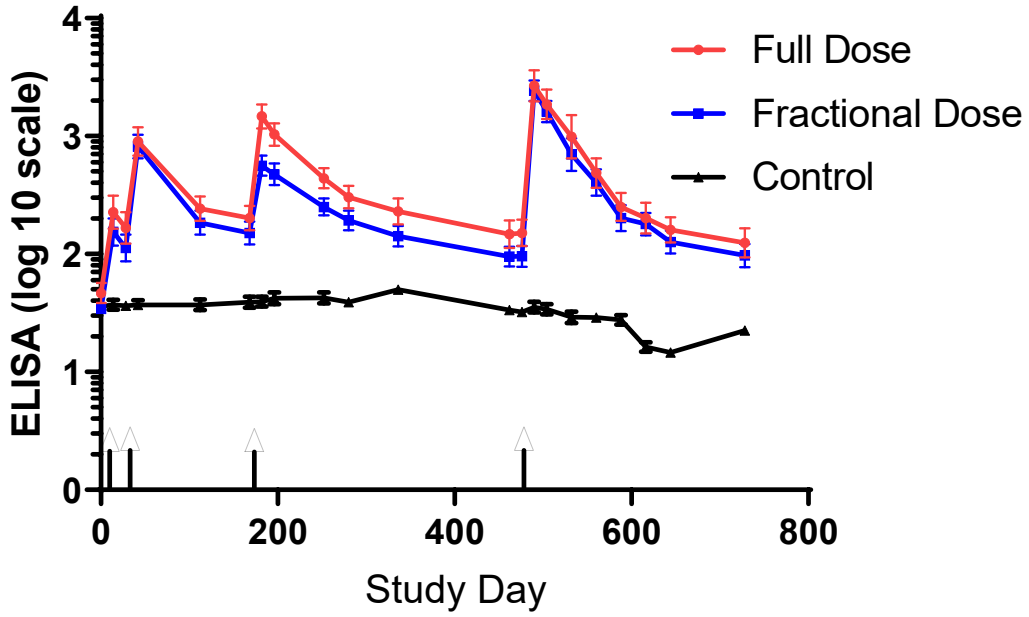


Figure 17. ELISA against Pfs230D1 antigen.
Titer increase was greater with full versus fractional regimen after the 3rd vaccine dose. The booster was full dose for both vaccination arms and antibody titers increased equally for full and fractional arms. Full dose = Pfs230D1M-EPA/AS01 at 40 µg at 0, 1, 6, 18 months. Fractional dose = Pfs230D1M-EPA/AS01 at 40 µg at 0, 1,18 months, receipt of fractional dose of 8 µg Pfs230D1-EPA/AS01 at dose #3 at 6 months.

The functional activity was also measured by SMFA at 12 weeks post both 2nd and 3rd vaccination. Both fractional and full dose arms were found to have significantly higher TRA (**Figure 18**), the decrease in the number of oocyst per infected mosquito, after both 2nd and 3rd vaccination, compared to control arms ($p<0.01$). As has been seen in the antibody titers, there was significantly higher TRA in the full dose arm ($p=0.04$) after 3rd vaccination compared to 2nd vaccination; this difference was not observed in the fractional dose arms (**Figure 18**). Together with the ELISA data (**Figure 16**), these results indicate that the full dose arm regimen may be inducing better antibody responses than the fractional dose regimen.

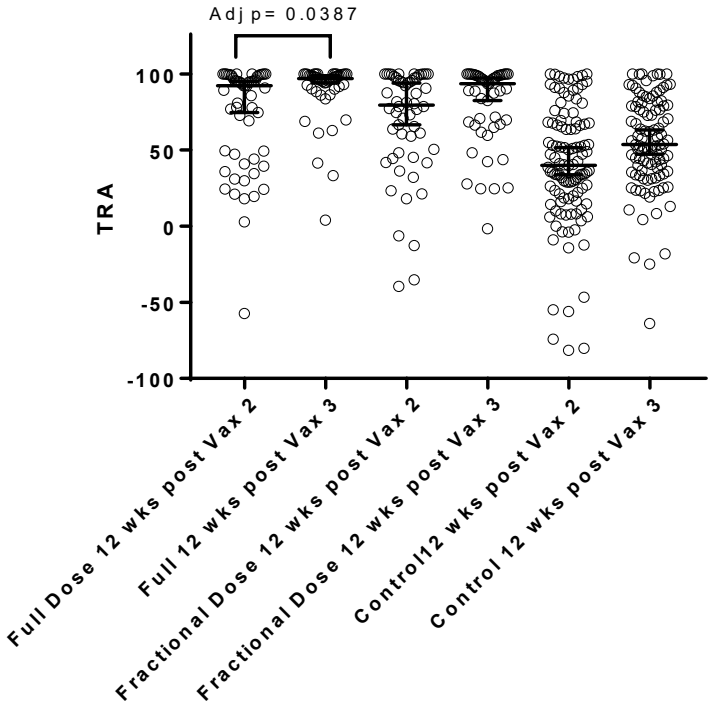


Figure 18. Transmission Reducing Activity Measured by SMFA 12 weeks post 2nd and 3rd Vaccinations in Bancoumana/Doneguebougou, Mali during Main Phase trial.

Note*:* Error bars are median and 95% CI.

Interestingly, when evaluating functional activity as TBA, it is not until after the 3rd vaccination that both arms were significantly different from control arm ($p<0.01$ for both; **Figure 19**). After the 2nd vaccination, both the full and fractional dose arms were not significantly different from controls.

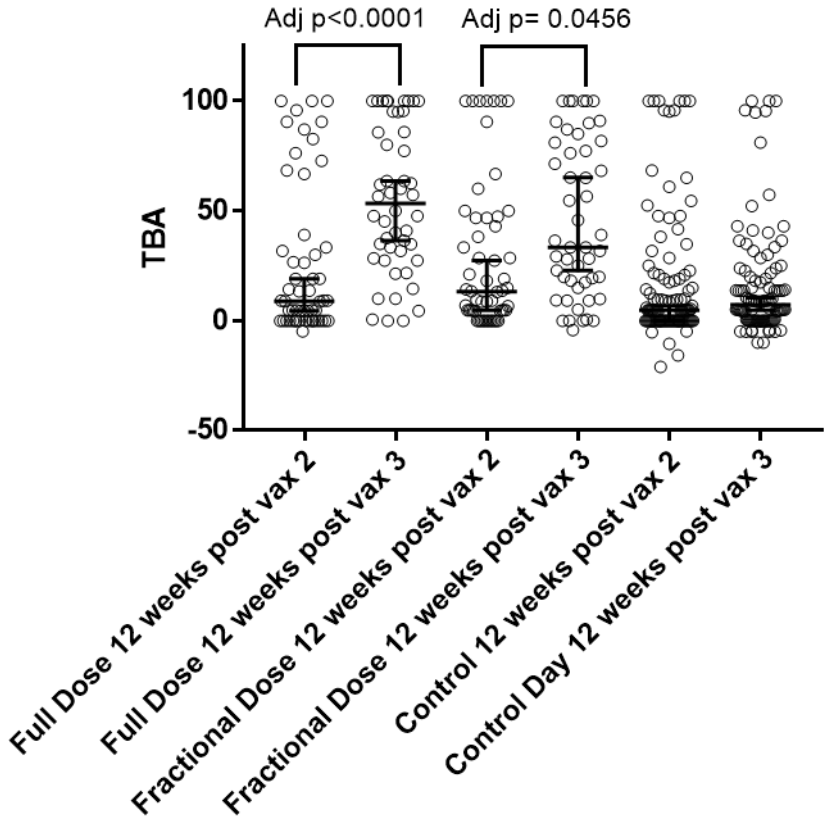


Figure 19. Transmission blocking activity measured by SMFA 12 weeks post 2nd and 3rd vaccinations in Bancoumana/Doneguebougou, Mali during Main Phase trial.

Note*:* Error bars are median and 95% CI

Functional activity, measured by SMFA by transmission reducing activity, was maintained after fourth dose at a high level of activity. Transmission blocking activity (reduction in infected mosquitoes) by SMFA was significantly higher in both Pfs230D1M vaccine arms compared to the comparator at three months post dose 4 but did not significantly differ between full and fractional dosing regimens (**Figure 20**).

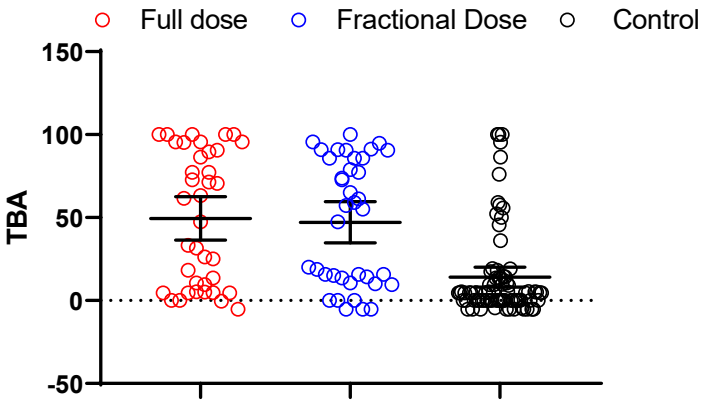


Figure 20. Serum transmission blocking activity (TBA) by SMFA 3 months after Pfs230/AS01 booster dose (Day 560).
TBA (% reduction in infected mosquitoes) was significantly higher in Pfs230D1 vaccine arms versus comparator group. There was no significant difference between full or fractional dosing.

These promising results demonstrate that Pfs230D1M-EPA/AS01 has the potential to provide transmission reducing activity as well as transmission blocking activity across two malaria seasons.

3.2.4 Functional Activity by Direct Skin Feeds

Direct skin feeds (DSF) were also used to assess vaccine activity. During the rainy season in 2017 (Year 1, Main Phase) and 2018 (Year 2, fourth dose), a total of 4861 DSFs were performed in 2017 and another 5056 DSFs were completed in 2018. There was a total of 40 positive DSFs (0.82%) from 19 unique individuals in 2017; and a total of 88 positive DSFs (1.74%) from 25 unique individuals in 2018 (**Table 6**). A trend of lower infections in the full dose regimen was observed in 2017 but not significant. Positive DSFs were significantly less frequent in the full dose regimen in 2018, a first and an extremely important achievement in the field of malaria transmission blocking vaccines – in vivo functional activity.

Table 6. Summary of Direct Skin Feed (DSF) results.
Twice weekly feeds for 12 weeks in 2017 and for 16 weeks in 2018. *, ^ proportion of infection between groups were significantly different by chi-square test.

Group	N. DSF Positive	N. feeds performed	% Positive
Comparator	**76**	**4960**	**1.53%**
2017	21	2462	0.85%
2018^	55	2498	2.20%
Pfs230/AS01 fractional dose	**42**	**2450**	**1.71%**
2017	14	1168	1.20%
2018*	28	1282	2.18%
Pfs230/AS01 full dose	**10**	**2516**	**0.40%**
2017	5	1231	0.41%
2018*^	5	1285	0.39%

3.3 Pfs230D1M-EPA/AS01 in Healthy Malian Children and Adults (19-I-N086)

The Phase 2 study of the safety, immunogenicity, vaccine activity, and vaccine efficacy of Pfs230D1M-EPA/AS01 against *Plasmodium falciparum* malaria in VUs in Doneguebougou, Mali and surrounding villages started the age de-escalation Pilot Phase in April 2019 (9-18 yo) and May 2019 (5-8 yo) followed by Main Phase enrollment and vaccinations in June 2019.

For the initial Pilot Phase safety arms, 30 subjects in each age group received Vaccinations #1, #2 on a 0, 28 day schedule and then were scheduled to receive Vaccination #3 along with the majority of their vaccine unit (VU) members, resulting in a final Vaccination schedule of 0, 28, 126 days. Main Phase subjects (vaccinees; Arms 3a-3f) started enrollment in June 2019 and completed enrollment in September 2019. Subjects in the Main Phase are scheduled to receive vaccinations on a 0, 28, 56 day schedule.

All study vaccinations for the primary series have been completed as of 30 November 2020. Vaccination #3 was completed in 97.9% of vaccinees (1078/1101) who received Vaccination #1 and 1066/1101 (97%) of participants had all three doses of the vaccine. Children aged 1-4 year old (Arm 4a/4b; n=192) enrolled with their VU prior to dose #3. Direct skin feeds started in 9-18 year old subjects (Arms 1a/1b/3c/3d) in Sep 2019 and ended in March 2020. Summary of study enrollment, vaccination, DSF progress is summarized below in **Table 7**.

Table 7. Summary of Study Progress for 19-I-N086

	Pilot Phase		Main Phase			
	Arm 2a/2b 5-8 yo	**Arm 1a/1b 9-18 yo**	**Arm 3a/3b 5-8 yo**	**Arm 3c/3d 9-18 yo**	**Arm 3e/3f ≥19 yo**	**Arm 4a/4b 1-4 yo**
Number subjects enrolled	30	30	185	369	495	192
Timing of enrollment	May 2019	Apr 2019	Jun to Sep 2019			Aug to Nov 2019
Number subjects received ≥1 vaccine	30	30	183	368	490	--
Timing of vaccination	May to Oct 2019	Apr to Sep 2019	Jun to Nov 2019			--
Number subjects AL dosed prior to Vax #3	29	--	181	--	--	--
Timing of AL dosing prior to Vax #3	Aug to Oct 2019	--	Aug to Nov 2019	--	--	--
Number subjects underwent DSF ≥1	--	26	--	358	--	--
Timing of start -end of DSFs	--	Sep to Dec 2019	--	Sep 2019 to Mar 2020	--	--

3.3.1 Safety in Healthy Malian Children and Adults

At the time of the fourth dose amendment (V5.0), safety data post dose 1, 2, and 3 as well as malaria episodes (patent parasitemia, symptomatic malaria) and direct skin feeding events were reviewed by the study team, Sponsor, NIAID DSMB, an external scientific advisory board, and internal scientific review committee without any reported safety concerns about proceeding with a fourth dose and repeat study procedures for an other year of evaluation.

3.3.1.1 1-4 Years Old (Unvaccinated)

At the time of the fourth dose amendment (V5.0), 192 children 1-4 years of age had enrolled in the study. Children in this age group did not nor will not receive vaccinations but did receive AL treatment at enrollment and underwent regular blood draws during the study. Most commonly reported AE has been malaria and rhinitis. Grade 3 AEs have been reported in 14 kids (14/192, 7.3%) with the most commonly reported Grade 3 AE being malaria, as expected. One SAE (malaria) has been reported with associated anemia; unrelated to study participation.

3.3.1.2 5-8 Years Old (Pilot + Main; Vaccinated)

At the time of the fourth dose amendment (V5.0), 213 subjects aged 5-8 years old have received at least one vaccination, with 210 receiving 2 doses, and 210 receiving 3 doses.

Vaccinations have been well tolerated with majority of the reported AEs being mild (Grade 1) and most being expected local site reactogenicity. For solicited AEs, as seen with similar vaccines with the adjuvant AS01 in young children (**Figure 7**) and our previous experience in adults, the number of solicited AEs within 7 days post vaccination, in particular interest fevers, increased in frequency and severity from Vaccination #1 (5/213 subjects, 2.3%; all Grade 1) to Vaccination #2 (18/210 subjects, 8.6%; Grade 1 to 3) and then decreased post Vaccination #3 (7/210 subjects, 3.3%; Grade 1 to 3). These fevers tend to occur within 24 hours post vaccination and resolved within 1-3 days. Majority of fevers experienced are mild/moderate, but Grade 3 fevers can be seen post vaccination as previously reported, but overall in a small proportion of the population (4/213, 1.9%). Most commonly reported unsolicited AE has been malaria and rhinitis, unrelated to vaccination.

No SAEs nor Grade 4 or higher AEs have been reported in this age group. Grade 3 AEs have been reported in 16 subjects (16/213, 7.5%) during the course of the study with only 4 related Grade 3 AEs, all of which were Grade 3 pyrexia (4/213, 1.9%) as noted above. Most commonly reported not related Grade 3 AE by subject (5/213, 2.3%), as expected, has been malaria.

3.3.1.3 9-18 Years Old (Pilot + Main, Vaccinated)

At the time of the fourth dose amendment (V5.0), 398 subjects aged 9-18 years old have received at least one vaccination, with 391 receiving 2 doses, and 386 receiving 3 doses.

Vaccinations were also well tolerated in this age group with majority of the reported AEs being mild and majority of Grade 1 AEs being expected local site reactogenicity. As noted in the 5-8 year olds, but in a smaller proportion of vaccinees and with decreased severity, pyrexia was more commonly reported post Vaccination #2, with 18 AEs reported (Grade 1: 10/18; Grade 2: 7/18; Grade 3: 1/18) than post dose 1 or subsequently for Vaccination #3. Most commonly reported unsolicited AE has also been malaria and rhinitis.

Grade 3 AEs have been reported in 23 subjects (23/398, 5.8%) during the course of the study with only 3 related Grade 3 AEs, including Grade 3 pyrexia, n=1 and Grade 3 neutropenia, n=2 (3/398, 0.8%). Most commonly reported not related Grade 3 AE by subject (5/213, 2.3%), as expected, has been malaria. One Grade 4 AE has been reported as a SAE (Retained Placenta or Membranes; not related to vaccination).

3.3.1.4 ≥19 Years Old (Main, Vaccinated)

At the time of the fourth dose amendment (V5.0), 490 subjects aged ≥19 years old have received at least one vaccination, with 479 receiving 2 doses, and 482 receiving 3 doses.

Vaccinations were well tolerated with majority of the reported AEs being mild and majority of Grade 1 AEs reported being expected local site reactogenicity. As noted in the pediatric age groups, adults also had an increase in pyrexia reporting post dose #2, though not as frequent or severe. As seen with the younger kids, as expected, most commonly reported unsolicited AEs have been malaria and rhinitis.

Grade 3 AEs have been reported in 11 subjects (11/490, 2.2%) during the course of the study with 1 related Grade 3 AE (neutropenia) reported. One SAE, which was also a Grade 4 AE, (increased creatinine, not related to vaccination) has been reported.

3.3.1.5 Clinical Malaria and Parasitemia

Malaria, measured by scheduled blood smears in 1-18 year olds, as well as unscheduled sick visits in all participants, was prospectively followed in vaccinated and unvaccinated population and summarized below for the first malaria transmission post dose 3 (2019-2020) (**Table 8**).

Table 8. Malaria AE and Parasitemia Summary

	Arms 4a/4b 1-4 yo	**Arm 2a/2b/13a/3b 5-8 yo**	**Arm 1a/1b/3c/3d 9-18 yo**	**Arm 3e/3f ≥19 yo**
	AL day -14	**AL day -7 + AL day 42 or 112**	**AL day -7**	**AL day -7**
Post Vaccination #1	N/A	n=213	n=398	n=490
Malaria AE	N/A	6 (2.8%)	13 (3.3%)	3 (0.6%)
Positive *P. falciparum*	N/A	17 (8.0%)	28 (7.0%)	19 (3.9%)
Post Vaccination #2	N/A	n=210	n=391	n=479
Malaria AE	N/A	25 (11.9%)	45 (11.5%)	21(4.4%)
Positive *P. falciparum*	N/A	30 (14.3%)	83 (21.2%)	43 (9.0%)
Post Vaccination #3		n=210	n=386	n=482
Malaria AE		135 (64.3%)	282 (73.1%)	129 (26.8%)
Positive *P. falciparum*		109 (51.9%)	275 (71.2%)	163 (33.8%)

[1] Numbers are unique individuals unless otherwise stated.

3.3.2 Immunogenicity in Healthy Malian Children

Anti-Pfs230 antibody testing was implemented to be conducted in Mali in early 2020. At the time of the fourth dose amendment (V5.0) ELISA assays from the Pilot Phase (Pediatric Arms, n=30 9-18 years old, n=30 5-8 years old; 1:1 randomization to Pfs230D1M-EPA/AS0, n=15/age arm and comparator, n=15/age arm) have been completed prior to premature cessation of laboratory activities secondary to COVID-19 pandemic. **Figure 21** presents the antibody responses seen in the evaluated population (both 9-18 yo Pilot Phase subjects and 5-8 yo Pilot Phase subjects). Children, on initial analysis, appear to have similar antibody responses to Pfs230D1M-EPA/AS01 vaccine as healthy adults.

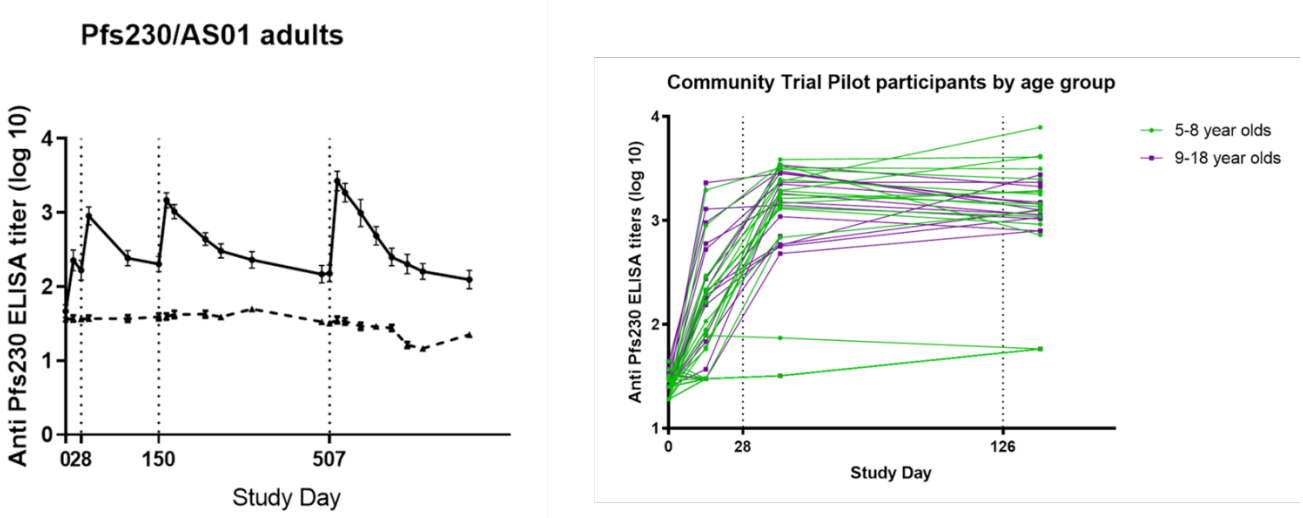


Figure 21. Comparison of Pfs230D1M-EPA/AS01, 40 ug, in Healthy Malian Adults (17-I-N006) to Healthy Malian Children [5-8 year olds (green), 9-18 year olds (purple)] (19-I-N086).

For 19-I-N086, only blinded data are presented given study status; figure represents only those values above the level of ELISA detection.

3.3.3 Functional Activity by DSF in Healthy Malian Children

Direct Skin Feeds (DSF) were completed in March 2020. The study remains blinded, but during the DSF follow-up there have been 70 positive DSFs (2.4%) in the 2875 DSFs completed. These positive DSFs occurred in 45 unique individuals and resulted in 550 infected mosquitoes. A plan for additional analysis of these positive DSFs, including oocyst speciation, sieving analysis of Pfs230, and parasite genotyping is currently pending.

4 Malaria Transmission Dynamics in Mali

As stated previously, in order for a TBV to have a significant impact on malaria transmission, it would have to be administered at a community level and include a high proportion of community participants that are significantly contributing to transmission events. Thus, it is important to understand malaria transmission dynamics in order to maximize the vaccine efficacy.

Transmission of malaria is influenced by environmental, social and behavioral factors of the host, vector, and parasite.[30-36] Mosquito factors that influence transmission include innate susceptibility to *Plasmodium*, host choice, mosquito longevity, and mosquito feeding and resting behavior. Once a mosquito has an established malaria infection in its salivary glands, the probability of transmitting malaria to a new human host is thought to be regulated by mosquito parasite density factors.[37] Human genetic factors also play a role. For example, different hemoglobinopathies, such as sickle cell trait, hemoglobin C and E, and α-thalassemia, are known to confer protection against malaria infection.[38,39]

DSF have been traditionally used to study malaria transmission dynamics across a number of field sites worldwide.[40-43] Several studies have been conducted in Mali enrolling hundreds of participants in or near Bancoumana with no significant adverse effects. Touré et al conducted DSF on 72 children as young as 2 years old in 1994-1995, with no safety risks identified.[44] Diallo et al. conducted DSF from 1996-1998 on 372 children 4-18 years of age, also with no safety issues.[45] DSF experiments had also been conducted in Bancoumana and a neighboring village in 2002-2003 on a total of 44 gametocyte carriers 6-18 years of age without any safety problems.[46]

By the end of 2017, MRTC/LMIV teams had performed nearly 10,000 DSFs on subjects 5 years and older under a number of different protocols completed in the past 6 years (NIAID Protocols 11-I-N143, 13-I-N109, 14-I-N159, and 15-I-0044) as summarized in **Table 9** and **Figure 22** below. All DSF participants were actively followed 24 hours post feed and were passively followed for 2 weeks post feed on the observational studies (11-I-N143, 14-I-N159) and seen frequently (at least 6 times over the course of 2 months) on the vaccine protocols (13-I-N109, 15-I-0044). Except for 1 case of definitely related grade 2 erythema at the DSF site that was resolved within 48 hours, there have been no other adverse events (AEs) recorded as related (definitely, probably, or possibly) to the feeding procedures in either study.

From 2011-2015, LMIV conducted a transmission-blocking assay study in Bancoumana (protocol 11-I-N143; NCT01360112) where 500 adults and children ages 3 months to 50 years were enrolled and underwent monthly visits to assess parasitaemia. When found to be parasite- and/or gametocyte-positive, individuals underwent DSF assays to explore transmission dynamics on an individual level as well as compare mosquito infectivity by DSF to that of mosquitoes fed in membrane feeding assays in Mali and the US. As part of this study, we optimized the parameters that contribute to successful mosquito feeding outcomes in DSF. Such parameters include the following: 1) mosquito age at time of feeding, 2) duration of mosquito starvation prior to feeding, 3) anatomical location of DSF feeding (arm, calf, and ankle), and 4) time of day for DSF (dawn or dusk). We found that younger mosquitoes were significantly associated with higher feeding, survival, and infection rates. Longer starvation times were positively, but not significantly, associated with higher infection rates, but starvation times of 20 hours were negatively associated with feeding and survival. Although dusk was found to be associated with higher infection rates, this may be confounded by the time from positive BS. Based on these findings, we make specific recommendations for optimal feeding parameters to maximize the chance of detecting parasite transmission in a standardized manner.

In 2013, LMIV employed DSFs in a vaccine study whereby gametocyte-positive vaccine participants were invited to undergo DSFs post third vaccination of a Pfs25 vaccine product (13-I-N109). In 2014, DSFs were expanded to cover all vaccine participants enrolled in the Pfs25 trial for a 6 week period post vaccination 4 to explore whether DSF assays could be used to measure functional activity endpoints.[47,48]

Also in 2014, LMIV conducted a PfSPZ vaccine trial in Doneguebougou, Mali, and conducted DSF assays on all vaccine participants for 6 weeks post vaccination 4 to determine if the vaccine had an effect on interrupting malaria transmission (14-I-N159).

In 2015, LMIV initiated a second TBV trial using the combination of Pfs25 and Pfs230 antigens (15-I-0044). Vaccine enrollees were again invited to undergo DSFs twice weekly post vaccinations 3 and 4 for a 6-week period. This represented the largest number of DSFs conducted by the Mali-LMIV team to date with 2,005 conducted in 2015 and 1,844 conducted in 2016.

A Phase 1 trial of Pfs25M-EPA/AS01 combined with Pfs230D1M-EPA/AS01 in adults in Bancoumana and Doneguebougou enrolled in April 2017, and an important secondary objective of the trial will be to compare mosquito infectivity in vaccinated subjects versus the comparator group. To date 4,865 DSFs have been performed in this study.

Research at LMIV examining what factors lead to a positive DSF are ongoing, but the strongest correlate to a positive DSF is host gametocyte density: Individuals with a higher gametocyte prevalence in their peripheral BS are more likely to give a positive DSF. This, however, is not always the case and some individuals with high gametocyte burdens do fail to infect mosquitoes. A possible explanation may be recent ingestion of antimalarial drugs which are known to ablate transmission.[49] Likewise, positive DSFs do occur from individuals who are BS–negative for gametocytes, indicating that sub-patent gametocyte levels are still able to infect mosquitoes in some cases.

Gametocyte carriage varies with age, with younger age groups (<10 years old) having higher gametocyte carriage rates and densities.[50] We have also been able to better understand the epidemiology of malaria transmission in this area. Based on data collected in the first 2 years (2011-2012) of our transmission-blocking assay study (11-I-N143), gametocyte carriage rates are higher in children 5-17 years old and in adults below the age of 35 years, compared to rates in children under 5 years. Mapping data has also identified housing clusters with higher carriage rates.

Table 9: Summary of LMIV Direct Skin Feed (DSF) Studies in Mali

Year	Site	Protocol No.	Total No. DSF	No. Positive	No. Unique Infected Individuals	Participant Age Range	Feeding Criteria	Time of Year
2011	Bancoumana	11-I-N143	73	13	12	5-50 years	Gametocyte-positive only	Jun - Dec
2012	Bancoumana	11-I-N143	41	17	17	5-50 years	Gametocyte-positive only	Jan - Dec
2013	Bancoumana	11-I-N143	16	1	1	5-50 years	Gametocyte-positive only	Jan - Jun
2014	Bancoumana	11-I-N143	17	7	6	5-50 years	Gametocyte-positive only	Jan - Oct
2015	Bancoumana	11-I-N143	2	0	0	5-50 years	Gametocyte-positive only	Jan - Feb
2013	Bancoumana	13-I-N109	109	10	8	18-45 years	Parasite- or gametocyte-positive	Jul - Dec
2014	Bancoumana	13-I-N109	516	16	12	18-45 years	All individuals	Jan - Dec
2015	Bancoumana	13-I-N109	9	0	0	18-45 years	All individuals	Feb - Mar
2014	Doneguebougou	14-I-N159	458	19	16	18-35 years	All individuals	Aug - Dec
2014	Doneguebougou	14-I-N159	42	1	1	18-35 years	All individuals	Jan
2015	Bancoumana	15-I-0044	2005	58	18	18-50 years	All individuals	Sept - Dec
2016	Bancoumana	15-I-0044	1844	30	18	18-50 years	All individuals	Sept – Nov
2017	Bancoumana/Doneguebougou	17-I-N006	4865	40	19	18-50 years	All individuals	Sept - Dec

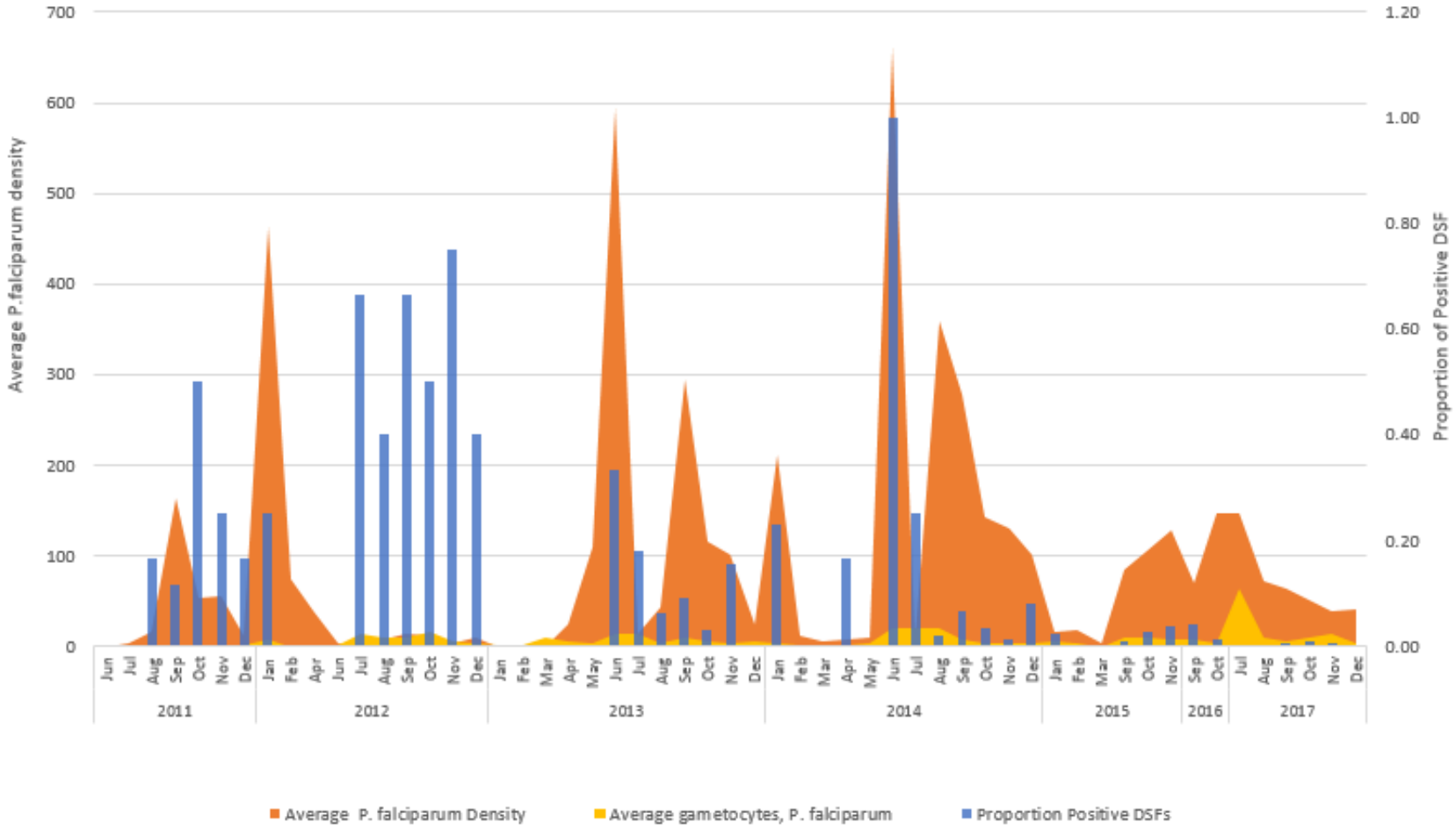


Figure 22: Proportion of positive feeds (blue) with *P. falciparum* parasite (orange) and gametocyte density (yellow) over six years of direct skin feed (DSF) assays in Mali.

Note: Feeding criteria changed over time with initial studies requiring individuals to be gametocyte-positive to participate in feeds; later studies including vaccine trials fed on all individuals irrespective of blood smear status.

We have initiated a study that surveys measures of malaria infection and transmission across the community in order to prepare for community trials of transmission-blocking vaccines. In this survey of unprecedented scope, all residents within selected compounds of Bancoumana village were invited to participate in the study, and a similar survey has been initiated in Doneguebougou village. Both Bancoumana and Doneguebougou villages have been the sites for initial evaluations of our transmission-blocking vaccines in adults. All study participants provide a monthly blood sample for microscopic evaluation of malaria infection gametocytemia by Giemsa-stained BS. All individuals 5 years and older are invited to undergo DSFs with laboratory-raised mosquitoes on a monthly basis, and all huts where study participants reside are visited monthly for capture of blood-fed *Anopheles* mosquitoes.

The study results have emphasized that children between the ages of 5-18 years are the primary target for a TBV, as well as the primary beneficiaries of tools such as TBV that will reduce the incidence of malaria infection across the community.

Studies commenced in Bancoumana with BS collections in February 2018, which is early in the dry season, and direct skin feeding assays were started in March 2018. Across the dry season and into the rainy season, which begins around July, children 5-18 years of age consistently have the highest prevalence of malaria infection (measured as the proportion of children who have detectable *P. falciparum* parasites on a BS at a scheduled visit or an unscheduled visit (due to illness) at least once during the month (**Figure 23**). Thus, these children appear to be the most likely of all the age groups to benefit by a reduction in the force of infection in the community.

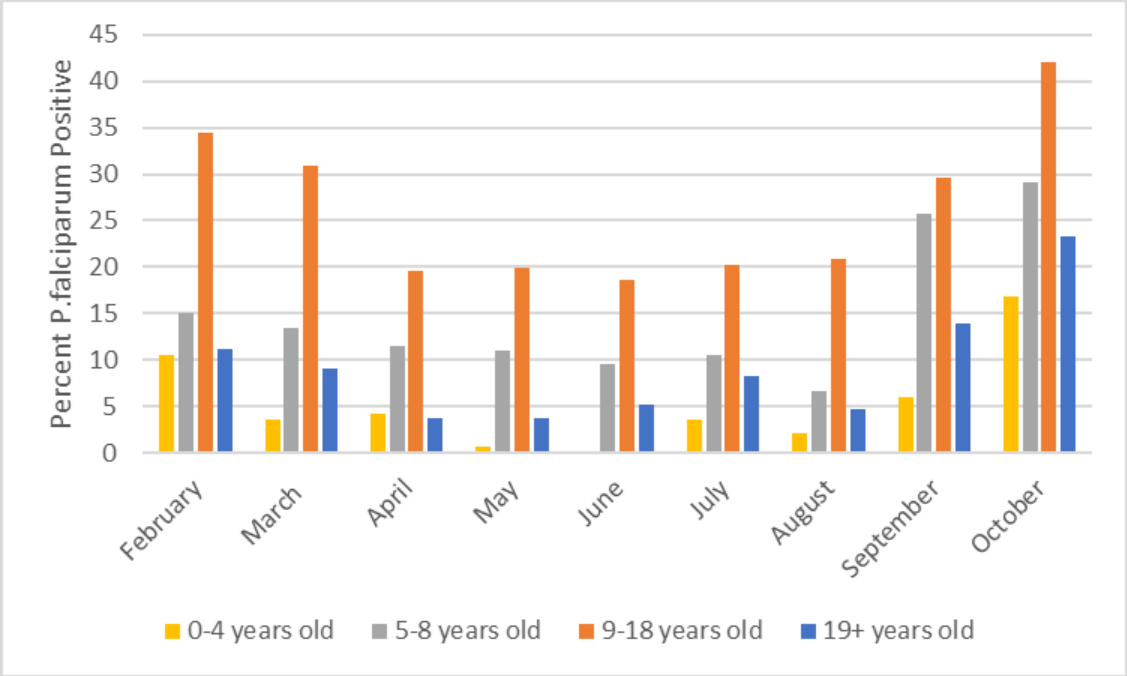


Figure 23. Percent *P. falciparum* Positive by Month and Age.

Children between 5-18 years of age also have higher measures of malaria transmission. Thus far in the study, children 5-18 years of age consistently have the highest prevalence of *P. falciparum* gametocytemia (**Figure 24**), suggesting they are a key reservoir of ongoing transmission to mosquitoes. Notably, children under 5 have a relatively low rate of *P. falciparum* gametocytemia throughout follow-up, and gametocytemia has been absent in this demographic from August onward (**Figure 24**). This latter effect of reduced gametocytemia may be the result of seasonal malaria chemoprevention (SMC), which entails monthly doses of sulfadoxine-pyrimethamine + amodiaquine given to all under 5 children in Mali during the malaria season; in Bancoumana, these treatments started at the end of July during the current study. The effect of SMC can also be seen in the relatively low rates of asexual *P. falciparum* parasitemia seen in under 5 children during the malaria season (**Figure 23**). The use of SMC implies that Malian children under 5 years of age may not be a key target for TBV administration during trials to measure vaccine activity and efficacy, and this notion is supported by the low rates of gametocytemia observed in this population during the malaria transmission season thus far.

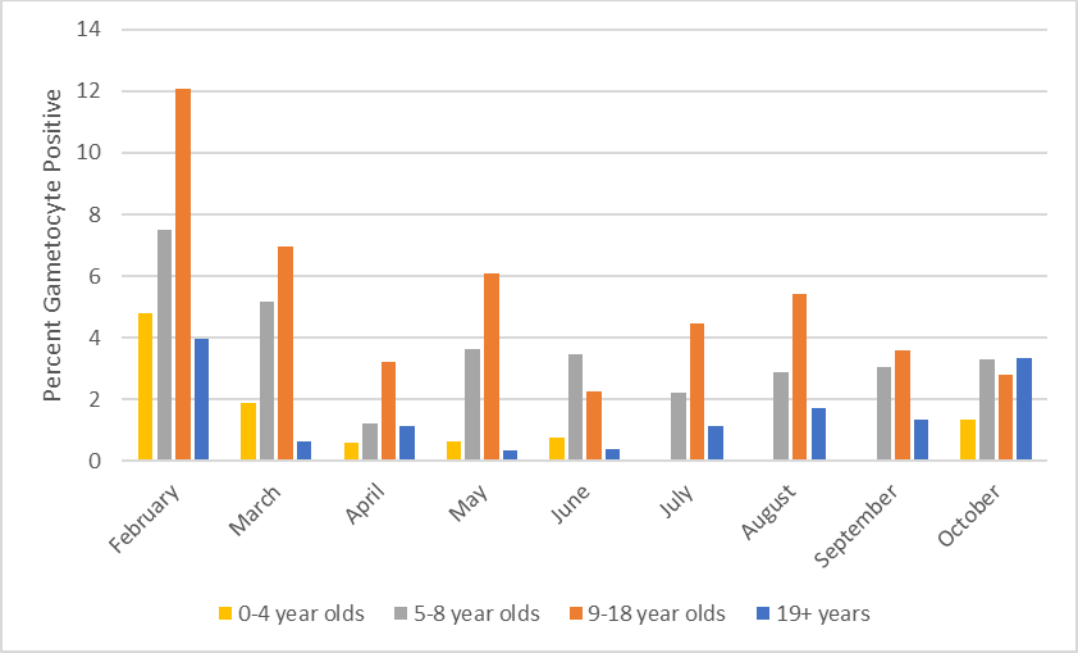


Figure 24. Percent *P. falciparum* Gametocyte Positive by Month and Age.

More strikingly, the DSF studies, which have thus far been limited to participants 5 years of age and older, have highlighted the disproportionate role of children 5-18 years of age in parasite transmission. From March through August 2018, there were 28 positive DSF assays out of 3,806 performed (**Figure 2**). Remarkably, 27 of these positive DSF assays were recorded in children 5-18 years of age, while only one was recorded in an adult. Notably, the rate of parasite transmission by DSF in children was higher in the dry season (2% positive in March) than was previously seen in adults during the rainy season (0.9% positive in 2017). The rates of DSF positivity in children 5-18 years of age in July-August suggest that we can expect an overall rate throughout the transmission season of 2.5% or higher during follow-up.

5 Experience in Children with AS01 Adjuvant

GlaxoSmithKline (GSK) has developed several vaccine adjuvants under the auspices of its Adjuvant System program, with 5 products being used in clinical trials to date and 3 currently used in licensed vaccines: AS01, AS03, and AS04 (**Table 10**). Our current trial is using the AS01 system, which contains 2 immunostimulatory components, 3-O-desacyl-4'-monophosphoryl lipid A (MPL) and the saponin QS-21 formulated with liposomes. AS01 was designed to provoke a strong humoral immune response with an acceptable safety profile. AS01 is prepared by GSK in 2 forms: $AS01_B$ (provided in 100 µg/mL in 1 mL to provide in 0.5 mL 50 µg MPL + 50 µg QS-21 in liposomal formulation), which is used in the licensed herpes zoster vaccine Shingrix®, and $AS01_E$, which contains half the dose of MPL and QS-21 as $AS01_B$ and is used in the candidate malaria vaccine Mosquirix™.

Table 10. AS01 Adjuvant Systems. Adapted from Garcon et al. 2017, From discovery to licensure, the Adjuvant System story.

Adjuvant System	Composition	Vaccines licensed or in Phase III trials	Vaccines in Phase I or II trials	Development discontinued
AS01	A combination of immunostimulants QS-21 and MPL with liposomes	Malaria vaccine Herpes zoster vaccine	Malaria next generation COPD exacerbations associated with non-typeable Haemophilus influenzae and Moraxella catarrhalis Tuberculosis vaccine HIV vaccine	—
AS02	A combination of immunostimulants QS-21 and MPL with an oil in water emulsion	—		HIV vaccine Tuberculosis vaccine Therapeutic melanoma vaccine Malaria vaccine.
AS03	A combination of an oil in water emulsion with alpha-tocopherol (Vitamin E) as immuno-enhancing component	Pre-pandemic H5N1 vaccine Pandemic H1N1 influenza vaccines (*Arepanrix™*, *Pandemrix™*)	—	
AS04	MPL is adsorbed onto aluminum hydroxide or aluminum phosphate, depending on the vaccine with which it is used	Human papillomavirus vaccine (*Cervarix™*) Hepatitis B for pre- and haemodialysis patients (*Fendrix™*)	—	Herpes simplex vaccine
AS15	A combination of immunostimulants CpG 7909, QS-21 and MPL with liposomes	—	—	MAGE-A3 Cancer Immunotherapeutics: melanoma and non-small-cell lung cancer vaccines

QS-21: Quillaja saponaria Molina: fraction 21. (Antigenics Inc., a wholly owned subsidiary of Agenus Inc., Lexington, MA, USA).
MPL: 3-deacylated monophosphoryl lipid.
CpG7909: an immunostimulatory nucleotide.
COPD: Chronic Obstructive Pulmonary Disease

5.1 RTS,S/AS01$_E$ Pediatric Safety Data

RTS,S/AS01$_E$ is the most studied investigational vaccine in infants and children that utilizes the AS01 adjuvant system. Following initial development, safety, immunogenicity, and protective efficacy assessment in adults in the US, Europe, and Africa, RTS,S has undergone subsequent 8 Phase 1/2 and 3 Phase 3 clinical studies in subjects aged at least 5 months and 4 Phase 1/2 and 2 Phase 3 clinical studies in infants less than 5 months of age have been completed.

In these studies, the AS01 adjuvant has been shown to be more immunogenic than the AS02 adjuvant used in the initial studies and RTS,S has continued to be well tolerated and efficacious in younger age groups.[51-54] These studies led to a large scale Phase 3 study, as previously noted, with the final product Mosquirix™ (RTS,S 25 μ g/AS01$_E$) evaluated in over 15,000 children across 11 sites in 7 countries.[55] Overall, the vaccine had an acceptable safety profile, even when given to young children and infants and in co-administration with the Expanded Programme on Immunization (EPI) routine vaccines. Following the large Phase 3 studies, imbalances were seen in the vaccinees versus controls that are now being further explored specifically in regards to cases of meningitis, febrile seizures, and all cause mortality in female children.[55-57]

The most common side effects seen in participants who are vaccinated with Mosquirix™ are low grade fever (27%), irritability (14%), injection site pain (16%) and injection site swelling (7%), and these occur at rates similar to when children receive standard vaccines as summarized in **Table 11** below. In children (5- 17 months of age), there was notably higher incidence of fever during the primary series of vaccination and during a booster 4th dose within 7 days of receiving the vaccine compared to comparator groups. Some of these fever incidences were associated with generalized convulsive seizures (2.5 per 1000 doses in those receiving the 4th booster dose at 20 months versus 1.2 per 1000 doses in those who received comparator vaccine at the 20-month timepoint vs 0.4 per 1000 doses in those who received only comparator vaccine doses at all 4 time points). In infants (6 to 12 weeks of age), the rate of febrile seizures was also noted to be 2.2 per 1000 doses following the 4th dose. All the seizure episodes resolved without long-term sequalae.

The frequency of overall SAEs was not significantly different between vaccinated participants and control participants. However, there was a significant difference in the number of meningitis SAEs, with 21 having occurred in vaccinated children versus 1 in control children.[56] No clear relationship between Mosquirix™ and meningitis has been identified and further analysis and investigation is ongoing. In question is still whether it is the antigen or the adjuvant that may be related to these (febrile seizures and meningitis) adverse events.

Table 11. Adverse reactions reported after 3 doses of the vaccine[58] Note: Very common ≥1/10; Common ≥1/100 to < 1/10; Uncommon ≥1/1000 to < 1/100

System Organ Class	Frequency	Adverse Reactions
Metabolism and nutrition disorders	Common	Decreased appetite
Psychiatric disorders	Very common	Irritability
	Common	Somnolence
Nervous system disorders	Uncommon	Febrile convulsions (within 7 days post vaccinations)
Gastrointestinal disorders	Common	Diarrhea
	Uncommon	Vomiting
General disorders at administration site conditions	Very common	Fever, injection site reactions (including swelling, erythema and pain)
	Uncommon	Injection site induration

The European Medicines Agency has reviewed the safety profile of Mosquirix™ and has stated that, "The safety profile of this vaccine is acceptable and quite similar to others apart from a higher risk for febrile convulsions in the older age group within 7 days after a dose (mostly the third dose) of Mosquirix™"[59]. Childhood febrile seizures are common in children between the

age of 6 months and 5 years with peak episodes between 12 and 18 months. Although investigations are ongoing in order to determine the relationship between Mosquirix™ and febrile seizures, due to ongoing concerns about this relationship, the proposed study which uses the same adjuvant AS01, will not enroll children under the age of 5 years.

A Phase I double-blind, randomised controlled, staggered, dose-escalation study using RTS,S/AS02A was completed in older children, 6-11 years, in Gambia. In this study, the older children were divided into 3 groups (n=20 in each group) to receive 10 µg RTS,S dose (10 µg RTS,S in 0.1 mL AS02A), 25 µg dose (25 µg RTS,S in 0.25 mL AS02A) or 50 µg dose (50 µg RTS,S in 0.5 mL AS02A) at 0, 1 and 3 months (**Figure 25**). A comparator group (n=30) received rabies vaccine. The vaccine was found to be safe and well tolerated, there were no reported SAEs related to the study. The most common AEs reported was injection site pain (>80% of the doses), headache (18-24% vs 12% in rabies vaccine) and fever (10% vs 6% in rabies vaccine) in all dose groups. There were few Grade 3 AEs that resolved within 24 hours. The rate of unsolicited AEs was similar between the vaccine groups and control group. There was only one SAE in the control group, bronchopneumonia, that resolved without sequelae (**Figure 25**).[60]

Symptom experienced	Rabies vaccine (*N*=90)	RTS,S/AS02A		
		10 µg dose (*N*=60)	25 µg dose (*N*=59)	50 µg dose (*N*=60)
Pain				
Any	74 (82.2%)	53 (88.3%)	53 (89.8%)	59 (98.3%)
Grade 3	0 (0.0%)	0 (0.0%)	4 (6.8%)	8 (13.3%)
Swelling				
Any	1 (1.1%)	5 (8.3%)	10 (16.9%)	12 (20.0%)
Grade 3	0 (0.0%)	1 (1.7%)	1 (1.7%)	4 (6.7%)
Fever[a]				
Any	5 (5.6%)	6 (10.0%)	5 (8.5%)	6 (10.0%)
Grade 3	0 (0.0%)	1 (1.7%)	0 (0.0%)	0 (0.0%)
Any Rel	4 (4.4%)	2 (3.3%)	4 (6.8%)	5 (8.3%)
Grade 3 Rel	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Limited arm motion				
Any	3 (3.3%)	5 (8.3%)	12 (20.3%)	11 (18.3%)
Grade 3	0 (0.0%)	0 (0.0%)	2 (3.4%)	2 (3.3%)
Malaise				
Any	4 (4.4%)	5 (8.3%)	8 (13.6%)	11(18.3%)
Grade 3	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (1.7%)
Any Rel	4 (4.4%)	5 (8.3%)	7 (11.9%)	10 (16.7%)
Grade 3 Rel	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (1.7%)
Nausea				
Any	2 (2.2%)	4 (6.7%)	2 (3.4%)	6 (10.0%)
Grade 3	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Any Rel	2 (2.2%)	3 (5.0%)	1 (1.7%)	6 (10.0%)
Grade 3 Rel	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Headache				
Any	11 (12.2%)	11 (18.3%)	14 (23.7%)	12 (20.0%)
Grade 3	0 (0.0%)	0 (0.0%)	0 (0.0%)	2 (3.3%)
Any Rel	10 (11.1%)	7 (11.7%)	10 (16.9%)	8 (13.3%)
Grade 3 Rel	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (1.7%)

N: number of documented doses; Rel: judged to be related to vaccination by investigator. Grade 3 limited arm motion: abduction at the shoulder ≤30°; Grade 3 pain at the injection site: spontaneously painful; Grade 3 swelling: >50 mm and persisting more than 24 hours; Grade 3 fever: axillary temperature ≥39.0 °C; Grade 3 for other symptoms: adverse event that prevents normal activity. *Note*: All local adverse events (i.e. pain and swelling at the injection site and limited arm motion) were always considered to be causally related to vaccination.

[a] Axillary temperature ≥37.5 °C.

Figure 25. Local and Systemic Symptoms Occurring 4 Days after Vaccination

Although there is sparse data regarding the safety of AS01 in children above the age of 5 years, the safety data from the study above with $AS02_A$ is encouraging. As had been seen in adults, the most common AEs were mild local reactogenicity and fever.

5.2 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 pre-erythrocytic malaria parasites to prevent human infection or sexual stage malaria parasites to prevent transmission to mosquitoes (TBV) in order to interrupt malaria transmission.

LMIV has the world's most advanced program for clinical development of TBVs. TBVs 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 showed a consistent increase in Pfs25 antibody response by ELISA following each subsequent vaccination, but these responses appear short lived. Data from these studies also showed that increasing antibody titers were correlated with increased functional transmission reducing activity by SMFA. Further studies in the US and Mali also showed 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 Pfs230D1M can achieve high functional activity by SMFA compared to regimens containing Pfs25. However, the duration of the antibodies was shorter than intended. Although there was no evidence of interference of activity when Pfs25 and Pfs230D1M were combined, it did not appear that Pfs25 enhanced the activity of Pfs230D1M.

A more potent adjuvant, AS01, was then evaluated in a Phase 1 clinical trial in Mali, induced higher titers as well as longer lasting functional antibodies for both Pfs25M and Pfs230D1M assessed individually or in combination. Since Pfs230D1M appeared superior for inducing functional antibody, it was selected to continue with the future community studies to assess the efficacy of this vaccine in reducing transmission of malaria at a community level. However, to assess vaccine efficacy that reduces malaria incidence in the community, we need to ensure that a large portion of the population is enrolled and vaccinated, especially individuals who transmit malaria at high rates. Ongoing LMIV studies have identified children as contributing disproportionately to transmission events, hence it will be essential to also include children in future community trials of a transmission-blocking vaccine.

At this time, we will evaluate for the first time the vaccine antigen Pfs230D1M formulated with AS01 to see whether it is safe in healthy Malian children, and if so, we will proceed to enroll all

willing residents within selected family compounds to receive vaccine or comparator to further assess safety, and to assess vaccine efficacy, activity and immunogenicity. Vaccine efficacy will be assessed by detecting *P. falciparum* infections by BS, vaccine activity will be assessed by DSF assays and SMFA, and lastly immunogenicity will be assessed by testing sera in ELISA. Given the promising results, in particular seen with DSF activity after a fourth dose of Pfs230D1M-EPA/AS01 in healthy adults, we will assess this vaccine regimen over two malaria seasons (2019-2020, 2020-2021).

6 Study Objectives

6.1 Pilot Objectives

6.1.1 Primary Objectives

- To assess safety and reactogenicity of administration of Pfs230D1M-EPA/AS01
- To assess vaccine activity of Pfs230D1M-EPA/AS01 against *P. falciparum* transmission by direct skin feeding assay (DSF) (Arms 1a/1b only) after receipt of 3rd and 4th vaccination and analyzed with the main group

6.1.2 Secondary Objectives

- To assess vaccine efficacy of Pfs230D1M-EPA/AS01 against *P. falciparum* infection measured by blood smear after receipt of 3rd and 4th vaccination and analyzed with the main group
- To assess immunogenicity as measured by enzyme-linked immunosorbent assay (ELISA) titer response to Pfs230D1M
- To assess functional antibody response by standard membrane feeding assay (SMFA) to Pfs230D1M

6.1.3 Exploratory Objectives

- To explore cellular and humoral responses to Pfs230D1M
- To identify host and parasite factors associated with transmission
- To track transmission of parasites from human to human using highly variant gene fragments
- To explore the impact of human movement on transmission and parasitemia

6.2 Main Objectives

6.2.1 Primary Objectives

- To assess vaccine activity of Pfs230D1M-EPA/AS01 against *P. falciparum* transmission by direct skin feeding assay (DSF) (Arms 3c/3d only)

6.2.2 Secondary Objectives

- To assess vaccine efficacy of Pfs230D1M-EPA/AS01 against *P. falciparum* infection measured by blood smear (All Arms except Arms 3e/3f)
- To assess safety and reactogenicity of administration of Pfs230D1M-EPA/AS01 (All Arms except 4a/4b)
- To assess immunogenicity as measured by enzyme-linked immunosorbent assay (ELISA) titer response to Pfs230D1M (All Arms except Arms 4a/4b)
- To assess functional antibody response by standard membrane feeding assay (SMFA) to Pfs230D1M (All Arms except Arms 4a/4b)

6.2.3 Exploratory Objectives

- To explore cellular and humoral responses to Pfs230D1M (All Arms except Arms 4a/4b)
- To identify host and parasite factors associated with transmission
- To track transmission of parasites from human to human using highly variant gene fragments
- To explore the impact of human movement on transmission and parasitemia

7 Study Design

7.1 Description of the Study Design

This is a Phase 2, double-blind, block (compound/vaccine unit)-randomized, comparator-controlled study designed to evaluate the safety, reactogenicity, immunogenicity, transmission-blocking activity, and subsequent vaccine efficacy of Pfs230D1M conjugate formulated on AS01. Subjects will be drawn from approximately 137 compounds/vaccine units (extended families/neighboring compounds) in Doneguebougou and surrounding villages that have agreed as a family/compound to participate in the study. Individuals within the compound will not be randomized to individual arm assignments, but rather an entire family/compound will be randomized as a whole to receive either Pfs230D1M-EPA/AS01 or comparator vaccine in eligible and willing residents in that family/compound (vaccine unit) as well as the enrolled young children (1-4 years of age) in the parasitemia cohort (Group 4).

As previously noted, all eligible and enrolled pilot and main phase subjects ≥5 years of age or older will receive AL treatment prior to first vaccination as well as either Pfs230D1M-EPA/AS01 or comparator vaccine at 0, 28, 56, and 392 days (main phase) and 0, 28, 126, and 448 days (pilot phase). All vaccinated subjects will be followed for safety and tolerability, as well as immunogenicity via ELISA and functional antibody responses via SMFA.

Subjects 5-8 years of age will also receive AL treatment ~14 days prior to vaccination #3 and ~7 days prior to vaccination #4 in their compound, regardless of other antimalarial treatments

administered prior, subject to clinician's discretion. Children 1-4 years of age will be enrolled just before the malaria transmission season and will receive AL concomitantly with children 5-8 years of age who will be treated ~14 days before the 3rd vaccine dose and ~7 days prior to vaccination #4. All enrolled subjects (except adults, ≥19 years old) will be monitored for patent parasitemia every 2 weeks, and all enrolled subjects during unscheduled visits if clinically indicated, for at least 6 months up to 12 months post vaccination, with time to patent parasitemia being evaluated as a vaccine efficacy timepoint in children 1-8 years of age as well as 9-18 years of age within the compounds (separate secondary evaluation given expected nighttime mobility and no pre-transmission season AL treatment). Preteen and adolescent subjects will also be assessed by DSF for vaccine activity starting 2 weeks post receipt of vaccination #3 and post receipt of vaccination #4; DSFs will be completed every 2 weeks for a total of 8 DSF (post vaccination #3) and for a total of 10 DSF (post vaccination #4).

7.2 Study Groups

7.2.1 Pilot Safety Group

We will enroll, drug treat with AL prior to vaccination #1, and vaccinate a total of 60 children. Subjects will be enrolled in 2 different age groups, as follows:

Group 1: Subjects 9 to 18 years of age (n=30)

- ***Arm 1a*** (n=15), to receive 40 μg of Pfs230D1M-EPA/AS01 on days 0, 28, 126, 448; receipt of AL on day -7
- ***Arm 1b*** (n=15), to receive HAVRIX (day 0, 448), TYPHIM Vi (day 28), Menactra (day 126); receipt of AL on day -7

Group 2: Subjects 5 to 8 years of age (n=30)

- ***Arm 2a*** (n=15), to receive 40 μg of Pfs230D1M-EPA/AS01 on days 0, 28, 126, 448; receipt of AL on day -7, 112, and 441
- ***Arm 2b*** (n=15), to receive HAVRIX (day 0, 448), TYPHIM Vi (day 28), Menactra (day 126); receipt of AL on day -7, 112, and 441

The age groups will be enrolled in descending order, oldest to youngest, with at least 2 weeks between the previous vaccination of one group (Group 1) and the first enrollment of the next group (Group 2). The age de-escalation design allows assessment of the risk level in pre-teens and teenagers based on data from adults and, subsequently, the risk level in younger children based on data from teenagers. Teenagers and older children who are able to provide assent will participate before the study opens to younger children who cannot assent.

After receipt of vaccination #1 in the older age group (Group 1: Arms 1a/1b), the DSMB will conduct an interim safety review of all safety data collected to date before the vaccination #1 is

administered in younger children (Group 2: Arms 2a/2b) and second vaccination in the older group (Group 1: Arms 1a/1b) age groups respectively. The DSMB will also review the safety data collected to date prior to administration of vaccination #2 in the younger age group (Group 2: Arms 2a/2b; **Figure 26**).

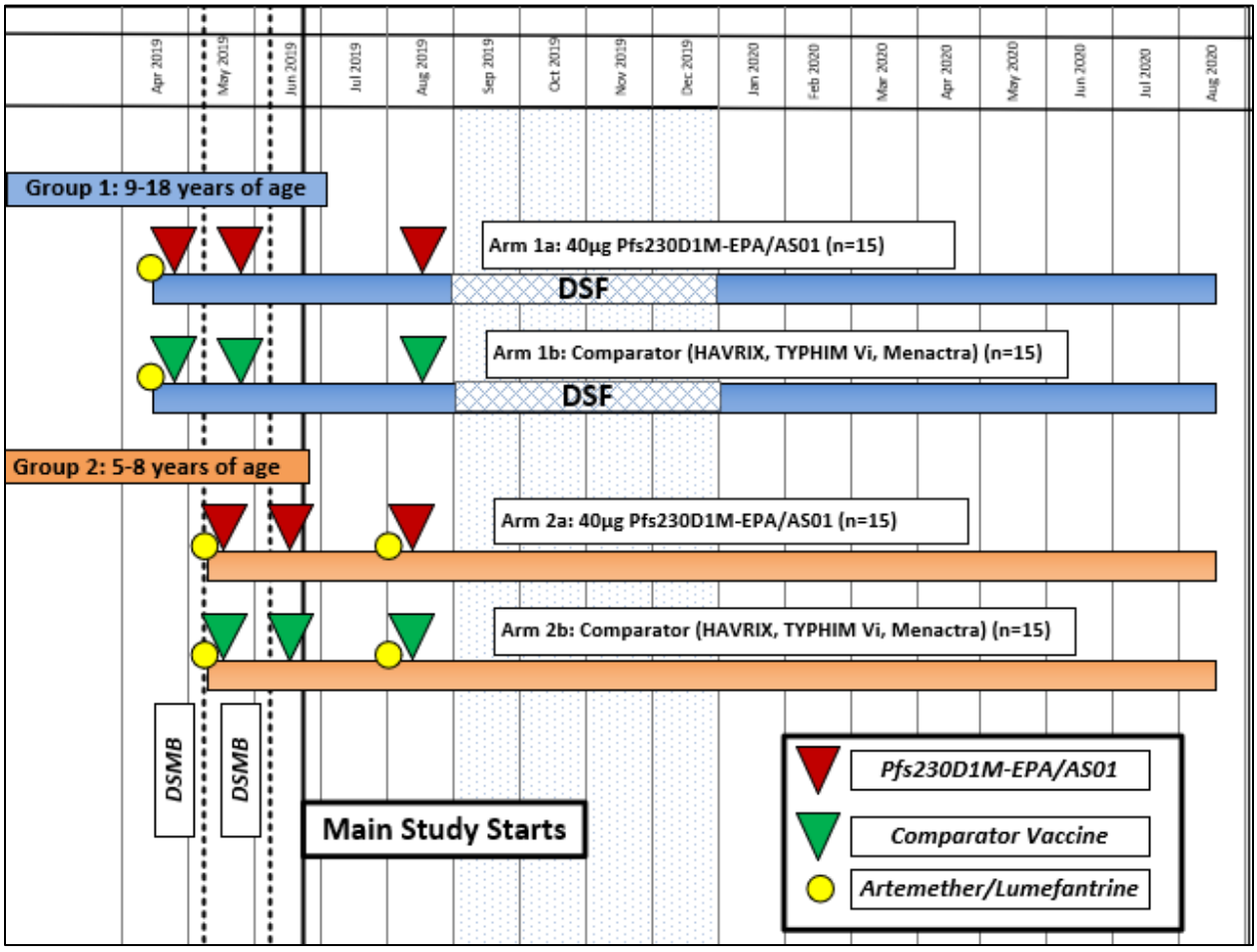


Figure 26. Pilot Safety Group Study Schema

Once the main phase of the study starts, children enrolled in the pilot study will join the main phase and complete their third vaccination with their respective family compounds. Those children enrolled in Group 2 (Arms 2a/2b), in alignment with all others 5-8 years of age, will also receive a treatment course of AL prior to vaccination #3 and those in Group 1 (Arms 1a/1b) will join the main phase Arms 3c/3d (9-18 years of age) for vaccination #3.

7.2.2 Main Study Group

We will enroll, drug treat with AL prior to vaccination #1, and vaccinate a total of approximately 137 compounds (~1500 subjects) that have approximately 80% consented, eligible subjects. Subjects will be enrolled and vaccinated by family compound and specified to Arms as noted below by age at time of enrollment (**Figure 27**). Younger children (Group 4; n~400) will also be enrolled at the same time as their corresponding family compound. While our goal is to enroll the maximum number of children in this age bracket, there are currently children 1-4 years of age in Doneguebougou per the most recent census (**Table 12**); enrollment may occur in adjacent villages if required to meet minimum sample sizes. If there are younger children in the compound that will reach the age of study participation prior to the family compounds' scheduled 3rd vaccination, they can be enrolled on a continuous basis until then once they become of age.

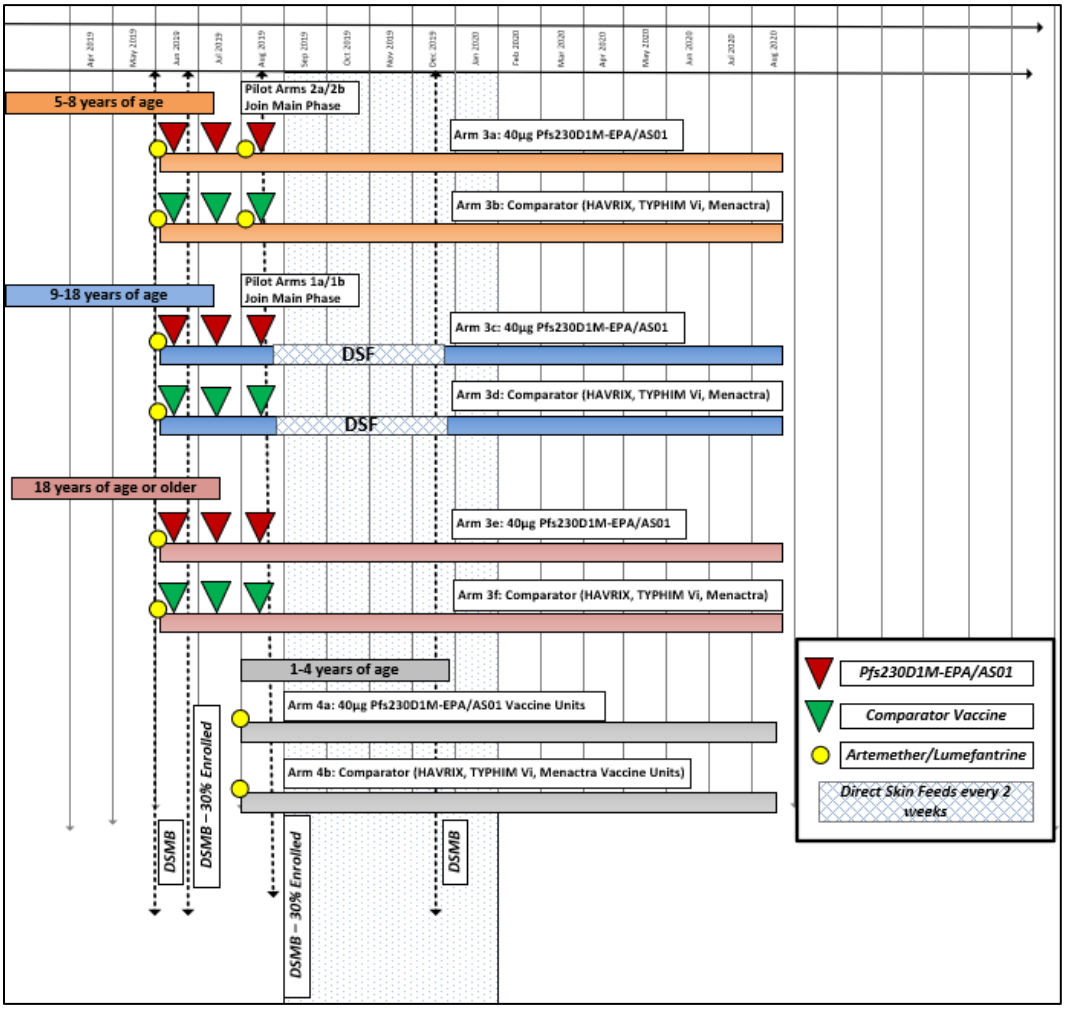


Figure 27. Main Phase Study Schema.

Table 12. Approximate Census Counts in Doneguebougou in June 2018.

Age (years)	<1	1-4	5-8	9-18	19+
Population	29	295	229	584	867
Actual Enrollment	0	192	215	399	495

Group 3 (n~1500): Pfs230D1M-EPA/AS01 or comparator vaccine

- ***Arm 3a:*** 5-8 years of age to receive 40 μg of Pfs230D1M-EPA/AS01 on days 0, 28, 56, 392; receipt of AL on day -7, 42, and 385
- ***Arm 3b:*** 5-8 years of age to receive HAVRIX (day 0, 392), TYPHIM Vi (day 28), Menactra (day 56); receipt of AL on day -7, 42, and 385
- ***Arm 3c:*** 9-18 years of age to receive 40 μg of Pfs230D1M-EPA/AS01 on days 0, 28, 56, 392; receipt of AL on day -7
- ***Arm 3d:*** 9-18 years of age to receive HAVRIX (day 0, 392), TYPHIM Vi (day 28), Menactra (day 56); receipt of AL on day -7
- ***Arm 3e:*** 19 years of age or older to receive 40 μg of Pfs230D1M-EPA/AS01 on days 0, 28, 56, 392; receipt of AL on day -7
- ***Arm 3f:*** 19 years of age or older to receive HAVRIX (day 0, 392), TYPHIM Vi (day 28), Menactra (day 56); receipt of AL on day -7

Group 4 (n=400): blood smear + AL treatment (parasitemia)

- ***Arm 4a***: 1-4 years of age residing with/in same families/compounds as Arms 1a, 2a, 3a, 3c, 3e (Pfs230D1M-EPA/AS01 compounds) receipt of AL on day -14, 385
- ***Arm 4b:*** 1-4 years of age residing with/in same families/compounds as Arms 1b, 2b, 3b, 3d, 3f (comparator compounds) receipt of AL on day -14, 385

To ensure further safety, family compounds will be enrolled in a staggered manner with acceleration over time, with a planned DSMB review after approximately 1/3 enrollment (~30 family compounds/vaccine units). Five compounds will be enrolled within the first 1-2 days of the main phase enrollment, then will await 2 days from first vaccination in that wave of enrollment until enrolling an additional 10 compounds over 1-2 days. Following waiting at least 2 days from the first vaccination during that second wave of enrollment, a final additional 15 compounds will be enrolled. Given our current estimates of family compound make up, this initial study enrollment (Pilot Phase) will result in an additional vaccination of a smaller subset of 5-8 years of age and 9-18 years of age for extra safety data prior to accelerated enrollment of the remaining family compounds ~1 week later.

Children in Arms 2a/2b, 3a/3b, and 4a/4b (1-8 years of age) will also receive a treatment course of AL ~14 days prior to the time of vaccination #3 in the relevant Arms (AL dosing prior to

Vaccination #3 = study day 112/42 for Arms 2a/2b, 3a/3b; for Arms 4a/4b this is study day -14). Preteen and adolescent subjects in Arms 1a/1b, 3c/3d will also be assessed by DSF for vaccine activity starting 2 weeks post receipt of vaccination #3; DSFs will be completed every 2 weeks for a total of 8 DSFs.

All enrolled subjects (Groups 1-4, except adults on a less regular active follow-up) from the family compound will be monitored for patent parasitemia every 2 weeks, and during unscheduled visits if clinically indicated, for at least 6 months up to 12 months post vaccination, with time to patent parasitemia being evaluated as a vaccine efficacy timepoint in children 1-8 years of age as well as 9-18 years of age within the compounds (separate secondary evaluation given expected nighttime mobility and no pre-transmission season AL treatment).

7.2.3 Year 2: Pilot and Main Study Groups

In Year 2, during the last scheduled follow-up visit from Year 1, each VU will be approached for reconsenting and focus rescreening to continue on for a 4th vaccination (Arms 1a/1b, 2a/2b, 3a/3b/3c/3d/3e/3f) and followed for the malaria season (2020) and for a year post last vaccination (**Figure 28**).

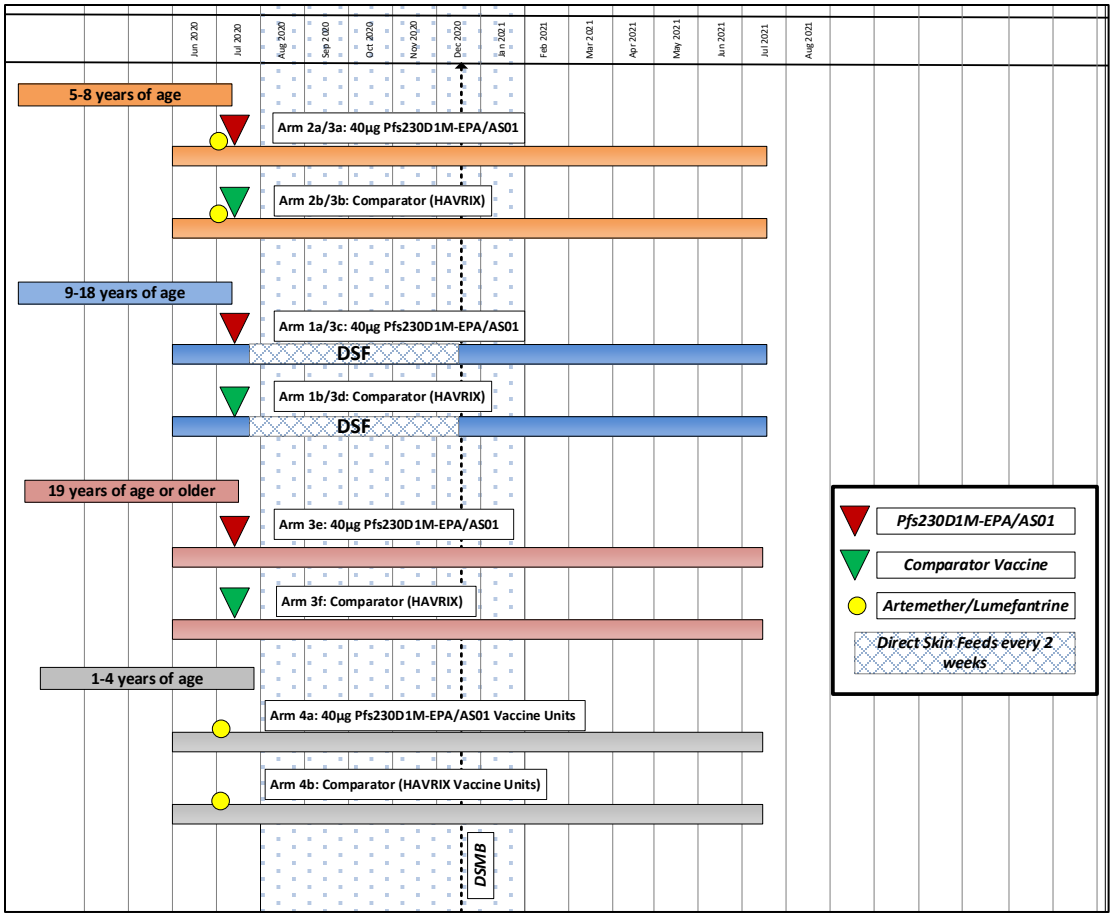


Figure 28. Fourth Dose (Year 2) Study Schema

Updated study schedule for fourth dose. Pilot and Main Phase will align with their VUs for timing of AL dosing and vaccination.

8 Study Endpoints

8.1 Pilot Endpoints

8.1.1 Primary Endpoints

- Incidence of local and systemic adverse events (AEs) and serious adverse events (SAEs)
- *P. falciparum* functional activity as measured by DSF (Arms 1a/1b only) after receipt of 3rd and 4th vaccination and analyzed with the main group

8.1.2 Secondary Endpoints

- *P. falciparum* asexual parasitemia as measured by blood smears after receipt of 3rd and 4th vaccination and analyzed with the main group
- Anti-Pfs230D1M immunoglobulin (Ig) G levels as measured by ELISA
- Transmission-reducing activity (TRA)/Transmission-blocking activity (TBA) of induced antibody in SMFA

8.1.3 Exploratory Endpoints

- Cellular immune responses and antibody repertoire of functional antibody responses to vaccination
- Measuring hemoglobin, hemoglobin variants, levels of parasitemia/gametocytemia, OMICS analyses (proteomics, genomics), clinical malaria infection and treatment
- Sequences of highly variant parasite gene fragments
- Evaluation of congregate movement measurements by GPS tracking on functional activity as measured by DSF and *P. falciparum* asexual parasitemia as measured by blood smears (subgroup from Arms 1a/1b only)

8.2 Main Endpoints

8.2.1 Primary Endpoints

- *P. falciparum* functional activity as measured by DSF (Arms 3c/3d)

8.2.2 Secondary Endpoints

- *P. falciparum* asexual parasitemia as measured by blood smears (All Arms except Arms 3e/3f)
- Incidence of local and systemic AEs and SAEs (All Arms except Arms 4a/4b)
- Anti-Pfs230D1M immunoglobulin (Ig) G levels as measured by ELISA (All Arms except Arms 4a/4b)
- Transmission-reducing activity (TRA)/Transmission-blocking activity (TBA) of induced antibody in SMFA (All Arms except Arms 4a/4b)

8.2.3 Exploratory Endpoints

- Cellular immune responses and antibody repertoire of functional antibody responses to vaccination (All Arms except Arms 4a/4b)
- Measuring hemoglobin, hemoglobin variants, levels of parasitemia/gametocytemia, OMICS analyses (proteomics, genomics), clinical malaria infection and treatment
- Sequences of highly variant parasite gene fragments
- Evaluation of congregate movement measurements by GPS tracking on functional activity as measured by DSF and *P. falciparum* asexual parasitemia as measured by blood smears (subgroup from Arms 3c/3d only)

8.3 Sample Size and Estimated Duration of Study

A total of 60 subjects will be vaccinated in the age de-escalation pilot phase of the trial. At the time of enrollment, all children will receive a treatment course of AL prior to first vaccination and will be randomized at the time of vaccination #1. Since children may be replaced if enrolled but not randomized, more subjects may be initially enrolled, but no more than 60 subjects will

receive at least 1 vaccine. Each subject will be monitored actively for at least 12 months after the last vaccination, for a total of 13 to 18 months of participation depending on timing of screening for the study. Up to 80 subjects will be screened to accommodate possible screening failures.

For the main phase of the study, approximately 137 total compounds/vaccine units will be approached for enrollment for an estimated total of ~1500 vaccinated subjects 5 years of age and older and ~400 subjects 1-4 years of age. Given each compound is of a variable population (ranging from 5-90+ years of age), this is an estimate of enrollment with expected enrollment in the total main phase to be ~1500 subjects +/- 15%. Subjects will be monitored actively for at least 12 months after the last vaccination, for a total of 13 to 18 months of study participation depending on timing of screening for the study. Up to 2380 subjects will be screened to accommodate possible screening failures.

For the fourth dose and continuation on study, the enrolled compounds/vaccine units will be approached for reconsenting and re-screening. No new enrollments will be permitted. Despite adjusted ages over the last year, subjects will remain in their assigned arms from enrollment for evaluation of study endpoints (e.g. DSFs, blood smears), receipt of AL, and receipt of vaccinations. All subjects who received at least 1 vaccination during 2019-2020 and are on study may be eligible for continuation. All enrolled children (1-4 year olds) who received antimalarial treatment with their VU will be eligible for continuation. Subjects not wanting to continue on study for another year or are found to be ineligible will not be unblinded until the end of Year 2, and will be provided the opportunity to complete a final visit with their VU at that time.

8.4 Study Definitions

Screened: Subjects will receive a screening identification number (SC-XXX-XXX) when the informed consent is signed, and will either be determined as “enrolled” or “screen failures” as noted below; subjects within the same vaccine unit will be noted by the same first 3 numbers (SC-**XXX-**xxx) of their screening identification.

- Screening may be completed over the course of multiple visits.
- Screening will occur within 56 days prior to enrollment into the study.
- If the screening visit is >56 days prior to enrollment (determined by the date of each component), then an updated medical review, physical exam/vital signs, repeat Malaria Comprehension Exam, and repeat laboratory evaluation (inclusive of safety labs, human immunodeficiency virus [HIV], EKG) will be completed to determine eligibility for enrollment.

Enrolled: Subjects will be considered enrolled beginning with the receipt of the first blood draw and first AL dose.

- Final study number assigned – MV-19-XXX-XXX-X [MV-19-(vaccine unit #)-(study # per age)-(letter for QC)]
  - Given the last three numbers of the study number are dependent on age and requirement by the electronic database to have a study number during screening, if a child/young adult changes age categorization (4→5-year-old, 8→9-year-old, 18→19-year old) between screening and enrollment a new study ID will be assigned categorizing the study subject as above. The screening identification number will not change.

Randomized: Subjects are considered randomized when their family compound/vaccine unit undergoes randomization collectively at the time of first vaccination of their first family member. A compound/family may be randomized once the following criteria are met:

- Approximately 80% of the family compound/vaccine unit preliminarily eligible to be vaccinated has signed consent, agreed to participate in the study, and has been found preliminarily eligible for enrollment
- Entire family/compound will be randomized as a collective whole at the time of first vaccination in any family compound/vaccine unit participant
- To note: prior to the start of the study, the study geographers, based off GPS coordinates and village census assigned family compounds into vaccine units which were randomized as a unit.

Re-enrolled: Subjects are considered re-enrolled for Year 2 of follow-up when they complete the following:

- Re-consent
- Successfully rescreen and meet inclusion/exclusion criteria for Year 2
- Receive either AL (1-8 years old only) and/or vaccine (scheduled dose #4)

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 11.1**
- Subject withdraws consent before being randomized.
- Family compound/Vaccine unit preliminary enrollment is significantly lower than approximately 80%, including low estimated pediatric participation.

Discontinued: Subjects 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.

9 Study Population

9.1 Study Site

The study will be carried out in collaboration between the LMIV/National Institutes of Health (NIH) and the MRTC headquartered in Bamako, Mali. The study will be conducted by the MRTC at one location in Mali, West Africa (see **Figure 29**). The MRTC staffing at the Mali site is employed by NIAID/ University of Sciences, Techniques, & Technologies of Bamako (USTTB) programs and assigned to the Doneguebougou site for the duration of the study to provide clinical care and execute the protocol.

Doneguebougou is a community located 30 km north of Bamako and has a population of about 2,000 people, with another 2,000 inhabitants in the surrounding villages. For the purpose of vaccine trials and epidemiology studies, adequate facilities have been put in place at Doneguebougou within walking distance to the residents' homes. At Doneguebougou, 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 one transmission season. There is a high study participation rate per compound in Doneguebougou, thus this site greatly fits the goal of a community-wide vaccination strategy such as what is being proposed for these TBV vaccines.

The additional nearby village to Doneguebougou, named Sirababougou, will also be approached for enrollment. Sirababougou is located at about 5km northeast of Doneguebougou village, the main site (see **Figure 29**). The population of Sirababougou is about 700 inhabitants based on the census performed at the beginning of this year by the study team. The population's primary occupation is mainly farming and the sociocultural behavior of the Sirababougou village is

similar to Doneguebougou. Malaria transmission intensity is expected to be similar to Doneguebougou village.

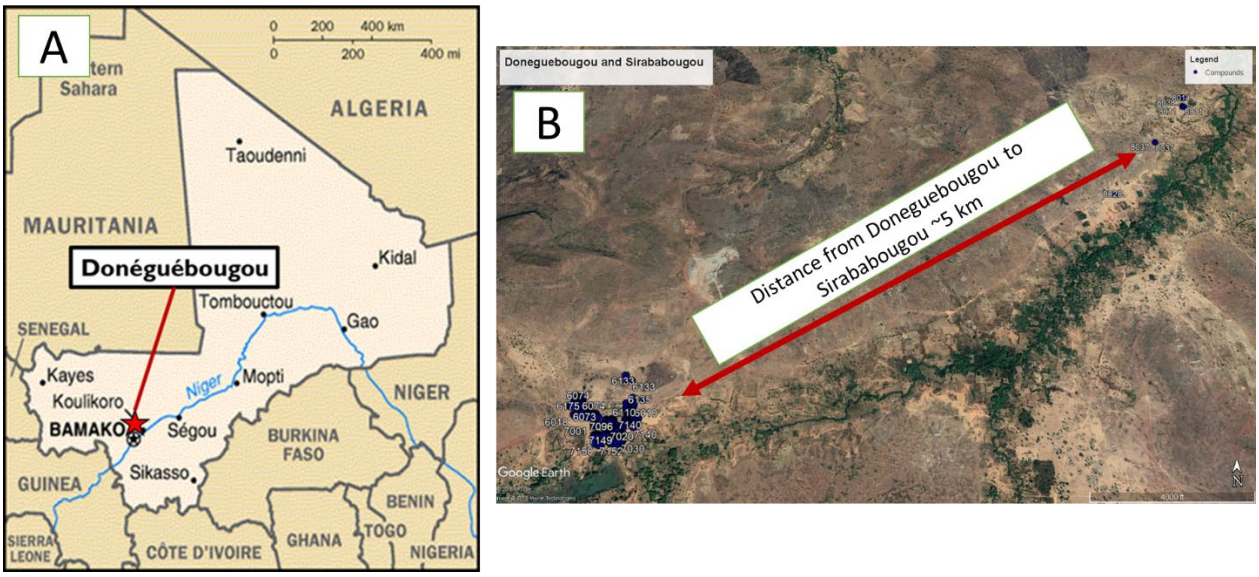


Figure 29: Maps Showing the Location of Doneguebougou, Mali.
(A) In reference to other West African countries and within Mali; (B) Context of location of Doneguebougou and Sirababougou to one another.

A family compound in Mali is a composite of different houses, and members are usually related by paternal relationship. The family compound can contain members of several related generations and be as large as 100+ or as small as 5-10. The family compound has a chief of family (usually the oldest man), while houses within the family compound are led by the chief of the household (usually the husband of the household). Important decisions are typically made by the chief of family. Spatial distribution and categorization of inhabitants by family compounds in Doneguebougou are provided below in **Figure 30**. As noted prior, a single family compound may compose a single vaccine unit or multiple family compounds may compose a single vaccine unit.

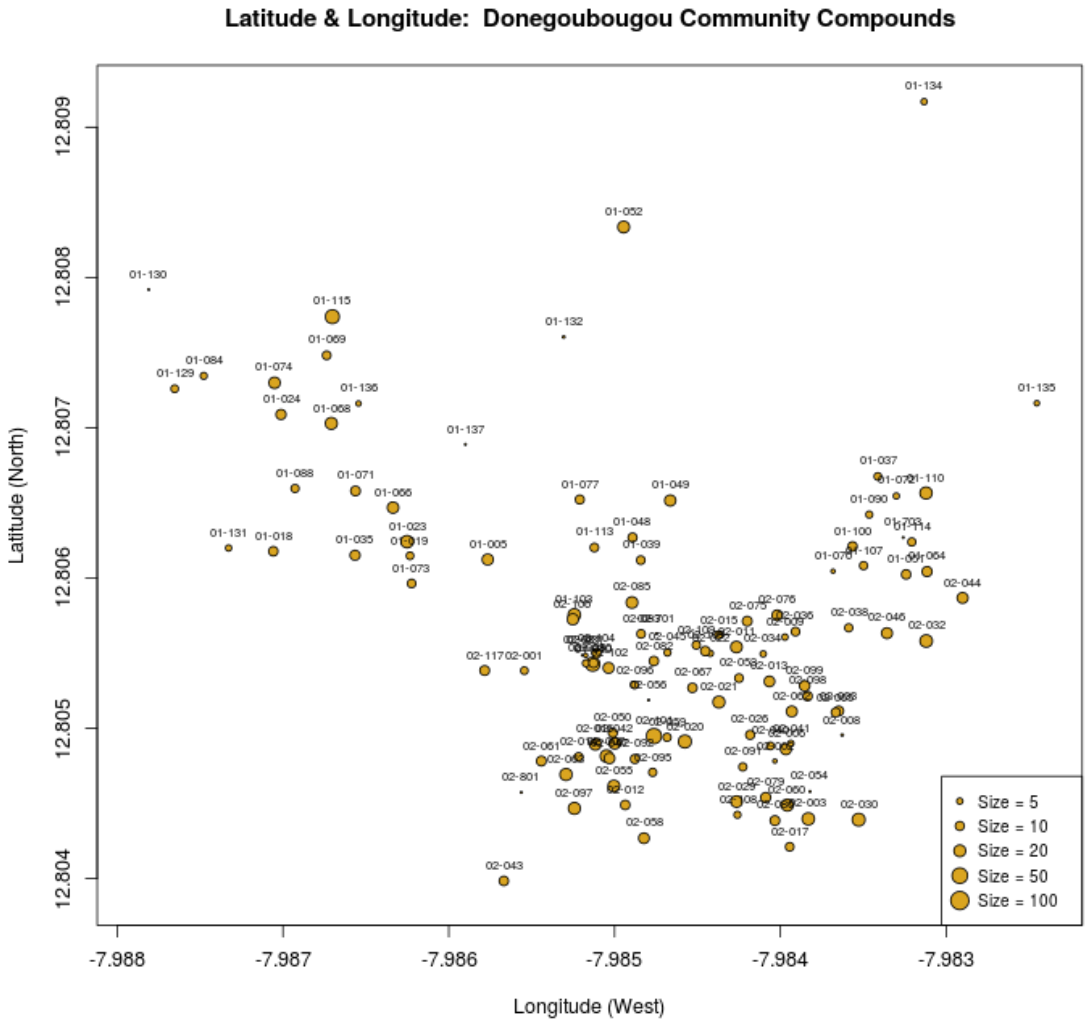


Figure 30: Aerial Map Showing Distribution of Family Compounds in Doneguebougou, Mali

9.2 Rationale for Subject Selection

TBVs must be given to a large proportion of any given community to be successful in preventing the spread of malaria. Teenagers and children, especially school-aged children, are major transmitters of the malaria parasite. Thus, it is important to study whether a candidate TBV is safe, well tolerated, and effective in children as well as adults. By enrolling young children who live and often stay within the confines of these compounds, the study will illustrate a measurable direct effect of the Pfs230D1M-EPA/AS01, as seen by a reduction in the rate of malaria infections.

To ensure adequate cohorting, healthy children living in compounds/vaccine units in which approximately 80% of those preliminarily eligible for vaccination have agreed to participate in

the study, signed informed consent, and are preliminarily eligible for enrollment (e.g. screening of available vaccine unit for major medical problems, pregnancy, and time availability) will be recruited for this study.

9.3 Recruitment Plan

Community permission will be obtained from village elders and other community members in Doneguebougou after explanation and discussion of the study at a community meeting. 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.

9.4 Inclusion Criteria for Groups 1, 2, 3

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

1. Meets age requirements for Arm currently being enrolled.
2. Available for the duration of the trial.
3. Family compound known resident or long-term resident (more than 1 year) of Doneguebougou, Mali or surrounding villages.
4. Able to provide proof of identity to the satisfaction of the study clinician completing the enrollment process.
5. In good general health and without clinically significant medical history in the opinion of the investigator.
6. Females of childbearing potential must be willing to use reliable contraception from 21 days prior to Study Day 0 and until 1 month after the last vaccination.
    - A reliable method of birth control includes **<u>one</u>** of the following:
        1. Confirmed pharmacologic contraceptives (parenteral) delivery;
        2. Intrauterine or implantable device

    EXCEPTIONS to required pregnancy prevention includes the following:

        1. Postmenopausal state: defined as no menses for 12 months without an alternative medical cause
        2. Surgical sterilization
        3. Unmarried AND not sexually active AND menstruating OR not menstruating females 12-17 years of age
        4. NOTE: if a female of childbearing potential's status changes during the course of vaccination through 1 month post vaccination (e.g. they become ≥18 years of age,

married, or sexually active), the female will be required to start reliable contraception

7. Willing to have blood samples stored for future research.

9.5 Inclusion Criteria for Group 4

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

1. Meets age requirements for Arm currently being enrolled.
2. Available for the duration of the trial.
3. Family compound known resident or long-term resident (more than 1 year) of Doneguebougou, Mali or surrounding villages.
4. Able to provide proof of identity to the satisfaction of the study clinician completing the enrollment process.
5. In good general health and without clinically significant medical history in the opinion of the investigator.
6. Willing to have blood samples stored for future research.

9.6 Inclusion Criteria for Groups 1, 2, 3, (Year 2)

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

1. Previously enrolled in the study and has received at least 1 vaccination.
2. Available for the duration of the trial.
3. In good general health and without clinically significant medical history in the opinion of the investigator.
4. Females of childbearing potential must be willing to use reliable contraception from 21 days prior to scheduled vaccine dose #4 and until 1 month after the 4th vaccination.
    - A reliable method of birth control includes **<u>one</u>** of the following:
        1. Confirmed pharmacologic contraceptives (parenteral) delivery;
        2. Intrauterine or implantable device

    EXCEPTIONS to required pregnancy prevention includes the following:

        1. Postmenopausal state: defined as no menses for 12 months without an alternative medical cause
        2. Surgical sterilization
        3. Unmarried AND not sexually active AND menstruating OR not menstruating females 12-17 years of age

4. NOTE: if a female of childbearing potential's status changes during the course of vaccination through 1 month post vaccination (e.g. they become ≥18 years of age, married, or sexually active), the female will be required to start reliable contraception
5. Willing to have blood samples stored for future research.

9.7 Inclusion Criteria for Group 4 (Year 2)

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

1. Previously enrolled in the study
2. Available for the duration of the trial.
3. In good general health and without clinically significant medical history in the opinion of the investigator.
4. Willing to have blood samples stored for future research.

9.8 Exclusion Criteria for Groups 1, 2, 3

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

1. Pregnant, as determined by a positive urine or serum beta human choriogonadotropin (β-hCG) test (***if female***).

   NOTE: Pregnancy is also a criterion for discontinuation of any further vaccine dosing
2. Menstruating females 11 years of age and younger. (In order to avoid cultural implications of further assessing pregnancy potential i.e. sexual activity in this age group.)
3. Behavioral, cognitive, or psychiatric disease that in the opinion of the investigator affects the ability of the subject to understand and comply with the study protocol at a level appropriate for the subject's age.
4. Hemoglobin, white blood cell (WBC), absolute neutrophil count, or platelet levels 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 2.)
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 2.)
6. Infected with HIV

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. Clinically significant prolonged QTc (>450 milliseconds) on screening EKG
9. History of receiving any investigational product within the past 30 days.
10. Current or planned participation in an investigational vaccine study until the last required protocol visit.
11. Medical, occupational, or family problems as a result of alcohol or illicit drug use during the past 12 months.
12. History of a severe allergic reaction or anaphylaxis.
13. Known:
    - 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.
    - Autoimmune or antibody-mediated disease including but not limited to: systemic lupus erythematosus, rheumatoid arthritis, multiple sclerosis, Sjögren's syndrome, or autoimmune thrombocytopenia.
    - Immunodeficiency syndrome.
    - Seizure disorder (exception: history of simple febrile seizures)
    - Asplenia or functional asplenia.
    - Use of chronic (≥14 days) oral or intravenous (IV) corticosteroids (excluding topical or nasal) at immunosuppressive doses (i.e., prednisone >10 mg/day) or immunosuppressive drugs within 30 days of Study Day 0.
    - Allergy to latex or neomycin
    - Adverse reaction to hepatitis A and/or typhoid and/or meningococcal vaccine in the past
    - Adverse reaction to artemether/lumefantrine in the past
14. Receipt of:
    - Live vaccine within the past 4 weeks or a killed vaccine within the past 2 weeks to enrollment.
    - Immunoglobulins and/or blood products within the past 6 months.
    - Investigational malaria vaccine in the last 2 years.
15. Any other condition that in the opinion of the investigator would jeopardize the safety or rights of a subject participating in the trial, interfere with the

evaluation of the study objectives, or would render the subject unable to comply with the protocol.

Co-enrollment guidelines: Co-enrollment in other trials is restricted, other than enrollment on observational studies. Consideration for co-enrollment in trials evaluating the use of a licensed medication will require the approval of the PI. Study staff should be notified of co-enrollment on any other protocol as it may require the approval of the investigator.

9.9 Exclusion Criteria for Group 4

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

1. Behavioral, cognitive, or psychiatric disease that in the opinion of the investigator affects the ability of the subject to understand and comply with the study protocol at a level appropriate for the subject's age.
2. 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.
3. Clinically significant prolonged QTc (>450 milliseconds) on screening EKG
4. History of adverse reactions to artemether/lumefantrine in the past
5. History of receiving any investigational product within the past 30 days.
6. Current or planned participation in an investigational vaccine study until the last required protocol visit.
7. Receipt of:
    - Investigational malaria vaccine in the last 2 years.
8. Any other condition that in the opinion of the investigator would jeopardize the safety or rights of a subject participating in the trial, interfere with the evaluation of the study objectives, or would render the subject unable to comply with the protocol.

9.10 Exclusion Criteria for Groups 1, 2, 3 (Year 2)

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

1. Pregnant, as determined by a positive urine or serum beta human choriogonadotropin (β-hCG) test (***if female***).

NOTE: Pregnancy is also a criterion for discontinuation of any further vaccine dosing

2. Menstruating females 11 years of age and younger. (In order to avoid cultural implications of further assessing pregnancy potential i.e. sexual activity in this age group.)
3. Behavioral, cognitive, or psychiatric disease that in the opinion of the investigator affects the ability of the subject to understand and comply with the study protocol at a level appropriate for the subject's age.
4. Hemoglobin, white blood cell (WBC), absolute neutrophil count, or platelet levels 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 2.)
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 2.)
6. Infected with HIV
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. Medical, occupational, or family problems as a result of alcohol or illicit drug use during the past 12 months.
10. History of a severe allergic reaction or anaphylaxis.
11. Known:

- 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.
- Autoimmune or antibody-mediated disease including but not limited to: systemic lupus erythematosus, rheumatoid arthritis, multiple sclerosis, Sjögren's syndrome, or autoimmune thrombocytopenia.
- Immunodeficiency syndrome.
- Seizure disorder (exception: history of simple febrile seizures)
- Asplenia or functional asplenia.
- Use of chronic (≥14 days) oral or intravenous (IV) corticosteroids (excluding topical or nasal) at immunosuppressive doses (i.e., prednisone >10 mg/day) or immunosuppressive drugs within 30 days of Study Day 0.

- Allergy to latex or neomycin
- Adverse reaction to hepatitis A and/or typhoid and/or meningococcal vaccine in the past
- Adverse reaction to artemether/lumefantrine in the past

12. Receipt of:
    - Live vaccine within the past 4 weeks or a killed vaccine within the past 2 weeks to enrollment.
    - Immunoglobulins and/or blood products within the past 6 months.
13. Any other condition that in the opinion of the investigator would jeopardize the safety or rights of a subject participating in the trial, interfere with the evaluation of the study objectives, or would render the subject unable to comply with the protocol.

9.11 Exclusion Criteria for Group 4 (Year 2)

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

1. Behavioral, cognitive, or psychiatric disease that in the opinion of the investigator affects the ability of the subject to understand and comply with the study protocol at a level appropriate for the subject's age.
2. 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.
3. History of adverse reactions to artemether/lumefantrine in the past
4. History of receiving any investigational product within the past 30 days.
5. Current or planned participation in an investigational vaccine study until the last required protocol visit.
6. Receipt of:
    - Investigational malaria vaccine in the last 2 years.
7. Any other condition that in the opinion of the investigator would jeopardize the safety or rights of a subject participating in the trial, interfere with the evaluation of the study objectives, or would render the subject unable to comply with the protocol.

9.12 Justification for Exclusion of Special Populations

9.12.1 Justification of Exclusion of Pregnant Women

This study will not vaccinate pregnant women during the course of the study; they will be excluded from this cohort. The effects of Pfs230D1M-EPA/AS01 on the developing human fetus are unknown with the potential for teratogenic or abortifacient effects. In a reproductive and developmental toxicity study in female rats who were administered SHINGRIX or the $AS01_B$ adjuvant alone, no adverse effects on preweaning development up to post-natal day 25 were observed, and there were no vaccine-related fetal malformations or variations.

In addition, hepatitis A vaccine (HAVRIX) and typhoid vaccine (TYPHIM Vi) and meningococcal vaccine (Menactra) are classified as FDA pregnancy risk category C. Though, according to the Advisory Committee on Immunization Practices (ACIP), administration of inactivated virus vaccines to pregnant women have not resulted in adverse effects in the fetus. The ACIP recommends vaccination during pregnancy when the likelihood of disease exposure is high, potential infection would cause harm to mother or fetus, and when the vaccine is unlikely to cause harm. The manufacturer recommends administration of the vaccine to pregnant women only if clearly needed.

9.12.2 Justification for Exclusion of Children Under the Age of Five (Vaccination)

Children under the age of 5 years are excluded from this study because there are insufficient data regarding dosing or AEs available in adults to judge the potential risk in children in this age group. Some evidence suggests an increased risk of febrile seizures with receipt of AS01 from RTS,S/AS01 studies in young children; since febrile seizures in the general population are most likely to occur in children under the age of 5 years, children under 5 years of age are excluded from vaccination. In addition, Malian children under 5 years of age receive monthly antimalarial treatments with long acting agents (termed Seasonal Malaria Chemoprevention), which limits their rates of parasitemia and especially gametocytemia, and as a consequence these children are not a primary target for transmission blocking vaccine assessment.

10 Study Agents

10.1 Pfs230D1M-EPA/AS01

10.1.1 Manufacturing

PpPfs230D1M and EcEPA lots, both manufactured at the Walter Reed Bioproduction facility (Silver Spring, Maryland) in current Good Manufacturing Practices (cGMP) compliance, were used to manufacture the conjugate. PpPfs230D1M is a *Pichia*-expressed recombinant subsegment (S542-G736) of Pfs230 with a molecular mass of 21,854 Daltons. EcEPA is an *Escherichia coli*-expressed recombinant protein with a 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 and July 2016.

The Pfs230D1M-EPA was formulated as conjugated Pfs230D1M in 4 mM phosphate-buffered saline (PBS) to a 2X dilution of the high dose (160 µg/mL in 0.5 mL volume) in cGMP compliance at Walter Reed Bioproduction facility in April 2016 or at the Biopharmaceutical Development Program (BDP) in December 2018 and is provided as a single use vial. $AS01_B$ adjuvant was manufactured for use in the SHINGRIX vaccine by GSK as 100 µg/mL MPL and 100 µg/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. The vaccine will be mixed and drawn up into the syringes directly prior to injection. A final dose of 0.5 mL will administer 40 µg conjugated Pfs230D1M, 31 µg or 36 µg conjugated EPA in AS01 (25 µg MPL+ 25 µg QS21 liposomal formulation).

10.1.2 Disposition and Dispensation

The 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, Walter Reed Army Institute of Research, Silver Spring, Maryland, where the materials were formulated and packaged, or from ThermoFisher BioServices in Gaithersburg, MD where additional product manufactured at BDP is stored. The $AS01_B$ vials will be purchased as manufactured for the SHINGRIX vaccine by GSK.

10.1.3 Formulation, Packaging, and Labeling

Each single-use vial of Pfs230D1M-EPA contains 160 µg/mL of conjugated Pfs230D1M, 124 µg/mL or 143 ug/mL of conjugated EPA in 4-mM PBS in a volume of 0.5 mL. The vial label reads: 160 µg/mL Conjugated Pfs230D1M in 4-mM PBS. Vaccines will be labeled "Caution: New Drug – Limited by Federal (or United States) law to investigational use."

There are 2 formulations in the AS01 family: $AS01_B$ and $AS01_E$. While $AS01_B$ contains 50 µg of MPL and 50 µg of QS21 per one human dose, $AS01_E$ contains half the quantity of each immune-enhancer (i.e., 25 µg of MPL and 25 µg of QS-21). For this study, the $AS01_B$ (50 µg of MPL and 50 µg of QS21 per one human dose) adjuvant will be used at a diluted concentration similar to the concentration of $AS01_E$ (i.e., 25 µg of MPL and 25 µg of QS-21), but not the same formulation as $AS01_E$.

10.1.4 Storage, Shipping, and Stability

Study vaccine Pfs230D1M-EPA must be stored at -65°C to -90°C except during vaccine transports (on days of vaccination/use), when the range must remain within -60°C to +9°C. This allows for the vaccine to thaw during transport to the field. The vaccine is then stored at 2-8°C

until use, and cannot be refrozen for later use. Once thawed, the vaccine vial must be labeled and quarantined as unusable. Shipping specifications in the standard operating procedures (SOPs) allow for a range of -90°C to -40°C for dry ice shipments. The long-term storage in a freezer cannot exceed -65°C, however. Vaccines are always shipped on dry ice, and generally remain below -70°C. Comparator vaccines HAVRIX® or TYPHIM Vi®, and adjuvant AS01B must be stored at temperatures of 2-8°C, except during vaccine transports, when the range must remain within 0.5°C to 9.0°C. There are ranges built into the individual SOPs for shipping, transport and direct use.

Vials will be transported and stored at temperature-controlled conditions, according to standard operating procedures (SOPs). Temperature data loggers will accompany the vaccines at all times to ensure storage temperature limits have not been violated. 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. 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.

10.1.5 Preparation, Dosage, and Administration of Study Agents

The TBV conjugates are stored at -80°C until just before transport to the field for use, then stored at 2 to 8°C as described above. AS01 adjuvant is stable in the refrigerator at +2°C to +8°C. The final vaccine for administration is obtained by admixing Pfs230D1M-EPA with AS01 and must be given via intramuscular (IM) injection within 4 hours of reconstitution. The formulation of the study vaccine is presented in **Table 13**.

Table 13. Transmission-Blocking Vaccine (TBV)

Vaccine	Product Name	Formulation	Volume to be administered	Number of doses
TBV/AS01 (Arms 1a, 2a, 3a, 3c, 3e)	Pfs230D1M-EPA AS01$_B$	Pfs230D1M = 40 µg EPA= 31 µg AS01 = 25 µg MPL + 25µg QS21 liposomal formulation	0.5 mL	4

10.1.6 Administration

Study agent or comparator vaccines will be administered as IM injections into the deltoid muscle or anterolateral thigh. Arms may be alternated with successive vaccinations. When choosing an arm for the vaccine injection, clinicians should consider whether there is an arm injury, local skin problems such as scarring 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.

10.2 Comparator vaccine

The comparator vaccines (HAVRIX® and TYPHIM Vi® and Menactra®) will be distributed to the study in single-use or multi-use vials per the manufacturer's recommendation and will be administered by IM injection on the schedules outlined in the Study Design (**Section 7**). MenAfriVac comparator (Menactra® was previously used but another meningitis vaccine, the MenAfriVac® will be used) vaccine will also be offered to participants randomized to the Pfs230 arms at the end of the study.

10.2.1 HAVRIX® (Hepatitis A Vaccine)

HAVRIX (Hepatitis A Vaccine; HAV) is produced by GSK and is a sterile suspension of inactivated virus for IM administration. The virus (strain HM175) is propagated in MRC-5 human diploid cells. After removal of the cell culture medium, the cells are lysed to form a suspension. This suspension is purified through ultrafiltration and gel permeation chromatography procedures. Treatment of this lysate with formalin ensures viral inactivation. It is then adsorbed on aluminum hydroxide. HAVRIX is FDA approved for active immunization against disease caused by HAV for persons 12 months of age and older. In children and adolescents (1-18 years of age) a single 0.5-mL dose will be administered on day 0, day 392/448. In adults (≥19 years of age) a single 1-mL dose will be administered on day 0, day 392/448.

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

If HAVRIX in not available for the study, another equivalent FDA approved or WHO certified HAV will be used and the package insert submitted to NIH IRB and FMPOS EC prior to administration.

10.2.2 TYPHIM Vi (*Salmonella typhi* vaccine)

TYPHIM Vi (Typhoid Vi Polysaccharide Vaccine), produced by Sanofi Pasteur SA, for IM use, is a sterile solution containing the cell surface Vi polysaccharide extracted from *Salmonella enterica* serovar *Typhi*, S typhi Ty2 strain (inactivated, subunit vaccine). TYPHIM Vi vaccine is indicated for active immunization for the prevention of typhoid fever caused by *S typhi* and is FDA approved for use in persons 2 years of age or older. The recommended immunizing dose for adults and children is a single injection of 0.5 mL, this is the dose that will be given on day 28. The dose for adults will be given by IM route in the deltoid, and the dose for children will be given IM either in the deltoid or the anterolateral thigh.

Additional information is provided in the TYPHIM Vi package insert provided.

If TYPHIM Vi is not available for the study, another equivalent FDA approved or WHO certified *Salmonella typhi* inactivated, subunit vaccine will be used and the package insert submitted to NIH IRB and FMPOS EC prior to administration.

10.2.3 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.

Previously, Advisory Committee on Immunization Practices recommended only the quadrivalent meningococcal polysaccharide vaccine (MPSV4) for use in adults age 56 years and older. The most recent recommendations recommend the use of Menactra® in adults age 56 years and older who were vaccinated previously with conjugated vaccine (e.g. Menactra® or MenAfriVac) and now need revaccination or are recommended to receive multiple doses. The use of these conjugated vaccines in people age 56 and older is off-label but recommended by ACIP. CDC expands that recommendation to any child or adult at increased risk for meningococcal disease (e.g. individuals in an epidemic or highly endemic country such as Mali). A single dose (0.5 mL) is recommended for all individuals regardless of age. The dose for adults will be given by IM route in the deltoid, and the dose for children will be given IM either in the deltoid or the anterolateral thigh.

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

10.3 Contraindications to Vaccination

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

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

Subjects will be encouraged to remain in the study for safety evaluation of the dose(s) already received and complete research visits for immunogenicity and functional activity if deemed safe by the PI.

10.4 Indications for Deferral of Vaccination

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

- Oral temperature >38.0ºC at the time of vaccination.
- Receipt of a prohibited medication/procedure as described in **Section 10.6**
- 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 individuals 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 (i.e., temperature ≤38.0ºC and/or lack of symptoms) within the vaccination window. Subjects will be encouraged to remain in the study for safety evaluation of the dose(s) already received and complete research visits for immunogenicity and functional activity if deemed safe by the PI.

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 subsequent immunizations, the investigator may elect to continue to follow them per protocol without additional vaccinations for the remainder of the study. Subjects who miss vaccinations after the first vaccination cannot be replaced.

10.5 Concomitant Medications and Procedures

All concomitant prescription and nonprescription (including over-the-counter) medications taken during study participation will be recorded. For this protocol, a prescription medication is defined as a medication that can be prescribed only by a properly authorized/licensed clinician.

10.6 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 2-week period prior to and following each vaccination or licensed live vaccines in the 4-week period prior to and following each vaccination.
- Receipt of immunoglobulins or any blood products up to 6 months prior to the first study vaccination and continuing for 30 days after administration of the last vaccination.

- Chronic oral or IV 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 during the study period is not exclusionary but will be documented by clinical staff.

10.7 Vaccine Accountability

After administration of a vaccine dose, the single-dose vials of antigen and adjuvant or comparator vaccine will be accounted for according to the site SOPs 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.

11 Study Schedule

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

11.1 Screening

The purpose of the screening visit is to determine volunteer eligibility for study participation. Screening procedures include the informed consent process, Malaria Comprehension Exam, laboratory assessments, and clinical assessments. Screening activities can occur over multiple visits if necessary, including the day of enrollment.

In the event that HIV infection or other chronic illnesses are 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 will be reported to the local health department according to applicable laws and appropriate medical referrals initiated.

11.1.1 Screening for Arms 1, 2, 3

The following screening evaluations must be completed for all subjects within the 56 days prior to first vaccination (Arms 1, 2, 3):

- Explain the study and informed consent/assent documents to the subject and their parent/guardian. (Year 1 + 2)
- Ensure that the subject (aged 7-17 years of age and unmarried) has acknowledged assent by signing or fingerprinting the assent document and that the parent/guardian acknowledges permission for their children or for themselves to participate by signing or fingerprinting the informed consent form. Ensure that the subject and parents/guardians receive a signed copy of the informed consent and assent (when applicable). (Year 1 + 2)
- Ensure the subject and/or parents/guardians have correctly answered ≥80% of the questions on the Malaria Comprehension Exam. (Year 1 only)
- Elicit a complete medical history, including menstrual and contraceptive history and/or history of surgical sterility for females, sexual activity and marital status for females 12-17 years of age, and medication use. (Year 1 + 2)
- Females of childbearing potential must be willing to use reliable contraception from at least 21 days prior to first and fourth vaccination through 1 month after the last vaccination. (Year 1 + 2)
    - EXCEPTIONS to required pregnancy prevention includes the following:
        - Postmenopausal state: defined as no menses for 12 months without an alternative medical cause
        - Surgical sterilization
        - Unmarried AND not sexually active AND menstruating OR not menstruating females 12-17 years of age

    NOTE: if a female of childbearing potential's status changes during the course of vaccination through 1 month post vaccination (e.g. they become ≥18 years of age, married, or sexually active), the female will be required to start reliable contraception
- Administer a complete physical examination, including vital signs (blood pressure, temperature, and heart rate), height/length, and weight. (Year 1 +2)
- 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. (Year 1 + 2)
- Obtain blood for complete blood count with differential (CBC w/diff) and platelet count, ALT, Cr, and HIV antibody. (Year 1 + 2)
- Obtain urine for urinalysis/urine dipstick for protein and blood and, for females of childbearing potential, obtain urine (or serum) for pregnancy testing. (UA, Year 1 only; Pregnancy testing Year 1 + 2)

- Pregnancy testing (urine or serum) will be completed on all females 9 years of age and older as indicated in **Appendix A.**

- Obtain a 12-lead electrocardiogram (EKG). (Year 1 only)

If screening laboratories are completed within ≤2 days prior to Study Day -7 prior to vaccination (AL dosing), these clinical laboratory values (CBC w/diff, ALT, Cr) may be used for Study Day -7 assessments and do not need to be repeated.

If initial screening is completed >56 days prior to Study Day 0 (day of vaccination #1), the following screening procedures (per procedure; only those >56 days prior to Study Day 0) will need to be repeated: repeat a new Malaria Comprehension Exam, updated medical history, repeat screening labs (except HIV unless determined change in risk), repeat physical exam, and repeat EKG. Subjects will then be reassessed for eligibility based on the re-screening information.

11.1.2 Screening for Arm 4

The following screening evaluations must be completed for all subjects within the 56 days prior to enrollment (Arm 4):

- Explain the study and informed consent documents to parent/guardian. (Year 1 + 2)
- Ensure that the parent/guardian has acknowledged consent by signing or fingerprinting the consent document and that the parent/guardian acknowledges permission for the subject to participate by signing or fingerprinting the informed consent form. Ensure that the parent/guardian receives a signed copy of the informed consent. (Year 1 + 2)
- Ensure the subject's parent/guardian has correctly answered ≥80% of the questions on the Malaria Comprehension Exam. (Year 1 only)
- Elicit a complete medical history and medication use. (Year 1 + 2)
- Administer a complete physical examination, including vital signs (blood pressure, temperature, and heart rate), height/length, and weight. (Year 1 + 2)
- Obtain a 12-lead electrocardiogram (EKG). (Year 1 only)

If initial screening is completed >56 days prior to Study Day 0, the following screening procedures will need to be repeated before enrollment: repeat a new Malaria Comprehension Exam, updated medical history, repeat physical exam, and repeat EKG. Subjects will then be reassessed for eligibility based on the re-screening information.

11.2 Assignment to Groups

11.2.1 Pilot Safety Study

The subjects in Pilot Safety Study will be assigned randomization per their family compound randomization. In order for a compound to qualify for randomization, approximately 80% of the residents within a family compound/vaccine unit who are preliminarily eligible for vaccination must have signed consent and be preliminarily eligible for enrollment (e.g. screening available vaccinee unit for major medical problems, pregnancy, and time availability). When the first child (<19 years of age) from the family compound is scheduled for vaccination, the family compound as a whole will then be randomized to Pfs230D1M-EPA/AS01 or comparator.

The Pilot Study cohorts will be enrolled in the following order:

1. Arms 1a (n=15) and 1b (n=15); then
2. Arms 2a (n=15) and 2b (n=15)

11.2.2 Main Study

If a family compound has already been randomized secondary to enrollment of a child in the Pilot Safety Study (Group 1/2), these family compounds will be prioritized for the start of the Main Study to enroll, undergo AL drug treatment, and receive their first vaccination as a unit. As new family compounds/vaccine units are considered preliminarily eligible for enrollment and randomization, the same procedures as above will be followed until approximately 137 compounds/vaccine units are enrolled into the study.

Enrollment occurs with receipt of AL treatment. Randomization into the study occurs with first vaccination scheduled in the first eligible member of a family compound. Subjects who are screened but not vaccinated may be replaced.

11.3 Early Termination Visit

If a subject withdraws or is withdrawn from the study after receipt of the first vaccination but before their final study visit, then they will be encouraged to return to the clinic for an Early Termination Visit, at which they will complete as many procedures of the end-of-study visit as possible.

12 Study Procedures/Evaluations

12.1 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.

12.2 Blood Draw

The amount of blood drawn will be within the limits allowed for adult and pediatric research subjects by the NIH (i.e., for children not exceeding 5 mL/kg in a single day or 9.5 mL/kg per 8-week period). Blood will be used for evaluations and assays described below and may be stored for future research.

12.3 Clinical Laboratory Testing

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

1. CBC with differential and platelet count
    - The following CBC parameters will be assessed for safety throughout the trial: WBC count, absolute neutrophil count (ANC)/absolute granulocyte count (AGC), hemoglobin, and platelet count.
    - Absolute lymphocyte count (ALC) is collected for research purposes
2. Serum Creatinine
3. ALT
4. HIV antibody test (can include rapid diagnostics, ELISA, western blot if indicated)
5. Urine dipstick/urinalysis
6. Urine and/or serum pregnancy testing (β-hCG) in females of childbearing potential

12.4 Electrocardiogram

Electrocardiograms (12-lead ECGs) will be performed during screening (Year 1 only given assessing genetic predisposition for prolonged QTc; can be repeated in Year 2 continuation if change in health or medication warranting re-evaluation) and as needed throughout the study by the study site team in Mali and read for QTc intervals by the site study team. Subjects with abnormally prolonged QTc (>450 milliseconds) will be excluded.

12.5 Malaria Diagnostics

12.5.1 Blood Smears (BS)

The gold standard for malaria diagnosis 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 A**. BS are prepared in duplicate according to standard procedures and evaluated by trained study microscopists.

For detection of gametocytemia, counts are reported per 1,000 WBCs. A positive gametocyte read is defined as a single, confirmed gametocyte seen by one reader and confirmed by the other microscopist per 1,000 WBCs.

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

Thin/thick BS will be used for detection of *P. falciparum* parasitemia as a primary efficacy endpoint for the main phase of the trial, and for malaria diagnosis throughout the study.

12.5.1.1 Symptomatic Malaria

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

Volunteers will be treated with either AL or another approved/licensed anti-malarial medication per Malian Government treatment guidelines.

12.5.2 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.

12.5.3 Malaria Polymerase Chain Reaction

While detection of parasites on thick BS remains the most common primary endpoint in human challenge trials, both PCR- and nucleic acid sequence–based amplification methods have been increasingly used to support BS data in malaria vaccine trials.[61,62] 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 BS.[63-65] 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 quantitative PCR (qPCR) that detects 18s of *P. falciparum* with a detection limit of at least 20 parasites/mL; this technique will be used during the study for comparison to traditional thick BS.

P. falciparum qPCR may be performed from all scheduled visits with a malaria BS noted (see **Appendix A**) 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 deoxyribonucleic acid (DNA) preservation, but venous blood draws can also be used.

12.6 Immunologic Laboratory Testing

12.6.1 ELISA

Anti-Pfs230 ELISAs will be performed on sera obtained from immunized subjects at MRTC in Bamako, Mali and may also be performed at collaborating laboratories.

For Pfs230D1M ELISAs, microwell plates are first 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. 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.

12.6.2 Transmission Assays

The transmission-blocking assay which will be conducted is summarized in **Table 14**.

Table 14. Transmission-Blocking Assay

Assay	Mosquitoes	Test Samples	Site
Standard Membrane-Feeding Assay	Lab strain (*Anopheles stephensi*)	Membrane feeds with lab cultured parasites mixed with test serum/plasma	LMIV LMVR
Direct Skin Feeding Assay	Colony strain (*Anopheles coluzzi*)	Mosquitoes feed directly on skin of study participants	MRTC

Feeding assays demonstrate biologic activity of transmission-blocking antibodies and are critical to selection of TBV candidates. Subjects will be screened periodically by BS (as seen in **Appendix A**) for the presence of asexual parasites and gametocytes.

12.6.3 Standard Membrane Feeding Assays

Membrane-feeding assays demonstrate biologic activity of transmission-blocking antibody and are critical to selection of vaccine candidates. SMFAs will be performed on sera obtained at baseline and periodically after vaccination as outlined in **Appendix A**. In a SMFA, test serum 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.[66] The SMFAs will be conducted at LMIV and Laboratory of Malaria and Vector Research (LMVR) in Rockville, Maryland.

SMFA will be conducted at LMIV using laboratory-strain mosquitoes and parasites. Assays will compare feedings with the following:

- 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-Pfs230-specific TBAs, SMFAs may also be conducted by the following methods:

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

12.6.4 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.[66] We believe DSFs, in which insectary-raised clean mosquitoes are directly fed on infected individuals, 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 2 years of age or more without any risks identified.[44] From 1996 to 1998, Diallo and colleagues conducted DSFs on 372 children 4 to 18 years of age without any safety problems.[45] In 2002 and 2003, DSFs were

carried out in Bancoumana and in a neighboring village on a total of 44 gametocyte carriers 6 to 18 years of age,[67] also without any reported safety problems.

More recently, as described in detail in **Section 4**, the study team in Mali has performed over 10,000 DSFs on subjects older than 5 years since June 2011. All DSF participants were actively followed 24 hours post feed and for as many as 24 DSFs. Except for 1 case of definitely related Grade 2 erythema that resolved within 48 hours and 5 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 protocol #11-I-N143, multiple DSFs during a 24-hour period have been completed in 18 subjects to date. In these 18 subjects, DSFs were well-tolerated, and there have been no reported AEs resulting from having multiple direct skin feeds completed in a short period of time.

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.

The total number of mosquitoes used for each DSF will be maintained at appropriately 60 mosquitoes, and no subject will undergo more than 8 DSFs during the study period in Year 1 and no more than 10 DSFs during the study period in Year 2. Note, if subjects 9-18 years of age are not willing to undergo a feed at the time of the DSF, they will not be withdrawn, but rather will be followed per protocol with periodic DSF or without undergoing further DSFs.

In our previous TBV protocols, DSFs started either 1 or 2 weeks post vaccination in order to time the DSFs with expected peak antibody responses. As antibody responses have significantly improved, in peak levels and more importantly durability, the question of functional activity no longer is focused on peak time periods, but rather duration of activity. Thus, at this time it is planned that post Vaccination #3, DSFs will start 14 days post Vaccination #3 and continue every 14 days for 8 total DSF assays. Post Vaccination #4, DSFs will also start 14 days post Vaccination #4 and continue every 14 days for 10 total DSF assays.

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.

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

12.6.4.1 Withdrawal from DSF

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 A**).
- 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 (CRFs) accordingly.

12.6.5 Entomological Procedures and Insectary Used in DSF

12.6.5.1 Mosquito Rearing at the Insectary

A laboratory colony of *A. gambiae (coluzzi)* 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 Rift Valley fever (RVF) virus 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.

12.6.5.2 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 the molecular form of the mosquito.

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

12.7 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.[68-70] 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[71] (stool PCR for 8 common gastrointestinal pathogens) may be completed at the time of AL dosing until first vaccination. Given that these tests are run for research purposes and retrospectively, if these results are available prior to the final study visit and parasites are detected, and if recommended, subjects will be treated with standard treatment and continued in the study. Urinary schistosomiasis testing may be completed as well at the time of screening if there is concern it may be the etiology of subject's proteinuria and/or hematuria; these subjects will receive treatment at no cost if they test positive.

12.8 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 may be used for clinical diagnostic purposes.
- qPCR may be used to detect gametocytes using whole blood collected on the day of positive BS.

- Filter paper filled with 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.[72]
- Typing of hemoglobin variants may be assessed by gel electrophoresis
- Hepatitis B and C status may be assessed by serology and/or PCR
- Cytokine levels may be evaluated during and following vaccination.

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

12.9 Collection of Malaria Prevention Measures During the Transmission Season

Following family compound/vaccine unit receipt of Vaccination #3 and #4, on a monthly basis enrolled subjects will be asked at one of their clinic visits about other malaria prevention measures being utilized by the individual or family over the last month, including use of bednets, indoor residual spraying, seasonal malaria chemoprophylaxis, personal use of insecticide, and/or intermittent preventive treatment.

12.10 Exploration of Human Movement During Transmission Season (Optional)

To maintain malaria transmission, the human reservoir, competent vector population, and the malaria parasite must overlap in space and time.[20,73-77] While there is evidence that this overlap is likely to take place in or near an individual's residence, which shares an environment with the vector, the dynamics of onward transmission from this focal point to others living further from competent vector populations is not fully understood.[78] Where individuals spend their time, how frequently they travel, and the duration of travel can inform where individuals are likely to acquire and transmit malaria.[77]

Commercially available GPS data loggers have the potential to measure human movement to capture and describe both small-scale and large-scale movement patterns simultaneously. Their low cost, availability and ease of use makes them ideal for tracking human movement to infer exposure to infection.[79] Commercially available GPS data loggers have previously been used to determine how human movement impacts other infectious diseases, such as schistosomiasis, hookworm, and dengue virus in various settings. [79-82] Commercially available GPS data loggers are able to track individual movements continuously over pre-determined period of time.[80]

Use of GPS data loggers, piloted in randomized subpopulation our DSF cohort (Arms 1a/1b, 3c/3d; 9-18 years of age) during the period of DSF assessments (starting 14 days post Vaccination #3 through 112 days post Vaccination #3) will allow for initial estimates of human

movement in rural areas of Doneguebougou, Mali and surrounding villages, which may aid in describing malaria transmission in this area.

Our targeted enrollment population, children/young adults 9-18 years of age, will have the option to participate in this portion of the study. Each participant approached for participation, if willing to participate, will be asked to carry the logger daily for up to 4 months total. Eligible participants will be provided information regarding their participation via an additional consent/assent forms which will include the use of GPS data loggers, how they can be worn or carried, and will have the opportunity to ask questions and raise concerns. Information about what the GPS logger can and cannot do will be provided for review prior to participation. Participation in this portion of the study will be optional and initial results descriptive during this pilot phase.

12.11 Human Movement (2019-2020)

The pilot study to assess the feasibility of using small GPS devices (IgotU ® GT-600) to track the movement of study participants enrolled in the study in Arms 1a/1b or 3c/3d (9-18 year-olds) was successfully implemented in August 2019. VUs with both enrolled and vaccinated male and female participants in the targeted age range were ranked and approached for participation; one male and one female from each unit were selected to facilitate comparison by sex.

Enrollment in the Optional Human Movement study is summarized in **Figure 31**.

	GPS cohort	Trial Overall*
N	101	403
Age (average, SD)	13.2, 2.5	13.2, 2.6
Sex, male (n,%)	51 (50%)	210 (35%)
Ethnicity (n, %)		
Malinke	2 (2%)	3 (1)%
Bambara	38 (38%)	156 (39%)
Peuth		13 (3%)
Sarakole	57 (56%)	226 (56%)
Other	1 (1%)	5 (1%)
Unknown	3 (3%)	
** At Enrollment*		

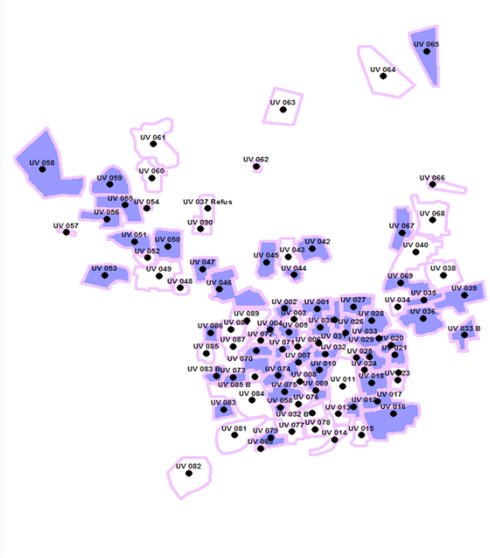


Figure 31. Summary of Human Movement Study Enrollment
Table summarizing key participant characteristics of GPS cohort (human movement subgroup) and overall trial enrollment of 9-18 year old children/young adults. Map representative of vaccine unit perimeters (abbreviated on this map = UV, referenced elsewhere as VU) in Doneguebougou, Mali with units participating in the human movement study (GPS cohort) colored in purple.

As planned, data from the units had to be pre-processed to remove coordinates from before distribution and after collection. Erroneous points, defined as the difference between two consecutive coordinates that indicated a velocity greater 10 km/hour, were flagged for removal Trajectories of individuals were built connecting the points in consecutive time points in ArcMap 10.3.1 Tracking Analyst.

Key logistical parameters of interest in this pilot were determining the failure rates of the units and the quality of the data collection over time, with failure rates seen ~10%. GPS units were well accepted by the community, with only one refusal, which was secondary to planned travel during the evaluation period. Data from Year 1 identified distinct differences in 9-18 year old male and female movements that may be informative to transmission dynamics in the community.

Given these preliminary results, the study team is proposing to continue GPS monitoring of the same children/young adults for Year 2. In addition to strengthening the analysis of movement's impact on malaria transmission and subsequent parasitemia, with the ongoing COVID-19 pandemic, any changes in movement from pre-COVID-19 and post COVID-19 will also be able to be assessed.

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

Intended Use: Samples, specimens, and data collected under this protocol may be used to study malaria and related diseases as well as vaccination. Genetic testing will be limited to hemoglobin typing and/or HLA testing. NIH researchers will have access to personally identifiable information.

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 College of American Pathologists (CAP)–certified 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). Data will be tracked as described in **Section 19.1**.

Disposition at the Completion of the Protocol: In the future, other investigators (both at NIH and outside) may wish to study these samples and/or data. Before any sharing of samples, data,

or clinical information, either IRB and EC approval must be obtained or the NIH Office of Human Subjects Research Protections (OHSRP) and FMPOS EC must determine that the research is exempt from IRB/EC oversight. OHSRP and the EC can make this determination for some research where the samples or data have no personal identifying information about the study subject and the researcher is not able to ascertain it.

At the time of protocol termination, samples will be either destroyed or, after IRB/EC approval, transferred to another existing protocol. Data will be archived by the study team in compliance with requirements for retention of research records; alternatively, after IRB/EC and study sponsor approval, the data may be either destroyed or transferred to another repository.

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 a protocol deviation, unanticipated problem (UP), and/or compromises the scientific integrity of the data collected for the study, will be reported to the NIH IRB and FMPOS EC.

Additionally, subjects may decide at any point not to have their samples stored. In this case, the PI will destroy all known remaining samples and report what was done to both the subject and to the IRB and EC. This decision will not affect the individual's participation in any other protocols at NIH.

14 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 subjects 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.

15 Assessment of Safety

15.1 Definitions

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

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

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

Serious Adverse Event (SAE): An SAE is an AE that results in one or more of the following outcomes:

- death
- a life-threatening event (places the participant at immediate risk of death from the event as it occurred)
- 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 should be exercised in deciding events that may not be immediately life threatening or result in death or 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 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 Investigational New Drug application (IND) sponsor to make this determination.

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

Unanticipated Problem: A UP is any event, incident, experience, or outcome that is

- unexpected in terms of nature, severity, or frequency in relation to
    - the research risks that are described in the IRB-approved research protocol and informed consent document, investigator's brochure, or other study documents; and
    - the characteristics of the subject population being studied; and
- possibly, probably, or definitely related to participation in the research; and
- 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): A UP that does not fit the definition of an AE, but which may, in the opinion of the investigator, involve risk to the participant, affect others in the research study, or significantly impact the integrity of research data. Such events would be considered non-serious UPs. 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 (NOCI) 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.

15.2 Documenting, Recording, and Reporting Adverse Events

At each contact with the participant, information regarding AEs 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, NIH IRB, FMPOS EC, U.S. Food and Drug Administration [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.

All abnormal laboratory findings will be reviewed on a routine basis by the PI to identify potential safety signals. An abnormal lab not included on the toxicity table should be assessed in a similar fashion to the criteria above.

15.3 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 15** below) and unsolicited AEs will be recorded through 7 days after each vaccination, including injection site reactions, or until resolved. After that period only, unsolicited AEs (including symptomatic malaria), SAEs, UPs, and NOCIs will be recorded. Note that a positive BS without associated clinical symptoms will not be reported as an AE.

Table 15. 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
Cr – increased Cr
Local reactogenicity
Injection pain/tenderness
Injection erythema/redness
Injection swelling
Injection induration
Injection pruritus
Limitation of arm movement

Abbreviations: ALT, alanine transaminase; ANC, absolute neutrophil count; AGC, absolute granulocyte count; CR, creatinine; WBC, white blood cell.

Additional laboratory abnormalities other than those specified as safety labs in the protocol should be reported as AEs 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 AE 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 C**, will be collected and graded through 7 days after each vaccination or until resolved.

The investigator will assess 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.

15.3.1 Severity

Severity of AEs will be assessed by the investigator as described in **Appendix C**. AEs not included in the Appendices will be graded for severity using the followings definitions as seen in **Table 16**.

Table 16: 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

15.3.2 Causality

Causality (likelihood that the event is caused by the study agent(s)) 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 (e.g., dechallenge, rechallenge) should 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 BS 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 IRB/EC is needed, AE relationship will be determined as noted above.

15.4 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 Safety Expedited Report Form (SERF).

SAEs that occur after the study follow-up period (e.g., after 3 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 Clinical Safety Office (CSO) as described below.

15.5 Investigator Reporting Responsibilities to the Sponsor

15.5.1 Adverse Events

Line listings, frequency tables and other summary AE data will be submitted to the IND sponsor when requested for periodic safety assessments, review of IND Annual Reports, review of IND Safety Reports, and preparation of final study reports.

15.5.2 Serious Adverse Events

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

CSO CONTACT INFORMATION:
Clinical Safety Office
5705 Industry Lane
Frederick, MD 21704
Phone: 301-846-5301
Fax: 301-846-6224
E-mail: rchspsafety@mail.nih.gov

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

SAEs that occur after the final study visit that are reported to and are assessed by the investigator to be possibly, probably, or definitely related to study drug must be reported to the CSO.

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
Email: patrick.duffy@nih.gov

15.5.3 Unanticipated Problems

UPs that are also AEs must be reported to the CSO by fax or e-mail attachment using the NIH Reportable Events Form no later than 7 calendar days of site awareness of the event. UPs that are not AEs are not reported to the CSO.

15.5.4 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 within 1 business day from site awareness.

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.

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

- Discontinue the study agent.
- Discuss with women who become pregnant continued participation in the study, including continued safety/research labs (at investigator's discretion given blood volumes), direct skin feeds, and clinical visits.
- Report to FMPOS EC as an informational item.
- Report to the DSMB, Sponsor Medical Monitor, and Malian Independent Safety Monitor (ISM).
- Advise research participant to notify the obstetrician of study participation and potential study agent exposure.

15.6 Investigator Reporting Procedures to the NIH IRB

15.6.1 Definitions

Protocol Deviation: Any change, divergence, or departure from the IRB-approved research protocol.

Major Deviations: Deviations from the IRB approved protocol that have, or may have the potential to, negatively impact, the rights, welfare or safety of the subject, or to substantially negatively impact the scientific integrity or validity of the study.

Minor Deviations: Deviations that do not have the potential to negatively impact the rights, safety, or welfare of subjects or others, or the scientific integrity or validity of the study.

Non-Compliance: Failure of investigator(s) to follow the applicable laws, regulations, or institutional policies governing the protection of human subjects in research, or the requirements or determinations of the IRB, whether intentional or not. Failure of subjects to comply with the research protocol does not represent non-compliance unless that failure is due to an action or omission of a member of the research team, for example, the failure to give adequate instruction to the subject.

Serious non-compliance: Non-compliance, whether intentional or not, that results in harm or otherwise materially compromises the rights, welfare and/or safety of the subject. Non-compliance that materially effects the scientific integrity or validity of the research may be considered serious non-compliance, even if it does not result in direct harm to research subjects.

Continuing non-compliance: A pattern of recurring non-compliance that either has resulted, or, if continued, may result in harm to subjects or otherwise materially compromise the rights, welfare and/or safety of subjects, affect the scientific integrity of the study or validity of the results. The pattern may comprise repetition of the same non-compliant action(s), or different noncompliant events. Such non-compliance may be unintentional (e.g. due to lack of understanding, knowledge, or commitment), or intentional (e.g. due to deliberate choice to ignore or compromise the requirements of any applicable regulation, organizational policy, or determination of the IRB).

15.6.1.1 Expedited Reporting to the NIH IRB and FMPOS EC

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.

15.6.1.2 Annual Reporting to the NIH IRB and FMPOS EC

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

15.7 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 the FMPOS EC. Investigators must also comply with all FMPOS EC reporting requirements, including expedited and annual reporting requirements.

15.8 Sponsor's Reporting Responsibilities

SUSARs, as defined in Title 21 of the United States Code of Federal Regulations (CFR) Part 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.

15.9 Pausing Rules for the Protocol

Pausing is the suspension of administration of study agent to a single subject or specified group until a decision is made whether or not to resume administration of the study agent.

15.9.1 Pausing Rules for an Individual Subject

The pausing criteria for a SINGLE subject in this study include any of the following; note Year 1 and Year 2 will be evaluated independently given time between dosing:

- A subject experiences one SAE that is determined as possibly, probably, or definitely related to a study agent
- A subject experiences ≥2 Grade 3 or greater AEs that are deemed not to be a SAE (solicited local/systemic or unsolicited; lasting 48 hours or more) that are possibly, probably, or definitely related to a study agent, within the 7 days post vaccination
- A subject experiences ≥2 Grade 2 or greater laboratory abnormality (see **Appendix C**) or any Grade 3 or higher laboratory abnormality that are not deemed to be a SAE and are possibly, probably, or definitely related to the study agent, within the 7 days post vaccination
- ≥1 subject experiences 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 necrosis at the injection site, within the 7 days post vaccination
- Any safety issue that the site investigator determines should pause administration of a study agent to a single subject.

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

15.9.2 Pausing Rules for a Particular Age Group

The pausing criteria for a SPECIFIC AGE group in this study include any of the following; note Year 1 and Year 2 will be evaluated independently given time between dosing:

- An age group enrolled (e.g. 5-8 years of age, 9-18 years of age) experiences one SAE that is determined as possibly, probably, or definitely related to a study agent
- ≥5% of a specific age group experiences Grade 3 or greater AEs (solicited local/systemic or unsolicited; lasting 48 hours or more) that are possibly, probably, or definitely related to a study agent, within the 7 days post vaccination
- ≥10% of a specific age group experiences Grade 2 or greater laboratory abnormality (see **Appendix C**) or ≥5% Grade 3 or higher laboratory abnormality that are possibly, probably, or definitely related to the study agent, within the 7 days post vaccination
- Any safety issue or identifiable trend in AE reporting that the site investigator determines should pause administration of a study agent to a specific age group

The CSO, in collaboration with the PI, may also pause a specific age group if a safety concern is identified during routine aggregate data analysis.

15.9.3 Reporting a Pause

If a pausing criterion is met, a description of the AE(s) or safety issue must be reported by the PI within 2 business days to the CSO, the IRB/EC, and the DSMB by fax or email.

15.9.4 Resumption of a Paused Study

The CSO, in collaboration with the PI and DSMB, will determine whether or not it is safe to resume administration of the study agent to the subject or a specific age group. The PI will notify the IRB/EC of the decision on resumption of the study agent. A subject who does not resume to receive the study agent will continue to be followed for safety.

15.10 Halting Rules for the Protocol

Halting the study requires immediate discontinuation of study agent administered for all subjects and suspension of enrollment until a decision is made whether or not to continue enrollment and study agent administration.

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); note Year 1 and Year 2 will be evaluated independently given time between dosing:

- One or more subjects experience death or any life-threatening SAE, **or**

- ≥2 subjects experience an SAE as defined in **Section 15.1** of this protocol that is determined to be possibly, probably, or definitely related to the vaccine, **or**
- ≥1 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**
- ≥1 subjects experience a hypersensitivity reaction (e.g. anaphylaxis, diffuse urticaria) that is determined to be possibly, probably, or definitely related to the vaccine, **or**
- ≥1 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 necrosis at the injection site, within the 7 days post vaccination, **or**
- ≥10% subjects enrolled 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**
- ≥10% subjects enrolled 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**
- ≥30% subjects enrolled experience the same Grade 2 or higher laboratory abnormality (see **Appendix C**) or ≥10% subjects enrolled experience the same Grade 3 or higher laboratory abnormality regardless of vaccine relationship, within the 7 days post vaccination, **or**
- ≥10% subjects enrolled 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**
- Any safety issue that the study PI or IND sponsor determines should halt the study.

The PI and/or CSO will determine if the study should be halted. In addition, the FDA may halt the study at any time following review of any safety concerns. The NIH IRB or FMPOS EC may also halt the study.

15.10.1 Reporting a Study Halt

If a halting rule is met, a description of the AE(s) or safety issue must be reported by the PI within 1 business day to the CSO, the IRB/EC, and the DSMB by fax or email.

15.10.2 Resumption of a Halted Study

The IND sponsor, in collaboration with the PI and the DSMB, will determine if it is safe to resume the study. The conditions for resumption of the study will be defined in this notification. The PI will notify the IRB/EC of the decision on resumption of the study.

15.11 Discontinuation of Study

The NIAID/Office of Clinical Research Policy and Regulatory Operations (OCRPRO) as the study sponsor, the NIH IRB, FMPOS EC, 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.

15.12 Withdrawal Criteria for an Individual Participant

A subject will not be considered to have completed the trial if any of the following reasons apply; note Year 1 and Year 2 will be evaluated independently given time between dosing:

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. The investigator will attempt to determine the reason for the subject's decision and document it in the study chart.
3. *Non-compliance with protocol* – applies to a subject who does not comply with protocol-specific visits or evaluations on a consistent basis, and to the extent that it is potentially harmful to the subject or to the integrity of the study data. 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.

15.12.1 Replacement of Withdrawn Participants or Participants Who Discontinue Study Agent

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.

15.13 Safety Oversight

15.13.1 Safety Review and Communications Plan

A Safety Review And Communications Plan (SRCP) has been developed for the protocol. The SRCP is an internal communications document between the PI and the 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.

15.13.2 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.

15.13.3 Independent 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 AEs 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 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 except for communication to the PI that the review has been completed (via in-person communication, phone, or email). All SAEs, all UPs, and all IND Safety Reports will be reported by the PI to the ISM at the same time they are submitted to the IRB/EC 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/EC on a biannual basis or more frequently if a safety concern is raised.

15.13.4 Data and Safety Monitoring Board (DSMB)

The NIAID intramural DSMB includes independent experts that do not have direct involvement in the conduct of the study and have no significant conflicts of interests as defined by NIAID policy. The DSMB will review the study prior to initiation and at the end of the study, and 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 as denoted in **Figure 23** and **Figure 24**. 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 board at the time pausing or 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/EC.

16 Site Monitoring Plan

According to the International Conference on Harmonisation (ICH) E6(R2) Good Clinical Practice (GCP) guidelines, section 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 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, DataFax data abstracts) and pertinent hospital or clinical records readily available for inspection by the FMPOS EC, FDA, the site monitors, and the NIAID staff for confirmation of the study data.

A specific protocol monitoring plan will be discussed with the PI 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.

17 Statistical Considerations

17.1 Pilot Study

17.1.1 Primary Objective

The primary objective of the pilot study is to assess the safety and reactogenicity of Pfs230D1M-EPA/AS01 in Malian children (Arms 1a, 2a) compared to comparator vaccine (Arms 1b, 2b).

- 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 study 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 (WSRT) 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, the Wilcoxon-Mann-Whitney test (WMW, also known as Wilcoxon rank sum test and Mann-Whitney U test) as well as linear regression may be used.
- SAEs occurring within the study period will be listed by relationship to vaccine.

17.1.2 Secondary Objective

The secondary objectives of this study are:

- To assess ELISA titer response to Pfs230D1M-EPA/AS01 in Malian children (Arms 1a, 2a)
- To assess functional antibody response by SMFA to Pfs230D1M-EPA/AS01 in Malian children (Arms 1a, 2a)

Anti-Pfs230 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. 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.

17.1.3 Exploratory Objective

The exploratory objective of this study is:

- To explore cellular responses and antibody repertoire of functional antibody responses to vaccination (all arms)

We will characterize and compare host immune responses and antibody repertoires to malarial antigens and to vaccine antigens in African children prior to and after vaccination with Pfs230D1M-EPA/AS01 by mixed effects model or WSRT depending on the type of immune response. We will also make figures depicting immune response based on the study arm.

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.

17.2 Main Study

17.2.1 Primary Objective

To assess vaccine activity of Pfs230D1M-EPA/AS01 against *P. falciparum* transmission by direct skin feeding assay (DSF) (Arms 1a/1b and 3c/3d only)

A Poisson type model with compound level clustering with offset for the number of DSF tests that were performed will be used. The primary analysis of activity measured by DSF will be the Poisson GEE, which will be performed on the data once with Pm/Po midguts, and once without. The proportion of subjects infecting at least 1 mosquito in at least 1 DSF over the primary analysis period following dose 3 will be compared by use of a GEE model for binary response with compound level clustering.

Each of the above analyses will be performed on the subsample of subjects that had a concurrent positive BS (any parasite sexual and/or asexual) or rapid test positive with their DSF results.

17.2.2 Secondary Objectives

To assess vaccine efficacy of Pfs230D1M-EPA/AS01 against *P. falciparum* infection measured by BS (All Arms except Arms 3e/3f)

The vaccine efficacy will be assessed by comparing the rates of positive BS in children 5-18 years of age receiving Pfs230D1M-EPA/AS01vs those receiving the comparator vaccines. An additional measure of vaccine protection will be assessed in the participants 1-4 years of age in Arms 4a/4b by comparing the rates of positive BS in the compounds receiving Pfs230D1M-EPA/AS01vs those receiving the comparator vaccines.

To assess safety and reactogenicity of administration of Pfs230D1M-EPA/AS01 (All Arms)

- 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 study 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 WSRT 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, the Wilcoxon-Mann-Whitney test (WMW, also known as Wilcoxon rank sum test and Mann-Whitney U test) as well as linear regression may be used.
- SAEs occurring within the study period will be listed by relationship to vaccine.

ELISA and SMFA assays will be conducted on a subset of samples deemed to be of scientific interest by DSF or BS assays due to the intensive laboratory analysis required to generate the data. Analysis of these data will take into account the compound randomization.

17.2.3 Exploratory Objectives

To explore cellular and humoral responses to Pfs230D1M (All Arms except Arms 4a/4b)
To identify host and parasite factors associated with transmission (All Arms)
To track transmission of parasites from human to human using highly variant gene fragments (All Arms)

GEE analyses with compound level clustering will be used to analyze ELISA, SMFA and safety endpoints.

17.3 Sample Size and Power Calculations

17.3.1 *Pilot Study*

17.3.1.1 Safety

The arms are sufficiently sized for safety. For each dose level (n=15) in Arms 1a, 2a, vaccination of 15 subjects gives a probability of at least 0.90 for detecting 1 or more serious or severe AEs that occur with a probability of 0.142 or more per subject.

If we combine all treated groups (arms 1a and 2a), 30 subjects, we have 95% power to detect 1 or more serious or severe AEs that occur with a probability of 0.095 or more per subject. Due to arms having different age ranges, 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.

17.3.1.2 ELISA Analysis

There are several questions of interest based on the antibody response information to include the change in ELISA values from baseline after a given number of doses of vaccine and the change in ELISA values between doses. For this we will use age-group-specific WSRT within the 15 subjects receiving a given vaccine regimen.

The data from Protocol #17-I-N006 on subjects who had Pfs230 ELISA measurements after receiving two doses allow us to estimate the standard deviation (SD) of the log transformed Pfs230 ELISA responses at baseline (mean 3.688401 sd 0.5490291) and 3 months post vaccination 2 (mean 5.356736 sd 0.8868963).

Assuming similar values in this trial, there would be over 0.99 power to detect the difference between baseline and 3 months post-vaccination 2. This approximate power is calculated counting the number of significant comparisons out of a thousand comparisons between random normal draws from N(3.68,0.55) and N(5.36, 0.88) using the Wilcoxon test.

Using the background information from #17-I-N006, 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 2.3-fold higher geometric mean than the level of detection in the vaccinated group post vaccination 2 (Note: 2.3-fold is lower than the 5.31-fold observed in #17-I-N006, so we treat these numbers as conservative). This approximate power is calculated counting the number of significant comparisons out of a thousand comparisons between random normal draws from N(3.68,0.55) and N(3.68+log(2.3), 0.88) using the Wilcoxon test.

17.3.1.3 SMFA Analysis

For a group of 15 participants we anticipate at least 60% power to detect a difference in TRAs of 32% (the observed difference in #17-I-N006) and 80% power for a 40% difference. These Power calculations come from a simulation assuming values similar to the SMFA data from Protocol #17-I-N006, where the control group had an average TRA of 38 (sd 39) and a treatment group with sd 34. Thus counting the number of significant comparisons out of a thousand comparisons between random normal draws from N(38,39) and N(38+40, 34) using the Wilcoxon test can give approximate power.

If TBAs are used, we will standardize the TBAs to a common target control mean first using the methods of Swihart, Fay, & Miura (2018). A nice feature of the standardized TBA is that its power is identical to those of the TRA.

17.3.2 Main Study

17.3.2.1 Vaccine Activity

Vaccine activity will be measured by DSFs in children 9-18 years of age. The Bancoumana Community Study assessed monthly DSFs in children 10-18 years of age from March to August with a seasonal DSF positivity rate (i.e. at least 1 positive DSF) of 6%. We anticipate that this seasonal DSF rate will be increased to 12-16% for children 9-18 years of age during the rainy season with twice-monthly DSF testing. Doneguebougou has 560 children 9-18 years of age. We conservatively estimate that about 400 of these will be randomized and not drop out of the study.

Since the study is randomized by compound, clustering due to compound needs to be addressed in the power calculations. The Bancoumana Community Study provided data on seasonal parasitemia positivity which we use as a proxy for seasonal DSF positivity. For parasitemia, the intra-compound-correlation (ICC) was estimated to be .01 using a beta-binomial model. This low ICC coupled with the large number of compounds and relatively small number of subjects 9-18 years of age per compound (median of 4) indicates that clustering will be negligible. Power was simulated for a study with 100 compounds and 400 subjects with monthly DSF testing for 4 months. Since testing will be twice-monthly, simulating monthly testing will be conservative. The intra-subject correlation (ISC) for monthly serial DSFs was estimated to be .14 using a beta-binomial model for children 10-18 years of age from the Bancoumana Community Study. Four months of DSFs per child were generated using a beta-binomial model with ISC=.14 and parameters chosen to achieve seasonal DSF positivity rates of 12-16% and seasonal vaccine activities (VAs) for the probability of a positive DSF of .50 or .60. A GEE model with compound level clustering and the number of positive DSFs as outcome was used for analysis. Ten

thousand clinical trials were simulated. Powers are provided in **Table 17** and **Table 18** which indicates we have power greater than .80 for scenarios with a seasonal DSF rate of 12-16%.

Data collection for the Age DE study during the 2019 season was encouraging for achievement of the primary endpoint, positive Direct Skin Feeds. As of March, there were 45 unique individuals with a positive DSF of the 369 participants monitored for this endpoint in the 9-18 year old cohort.

Our initial sample size for the power calculations for the VA in the Main Study was based on an assumption about seasonal infection rates ranging from 0.12-0.16 and power to reject a null hypothesis of no difference in rate of DSF positivity by arm is ultimately a function of the overall rate of DSF positivity and the true vaccine activity (**Table 17**). The current data indicates that the seasonal infection rate for the period monitored by DSF was 12% which was below the desired power threshold of 0.8.

Table 17. Sample Size Power Calculations Based on Seasonal DSF Infection Rates and Seasonal Vaccine Activity

	Number of DSF+ Individuals Needed		
Power	**VA = 0.4**	**VA = 0.5**	**VA = 0.6**
0.8	115	64	40
0.9	153	87	53

As we remain blinded, we simulate power under three different scenarios for vaccine activity (**Figure 32)**. In the first scenario, we assume that vaccine activity is 0.4. In this simulation, 115 unique individuals would need to experience a DSF+ in order to reject the null hypothesis of no difference at alpha= 0.5 with a type 1 error rate of 0.8. In the scenario of 0.5 vaccine activity, 64 unique individuals would need to experience a positive feed. In the scenario with 0.6 VA, 40 unique individuals would need to experience a positive feed to achieve 0.8 power.

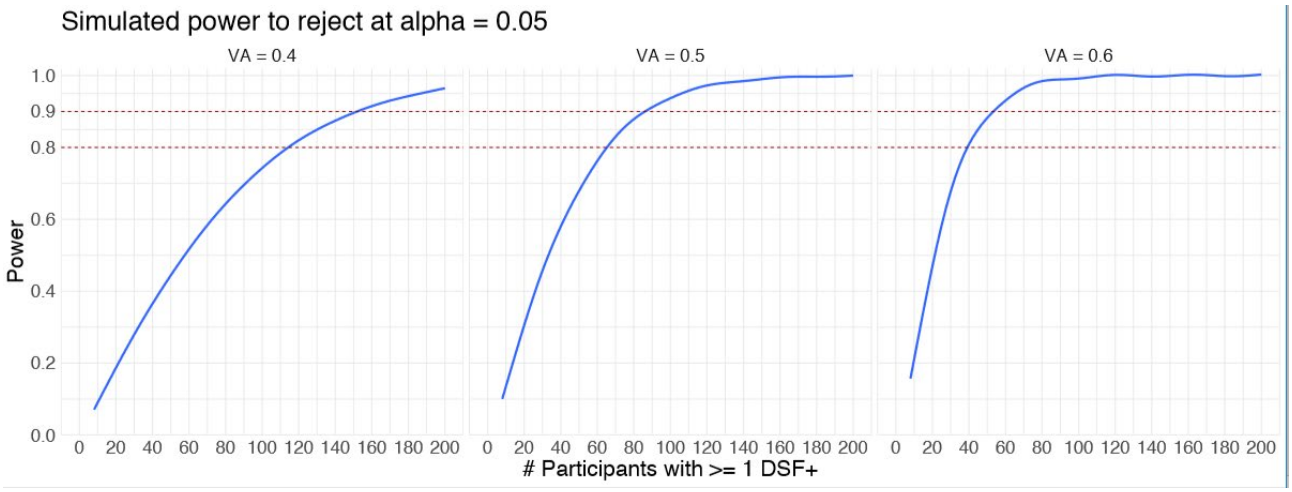


Figure 32. Simulated power under assumptions of 0.4, 0.5, and 0.6 vaccine activity.

These simulations would indicate that a second year of study is required in order to capture more of the seasonal infections, as the DSFs were monitored only from September – March. To guard against scenarios in which the VA falls in the lower ranges, we intend to combine the endpoints from Year 1 and Year 2 to achieve the needed power to distinguish vaccine activity. With additional follow up time, it appears feasible to have power for the all above scenarios.

17.3.2.2 Vaccine Efficacy

Vaccine efficacy will be measured by blood smear (BS) and detection of asexual Pf parasitemia. The background infection rate in the population is measured as the proportion of individuals from a recent PfSPZ trial (NIAID Protocol #16-I-N004) with any BS+ during follow up. Using this as the rate of background infection we can calculate power under various scenarios using as endpoint parasitemia positivity over the season. We use simple binomial tests with alpha =.05 two-sided and ignore the anticipated negligible effects of compound level clustering. Sample sizes are multiplied by an inflation factor of two to adjust for potential compound level contamination of the vaccine effects due to mixing of mosquitoes or movement of vaccinees. We see that with a minimum of 350 total subjects 1-8 years of age that are randomized and do not drop out, we have power to detect VEs of 30% or greater within this age group alone based on the risk of first infection. We will analyze subjects 9-18 years of age based on the proportion of positive blood smears.

Table 18. Vaccine Efficacy Sample Size Power Calculations Based on Predicted Vaccine Efficacy

Background Infection Rate	VE	Power	Total Sample Size	Total Sample Size per Arm After Adjustment for mosquito-human contamination
70%	20%	80%	375	750
70%	25%	80%	242	484
70%	30%	80%	172	343
70%	35%	80%	128	256
70%	40%	80%	98	196

17.3.2.3 Analysis

The primary DSF analysis will use a GEE model with compound level clustering and the number of positive DSFs as outcome will be used for analysis with an offset for the number DSF tests. The primary parasitemia analysis will be analogous. The primary analysis population will follow the intention-to-treat principle. Additional analyses will explore the effects of different covariates such as time since start of the rainy season, SMC treatment, compound-level vaccine coverage, or proximity to a control compound on vaccine efficacy.

The primary safety and immunogenicity analyses will compare the rates of AEs or levels of antibody/activity between vaccinated and control individuals using standard statistical methods that respect compound-based randomization, as appropriate.

17.4 Randomization

See **Section 11.2** for more information on individual study schedule.

This study will be cluster-randomized with family compounds, or groups of compounds (vaccine units) serving as the unit of comparison. A listing of the compounds in the study area with the numbers of individuals residing in their compounds will be created and used for randomization. The number of vaccine units will be the similar for each group. Enumerated compounds should have a target of approximately 80% participation rate of eligible participants for inclusion as a study unit, but consideration will be given to the number of children to finalize the decision for inclusion. Randomization for the full trial needs to be completed prior to the age de-escalation so that the randomization for vaccinated participants in the pilot phase study holds through the main phase.

18 Ethics/Protection of Human Subjects

This research will be conducted in compliance with the protocol, GCP, and all applicable regulatory requirements.

18.1 Institutional Review Board (IRB) and Ethics Committee (EC)

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 IRB and EC for written approval. The investigator must submit and obtain approval from the IRB/EC for all subsequent amendments to the protocol, informed consent documents, and other study documentation referenced above. The investigator will be responsible for obtaining IRB/EC approval of the annual continuing review throughout the duration of the study. The investigators will notify the reviewing IRB/EC of protocol violations and SAEs as specified in the relevant sections of the protocol.

18.2 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 ongoing 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 and assent forms will be approved by the participating IRB/EC. The subject's parent/guardian will sign the informed consent document prior to undergoing any research procedures. Children aged 7-17 years old (unless emancipated per Malian law, then will sign consent) will sign the assent document prior to undergoing any research procedures. The subjects or their parents/guardians may withdraw assent or 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's parent/guardian or the subject and the person who conducted the informed consent discussion. The original signed informed consent form, and if applicable the assent, will be retained in the subject's chart and a signed and dated copy will be provided to the subject and/or their parent/guardian. 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.

18.2.1 Mali Site Community Permission and Individual Informed Consent Process

18.2.1.1 Community Permission

Community permission will be obtained from village elders, family heads, and other community members after explanation and discussion of the study.[83] 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 forms 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 enrollment.

18.2.1.2 Individual Informed Consent

Local households and families will be invited to come to the study clinic for review of the informed consent. Potential subjects will be included along with their parent(s)/legal guardian(s) in discussions about the study. Written assent will be obtained if the child is at least 7 years old and has the maturity and psychological ability to give assent, as judged by the study member obtaining consent. Two parents/legal guardians will provide permission for the minor subject to participate by signing/fingerprinting the consent form.

At the consenting visit, the potential subject and their parent/guardian will read the consent or assent 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 and/or subjects' parents will be encouraged to ask questions and then will take a multiple-choice questionnaire (true/false; Malaria Comprehension Exam) 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.

18.2.2 Informed Consent Process for Minors Who Reach the Age of Consent

Legally effective informed consent will be obtained from subjects who initially enrolled on the study as a minor and remain on study when they reach the age of consent, even if the research does not involve any ongoing interactions or interventions with the subject but continues to meet the regulatory definition of "human subjects research" (e.g., it involves the continued analysis of identifiable specimens or data). Subjects and their families will be told of this requirement at the time of initial enrollment. We will make a reasonable attempt to reach subjects who have completed study participation or are lost to follow-up; however, if we are unable to contact the subject for reconsent, we request that the requirements for obtaining informed consent are waived, provided the IRB/EC agrees that the following conditions are met:

1. The research involves no more than minimal risk to the subjects.
    - The research samples and data will have already been collected and thus the ongoing analysis will not require additional contact with the former subject.
2. The waiver will not adversely affect the rights and welfare of the subjects.
    - This study focuses on a better understanding of malaria, vaccination, and immunology. Participation in this study should in no way affect the rights and welfare of subjects.
3. The research could not be practicably carried out without the waiver.
    - Ongoing analysis of the samples and data collected in this study is needed to address the study objectives. Thus, research would be hindered if analysis were halted.
4. Whenever appropriate, the subjects must be provided with additional pertinent information after participation.

- If former subjects are recontacted in the future, any new results obtained since the last contact will be provided to them.

18.2.3 Informed Consent Process for Emancipated Minors

A legally married minor female 16 years old and above is considered emancipated according to Malian law. Therefore, married female minors 16 years old and above will be able to provide legally effective informed consent to volunteer in this study.

18.3 Subject Confidentiality

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, including but not limited to, medical records. Records will be kept locked and data will be coded. Any personally identifiable information maintained for this study will be kept on restricted-access computers and networks. Personally identifiable information will only be shared with individuals authorized to receive it under this protocol. Individuals not authorized to receive personally identifiable information will be provided with coded information only, as needed. Clinical information will not be released without written permission of the subject, except as necessary for monitoring by NIH IRB, FMPOS EC, the FDA, NIAID, OHRP, or the sponsor's designee.

18.4 Potential Risks

Risks to the subjects are associated with vaccination and venipuncture. These risks are outlined below:

18.4.1 Study Vaccine

18.4.1.1 Intramuscular (IM) Vaccinations

Possible local vaccine reactions resulting from IM 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 effects will be monitored but are generally mild and self-limiting.

18.4.1.2 Immunization with Pfs230D1M-EPA/AS01

18.4.1.3 Pfs230D1M

Common solicited AEs during past immunization with Pfs230D1M-EPA/Alhydrogel and Pfs230D1M-EPA/AS01 include injection site pain, redness, induration, and swelling. Other commonly reported solicited AEs include headache, malaise, diarrhea, nausea, and fever.

While several subjects in the US and the Mali studies experienced changes in hematologic 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, no neutropenia events were 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 6 days following receipt of her fourth study vaccination with an acute onset of hemiplegia. She was diagnosed as having a cerebrovascular accident via computerized tomography (CT) scan and neurological assessment at the hospital in Bamako, Mali and was hospitalized. Her symptoms worsened overnight, and she died the following day. This SAE was reviewed at length by the study team, ISM, 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. At this time, we do not believe that arterial or venous occlusion are possible risk factors associated with vaccination, but we will provide as much available information to future study subjects as possible.

18.4.1.4 AS01

Overall, with the exception of local reactogenicity (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.

18.4.1.5 EPA

EPA has been studied in both malaria transmission vaccine studies (as noted with Pfs230D1M) and other vaccination studies.[11-13,84,85] 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.

18.4.1.6 Other Risks of the Study Vaccine

The study vaccine can cause non-specific inflammation and may be harmful while pregnant (**Section 9.8**). Women of reproductive potential will be required to agree to use birth control as outlined in **Section 9.4**. 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 and periodically throughout the course of the study as outlined in **Appendix A**.

18.4.1.7 Comparator vaccine

18.4.1.7.1 HAVRIX

In studies of adults and children 2 years of age and older, the most common solicited adverse events were injection-site soreness (56% of adults and 21% of children) and headache (14% of adults and less than 9% of children). Syncope (fainting) can occur in association with administration of injectable vaccines.

Data are limited regarding use of the hepatitis A vaccine during breast feeding and its excretion in human milk is unknown. The manufacturer recommends caution when administering to nursing mothers; however according to the ACIP, inactivated vaccines pose no risk to breast-feeding mothers or their infants and is not contraindicated for administration.

For complete safety details, please refer to the package insert provided. If for some unexplained reason, HAVRIX is not available, another Hepatitis A inactivated vaccine approved, either by FDA and/or WHO, in all enrolled age groups will be used instead. The package insert for that particular Hepatitis A vaccine will be submitted to NIH IRB/FMPOS EC, FDA, and Sponsor prior to use.

18.4.1.7.2 TYPHIM Vi

TYPHIM Vi can commonly (≥10%) cause fevers, malaise, headache, myalgias and local injection site reactions, in particular during the first 48 hours post vaccination. Less commonly (1-10%), it can cause additional systemic symptoms, including abdominal pain, nausea, vomiting, diarrhea, rash, pruritus.

Data are limited regarding use of the TYPHIM Vi and/or inactivated, subunit vaccines in general during breast feeding and its excretion in human milk is unknown. The manufacturer recommends caution when administering to nursing mothers; however, according to the ACIP, inactivated vaccines pose no risk to breast-feeding mothers or their infants and are not contraindicated for administration.

For complete safety details, please refer to the package insert provided. If for some unexplained reason, TYPHIM Vi is not available, another typhoid inactivated vaccine approved, either by FDA and/or WHO, in all enrolled age groups will be used instead. The package insert for that particular typhoid vaccine will be submitted to NIH IRB/FMPOS EC, FDA, and Sponsor prior to use.

18.4.1.7.3 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.

Data are limited regarding use of the Menactra® and/or inactivated, subunit vaccines in general during breast feeding and its excretion in human milk is unknown. The manufacturer recommends caution when administering to nursing mothers; however, according to the ACIP, inactivated vaccines pose no risk to breast-feeding mothers or their infants and are not contraindicated for administration.

For complete safety details, please refer to the package insert provided. If for some unexplained reason, Menactra® is not available, another meningococcal vaccine approved, either by FDA and/or WHO, in all enrolled age groups will be used instead. The package insert for that particular meningococcal vaccine will be submitted to NIH IRB/FMPOS EC, FDA, and Sponsor prior to use.

18.4.2 Artemether/Lumefantrine (AL)

All enrolled subjects will receive either one or two treatment courses with AL (e.g. Coartem) during the study regardless of their malaria status. A treatment course of AL for all ages is daily administration of AL over 3 consecutive days. AL is a registered, licensed, proven, and highly efficacious oral treatment. AL will be provided by the study team or designee for directly observed treatment by all study participants. This may occur outside of the clinical research site, but dosing and timing of dosing will be documented by a trained study team member.

AL 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%) are: headache, anorexia, dizziness, asthenia, arthralgia, and myalgia. Discontinuation of AL due to AE is rare (0.2%). Rare but serious hypersensitivity reactions (urticarial and angioedema) and skin reactions (bullous eruption) have been reported post marketing. AL should be taken with milk or another fatty food.

AL is a Category C pregnancy drug. Thus, all female participants will undergo pregnancy testing prior to receipt of the investigational dose of AL.

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

18.4.3 Venipuncture

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

18.4.4 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 12.6.5**) 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 RVF virus, and RVF has been reported to transovarially infect anopheles.[86] 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.[87] 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 RVF virus using molecular methods. Testing conducted over the last 2 years in Mali on LMIV/MRTC clinical trials have all been negative for RVF virus. 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 12.6.5**).

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 4 and 12.6.4),** hundreds of subjects in Bancoumana or neighboring areas have been enrolled in DSFs. Except for 1 case of definitely related Grade 2 erythema that was resolved within 48 hours and 5 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.

18.4.5 Other Risks

Women of reproductive potential will be required to agree to use birth control as outlined in **Section 9.4**. 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 A**.

18.5 Potential Benefits

Given the family compound design of the study, broad family compound/vaccine unit coverage (approximately 80%), and supporting evidence that malaria transmission is local, there is the potential benefit to all enrolled in regard to decreased malaria infection incidence where they live, including their own risk of future infection in the same transmission season.

18.6 Compensation

Subjects (adults or parents) will be given in kind (such as rice and/or millet) or cash equivalent, in multiple installments as outlined in **Table 19** and **Table 20**, 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 FMPOS EC recommends compensating the study subject for their time lost for study procedures. The amount equivalent to $6 in United States dollars (USD) for each scheduled visit with laboratory procedures/feeding procedures and equivalent to USD $3 for each scheduled visit without laboratory procedures.

Table 19: Estimated Compensation Schedule (Year 1)[1]

Study Activity	Number of Visits	US Dollar	Local Currency (CFA)[2]
1-4 years old			
Screening	1	$3	1500
Study Visits	17	$102	51000
Extra 9, 12 month visits[4]	1	$6	3000
Total	**19**	**$111**	**55500**
5-8 years old			
Screening	1	$6	3000
Vaccination #1	6	$30	15000
Vaccination #2	5	$24	12000
Vaccination #3	16	$84	42000
Extra Pilot Arm Visits	3	$12	**6500**
Extra 9, 12 month visit[4]	1	$6	3000
Total	**32/29**	**$162/$150**	**81000/75000**
9-18 years old			
Screening	1	$6	3000
Vaccination #1	6	$30	15000
Vaccination #2	5	$24	12000
Vaccination #3	16	$93	46500
Extra Pilot Arm Visits	3	$12	**6500**
Extra 9, 12 month visit[4]	1	$6	3000
Total	**32/29**	**$171/$159**	**85500/79500**
19 years of age and older			
Screening	1	$6	3000
Vaccination #1	6	$30	15000
Vaccination #2	5	$24	12000
Vaccination #3	11	$54	27000
Extra 9, 12 month visit[4]	1	$6	3000
Total	**24**	**$120**	**60000**
Unscheduled visits (all ages)			
Unscheduled visits[3]	TBD	**$3-6**	**1500-3000**
GPS Participation			
Per month	6	$36	18000

[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
[3] Compensation for unscheduled visits will be dependent if blood draw is indicated for research purposes
[4] 12 month follow-up visit = rescreening visit, so compensation included on Year 2 schedule now.

Table 20: Estimated Compensation Schedule(Year 2)[1]

Study Activity	Number of Visits	US Dollar	Local Currency (CFA)[2]
1-4 years old (Arms 4a/4b)			
Rescreening	1	$3	1500
Study Visits	18	$108	54000
Total	**19**	**$111**	**55500**
5-8 years old (Arms 2a/2b, 3a/3b)			
Rescreening	1	$6	3000
Study Visits	18	$108	54000
Total	**19**	**$114**	**57000**
9-18 years old (Arms 1a/1b, 3c/3d)			
Rescreening	1	$6	3000
Study Visits	17	$102	51000
Total	**18**	**$108**	**54000**
19 years of age and older (Arms 3e/3f)			
Rescreening	1	$6	3000
Study Visits	10	$57	28500
Total	**11**	**$63**	**31500**
Unscheduled visits (all ages)			
Unscheduled visits[3]	TBD	**$3-6**	**1500-3000**
GPS Participation			
Per month	5	$30	15000

[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
[3] Compensation for unscheduled visits will be dependent if blood draw is indicated for research purposes

19 Data Handling and Record Keeping

19.1 Data Capture and Management

In Mali, study data will be entered directly into a study-specific DataFax electronic database. Data from electronic CRFs will be collected directly from subjects during study visits and telephone calls or will be abstracted from subjects' medical records. Electronic CRFs and supporting laboratory, entomology documentation will be used as source. Any type of

corrections to the electronics CRFs will be documented and tracked. 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. NIH researchers will have access to personally identifiable information.

19.2 Record Retention

The investigator is responsible for retaining all essential documents listed in the ICH GCP guidelines. Study records will be maintained by the PI according to the timelines specified in 21 CFR 312.62 or a minimum of 5 to 7 years, and in compliance with institutional, IRB/EC, 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 and the FMPOS EC with the name of the person who will accept responsibility for the transferred records and/or their new location. NIAID and the FMPOS EC will be notified in writing and written OCRPRO/NIAID and FMPOS EC permission shall be obtained by the site prior to destruction or relocation of research records.

19.1 Protocol Revisions

No revisions to this protocol will be permitted without documented approval from the NIH IRB/FMPOS EC that granted the original approval for the study. Any change to the protocol will be submitted to the Sponsor and to the participating IRB/EC 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: Schedule of Procedures/Evaluations

Year 1: Arms 1a/1b (9-18 years of age; PILOT):

Arms 1a/1b (9-18 year olds)		Months			0					1					2	3	4					5		6		7		8		9		10		13
		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	28	29	30	31
Procedures	Blood Volume	Study Day	Screening (-56)	-7	0	1	3	7	14	28	29	31	35	42	56	84	112	126	127	129	133	140	154	168	182	196	210	224	238	252	266	280	294	378
		Days post Vac		-7	0	1	3	7	14	0	1	3	7	14	28	56	84	0	1	3	7	14	28	42	56	70	84	98	112	126	140	154	168	252
		Visit Windows (days) (1)		±7		0	±1	±2	±3	±7	0	±1	±2	±3	±7	±14	±28	±56	0	±1	±2	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±28	±56
Clinical Procedures																																		
Complete medical history/ physical			X																														
Informed consent			X																														
EKG			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
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
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
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
VACCINATION					X					X								X															
Artemether/Lumefantrine				X																													
Unblinding																																		
Feeding Evaluation (1)																						X	X	X	X	X	X	X	X					
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		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		3.0												
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					
Schistosomiasis +/- 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
Malaria PCR/Genotyping/ Gametocyte	CAP/EDTA			1.0	1.0				1.0	1.0				1.0			1.0	1.0				1	1	1	1	1	1	1	1	1.0	1.0	1.0	1.0	1.0
Pfs230 ELISA	SST				10				5	5				5			5	5			5	10			5				5			5	5
SMFA (2)																																		
DSF (3)	X																					X	X	X	X	X	X	X	X					
Transcriptional analysis	PAXGene			1	1		1					1								1		1											1	
Daily blood draw volume in mL			10.0	7.5	17.5	0.0	6.0	0.0	11.5	11.5	0.0	6.0	0.0	11.5	0.0	0.0	11.5	11.5	0.0	6.0	5.0	17.5	1.5	1.5	6.5	1.5	1.5	1.5	6.5	1.5	1.5	1.5	7.5	6.5
Cumulative blood volume in mL			10.0	17.5	35.0	35.0	41.0	41.0	52.5	64.0	64.0	70.0	70.0	81.5	81.5	81.5	93.0	104.5	104.5	110.5	115.5	133.0	134.5	136.0	142.5	144.0	145.5	147.0	153.5	155.0	156.5	158.0	165.5	172.0

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

2 SMFA may be completed on 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 monthly post Vaccination #3 through day 112 post Vaccination #3 (total = 8 feeds).

Year 1: Arms 3c/3d (9-18 years of age, MAIN):

Arms 3c/3d (9-18 year olds)		Months			0					1					2					3		4		5		6		7		8	11
		Visits		1	2	3	4	5	6	7	8	9	10	11 (4)	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
Procedures	Blood Volume	Study Day	Screening (-56)	-7	0	1	3	7	14	28	29	31	35	42	56	57	59	63	70	84	98	112	126	140	154	168	182	196	210	224	308
		Days post Vac		-7	0	1	3	7	14	0	1	3	7	14	0	1	3	7	14	28	42	56	70	84	98	112	126	140	154	168	252
		Visit Windows (days) (1)		±7		0	±1	±2	±3	±7	0	±1	±2	±28	±56	0	±1	±2	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±28	±56
Clinical Procedures																															
Complete medical history/ physical			X																												
Informed consent			X																												
EKG			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
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
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
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
VACCINATION					X					X					X																
Artemether/Lumefantrine				X																											
Unblinding																															
Feeding Evaluation (1)																			X	X	X	X	X	X	X	X					
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												
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						
Schistosomiasis +/- 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
Malaria PCR/Genotyping/ Gametocyte	CAP/EDTA			1.0	1.0				1.0	1.0				1.0	1.0				1	1	1	1	1	1	1	1.0	1.0	1.0	1.0	1.0
Pfs230 ELISA	SST				10				5	5				5	5			5	10			5				5				5	5
SMFA (2)																															
DSF (3)	X																		X	X	X	X	X	X	X	X					
Transcriptional analysis	PAXGene			1	1		1					1					1		1											1	
Daily blood draw volume in mL			10.0	7.5	17.5	0.0	6.0	0.0	11.5	11.5	0.0	6.0	0.0	11.5	11.5	0.0	6.0	5.0	17.5	1.5	1.5	6.5	1.5	1.5	1.5	6.5	1.5	1.5	1.5	7.5	6.5
Cumulative blood volume in mL			10.0	17.5	35.0	35.0	41.0	41.0	52.5	64.0	64.0	70.0	70.0	81.5	93.0	93.0	99.0	104.0	121.5	123.0	124.5	131.0	132.5	134.0	135.5	142.0	143.5	145.0	146.5	154.0	160.5

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

2 SMFA may be completed on 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 monthly post Vaccination #3 through day 112 post Vaccination #3 (total = 8 feeds).

4 Note this visit may occur twice depending on the approval of the V3.0 protocol; the first D42 visit (D42A) will include all D42 procedures EXCEPT AL dosing. This will visit will occur to maintain safety labs and assessments are completed on ALL subjects approximately 14 days post Vaccination #2 (±3 days). If this visit occurs, the next visit will be D42B for AL dosing and completed of ALL procedures as outlined in the visit (if D42A completed >14 days from D42B). This is to ensure the AL dosing in participants occurs approximately 14 days prior to Vaccination #3. If both visits are completed, subjects will receive an additional 3000 CFA ($6 USD).

Year 1: Arms 2a/2b (5-8 years of age; PILOT):

Arms 2a/2b (5-8 year olds)		Months			0					1					2	3	4					5		6		7		8		9		10		13
		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	28	29	30	31
Procedures		Study Day	Screening (-56)	-7	0	1	3	7	14	28	29	31	35	42	56	84	112	126	127	129	133	140	154	168	182	196	210	224	238	252	266	280	294	378
	Blood Volume	Days Post Vac		-7	0	1	3	7	14	0	1	3	7	14	28	56	84	0	1	3	7	14	28	42	56	70	84	98	112	126	140	154	168	252
		Visit Windows (days) (1)		±7		0	±1	±2	±3	±7	0	±1	±2	±3	±7	±14	±28	±56	0	±1	±2	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±28	±56
Clinical Procedures																																		
Complete medical history/ physical			X																														
Informed consent			X																														
EKG			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
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
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
Artemether/Lumefantrine				X														X																
VACCINATION					X					X								X																
Unblinding																																		
Laboratory Procedures																																		
CBC with differential	EDTA		2	2	2		2		2	2		2		2			2	2		2		2												
ALT/ Creatinine	SST		3	3	3		3		3	3		3		3			3	3		3		3												
HIV testing	SST		5																															
Urine dipstick or Urinalysis	Urine Container		X																															
Hemoglobin Typing (2)	EDTA			(X)																														
Schistomiasis testing	Urine Container			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
Malaria PCR/Genotyping/ Gametocyte	CAP/EDTA			1	1				1	1				1			1	1				1	1	1	1	1	1	1	1	1	1	1	1	1
Pfs230 ELISA	SST				10				5	5				5			5	5				5			5				5				5	5
SMFA (3)																																		
Transcriptional Analysis	PaxGene			1	1		1					1								1													1	
Daily blood draw volume in mL			10	7.5	17.5	0	6	0	11.5	11.5	0	6	0	11.5	0	0	11.5	11.5	0	6	0	11.5	1.5	1.5	6.5	1.5	1.5	1.5	6.5	1.5	1.5	1.5	7.5	6.5
Cumulative blood volume in mL			10	17.5	35	35	41	41	52.5	64	64	70	70	81.5	81.5	81.5	93	104.5	104.5	110.5	110.5	122	123.5	125	131.5	133	134.5	136	142.5	144	145.5	147	154.5	161

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

2 Hemoglobin typing, if not completed at screening, given completed retrospectively, can be completed at any time when there's a scheduled blood draw with an EDTA tube

3 SMFA may be completed on 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.

Year 1: Arms 3a/3b (5-8 years of age; MAIN):

Arms 3a/3b (5-8 year olds)		Months			0					1					2					3		4		5		6		7		8	11
		Visits		1	2	3	4	5	6	7	8	9	10	11 (4)	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
Procedures		Study Day	Screening (-56)	-7	0	1	3	7	14	28	29	31	35	42	56	57	59	63	70	84	98	112	126	140	154	168	182	196	210	224	308
	Blood Volume	Days Post Vac		-7	0	1	3	7	14	0	1	3	7	14	0	1	3	7	14	28	42	56	70	84	98	112	126	140	154	168	252
		Visit Windows (days) (1)		±7		0	±1	±2	±3	±7	0	±1	±2	±28	±56	0	±1	±2	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±28	±56
Clinical Procedures																															
Complete medical history/ physical			X																												
Informed consent			X																												
EKG			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
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
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
Artemether/Lumefantrine				X										X (4)																	
VACCINATION					X					X					X																
Unblinding																															
Laboratory Procedures																															
CBC with differential	EDTA		2	2	2		2		2	2		2		2	2		2		2												
ALT/ Creatinine	SST		3	3	3		3		3	3		3		3	3		3		3												
HIV testing	SST		5																												
Urine dipstick or Urinalysis	Urine Container		X																												
Hemoglobin Typing (2)	EDTA			(X)																											
Schistomiasis testing	Urine Container			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
Malaria PCR/Genotyping/ Gametocyte	CAP/EDTA			1	1				1	1				1	1				1	1	1	1	1	1	1	1	1	1	1	1	1
Pfs230 ELISA	SST				10				5	5				5	5				5			5				5				5	5
SMFA (3)																															
Transcriptional Analysis	PaxGene			1	1		1					1					1													1	
Daily blood draw volume in mL			10	7.5	17.5	0	6	0	11.5	11.5	0	6	0	11.5	11.5	0	6	0	11.5	1.5	1.5	6.5	1.5	1.5	1.5	6.5	1.5	1.5	1.5	7.5	6.5
Cumulative blood volume in mL			10	17.5	35	35	41	41	52.5	64	64	70	70	81.5	93	93	99	99	110.5	112	113.5	120	121.5	123	124.5	131	132.5	134	135.5	143	149.5

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

2 Hemoglobin typing, if not completed at screening, given completed retrospectively, can be completed at any time when there's a scheduled blood draw with an EDTA tube

3 SMFA may be completed on 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 Note this visit may occur twice depending on the approval of the V3.0 protocol; the first D42 visit (D42A) will include all D42 procedures EXCEPT AL dosing. This will visit will occur to maintain safety labs and assessments are completed on ALL subjects approximately 14 days post Vaccination #2 (±3 days). If this visit occurs, the next visit will be D42B for AL dosing and completed of ALL procedures as outlined in the visit (if D42A completed >14 days from D42B). This is to ensure the AL dosing in participants occurs approximately 14 days prior to Vaccination #3. If both visits are completed, subjects will receive an additional 3000 CFA ($6 USD).

Year 1: Arms 3e/3f (Adults 19 years of age and older):

Arms 3e/3f (Adults 19 years of age and older)		Months			0					1					2					3	4	5	6	7	8	11
		Visits		1	2	3	4	5	6	7	8	9	10	11 (3)	12	13	14	15	16	17	18	19	20	21	22	23
Procedures	Blood Volume	Study Day	Screening (-56)	-7	0	1	3	7	14	28	29	31	35	42	56	57	59	63	70	84	112	140	168	196	224	308
		Days post Vac		-7	0	1	3	7	14	0	1	3	7	14	0	1	3	7	14	28	56	84	112	140	168	252
		Visit Windows (days) (1)		±7		0	±1	±2	±3	±7	0	±1	±2	±28	±56	0	±1	±2	±7	±7	±7	±7	±7	±7	±28	±56
Clinical Procedures																										
Complete medical history/ physical			X																							
Informed consent			X																							
EKG			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
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
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
VACCINATION					X					X					X											
Artemether/Lumefantrine				X																						
Unblinding																										
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							
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											
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
Malaria PCR/Genotyping/ Gametocyte	CAP/EDTA			1.0	1.0				1.0	1.0				1.0	1.0				1		1		1		1.0	1.0
Pfs230 ELISA	SST				10				5	5				5	5			5	10		5		5		5	5
SMFA (2)																										
Transcriptional analysis	PAXGene			1	1		1					1					1		1						1	
Daily blood draw volume in mL			10.0	7.5	17.5	0.0	6.0	0.0	11.5	11.5	0.0	6.0	0.0	11.5	11.5	0.0	6.0	5.0	17.5	0.0	6.5	0.0	6.5	0.0	7.5	6.5
Cumulative blood volume in mL			10.0	17.5	35.0	35.0	41.0	41.0	52.5	64.0	64.0	70.0	70.0	81.5	93.0	93.0	99.0	104.0	121.5	121.5	128.0	128.0	134.5	134.5	142.0	148.5

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

2 SMFA may be completed on 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.

4 Note this visit may occur twice depending on the approval of the V3.0 protocol; the first D42 visit (D42A) will include all D42 procedures EXCEPT AL dosing. This will visit will occur to maintain safety labs and assessments are completed on ALL subjects approximately 14 days post Vaccination #2 (±3 days). If this visit occurs, the next visit will be D42B for AL dosing and completed of ALL procedures as outlined in the visit (if D42A completed >14 days from D42B). This is to ensure the AL dosing in participants occurs approximately 14 days prior to Vaccination #3. If both visits are completed, subjects will receive an additional 3000 CFA ($6 USD).

Year 1: Arms 4a/4b (1-4 years of age):

Arms 4a/4b (1-4 year olds)		Months		(1)	0					1		2		3		4		5		6	9	12
		Visits		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19
Procedures	Blood Volume	Study Day	Screening (-56)	-14	0	1	3	7	14	28	42	56	70	84	98	112	126	140	154	168	252	336
		Visit Windows (days)		0	±7	0	±1	±2	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±28	±56	±56
Clinical Procedures																						
Complete medical history/ physical			X																			
Informed consent			X																			
EKG			X																			
Interim clinical evaluation				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
Conmed review			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Artemether/Lumefantrine				X																		
Laboratory Procedures																						
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
Malaria PCR/Genotyping/ Gametocyte	CAP/EDTA			1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Daily blood draw volume in mL			0	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Cumulative blood volume in mL			0	1.5	3	4.5	6	7.5	9	10.5	12	13.5	15	16.5	18	19.5	21	22.5	24	25.5	27	28.5

1 Note this visit will occur when the majority of the child's VU present for their study day 42 visit (14 days prior to vaccination #3)

Year 2: Arms 4a/4b (1-4 years of age)

Arms 2a/2b (5-8 year olds)		Months	13.5		14		15		16		17		18		19		20	23	26
		Visits	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45
Procedures	Blood Volume	Study Day	378 (Rescreening)	385	392	406	420	434	448	462	476	490	504	518	532	546	560	644	728
		Days Post Vac	322	329	336/0	14	28	42	56	70	84	98	112	126	140	154	168	252	336
		Visit Windows (days) (1)	±84	±14	±112	±14	±14	±14	±14	±14	±14	±14	±14	±14	±14	±14	±14	±56	±56
Clinical Procedures																			
Updated medical history/ physical			X																
Reconsent			X																
Interim clinical evaluation				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
Conmed review			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
Artemether/Lumefantrine				X															
Unblinding (VU)																	X		
Laboratory Procedures																			
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
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	0.5	0.5	0.5	0.5
Daily blood draw volume in mL			1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1	1
Cumulative blood volume in mL			150.5	151.5	152.5	153.5	154.5	155.5	156.5	157.5	158.5	159.5	160.5	161.5	162.5	163.5	164.5	165.5	166.5

1 Visit windows are based off timing of days post the preceding vaccination; broad visit window in place for Booster dose in attempt to group VU together and provide flexibility given the ongoing COVID pandemic; increased visit windows for visits to align with parental visits

Year 2: Arms 2a/2b (5-8 years of age; Pilot Phase)

Arms 2a/2b (5-8 year olds)		Months	15.5		16				17		18		19		20		21		22	25	28
		Visits	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50
Procedures	Blood Volume	Study Day	434 (Rescreening)	441	448	451	455	462	476	490	504	518	532	546	560	574	588	602	616	700	784
		Days Post Vac	322	329	336/0	3	7	14	28	42	56	70	84	98	112	126	140	154	168	252	336
		Visit Windows (days) (1)	±84	±7	±112	±1	±2	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±56	±56
Clinical Procedures																					
Updated medical history/ physical			X																		
Reconsent			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
AE/SAE assessment			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
Artemether/Lumefantrine				X																	
VACCINATION					X																
Unblinding																			X		
Laboratory Procedures																					
CBC with differential	EDTA		2		2	2		2													
ALT/ Creatinine	SST		5		3	3		3													
HIV testing	SST																				
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
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	0.5	0.5	0.5
Pfs230 ELISA	SST				5			5			5				5				5	5	5
SMFA (3)																					
Transcriptional Analysis	PaxGene		1			1		1											1		
Daily blood draw volume in mL			9	0	11	6	0	11	1	1	6	1	1	1	6	1	1	1	7	6	6
Cumulative blood volume in mL			170	170	181	187	187	198	199	200	206	207	208	209	215	216	217	218	225	231	237

1 Visit windows are based off timing of days post the preceding vaccination; broad visit window in place for Booster dose in attempt to group VU together and provide flexibility given the ongoing COVID pandemic

2 SMFA may be completed on subjects at Study Day 448 and 504; other SMFA time points indicated above will be completed depending on associated ELISA results at that time point.

Year 2: Arms 3a/3b (5-8 years of age; Main Phase)

Arms 3a/3b (5-8 year olds)		Months	13.5		14				15		16		17		18		19		20	23	26
		Visits	29	30	31	32	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47
Procedures	Blood Volume	Study Day	378 (Rescreening)	385	392	395	399	406	420	434	448	462	476	490	504	518	532	546	560	644	728
		Days Post Vac	322	329	336/0	3	7	14	28	42	56	70	84	98	112	126	140	154	168	252	336
		Visit Windows (days) (1)	±84	±7	±112	±1	±2	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±56	±56
Clinical Procedures																					
Updated medical history/ physical			X																		
Reconsent			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
AE/SAE assessment			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
Artemether/Lumefantrine				X																	
VACCINATION					X																
Unblinding																			X		
Laboratory Procedures																					
CBC with differential	EDTA		2		2	2		2													
ALT/ Creatinine	SST		5		3	3		3													
HIV testing	SST																				
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
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	0.5	0.5	0.5
Pfs230 ELISA	SST				5			5			5				5				5	5	5
SMFA (3)																					
Transcriptional Analysis	PaxGene		1			1		1											1		
Daily blood draw volume in mL			9	0	11	6	0	11	1	1	6	1	1	1	6	1	1	1	7	6	6
Cumulative blood volume in mL			158.5	158.5	169.5	175.5	175.5	186.5	187.5	188.5	194.5	195.5	196.5	197.5	203.5	204.5	205.5	206.5	213.5	219.5	225.5

1 Visit windows are based off timing of days post the preceding vaccination; broad visit window in place for Booster dose in attempt to group VU together and provide flexibility given the ongoing COVID pandemic

2 SMFA may be completed on subjects at Study Day 336 and 448; other SMFA time points indicated above will be completed depending on associated ELISA results at that time point.

Year 2: Arms 1a/1b (9-18 years of age; Pilot Phase)

Arms 1a/1b (9-18 year olds)		Months	15.5	16				17		18		19		20		21		22	25	28
		Visits	33	34	35	36	37	38	39	40	41	42	43	44	45	46	47	48	49	50
Procedures	Blood Volume	Study Day	434 (Rescreening)	448	451	455	462	476	490	504	518	532	546	560	574	588	602	616	700	784
		Days post Vac	322	336/0	3	7	14	28	42	56	70	84	98	112	126	140	154	168	252	336
		Visit Windows (days) (1)	±84	±112	±1	±2	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±56	±56
Clinical Procedures																				
Updated medical history/ physical																				
Reconsent			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
AE/SAE assessment			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
Conmed review			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
VACCINATION				X																
Unblinding																		X		
Feeding Evaluation (1)							X													
Laboratory Procedures																				
CBC with differential	EDTA		2.0	2.0	2.0		2.0													
ALT/ Creatinine	SST		5.0	3.0	3.0		3.0													
HIV testing	SST																			
Urine/Serum pregnancy test (females only)	Urine Container or SST		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
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	0.5	0.5	0.5
Pfs230 ELISA	SST		5	5		5	10			5				5				5	5	5
SMFA (2)																				
DSF (3)	X						X	X	X	X	X	X	X	X	X	X				
Transcriptional analysis	PAXGene		1		1		1											1		
Daily blood draw volume in mL			14.0	11.0	6.0	5.0	17.0	1.0	1.0	6.0	1.0	1.0	1.0	6.0	1.0	1.0	1.0	7.0	6.0	6.0
Cumulative blood volume in mL			186.0	197.0	203.0	208.0	225.0	226.0	227.0	233.0	234.0	235.0	236.0	242.0	243.0	244.0	245.0	252.0	258.0	264.0

1 Visit windows are based off timing of days post the preceding vaccination; broad visit window in place for Booster dose in attempt to group VU together and provide flexibility given the ongoing COVID pandemic

2 SMFA may be completed on subjects at Study Day 448 and 504; 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 monthly post Vaccination #4 from day 463 through 588 (total = 10 feeds).

Year 2: Arms 3c/3d (9-18 years of age; Main Phase)

Arms 3c/3d (9-18 year olds)		Months	13.5	14				17		18		19		20		21		22	25	28
		Visits	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	41
Procedures	Blood Volume	Study Day	378 (Rescreening)	392	395	399	406	420	434	448	462	476	490	504	518	532	546	560	644	728
		Days post Vac	322	336/0	3	7	14	28	42	56	70	84	98	112	126	140	154	168	252	336
		Visit Windows (days) (1)	±84	±112	±1	±2	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±7	±56	±56
Clinical Procedures																				
Updated medical history/ physical																				
Reconsent			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
AE/SAE assessment			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
Conmed review			X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X	X
VACCINATION				X																
Unblinding																		X		
Feeding Evaluation (1)							X													
Laboratory Procedures																				
CBC with differential	EDTA		2.0	2.0	2.0		2.0													
ALT/ Creatinine	SST		5.0	3.0	3.0		3.0													
HIV testing	SST																			
Urine/Serum pregnancy test (females only)	Urine Container or SST		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
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	0.5	0.5	0.5
Pfs230 ELISA	SST		5	5		5	10			5				5				5	5	5
SMFA (2)																				
DSF (3)	X						X	X	X	X	X	X	X	X	X	X				
Transcriptional analysis	PAXGene		1		1		1											1		
Daily blood draw volume in mL			14.0	11.0	6.0	5.0	17.0	1.0	1.0	6.0	1.0	1.0	1.0	6.0	1.0	1.0	1.0	7.0	6.0	6.0
Cumulative blood volume in mL			174.5	185.5	191.5	196.5	213.5	214.5	215.5	221.5	222.5	223.5	224.5	230.5	231.5	232.5	233.5	240.5	246.5	252.5

1 Visit windows are based off timing of days post the preceding vaccination; broad visit window in place for Booster dose in attempt to group VU together and provide flexibility given the ongoing COVID pandemic

2 SMFA may be completed on subjects at Study Day 336 and 448; 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 monthly post Vaccination #4 from day 406 through 532 (total = 10 feeds).

Year 2: Arms 3e/3f (≥19 years of age)

Arms 3e/3f (Adults 19 years of age and older)		Months	13.5	14				15	16	18	20	23	26
		Visits	24	25	26	27	28	29	30	31	32	33	34
Procedures	Blood Volume	Study Day	378 (Rescreening)	392	395	399	406	420	448	504	560	644	728
		Days post Vac	322	336/0	3	7	14	28	56	112	168	252	336
		Visit Windows (days) (1)	±84	±112	±1	±2	±7	±7	±7	±7	±28	±56	±56
Clinical Procedures													
Updated medical history/ physical													
Reconsent			X										
Pre-test/Post-test HIV counseling			X										
Interim clinical evaluation				X	X	X	X	X	X	X	X	X	X
AE/SAE assessment			X	X	X	X	X	X	X	X	X	X	X
Pregnancy Prevention			X	X	X	X	X	X	X	X	X	X	X
Conmed review			X	X	X	X	X	X	X	X	X	X	X
VACCINATION				X									
Unblinding											X		
Laboratory Procedures													
CBC with differential	EDTA		2	2	2		2						
ALT/ Creatinine	SST		5	3	3		3						
HIV testing	SST												
Urine/Serum pregnancy test (females only)	Urine Container or SST		0.5	X									
Malaria Blood Smear	CAP/EDTA		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
Pfs230 ELISA	SST		5	5		5	10		5	5	5	5	5
SMFA (2)													
Transcriptional analysis	PAXGene		1		1		1				1		
Daily blood draw volume in mL			12.5	11.0	6.0	5.0	17.0	0.0	6.0	6.0	7.0	6.0	6.0
Cumulative blood volume in mL			161.0	172.0	178.0	183.0	200.0	200.0	206.0	212.0	219.0	225.0	231.0
1 Visit windows are based off timing of days post the preceding vaccination; broad visit window in place for Booster dose in attempt to group VU together and provide flexibility given the ongoing COVID pandemic													
2 SMFA may be completed on subjects at Study Day 392 and 448; other SMFA time points indicated above will be completed depending on associated ELISA results at that time point.													

Appendix B: Day to Day Schedule by Arms

Arms 4a/4b (1-4 year olds): No Vaccination

Study Day -14 (±0 days; Drug Treatment Visit; should align with Vaccine Unit visit prior to Vaccination #3)

The following evaluations/procedures will be completed before vaccination:

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 1.5 mL of blood for malaria blood smear and malaria PCR.
5. Administer first dose of artemether/lumefantrine (AL).
    a. Additional doses of AL will occur on study days -13, -12

Study Day 0 (±7 day)

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 1.5 mL of blood for malaria blood smear and malaria PCR.

Study Day 1 (±0 day)

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 1.5 mL of blood for malaria blood smear and malaria PCR.

Study Day 3 (±1 day)

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 1.5 mL of blood for malaria blood smear and malaria PCR.

Study Day 7 (±2 day)

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 1.5 mL of blood for malaria blood smear and malaria PCR.

Study Day 14 (±7 day)

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 1.5 mL of blood for malaria blood smear and malaria PCR.

Study Day 28 (±7 day)

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 1.5 mL of blood for malaria blood smear and malaria PCR.

Study Day 42 (±7 day)

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 1.5 mL of blood for malaria blood smear and malaria PCR.

Study Day 56 (±7 day)

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 1.5 mL of blood for malaria blood smear and malaria PCR.

Study Day 70 (±7 day)

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 1.5 mL of blood for malaria blood smear and malaria PCR.

Study Day 84 (±7 day)

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 1.5 mL of blood for malaria blood smear and malaria PCR.

Study Day 98 (±7 day)

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 1.5 mL of blood for malaria blood smear and malaria PCR.

Study Day 112 (±7 day)

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 1.5 mL of blood for malaria blood smear and malaria PCR.

Study Day 126 (±7 day)

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 1.5 mL of blood for malaria blood smear and malaria PCR.

Study Day 140 (±7 day)

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 1.5 mL of blood for malaria blood smear and malaria PCR.

Study Day 154 (±7 day)

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 1.5 mL of blood for malaria blood smear and malaria PCR.

Study Day 168 (±28 day)

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 1.5 mL of blood for malaria blood smear and malaria PCR.

Study Day 252 (±56 day)

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 1.5 mL of blood for malaria blood smear and malaria PCR.

Study Day 378 (±84 day)

1. Reconsent for continuation on study.
2. Perform updated medical history and physical examination, emphasizing examination of any acute complaints.
3. Record vital signs (weight, length, blood pressure, temperature, and heart rate).
4. Record AEs and concomitant medications, if applicable.
5. Obtain approximately 1 mL of blood for malaria blood smear and malaria PCR.
6. Administer first dose of artemether/lumefantrine (AL).
    a. Additional doses of AL will occur on study days -13, -12.

Study Day 385 (±14 day)

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.
5. Administer first dose of artemether/lumefantrine (AL).
6. Additional doses of AL will occur on study days 386, 387.

Study Day 392 (±112 day)

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 406 (±14 day)

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 420 (±14 day)

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 434 (±14 day)

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 448 (±14 day)

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 462 (±14 day)

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 476 (±14 day)

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 490 (±14 day)

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 504 (±14 day)

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 518 (±14 day)

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 532 (±14 day)

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 546 (±28 day)

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.
5. Scheduled unblinding of VU

Study Day 560 (±14 day)

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 644 (±56 day)

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 728 (±56 day)

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.

Arms 2a/2b (PILOT), 3a/3b (MAIN) (5-8 year olds): 3 Vaccinations (0, 1, 4, 16 months—PILOT; 0, 1, 2, 14 months—MAIN)

Study Day -7 (±7 days; Drug Treatment Visit)

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 7.5 mL of blood for CBC with differential and platelet count, ALT, creatinine, hemoglobin typing, malaria blood smear, malaria PCR, and transcriptional analysis (NOTE: if hemoglobin typing not obtained, can be obtained later during study at anytime when an EDTA tube is drawn). If safety labs were obtained for screening within ≤ 2 days prior to day -7, can use for study day -7 visit and do not need to repeat.
5. Obtain urine for Schistosomiasis testing (permittable to be collected any day between start of AL dosing and first vaccination; Main Phase only)
6. Administer first dose of artemether/lumefantrine (AL).
    a. Additional doses of AL will occur on study days -6, -5

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, and urine results from screening are within protocol-defined limits (see Exclusion Criteria above) before vaccinating
4. Perform basic history and focused physical examination, emphasizing examination of 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. Confirm that all criteria for vaccination have been met according to inclusion/exclusion criteria
8. Confirm continued eligibility to receive vaccination
9. Obtain approximately 17.5 mL of blood for CBC with differential and platelet count, ALT, creatinine, malaria blood smear, malaria PCR, anti-Pfs230 antibody ELISA and standard membrane feeding assay (SMFA), and transcriptional analysis.
10. Obtain urine for Schistosomiasis testing (permittable to be collected any day between start of AL dosing and first vaccination; Main Phase only)
11. 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 6 mL of blood for CBC with differential and platelet count, ALT, and creatinine, 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 11.5 mL of blood for CBC with differential and platelet count, ALT, creatinine, malaria blood smear, malaria PCR, anti-Pfs230 antibody ELISA and standard membrane feeding assay (SMFA).

Study Day 28 (±7 days; 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. Obtain approximately 11.5 mL of blood for CBC with differential and platelet count, ALT, creatinine, malaria blood smear, malaria PCR, anti-Pfs230 antibody ELISA, and standard membrane feeding assay (SMFA).
5. Confirm continued eligibility to receive vaccination
6. 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 6 mL of blood for CBC with differential and platelet count, ALT and creatinine, 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.

PILOT ONLY

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 11.5 mL of blood for CBC with differential and platelet count, ALT, creatinine, malaria blood smear, malaria PCR, anti-Pfs230 antibody ELISA, and standard membrane feeding assay (SMFA).

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.

PILOT AND MAIN PHASE JOIN SCHEDULES (STUDY DAYS OFF BY 70 DAYS)

Study Day 112 (PILOT) + 42 (MAIN) (±28 days; 84 days after Vaccination #2 in Pilot; 14 days after Vaccination #2 in Main)

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 11.5 mL of blood for CBC with differential and platelet count, ALT, creatinine, malaria blood smear and malaria PCR, anti-Pfs230 antibody ELISA, and standard membrane feeding assay (SMFA).
5. Administer first dose of artemether/lumefantrine (AL). (NOTE if D42A, no AL dosing; if D42B AL dosing to be completed)
    a. Pilot: Additional doses of AL will occur on study days 113, 114
    b. Pilot: Additional doses of AL will occur on study days 43, 44

Study Day 126 (PILOT) + 56 (MAIN) (±56 days; 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. Obtain approximately 11.5 mL of blood for CBC with differential and platelet count, ALT, creatinine, malaria blood smear, malaria PCR, anti-Pfs230 antibody ELISA and standard membrane feeding assay (SMFA).
5. Confirm continued eligibility to receive vaccination
6. 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 127 (PILOT) + 57 (MAIN) (±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 129 (PILOT) + 59 (MAIN) (±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 6 mL of blood for CBC with differential and platelet count, ALT, creatinine, transcriptional analysis.

Study Day 133 (PILOT) + 63 (MAIN) (±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 140 (PILOT) + 70 (MAIN) (±7 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 11.5 mL of blood for CBC with differential and platelet count, ALT, creatinine, malaria blood smear, malaria PCR, anti-Pfs230 antibody ELISA and standard membrane feeding assay (SMFA).

Study Day 154 (PILOT) + 84 (MAIN) (±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 1.5 mL of blood for malaria blood smear and malaria PCR.

Study Day 168 (PILOT) + 98 (MAIN) (±7 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 1.5 mL of blood for malaria blood smear and malaria PCR.

Study Day 182 (PILOT) + 112 (MAIN) (±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 6.5 mL of blood for malaria blood smear, malaria PCR, anti-Pfs230 antibody ELISA and standard membrane feeding assay (SMFA).

Study Day 196 (PILOT) + 126 (MAIN) (±7 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 1.5 mL of blood for malaria blood smear and malaria PCR.

Study Day 210 (PILOT) + 140 (MAIN) (±7 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 1.5 mL of blood for malaria blood smear and malaria PCR.

Study Day 224 (PILOT) + 154 (MAIN) (±7 days; 98 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 1.5 mL of blood for malaria blood smear, malaria PCR.

Study Day 238 (PILOT) + 168 (MAIN) (±7 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 6.5 mL of blood for malaria blood smear, malaria PCR, anti-Pfs230 antibody ELISA and standard membrane feeding assay (SMFA).

Study Day 252 (PILOT) + 182 (MAIN) (±7 days; 126 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 1.5 mL of blood for malaria blood smear and malaria PCR.

Study Day 266 (PILOT) + 196 (MAIN) (±7 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 1.5 mL of blood for malaria blood smear and malaria PCR.

Study Day 280 (PILOT) + 210 (MAIN) (±7 days; 154 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 1.5mL of blood for malaria blood smear and malaria PCR.

Study Day 294 (PILOT) + 224 (MAIN) (±28 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 7.5 mL of blood for malaria blood smear, malaria PCR, anti-Pfs230 antibody ELISA and standard membrane feeding assay (SMFA), and transcriptional analysis.

Study Day 378 (PILOT) + 308 (MAIN) (±56 days; 252 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 6.5 mL of blood for malaria blood smear, malaria PCR, anti-Pfs230 antibody ELISA and standard membrane feeding assay (SMFA).

Study Day 434 (PILOT- 308 days post Vaccination #3) + 378 (MAIN) 322 days after Vaccination #3) (±84 days);

1. Reconsent for continuation on study.
2. Perform basic history and focused physical examination, emphasizing examination of any acute complaints.
3. Record vital signs (blood pressure, temperature, and heart rate).
4. Record AEs and concomitant medications, if applicable.
5. Obtain approximately 9 mL of blood for HIV, CBC with differential and platelet count, ALT, creatinine, malaria blood smear and malaria PCR and transcriptional analysis.

Study Day 441 (PILOT 315 days post Vaccination #3) + 385 (MAIN 329 days post Vaccination #3) (±7 days; day of Vaccination #4)

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. Administer first dose of artemether/lumefantrine (AL).
   a) Pilot: Additional doses of AL will occur on study days 442, 443
   b) Pilot: Additional doses of AL will occur on study days 386, 387

Study Day 448 (PILOT) + 392 (MAIN) (±112 days; day of 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. 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. Confirm continued eligibility to receive vaccination.
5. Obtain approximately 11 mL of blood for CBC with differential and platelet count, ALT, creatinine, malaria blood smear, malaria PCR, anti-Pfs230 antibody ELISA and standard membrane feeding assay (SMFA).
6. 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 451 (PILOT) + 395 (MAIN) (±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 6 mL of blood for CBC with differential and platelet count, ALT, creatinine, transcriptional analysis.

Study Day 455 (PILOT) + 399 (MAIN) (±2 days; 7 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.

Study Day 462 (PILOT) + 406 (MAIN) (±7 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 11 mL of blood for CBC with differential and platelet count, ALT, creatinine, malaria blood smear, malaria PCR, anti-Pfs230 antibody ELISA, standard membrane feeding assay (SMFA) and transcriptional analysis.

Study Day 476 (PILOT) + 420 (MAIN) (±7 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 1 mL of blood for malaria blood smear and malaria PCR.

Study Day 490 (PILOT) + 434 (MAIN) (±7 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 1 mL of blood for malaria blood smear and malaria PCR.

Study Day 504 (PILOT) + 448 (MAIN) (±7 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 6 mL of blood for malaria blood smear, malaria PCR, anti-Pfs230 antibody ELISA and standard membrane feeding assay (SMFA).

Study Day 518 (PILOT) + 462 (MAIN) (±7 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 1 mL of blood for malaria blood smear and malaria PCR.

Study Day 532 (PILOT) + 476 (MAIN) (±7 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 1 mL of blood for malaria blood smear and malaria PCR.

Study Day 546 (PILOT) + 490 (MAIN) (±7 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 1 mL of blood for malaria blood smear, malaria PCR.

Study Day 560 (PILOT) + 504 (MAIN) (±7 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. Obtain approximately 6 mL of blood for malaria blood smear, malaria PCR, anti-Pfs230 antibody ELISA and standard membrane feeding assay (SMFA).

Study Day 574 (PILOT) + 518 (MAIN) (±7 days; 126 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 1 mL of blood for malaria blood smear and malaria PCR.

Study Day 588 (PILOT) + 532 (MAIN) (±7 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 1 mL of blood for malaria blood smear and malaria PCR.

Study Day 602 (PILOT) + 546(MAIN) (±28 days; 154 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 1 mL of blood for malaria blood smear and malaria PCR.
5. Scheduled unblinding of VU
    a. If received Pfs230D1M-EPA/ASO1 offer meningitis comparator vaccination

Study Day 616 (PILOT) + 560 (MAIN) (±28 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 7 mL of blood for malaria blood smear, malaria PCR, anti-Pfs230 antibody ELISA and standard membrane feeding assay (SMFA), and transcriptional analysis.

Study Day 700 (PILOT) + 644 (MAIN) (±56 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 6 mL of blood for malaria blood smear, malaria PCR, anti-Pfs230 antibody ELISA and standard membrane feeding assay (SMFA).

Study Day 784 (PILOT) + 728 (MAIN) (±56 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 6 mL of blood for malaria blood smear, malaria PCR, anti-Pfs230 antibody ELISA and standard membrane feeding assay (SMFA).

Arms 1a/1b (PILOT), 3c/3d (MAIN) (9-18 year olds): 3 Vaccinations (0, 1, 4 months—PILOT; 0, 1, 2 months—MAIN)

Study Day -7 (±7 days; Drug Treatment Visit)

1. Perform basic history and focused physical examination, emphasizing examination of any acute complaints.
1. Record vital signs (blood pressure, temperature, and heart rate).
2. Record AEs and concomitant medications, if applicable.
3. Obtain approximately 7.5 mL of blood for CBC with differential and platelet count, ALT, creatinine, hemoglobin typing. malaria blood smear and malaria PCR, and transcriptional analysis. (NOTE: if hemoglobin typing not obtained, can be obtained later during study at anytime when an EDTA tube is drawn). If safety labs were obtained for screening within ≤ 2 days prior to day -7, can use for study day -7 visit and do not need to repeat.
4. Obtain Urine +/- Stool Sample for Schistosomiasis +/- Helminth testing (permittable to be collected any day between start of AL dosing and first vaccination)
5. For females, obtain a urine or serum sample for β-hCG testing. Ensure that the test is negative before proceeding.
6. For females, ensure agreement and compliance with pregnancy prevention before AL dosing.
7. Administer first dose of artemether/lumefantrine (AL).
    a. Additional doses of AL will occur on study days -6, -5

Study Day 0 (day of Vaccination #1)

The following evaluations/procedures will be completed before vaccination:

1. Ensure that all inclusion/exclusion criteria are met.
2. Ensure that CBC, ALT, creatinine, HIV, and urine results from screening are within protocol-defined limits (see Exclusion Criteria above) before vaccinating
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. 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.
6. For females, obtain a urine or serum sample for β-hCG testing. Ensure that the test is negative before vaccinating.
7. For females, ensure agreement and compliance with pregnancy prevention before vaccinating.
8. Confirm that all criteria for vaccination have been met according to inclusion/exclusion criteria
9. Confirm continued eligibility to receive vaccination
10. Obtain approximately 17.5 mL of blood for CBC with differential and platelet count, ALT, creatinine, malaria blood smear, malaria PCR, anti-Pfs230 antibody ELISA, standard membrane feeding assay (SMFA), and transcriptional analysis.
11. Obtain Urine +/- Stool Sample for Schistosomiasis +/- Helminth testing (permittable to be collected prior to day 0; any day between start of AL dosing and first 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 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 6.0 mL of blood for CBC with differential and platelet count, ALT, creatinine, 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 11.5 mL of blood for CBC with differential and platelet count, ALT, creatinine, malaria blood smear, malaria PCR, anti-Pfs230 antibody ELISA and standard membrane feeding assay (SMFA).

Study Day 28 (±7 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.

5. For females, ensure agreement and compliance with pregnancy prevention before vaccinating
6. Obtain approximately 11.5 mL of blood for CBC with differential and platelet count, ALT, creatinine, malaria blood smear, malaria PCR, 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 6.0 mL of blood for CBC with differential and platelet count, ALT, creatinine, 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.

PILOT ONLY

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.
5. Obtain approximately 11.5 mL of blood for CBC with differential and platelet count, ALT, creatinine, malaria blood smear, malaria PCR, anti-Pfs230 antibody ELISA and standard membrane feeding assay (SMFA).

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.

PILOT AND MAIN PHASE JOIN SCHEDULES (STUDY DAYS OFF BY 70 DAYS)

Study Day 112 (PILOT) + 42 (MAIN) (±28 days; 84 days after Vaccination #2 in Pilot; 14 days after Vaccination #2 in Main)

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 11.5 mL of blood for CBC with differential and platelet count, ALT, creatinine, malaria blood smear, malaria PCR, anti-Pfs230 antibody ELISA, and standard membrane feeding assay (SMFA).

Study Day 126 (PILOT) + 56 (MAIN) (±56 days; 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.
5. For females, ensure agreement and compliance with pregnancy prevention before vaccinating.
6. Obtain approximately 11.5 mL of blood for CBC with differential and platelet count, ALT, creatinine, malaria blood smear, malaria PCR, 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 127 (PILOT) + 57 (MAIN) (±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 129 (PILOT) + 59 (MAIN) (±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 6.0 mL of blood for CBC with differential and platelet count, ALT, creatinine, and transcriptional analysis.

Study Day 133 (PILOT) + 63 (MAIN) (±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 5.0 mL of blood for anti-Pfs230 antibody ELISA, and standard membrane feeding assay (SMFA).

Study Day 140 (PILOT) + 70 (MAIN) (±7 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. For females, obtain a urine or serum sample for β-hCG testing. Ensure that the test is negative before proceeding.
5. Obtain approximately 17.5 mL of blood for CBC with differential and platelet count, ALT, creatinine, malaria blood smear, malaria PCR, anti-Pfs230 antibody ELISA, standard membrane feeding assay (SMFA) and Transcriptional analysis.
6. Feeding evaluations (DSF) will also be performed at this visit.
    a. Confirm eligible for DSF

Study Day 154 (PILOT) + 84 (MAIN) (±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 1.5 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 168 (PILOT) + 98 (MAIN) (±7 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 1.5 mL of blood for malaria blood smear, malaria PCR
5. For females, obtain a urine or serum sample for β-hCG testing. Ensure that the test is negative before proceeding.
6. Feeding evaluations (DSF) will also be performed at this visit.
    a. Confirm eligible for DSF

Study Day 182 (PILOT) + 112 (MAIN) (±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 6.5 mL of blood for malaria blood smear, malaria PCR, anti-Pfs230 antibody ELISA, standard membrane feeding assay (SMFA).
5. Feeding evaluations (DSF) will also be performed at this visit.
    a. Confirm eligible for DSF

Study Day 196 (PILOT) + 126 (MAIN) (±7 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 1.5 mL of blood for malaria blood smear, malaria PCR
5. For females, obtain a urine or serum sample for β-hCG testing. Ensure that the test is negative before proceeding.
6. Feeding evaluations (DSF) will also be performed at this visit.
    a. Confirm eligible for DSF

Study Day 210 (PILOT) + 140 (MAIN) (±7 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 1.5 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 (PILOT) + 154 (MAIN) (±7 days; 98 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 1.5 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 proceeding.
6. Feeding evaluations (DSF) will also be performed at this visit.
    a. Confirm eligible for DSF

Study Day 238 (PILOT) + 168 (MAIN) (±7 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 6.5 mL of blood for malaria blood smear, malaria PCR, anti-Pfs230 antibody ELISA, standard membrane feeding assay (SMFA).
5. Feeding evaluations (DSF) will also be performed at this visit.
    a. Confirm eligible for DSF

Study Day 252 (PILOT) + 182 (MAIN) (±7 days; 126 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 1.5 mL of blood for malaria blood smear and malaria PCR.

Study Day 266 (PILOT) + 196 (MAIN) (±7 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 1.5 mL of blood for malaria blood smear and malaria PCR.

Study Day 280 (PILOT) + 210 (MAIN) (±7 days; 154 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 1.5 mL of blood for malaria blood smear and malaria PCR.

Study Day 294 (PILOT) + 224 (MAIN) (±28 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 7.5 mL of blood for malaria blood smear, malaria PCR, anti-Pfs230 antibody ELISA, standard membrane feeding assay (SMFA), and transcriptional analysis.

Study Day 378 (PILOT) + 308(MAIN) (±56 days; 252 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 6.5 mL of blood for malaria blood smear, malaria PCR, anti-Pfs230 antibody ELISA, standard membrane feeding assay (SMFA).

Study Day 462 (PILOT) + 392 (MAIN) (±56 days; 336 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 6.5 mL of blood for malaria blood smear, malaria PCR, anti-Pfs230 antibody ELISA, standard membrane feeding assay (SMFA).

Study Day 434 (PILOT 308 days post Vaccination #3) + 378 (MAIN 322 days after Vaccination #3) (±84 days)

1. Reconsent for continuation on study.
2. Perform basic history and focused physical examination, emphasizing examination of any acute complaints.
3. Record vital signs (blood pressure, temperature, and heart rate).
4. Record AEs and concomitant medications, if applicable.
5. Obtain approximately 14 mL of blood for CBC with differential and platelet count, ALT, creatinine, HIV, malaria blood smear, malaria PCR, anti-Pfs230 antibody ELISA, standard membrane feeding assay (SMFA) and transcriptional analysis.
6. For females, obtain a urine or serum sample for β-hCG testing. Ensure that the test is negative before proceeding.

Study Day 448 (PILOT) + 392 (MAIN) (±112 days; day of Vaccination #4)

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.
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-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 451 (PILOT) + 395 (MAIN) (±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 6.0 mL of blood for CBC with differential and platelet count, ALT, creatinine, and transcriptional analysis.

Study Day 455 (PILOT) + 399 (MAIN) (±2 day; 7 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 5.0 mL of blood for standard membrane feeding assay (SMFA).

Study Day 462 (PILOT) + 406 (MAIN) (±7 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. For females, obtain a urine or serum sample for β-hCG testing. Ensure that the test is negative before proceeding.
5. Obtain approximately 17 mL of blood for CBC with differential and platelet count, ALT, creatinine, malaria blood smear, malaria PCR, anti-Pfs230 antibody ELISA, standard membrane feeding assay (SMFA) and Transcriptional analysis.
6. Feeding evaluations (DSF) will also be performed at this visit.
    a. Confirm eligible for DSF

Study Day 476 (PILOT) + 420 (MAIN) (±7 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 1 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 (PILOT) + 434(MAIN) (±7 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. For females, obtain a urine or serum sample for β-hCG testing. Ensure that the test is negative before proceeding.
5. Obtain approximately 1 mL of blood for malaria blood smear, and malaria PCR.
6. Feeding evaluations (DSF) will also be performed at this visit.
    a. Confirm eligible for DSF

Study Day 504 (PILOT) + 448 (MAIN) (±7 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 6 mL of blood for malaria blood smear, malaria PCR, anti-Pfs230 antibody ELISA, standard membrane feeding assay (SMFA).
5. Feeding evaluations (DSF) will also be performed at this visit.
    a. Confirm eligible for DSF

Study Day 518 (PILOT) + 462 (MAIN) (±7 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. For females, obtain a urine or serum sample for β-hCG testing. Ensure that the test is negative before proceeding.
5. Obtain approximately 1 mL of blood for malaria blood smear, and malaria PCR.
6. Feeding evaluations (DSF) will also be performed at this visit.
    b. Confirm eligible for DSF

Study Day 532 (PILOT) + 476 (MAIN) (±7 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 1 mL of blood for malaria blood smear, malaria PCR.
5. Feeding evaluations (DSF) will also be performed at this visit.
    a. Confirm eligible for DSF

Study Day 546 (PILOT) + 490 (MAIN) (±7 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. For females, obtain a urine or serum sample for β-hCG testing. Ensure that the test is negative before proceeding.
5. Obtain approximately 1 mL of blood for malaria blood smear and malaria PCR.
6. Feeding evaluations (DSF) will also be performed at this visit.
    a. Confirm eligible for DSF

Study Day 560 (PILOT) + 504 (MAIN) (±7 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. Obtain approximately 6 mL of blood for malaria blood smear, malaria PCR, anti-Pfs230 antibody ELISA, standard membrane feeding assay (SMFA).
5. Feeding evaluations (DSF) will also be performed at this visit.
    a. Confirm eligible for DSF

Study Day 574 (PILOT) + 518(MAIN) (±7 days; 126 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 proceeding.
5. Obtain approximately 1 mL of blood for malaria blood smear and malaria PCR.
6. Feeding evaluations (DSF) will also be performed at this visit.
    a. Confirm eligible for DSF

Study Day 588 (PILOT) + 532 (MAIN) (±7 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 1 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 602 (PILOT) + 546 (MAIN) (±28 days; 154 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 1 mL of blood for malaria blood smear and malaria PCR.
5. Scheduled unblinding of VU
    a. If received Pfs230D1M-EPA/ASO1 offer meningitis comparator vaccination

Study Day 616 (PILOT) + 560 (MAIN) (±7 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 7 mL of blood for malaria blood smear, malaria PCR, anti-Pfs230 antibody ELISA, standard membrane feeding assay (SMFA), and transcriptional analysis.

Study Day 700 (PILOT) + 644 (MAIN) (±56 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 6 mL of blood for malaria blood smear, malaria PCR and, anti-Pfs230 antibody ELISA, standard membrane feeding assay (SMFA).

Study Day 588 (PILOT) + 518 (MAIN) (±56 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 6 mL of blood for malaria blood smear, malaria PCR, anti-Pfs230 antibody ELISA, standard membrane feeding assay (SMFA).

Arms 3e/3f (Adults 19 years of age and older): 3 Vaccinations (0, 1, 2 months)

Study Day -7 (±7 days; Drug Treatment Visit)

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 7.5 mL of blood for CBC with differential and platelet count, ALT, creatinine, hemoglobin typing, malaria blood smear and malaria PCR, and transcriptional analysis.
5. For females, obtain a urine or serum sample for β-hCG testing. Ensure that the test is negative before proceeding.
6. For females, ensure agreement and compliance with pregnancy prevention before AL dosing.
7. Administer first dose of artemether/lumefantrine (AL).
    a. Additional doses of AL will occur on study days -6, -5

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, and urine results from screening are within protocol-defined limits (see Exclusion Criteria above) before vaccinating
4. Perform basic history and focused physical examination, emphasizing examination of 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.
8. For females, ensure agreement and compliance with pregnancy prevention before vaccinating.
9. Confirm that all criteria for vaccination have been met according to inclusion/exclusion criteria
10. Confirm continued eligibility to receive vaccination
11. Obtain approximately 17.5 mL of blood for CBC with differential and platelet count, ALT, creatinine, malaria blood smear, malaria PCR, anti-Pfs230 antibody ELISA, and standard membrane feeding assay (SMFA), and transcriptional analysis.
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 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 6.0 mL of blood for CBC with differential and platelet count, ALT, creatinine, 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 11.5 mL of blood for CBC with differential and platelet count, ALT, creatinine, malaria blood smear, malaria PCR, anti-Pfs230 antibody ELISA and standard membrane feeding assay (SMFA).

Study Day 28 (±7 days; 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. Obtain approximately 11.5 mL of blood for CBC with differential and platelet count, ALT, creatinine, malaria blood smear, malaria PCR, anti-Pfs230 antibody ELISA, and standard membrane feeding assay (SMFA).
5. For females, obtain a urine or serum sample for β-hCG testing. Ensure that the test is negative before vaccinating.
6. For females, ensure agreement and compliance with pregnancy prevention before vaccinating.
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 6.0 mL of blood for CBC with differential and platelet count, ALT, creatinine, 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.

Study Day 42 (±28 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 11.5 mL of blood for CBC with differential and platelet count, ALT, creatinine, malaria blood smear, malaria PCR, anti-Pfs230 antibody ELISA and standard membrane feeding assay (SMFA).

Day 56 (±56 days; 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.

5. For females, ensure agreement and compliance with pregnancy prevention before vaccinating.
6. Obtain approximately 11.5 mL of blood for CBC with differential and platelet count, ALT, creatinine, malaria blood smear, malaria PCR, 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 57 (±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 59 (±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 6.0 mL of blood for CBC with differential and platelet count, ALT, creatinine, and transcriptional analysis.

Study Day 63 (±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 5.0 mL of blood for anti-Pfs230 antibody ELISA, and standard membrane feeding assay (SMFA).

Study Day 70 (±7 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 17.5 mL of blood for CBC with differential and platelet count, ALT, creatinine, malaria blood smear, malaria PCR, anti-Pfs230 antibody ELISA, standard membrane feeding assay (SMFA) and transcriptional analysis.

Study Day 84 (±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.

Study Day 112 (±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 6.5 mL of blood for malaria blood smear, malaria PCR, anti-Pfs230 antibody ELISA, standard membrane feeding assay (SMFA).

Study Day 140 (±7 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 168 (±7 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 6.5 mL of blood for malaria blood smear, malaria PCR, anti-Pfs230 antibody ELISA, standard membrane feeding assay (SMFA).

Study Day 196 (±7 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 224 (±28 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 7.5 mL of blood for malaria blood smear, malaria PCR, anti-Pfs230 antibody ELISA, standard membrane feeding assay (SMFA), and transcriptional analysis.

Study Day 308 (±56 days; 252 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 6.5 mL of blood for malaria blood smear, malaria PCR, anti-Pfs230 antibody ELISA, standard membrane feeding assay (SMFA).

Study Day 392 (±56 days; 336 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 6.5 mL of blood for malaria blood smear, malaria PCR, anti-Pfs230 antibody ELISA, standard membrane feeding assay (SMFA).

Study Day 378 (±84 days; 322 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. For females, obtain a urine or serum sample for β-hCG testing. Ensure that the test is negative before vaccinating.
5. Obtain approximately 12.5 mL of blood for CBC with differential and platelet count, ALT, creatinine, malaria blood smear, malaria PCR, anti-Pfs230 antibody ELISA and standard membrane feeding assay (SMFA) and transcriptional analysis.

Day 392 (±112 days; day of Vaccination #4)

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.
5. For females, ensure agreement and compliance with pregnancy prevention before vaccinating.
6. Obtain approximately 11.0 mL of blood for CBC with differential and platelet count, ALT, creatinine, malaria blood smear, malaria PCR, 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 395 (±1 day; 3 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.
4. Obtain approximately 6.0 mL of blood for CBC with differential and platelet count, ALT and transcriptional analysis.

Study Day 399 (±2 days; 7 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.
4. Obtain approximately 5.0 mL of blood anti-Pfs230 antibody ELISA, and standard membrane feeding assay (SMFA).

Study Day406 (±7 day; 14 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 17.0 mL of blood for CBC with differential and platelet count, ALT, creatinine, malaria blood smear, malaria PCR, anti-Pfs230 antibody ELISA, standard membrane feeding assay (SMFA) and transcriptional analysis.

Study Day 420 (±7 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.

Study Day 448 (±7 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 6.0 mL of blood for malaria blood smear, malaria PCR, anti-Pfs230 antibody ELISA, standard membrane feeding assay (SMFA).

Study Day 504 (±7 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. Obtain approximately 6.0 mL of blood for malaria blood smear, malaria PCR, anti-Pfs230 antibody ELISA, standard membrane feeding assay (SMFA).

Study Day 546 (±28 days; 154 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 7.0 mL of blood for malaria blood smear, malaria PCR, anti-Pfs230 antibody ELISA, standard membrane feeding assay (SMFA) and transcriptional analysis.
5. Scheduled unblinding of VU
    a. If received Pfs230D1M-EPA/ASO1 offer meningitis comparator vaccination

Study Day 644 (±56 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 6.0 mL of blood for malaria blood smear, malaria PCR, anti-Pfs230 antibody ELISA, standard membrane feeding assay (SMFA).

Study Day 728 (±56 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 6.0 mL of blood for malaria blood smear, malaria PCR, anti-Pfs230 antibody ELISA, standard membrane feeding assay (SMFA).

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Appendix C: Toxicity Tables

Local Reactogenicity Grading[1]

Local Reaction to Injectable Product	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)
Pain at site	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	ER visit or hospitalization
Erythema/Redness at site[2]				
>15 years of age	2.5 – 5 cm	5.1 – 10 cm	> 10 cm	Necrosis or exfoliative dermatitis
≤ 15 years of age	≤ 2.5 cm in diameter	> 2.5 cm in diameter with < 50% surface area of the extremity segment involved (e.g., upper arm or thigh)	≥ 50% surface area of the extremity segment involved (e.g., upper arm or thigh)	Potentially life-threatening consequences (e.g., abscess, exfoliative dermatitis, necrosis involving dermis or deeper tissue)
Induration/Swelling at site[3]				
>15 years of age	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
≤ 15 years of age	≤ 2.5 cm in diameter	> 2.5 cm in diameter with < 50% surface area of the extremity segment involved (e.g., upper arm or thigh)	≥ 50% surface area of the extremity segment involved (e.g., upper arm or thigh)	Potentially life-threatening consequences (e.g., abscess, exfoliative dermatitis, necrosis involving dermis or deeper tissue)
Bruising at site[2]				

>15 years of age	2.5 – 5 cm	5.1 – 10 cm	> 10 cm	Necrosis or exfoliative dermatitis
≤ 15 years of age	≤ 2.5 cm in diameter	> 2.5 cm in diameter with < 50% surface area of the extremity segment involved (e.g., upper arm or thigh)	≥ 50% surface area of the extremity segment involved (e.g., upper arm or thigh)	Potentially life-threatening consequences (e.g., abscess, exfoliative dermatitis, necrosis involving dermis or deeper tissue)
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

Abbreviations: ER, emergency room.

[1] The definitions provided in the table are modified versions 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 and DAIDS Table for Grading the Severity of Adult and Pediatric Adverse Events version 2.1 July 2017.

[2] In addition to grading the measured local reaction at the greatest single diameter, the measurement should be recorded as a continuous variable.

[3] Induration/Swelling should be evaluated and graded using the functional scale as well as the actual measurement.

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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; at rest + calm				
Adult (≥13 yo)	101 – 115	116 – 130	> 130	ER visit or hospitalization for arrhythmia
Pediatric (≤ 12 yo)	121 – 135	136 -- 150	>150	ER visit or hospitalization for arrhythmia
Bradycardia - beats per minute[4]; at rest + calm				
Adult (≥13 yo)	50 – 54	45 – 49	< 45	ER visit or hospitalization for arrhythmia
Pediatric (≤ 12 yo)	55 -- 59	50 – 54	< 50	ER visit or hospitalization for arrhythmia
Hypertension (systolic) -mm Hg; at rest + calm				
Adult (≥13 yo)	141 – 150	151 – 155	> 155	ER visit or hospitalization for malignant hypertension
Pediatric (≤ 12 yo)	131 -- 140	141 -- 150	> 150	ER visit or hospitalization for malignant hypertension

Hypertension (diastolic) -mm Hg; at rest + calm				
Adult (≥13 yo)	91 – 95	96 – 100	> 100	ER visit or hospitalization for malignant hypertension
Pediatric (≤ 12 yo)	81 -- 90	91 – 95	> 95	ER visit or hospitalization for malignant hypertension
Hypotension (systolic) -mm Hg; at rest + calm				
Adult (≥13 yo)	85 – 89	80 – 84	< 80	ER visit or hospitalization for hypotensive shock
Pediatric (≤ 12 yo)	80 – 84	75 – 79	< 75	ER visit or hospitalization for hypotensive shock

Abbreviations: ER, emergency room.

[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 > 800 gms/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

Systemic AEs	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)
Seizures- <18 years of age; includes new or pre-existing febrile seizures	Seizure lasting < 5 minutes with < 24 hours postictal state	Seizure lasting 5 to < 20 minutes with < 24 hours postictal state	Seizure lasting ≥ 20 minutes OR > 24 hours postictal state	Prolonged and repetitive seizures (e.g., status epilepticus) OR Difficult to control (e.g., refractory epilepsy)

Abbreviations: ER, emergency room; IV, intravenous; PO, "per os" or oral administration.

[1] The definitions provided in the table are modified versions 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 and DAIDS Table for Grading the Severity of Adult and Pediatric Adverse Events version 2.1 July 2017.

Mali Laboratory AE Grading ≥15 years of age

Hematology and Biochemistry Values1, 2	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)
Hemoglobin (Male) - gm/dL	9.5 – 10.3	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 - 103/µL	11.5 – 15.0	15.1 – 20.0	20.1 – 25.0	> 25.0
WBC Decrease - 103/µL	2.5 – 3.3	1.5 – 2.4	1.0 – 1.4	< 1.0 with fever
Neutrophil/Granulocyte Decrease3 - 103/µL	0.80 – 1.00	0.50 – 0.79	< 0.50	< 0.50 with fever
Platelets Decreased - 103/µL	100 – 110	70 – 99	25 – 69	< 25
Creatinine (Male) - µmol/L	124.00 – 150.99	151.00 – 176.99	177.00 – 221.00	> 221.00 and requires dialysis
Creatinine (Female) - µmol/L	107.00 – 132.99	133.00 – 159.99	160.00 – 215.99	> 216.00 and requires dialysis
Liver Function Tests/ALT - U/L	75.0 – 150.9	151.0 – 300.9	301.0 – 600.0	> 600.0

Abbreviations: ALT, alanine transaminase; WBC, white blood cell.

[1] The laboratory values provided in the tables serve as guidelines and are dependent upon institutional normal parameters. 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.[88,89]

Mali Laboratory AE Grading <15 years of age

Hematology and Biochemistry Values1, 2	Mild (Grade 1)	Moderate (Grade 2)	Severe (Grade 3)	Potentially Life Threatening (Grade 4)
Hemoglobin (Male/Female) - gm/dL	7.5 – 8.4	6.1 – 7.4	5.0 – 6.0	< 5.0 g/dL
WBC Increase - 10^3/µL	14.5 – 16.0	16.1 – 20.0	20.1 – 30.0	>30.0
WBC Decrease - 10^3/µL	2.5 – 3.3	1.5 – 2.4	1.0 – 1.4	< 1.0 with or without fever
Neutrophil/Granulocyte Decrease3 - 10^3/µL	0.75 – 0.99	0.50 – 0.74	< 0.50	< 0.50 with fever
Platelets Decreased - 103/µL	100 – 120	70 – 99	25 – 69	< 25
Creatinine (Male/Female) - µmol/L	95.00 – 119.99	120.00 – 149.99	150.00 – 200.00	> 200.00 and requires dialysis
Liver Function Tests/ALT - U/L	75.0 – 150.9	151.0 – 300.9	301.0 – 600.0	> 600.0

Abbreviations: ALT, alanine transaminase; WBC, white blood cell.

[1] The laboratory values provided in the tables serve as guidelines and are dependent upon institutional normal parameters. 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.[88,89]

Appendix D: Institutional Normal Laboratory Values

Mali Adult (≥15 years of age): Institutional Normal Parameters

Chemistry

Chemistry[1]	Reference Range
Creatinine (Female) - µmol/L	< 72
Creatinine (Male) -µmol/L	48 – 98
ALT - U/L	< 41

Abbreviations: ALT, alanine transaminase.

[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

Abbreviations: ALT, alanine transaminase; WBC, white blood cell.

[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) – RBC/HPF	None or Trace < 5

Abbreviations: HPF, high power field; RBC, red blood cell.

[1] The laboratory values provided in the table are based on Bancoumana, Malian adults (age 18-45 years old)

Children (5-15 years of age)

Hematology Reference Intervals*

Hematology	Reference Interval	Units
WBC- Leukocytes	4.5 – 10.5	$10^3/\mu L$
Hemoglobin	9.6 – 13.5	g/dL
Platelet Count	138– 455	$10^3/\mu L$
Absolute Lymphocyte Count	1.8 – 5.4	$10^3/\mu L$
Absolute Granulocyte Count**	1.12 – 6.86	$10^3/\mu L$

Based on children ranging in age from 6 to 14 years old (based on sampling done at Doneguebougou site, Mali)
**Based on reference intervals for a Ugandan population,

Biochemistry Reference Intervals*

Chemistry	Reference Interval	Units
ALT	5.06 – 53.4	U/L
Creatinine	< 48.9	μM/L

Parameters from Pediatric Tables in Mali; MRTC, Dept of Epidemiology and Parasitic Diseases, Faculty of Medicine, Pharmacy and Odontostomatology, University of Bamako, Mali

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Appendix E: References

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